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**Volume 6 Number 1 & 2 October 2019 - February 2020**

# Academia and Society

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### Articles

# A Comparative Study on the Composting Performance of Garden Leaf Litter into Vermicompost using *Eudrilus eugeniae*

[1]*Dr. Jayanthi,* [2]*Dr. Cecily Rosemary Latha R, and* [3]*Dr. Rajathi Modilal*
[1,2,31]*Assistant Professor*
*PG and Research Department of Zoology,*
*Holy Cross College (Autonomous)*
*Tiruchirappalli- 620-002.*

**Abstract**

Vermicomposting is a simple biotechnological process of composting, in which certain species of earthworms are used to enhance the process of waste conversion and produce a better nutrient enriched product. The organic manure of vermicompost produced by using garden leaf litter such as *Couroupita guianensis* (Nagalinga tree), *Ficus religiosa* (Peepal tree) and *Prunus amygdalus* (Badam tree) with cow dung in 50:50 proportion and the epigeic earthworm species of *Eudrilus eugeniae*. The composting performance of garden leaf litter and reproductive potential of earthworms were observed. The physico-chemical parameters were analysed. The outcome of the present study perceptibly suggested that all the three garden leaf litter (*Couroupita guianensis, Ficus religiosa* and *Prunus amygdalus*) with cowdung in 50:50 proportions was a suitable substrate for the production of vermicompost.

**Key words:** Vermicompost, *C. guianensis, F. religiosa, P. amygdalus* and *E. eugeniae*

## Introduction

The potential of vermicomposting as a viable alternative method for waste management is gaining momentum all over India and globally. In recent years, earthworms in addition to microorganisms are important to process the biodegradable organic matters and to produce a nutrient rich organic fertilizer. Fallen dry leaves are available in large quantities throughout the year. The leaf litter is a major source of global organic matter. It has an important bearing on soil formation and its fertility. Leaf litter decomposition is an essential progression of the nutrient cycling in ecosystems (Satchell, 1983). Garden leaf litter is one of the most important plant wastes and used for the preparation of food materials for earthworms all over the world. Traditionally, the fallen leaf litter were left in the campus for natural degradation, which usually takes several months for conversion. Besides the leaves of these trees, have medicinal properties to cure various ailments. In the present investigation all the three medicinal plant leaves were subjected to vermicomposting process in order to obtain a valuable product *i.e.,* vermicompost.

*C. guianensis* is a traditional medicinal plant. The flowers are used to make perfume products. The fruit is edible, but not usually eaten by people because it has an unpleasant smell. It is otherwise called C*annonball* tree, *Jambolana* tree and *Nagalinga* tree (Sivakumar *et al.,* 2012). Peepal trees are native in Indi, which thrive in hot and humid conditions. They prefer full sunlight and grow in moist soil types, though loam is the best. *F. religiosa* has been used in traditional medicine for about 50 types of disorders. It's otherwise called *Bodhi* tree, *Ashwatha* and Peepal tree (Chandrasekar *et al.,* 2010). *P. amygdalus* is a deciduous tree. It is otherwise called Badam tree and Almond tree (Ali and Jamei, 2012). The aim of the present investigation is to find out the suitable substrates for vermicomposting process using garden leaf litter and analyze the physico-chemical parameters of vermicompost.

## Methodology

The garden leaf litter of *C. guianensis, F. religiosa* and *P. amygdalus* were separately collected. The collected garden leaf litter were cured in both open and shade places for 10 days. Simultaneously a similar method was adapted for cow dung. The vermibeds were prepared in 50:50 proportion *i.e.,* cured leaf litter mixed with cured cow dung and they filled in plastic trays separately. Water was sprinkled twice in a day. After 10 days mature, clitellate earthworm species of *E. eugeniae* were inoculated into the vermibed. During the entire composting period the moisture and temperature condition of the vermibed was maintained. Further, the vermicompost was collected and enumeration of cocoons and juveniles are done in each tray.

The physico-chemical parameters of vermicompost were analysed by the following method suggested by Tandon, 2005 and the organic carbon was estimated by the method of Walkey and black, 1974. All the statistical analyses were made by using SPSS (Statistical Package for Social Science) program version 16.0.for windows.

**Results and Discussion** The composition of pre-digested *C. guianensis, F. religiosa* and *P.amygdalus* leaf litter and cow dung in 50:50 proportion and their bioconversion into vermicompost by using *E.*

*eugeniae* is given in Table 1. The total weight of the pre-digested food mixture taken for the present study was 3000g. The experimental trays of *F. religiosa* produced maximum quantity of vermicompost (2548g) and took minimum number of days (33days) for bioconversion of leaf litter into vermicompost compared with other leaf litter. The *F. religiosa* leaf litter produced highest number of cocoons and juveniles than others (Fig. 2). The result of the present study is in concordance with the previous report of Darwin (1881) depicting the role of earthworms in the rapid decomposition of plant leaf litter and animal residues in the soil.

**Table 1: Composition of vermicompost produced from the garden leaf litter of *C. guianensis, F.religiosa* and *P.amygdalus* using *Eudrilus eugeniae*.**

| Particulars | *C.guianensis* | *F.religiosa* | *P.amygdalus* |
|---|---|---|---|
| Weight of garden litter (g) in each tray | 1500 | 1500 | 1500 |
| Weight of Cow dung(g) in each tray | 1500 | 1500 | 1500 |
| Total weight of predigested mixture (g) in each tray | 3000 | 3000 | 3000 |
| Mean number of days taken for Predigestion in each tray | 14 | 12 | 18 |
| Number of adult earthworms introduced in each tray | 105 | 105 | 105 |
| Mean number of days taken for Composting in each tray | 35 | 33 | 42 |
| Mean total weight of vermicompost obtained in each tray (g) | 2374 | 2548 | 2480 |

**# Experiments were conducted in triplicates in each tray.**

**Figure1: Comparison of Cocoons & Juveniles production in vermicompost from selected leaf litter of *C. guianensis, F.religiosa* and *P.amygdalus* in 50:50 concentrations with cow dung using *E. eugeniae***

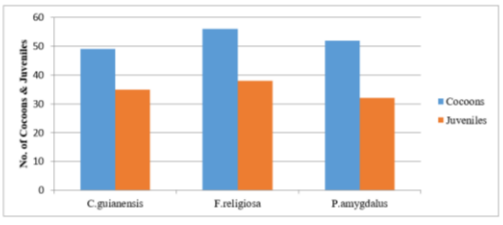


**Fig1. Enumeration of cocoons and juveniles in vermicompost**

The reproductive potential of earthworms is influenced by the quality and availability of food (Neuhauser *et al.*, 1979). Vermicast contains rich nutrients and promotes microbial activity required by the plants (Ramalingam and Ranganathan, 2001). It is understood from the results that the pH of the vermicompost harvested from all the trays were found to be within the standard values of vermicompost.

**Table 2: Magnitude of physico chemical parameters of vermicompost produced by selected leaf litter (*C. guianensis, F.religiosa* and *P.amygdalus*) with *E.eugeniae* in 50:50 concentration.**

| Parameters | *C.guianensis* M±SD | *F.religiosa* M±SD | *P.amygdalus* M±SD |
|---|---|---|---|
| pH | 7.04±0.1 | 7.0±0.1 | 7.03±0.01 |
| Electrical Conductivity ($dSm^{-1}$) | 3.78±0.06 | 1.91±0.01 | 2.14±0.04 |
| Organic Carbon (%) | 16.5±0.02 | 15.96±0.04 | 16.4±0.02 |
| Total Nitrogen (%) | 1.44±0.04 | 1.55±0.04 | 1.27±0.01 |
| Total Phosphorous (%) | 4.1±0.02 | 5.25±0.02 | 3.96±0.02 |
| Total Potassium (%) | 3.56±0.04 | 4.28±0.08 | 4.03±0.02 |
| Calcium (%) | 10.5±0.02 | 14.1±0.03 | 6.80±0.03 |
| Magnesium (%) | 5.24±0.02 | 7.04±0.1 | 4.95±0.03 |
| C:N | 11:1 | 10:1 | 12:1 |

The pH is an important factor because it's affect the availability of nutrients in the soil (Jayanthi and Neelanarayanan, 2010). The mean Electrical Conductivity and Organic Carbon values were observed to be low in *F. religiosa* produced vermicompost and high in others. Organic carbon is very essential for soil health (Karmegam and Daniel, 2012). The vermicompost collected from *F. religiosa* showed greater content of other nutrients namely total Nitrogen, total Phosphorous, total Potassium, Calcium and Magnesium than others. The micronutrients namely total Zinc (Zn), total Copper (Co) and total Manganese (Mn) content were also observed high in *F. religiosa* produced vermicompost.

**Figure 2: Comparison of Micronutrients of vermicompost produced by using selected leaf litter (*C. guianensis, F.religiosa* and *P.amygdalusw*) with *E.eugeniae***

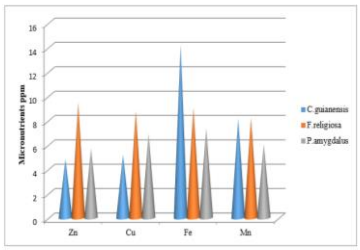


**Fig. 2 Micronutrients of vermicompost**

The maximum level of total Iron (Fe) was noticed in *C. guianensis* leaf litter produced vermicompost compared with others (Fig.2). The C:N

ratio of vermicompost was noticed minimum in *F. religiosa* and maximum in *P.amygdalus*. C:N ratio is the main condition that determines the quality of vermicompost. High nutrient content and other valuable properties of vermicompost that helps in improve the healthy crops. It is necessary for the development of good root system. The garden leaf litter of *C. guianensis, F. religiosa* and *P.amygdalus* also produce a good quality of vermicompost. The vermicomposting process helps in improving soil fertility and minimizes the use of chemical fertilizers. This eco-friendly technology makes organic recycling much more active and enhances plant growth. Vermitechnology also provides opportunities to the rural people for self-employment by utilizing the available agricultural resources.

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## A Survey on Cluster Based Routing Protocols in Mobile Wireless Sensor Networks


***[1]Dr. K. Juliet Catherine Angel, [2]Dr. N. Karpagavalli, [3]Dr. P. Revathi***

*[1,2,3]Assistant Professors,*
*Department of Computer Science,*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002*



**Abstract**

With the developments in the Mobile Technology, Mobile Wireless Sensor Networks (MWSN) has evolved as a new trend in Wireless Technology. The mobility of the node and the sink in the Wireless Sensor Network technology has paved way to Mobile Wireless Sensor Network. The dynamic nature of the network changes the topological structures while the number of nodes increases or decreases due to mobility in order to balance the network performance. A number of routing protocols have been developed to reduce the energy consumption and increase the network lifetime. This paper presents focuses on few routing protocols and provides a comparative analysis based on metrics like Mobility Pattern, Mobile Element, Network Architecture, Intra/Inter Cluster Routing, Stability, Protocol Operation, Communication Paradigm and Protocol Objectives.



**Keywords**

MWSN, Clustering, Routing, Communication


**Introduction**

Mobile Wireless Sensor Networks has evolved due to the rapid development in the mobile technology and Wireless Sensor Network Technology. MWSN[1] is a Wireless Sensor Network with the nodes in mobility which has an increased coverage, connectivity, scalability, reliability, balanced energy consumption and network lifetime. The flexibility of the network results in powerful computing capacity, communication capacity, and storage capacity. The mobility of the nodes and the sink in the Mobile

Wireless Sensor Network[2] improves the coverage rate efficiently, connectivity, scalability, reliability, reduced energy consumption and prolongs the network's lifetime. Hence MWSN is greatly used in real time applications like military, industrial control, nuclear radiation, environment for safety, monitoring systems, wearable medical monitoring systems, large-scale agricultural applications, and mobile forest fire warning system.

**Clustering in MWSN**

Clustering [3][4] is an energy efficient technique where the mobile sensor nodes are grouped into non-overlapping groups. Each cluster will be governed and monitored by a head node, Cluster Head (CH) which collects the data from all the cluster members and aggregates the same. The aggregated data will then be forwarded to the Base Station. This reduces the energy consumption and increases the network lifetime.

**Related Work**

**LEACH Mobile Protocol**

LEACH-Mobile protocol [5] was developed to providing data communication within the MWSN. The protocol confirms the presence of a mobile node within a cluster and thereby the node transmits the data to the CH within the allotted time slot. in TDMA schedule. The node that left the cluster due to mobility joins a new cluster by broadcasting a cluster join request message for which it will receive an ACK message from the new CH. As LEACH-Mobile assumes that the CHs are stationary it is not efficient in terms of energy dissipation and data delivery.

**Cluster-Based Routing Protocol**

Cluster Based Routing Protocol was designed to adapt mobility and traffic by maintaining separate databases for MWSNs (CBR Mobile) [6] for collecting data from the mobile sensor nodes. It maximizes the packet delivery ratio and minimize the average delay. The protocol is based on MAC design which reuses the free or unused timeslots for data transmission. The disconnected Cluster Members rejoins the network through another CH within a short span of time. Thus the protocol reduces packet loss compared to the LEACH-Mobile protocol but as it has high control overhead it consumes more energy than LEACH-Mobile and reduces the network lifetime.

**Mobility-Based Clustering Protocol**

Mobile Based Clustering(MBC) Protocol [7] consists of two phases namely a setup phase and a steady-state phase in each round similar to the LEACH protocol. Based on the energy and the mobility a sensor node is elects itself as a CH in the setup phase. The protocol depends upon the stability and the availability of the link between a Member Node and CH for building a cluster with reliable paths. The intra-cluster and inter-cluster communication performs the data transfer to the sink in every steady state phase. The protocol outperforms the other protocols during mobility but fails in addressing the issues which causes link breakage, packet dropping, and reducing of the network utilization.

**Energy-Efficient Competitive Clustering Algorithm**

An Energy-Efficient Competitive Clustering (EECC) algorithm[8] is a distributed clustering algorithm which uses a controlled mobile sink in order to improve the performance of MWSNs and mitigates the hotspot problem. The network is divided into clusters with a CH based on the residual energy. CH collects and aggregates data from its members. The mobile sink moves at a certain speed along a predefined line path, which is located at the center of the sensor field, and sojourns at some certain equidistant locations to collect data packets from neighboring CHs. EECC algorithm outperforms the LEACH protocol [24] in terms of energy consumption and network lifetime.

***3.1.5 Enhanced Cluster-Based Routing Protocol***

Enhanced Cluster Based Routing Protocol ECBR[9] for MWSN consists of five phases. They are initialization phase, cluster formation phase, CHs selection phase, data transmission phase, and reclustering and rerouting phase. A CH is selected based on the highest remaining energy, lowest mobility factor and least distance to the sink. It enhances the lifetime of the MWSNs by balancing the energy consumption. ECBR-MWSN clustering approach is more energy-efficient and hence effective in prolonging the network lifetime.

**Optimizing LEACH Clustering Algorithm.**

Optimizing LEACH Clustering Algorithm combines the use of the LEACH algorithm, mobile sink, and rendezvous points [10] to reduce the consumption energy. The operation of optimizing LEACH was divided into rounds. Each round starts with a setup

phase followed by a steady state phase. The setup phase consists of three stages: task ordination, cluster setup, and scheduling. In the task ordination, CHs and Rendezvous Nodes (RNs) are selected. Based on the distance between sensor nodes and CHs clusters are developed in the setup stage. Each CH broadcasts a schedule to its MNs to organize node transmission timing in the scheduling stage. In the steady-state phase, CHs collect the data from its MNs and forwards it to the mobile sink or to the nearest living RN. The mobile sink sends signals called beacons to notify the RNs about its arrival by moving along a predetermined line trajectory. The protocol decreases the energy consumption in MWSNs when compared to the traditional LEACH, particularly when the network is large.

**Comparative Analysis**

The Clustering Protocols namely LEACH-M, CBR-Mobile, MBC, EECC, ECBR and OLCA are compared based on Mobility Pattern, Mobile Element, Network Architecture, Intra/Inter Cluster Routing, Stability, Protocol Operation, Communication Paradigm and Protocol Objectives.

***Table 1: Comparative Analysis***

| Protocol | Mobility Pattern | Mobile Element | Network Architecture | Intra/Inter Cluster Routing | Stability | Protocol Operation | Communication Paradigm | Protocol Objectives |
|---|---|---|---|---|---|---|---|---|
| LEACH-M | N/A | Sensor Nodes | Block Based | SH/SH | V | Coherent Based | Node Centric | Maximize Lifetime, Supports Mobility |
| CBR-Mobile | Random using RWP | Sensor Nodes | Block Based | SH/SH | V | Coherent Based | Node Centric | Maximize Delivery Ratio; Minimize Average Delay |
| MBC | Random using RWP | Sensor Nodes | Tree Based | SH/MH | V | Coherent Based | Node Centric | Improves Packet Delivery Rate, Energy Consumption, Control Overhead |
| EECC | Predefined | One Sink | Block Based | SH/MH | V | Coherent Based | Node Centric | Maximize Lifetime; Solves The Hotspot |
| ECBR | Random using RWP | Sensor Nodes | Tree Based | SH/MH | V | Coherent Based | Node Centric | Maximize Lifetime; Balances Load |
| OLCA | Predefined | One Sink | Block Based | SH/SH | V | Coherent Based | Node/Location Centric | Maximize Lifetime; Minimize Energy Consumption |

**Conclusion**

Even though many routing protocols have been developed this paper reviews a few cluster based routing protocols developed for MWSN. A comparative analysis is done among the cluster based routing protocols namely LEACH-M, CBR-Mobile, MBC, EECC, ECBR and OLCA based on Mobility Pattern, Mobile Element, Network Architecture, Intra/Inter Cluster Routing, Stability, Protocol Operation, Communication Paradigm and Protocol Objectives. The reviewed protocols are evaluated and compared on the basis of delay, network size, and energy efficiency while highlighting the features and drawbacks of each protocol.

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# Study of Fuzzy Clustering in Temporal Data Mining

**[1]Dr. J. Mercy Geraldine, [2]Dr. G. Arockia Sahaya Sheela, [3]N. Kannammal**

[1,2,3]*Assistant Professor,*
*Department of Computer Science,*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002*

**Abstract**

Huge volumes of data are widely available in many organizations and there is an ardent need for deriving useful information from these data. Data mining is one of the efficient techniques for extraction of meaningful information from large volumes of data. It searches for relationships and frequently occurring patterns that are hidden in the data. In real life, data is associated with time and is denoted as temporal data. There are many issues related with mining useful information of interest to the user from large volumes of data. One of them is identifying frequently occurring patterns from temporal databases which is a mammoth task. Searching of an item and the time taken for it are improved by clustering. Fuzzy clustering results in accurate clusters. High quality clusters are obtained by clustering which leads to accurate temporal patterns.

**Keywords**

Temporal data mining, Fuzzy clustering, Frequent itemset, High quality clusters

## Introduction

Huge amount of data is accumulated in most of the organizations and there is a lack of techniques to derive useful information from them. Data mining is one of the efficient techniques for extraction of meaningful information from large volumes of data. It is concerned with analyzing large volumes of unstructured data to automatically discover interesting regularities or relationships which are hidden in them [1]. The information and knowledge gained by data mining can be used in a wide range of applications including fraud detection, market analysis, weather forecasting, remote sensing and many more. For example, the weather forecasting dataset which includes various parameters such as, temperature, cloud cover, rainfall, relative humidity, wind speed, and wind direction can be used to identify the climatic changes in the near future. This knowledge helps people to be prepared to face the natural calamities and protect themselves from its harmfulness. Data mining helps to identify potential locations of undetected natural resources using large volumes of remote sensing image data gathered by satellites. Also, it can be used in the analysis of medical data to identify the remarkable outbreak of infectious diseases. The application areas of data mining are huge in number and are bound to increase rapidly in the forthcoming years. The conventional methods of mining involve static databases. In real life, data is associated with time and is denoted as temporal data. Temporal database consists of data that implies the presence of time as a mandatory field. Applications which use temporal mining are important because they deal with time related data called as temporal data. Temporal mining performs association rule mining on temporal data, that is, time-based data.

## Literature Review

The refinement of clusters using the combination of K-means and ACO was introduced by Mary & Raja (2009). It had certain drawbacks which were overcome by their proposed method stated as follows. Mary & Raja (2010) described a cluster refinement method to improve the clusters formed by FCM. These clusters were validated using Xie-Beni validity index. The ant-based clustering algorithm left space for improvement in many ways. Also, the algorithm was dependent on many of the parameters such as pheromone, agent memory, number of agents, number of iterations, cluster retrieval and so on. Ants were used to refine the clusters rather than clustering the data points, for which they have been used so far. Wang & Zhang (2007) introduced the fundamental concepts of cluster validity and presented a review of the available fuzzy cluster validity indices. An analysis of the experimental results of the various validity indices in conjunction with FCM algorithm on various datasets had been presented. This helped in identifying the validity index suitable for the particular algorithm with specific parameters and datasets with unique properties.

## Temporal Data Types

**A. Fully Temporal:** It is time dependent. Data and information derived from it are completely dependent on time. Ex: Transactional data in databases [2].

**B. Time Series:** This is a special case of time stamped data. It is similar to a number line. The events are uniformly separated in time dimension. Time series and temporal sequences, are seen in a variety of domains like engineering, research, medicine and finance.

**C. Time Stamped:** It has explicit information related to time. Temporal distance between data elements can be found. Inferences made can be temporal or non-temporal. Ex: data from stock exchange, inventory management.

**D. Sequences:** Sequences are ordered events with or without a concrete notion of time. Ex: customer shopping sequences, biological sequences. If an event appears before another, it means that the former event has occurred before the latter.

**Temporal Data Mining Tasks**

Data mining has a wide range of applications. Tasks of data mining can be classified into some broad groups. In case of temporal data mining, these groups are Prediction, Classification, Clustering, Search and retrieval, Association.

**A. Association Analysis**

Association analysis is the discovery of relationships between attributes and value pairs showing attribute value conditions that occur frequently together in a given set of data [1][2][3]. The relationship is represented as an association rule of the form $X => Y$ where $X$ and $Y$ are sets of items and this rule must satisfy two interesting measures, support and confidence. Support measures the frequency of two items occurring together as a percentage of the total transactions. Confidence measures the dependency of a particular item on another. Given a database, the goal is to discover all the rules that have the support and confidence greater than or equal to the minimum support and confidence respectively. For example, a grocery store would like to analyze the buying nature of its customers with respect to the attributes like income, age, locality, job, etc. Association rule mining can be applied to temporal association as well. The general association rule X =>Y i.e. if X occurs then Y occurs, can be extended to a new rule X => tY i.e. if X occurs then Y will occur within time t. This new rule enables us to control the occurrence of an event to another event, within a time interval.

**B. Cluster Analysis**

Clustering is a method of grouping data into different groups, so that data in each group share similar trends and patterns. Clustering groups the data on the basis of a similarity measure, like Manhattan distance, Euclidian distance. The objectives of clustering include, uncovering natural groupings, initiating hypothesis about the data, and finding consistent and valid organization of the data. The principle of clustering is to maximize intra-class similarity and minimize inter-class similarity [3]. Clustering analyses data objects without considering known class label. Clustering finds its use in grouping people on the basis of their common behavior and identifying areas affected by harmful diseases.

**Clustering of Temporal Data**

Clustering is the process of assigning data into groups or clusters in such a manner that similar data items are present in the same group and dissimilar data items belong to different groups. Clustering algorithms are of two types, hard (crisp) clustering and soft (fuzzy) clustering. In hard clustering, the data elements are divided into unique clusters, where each data element is present exactly in one cluster. In soft clustering, data elements are present in more than one cluster, and a set of membership levels is associated with each data element. The fuzzy clustering algorithms are of two types, classical fuzzy clustering algorithms and shape based fuzzy clustering algorithms. One of the classical fuzzy clustering algorithms is the Fuzzy C Means (FCM) algorithm [4].

The main idea of fuzzy clustering is to partition the data into a group of clusters in a non-unique manner [5]. The data points are given membership values with regard to each cluster and fuzzy clustering algorithm is responsible for clusters growing into their different shapes. Data elements on the boundaries between several classes are not considered to entirely belong to one of the classes, but instead are allotted membership degrees between 0 and 1 indicating their extent of membership.

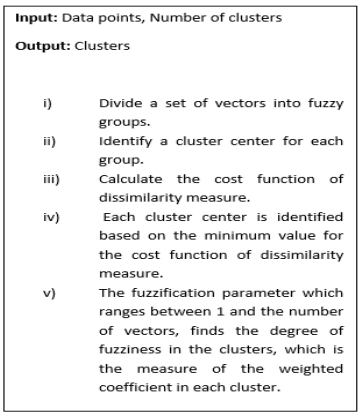


**Fig. 1. General concept of FCM**

**Quality of Clusters**

Clustering is the process of collecting the data into groups based on their similarity. The principle of clustering is to attain "high intra group similarity and low inter group similarity". A cluster is said to be of high quality if it satisfies the above principle. There are numerous cluster validity indices like Partition Coefficient, Partition Entropy, Fukuyama and Sugeno, Xie and Beni, Separation and Compactness etc., that are available to assess the quality of the cluster [6]. And also use of such validity indices does not need the predefinition of the number of clusters or fixed number of clusters. This helps in attaining enhanced clusters.

The cluster efficiency is evaluated using squared error, an intra-cluster measure and is explained as follows. Optimal clusters should minimize distance within clusters (intra-cluster) and maximize distance between clusters (inter-cluster). Squared error (*se*) is an intra-cluster measure [7][8]. It is denoted by Equation (1). Lesser value of *se* denotes efficient clusters.

$$se = \sum_{i=1}^{k} \sum_{p \in c_i} \left\| p - m_i \right\|^2 \qquad (1)$$

where

$m_i$ - mean of all instances in cluster $c_i$

$k$ - number of clusters

$p$ - instance of cluster $c_i$

The PCAES validity index is best suited for fuzzy clustering algorithms as it validates clusters based on the compactness and separation measures with proven accuracy and it is also capable of identifying noise in the clusters.

Cluster validity is used to evaluate the cluster quality. In the case when the number of clusters is not known apriori, the validity index may be used to find the optimal number of clusters. Although many validity indices are available, PCAES has been chosen to validate the clusters because of the drawbacks present in other validity indices which are stated as follows.

Partition Coefficient (*PC*) and Partition Entropy (*PE*) considered only the compactness of each cluster and not the data structure. It did not take into account the geometrical structure of the data. In the validity index given by Fukuyama and Sugeno (*FS*), a good separation measure was neither available for the cluster nor for the data structure and had the possibility of resulting in unexpected results. The validity index of Xie and Beni (*XB*) measured the compactness of each cluster and the data structure using the FCM objective function and the separation measure was calculated for all clusters and not for each cluster. The Separation and Compactness (*SC*) validity index was a complicated measure and it considered the data structure and not the individual clusters for defining the compactness and separation measures [6][9]. PCAES validity index is chosen because of its ability to identify compact and well separated clusters and data structure as well as it identifies noisy points in the clusters. The larger the PCAES index value the more compact and well separated are the clusters. PCAES index for a cluster '*i*' is defined by Equation (2) as

$$\mathrm{PCAES}_i = \sum_{j=1}^{n} \mu_{ij}^2 / \mu_M - \exp\left( - \min_{k \neq i} \{ \|a_i - a_k\|^2 \} / \beta_{\mathrm{T}} \right) \qquad (2)$$

where

$$\mu_M = \min_{1 \leqslant i \leqslant c} \left\{ \sum_{j=1}^{n} \mu_{ij}^2 \right\} \qquad (3)$$

$$\beta_{\mathrm{T}} = \frac{\sum_{l=1}^{c} \|a_l - \bar{a}\|^2}{c} \qquad (4)$$

The normalized partition coefficient is given by

$$\sum_{j=1}^{n} \mu_{ij}^2 / \mu_M$$

The exponential separation value is represented as

$$\exp\left( - \min_{k \neq i} \{ \|a_i - a_k\|^2 \} / \beta_{\mathrm{T}} \right)$$

Therefore, the PCAES validity index is given by Equation (5) as

$$\mathrm{PCAES}(c) = \sum_{i=1}^{c} \mathrm{PCAES}_i \qquad (5)$$

**Conclusion**

There are many issues related with mining useful information of interest to the user from large volumes of data. One of them is identifying frequently occurring patterns from temporal databases which is a mammoth task. Also optimizing the process of

temporal mining seems to be an unsolved problem Therefore, there is a need for an efficient algorithm for enhancement of temporal mining. Searching of an item and the time taken for it are important criteria in frequent itemset mining for determining the efficiency of the temporal mining process.

Clustering of temporal data prior to mining increases the efficiency of the frequent itemsets. The efficiency of the frequent itemsets greatly depends on the quality of the clusters extracted. The resulting clusters are validated using the Partition Coefficient and Exponential Separation (PCAES) validity index which portrays the quality of the clusters. Thus, Temporal mining on high quality clusters may lead to effective and efficient extraction of temporal rules.

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## A Review on Hierarchical Clustering Algorithm


***[1]V. Amala Deepa, [2]M.Amutha Gracy Alexis, and [3]A.Emima***

*[1,2,3]Assistant Professors*
*Department of Computer Science,*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*



**Abstract**

Clustering is a task of assigning a set of objects into groups called clusters. In data mining, hierarchical clustering is a method of cluster analysis which seeks to build a hierarchy of clusters. Strategies for hierarchical clustering generally fall into two types: Agglomerative: This is a "bottom up" approach: each observation starts in its own cluster, and pairs of clusters are merged as one moves up the hierarchy. Divisive: This is a "top down" approach: all observations start in one cluster, and splits are performed recursively as one moves down the hierarchy. This paper presents the review of Hierarchical Clustering Algorithms which are used for clustering data.



**Keywords**

Clustering, Agglomerative, Divisive.


**Introduction**

Clustering is the process which divides the data into groups called clusters such that one cluster's object are identical to each other and different cluster's object are unlike to each other [1].

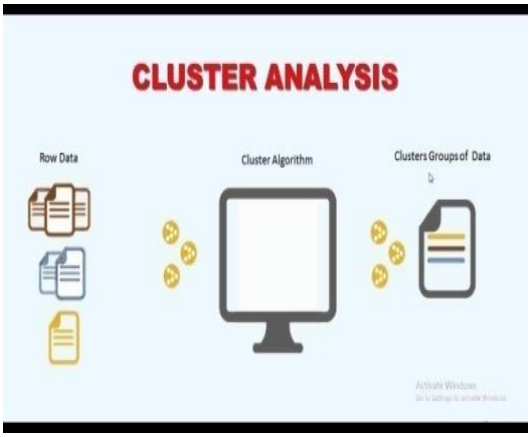



**Fig 1: Outline of Clustering**

Clustering is a common tactic for statistical data analysis, which is used in many fields, including machine learning, data mining, pattern recognition, image analysis, document retrieval and bioinformatics. Clustering can be considered the most

important unsupervised learning problem. There are several methods to do clustering, and the criteria of choosing a specific method essentially relies upon three considerations - data set size, data dimensionality and time complexity.

**Applications of Clustering Algorithms:**

a) Marketing: finding groups of customers with similar behaviour given a large database of customer data containing their properties and past buying records
b) Biology: classification of plants and animals given their features
c) Libraries: book ordering
d) Insurance: identifying groups of motor insurance policy holders with a high average claim cost; identifying frauds
e) City-planning: identifying groups of houses according to their house type, value and geographical location
f) Earthquake studies: clustering observed earthquake epicentres to identify dangerous zones
g) WWW: document classification; clustering weblog data to discover groups of similar access patterns

**Requirements to be satisfied:**

The main requirements that a clustering algorithm should satisfy are:

a) scalability
b) dealing with different types of attributes
c) discovering clusters with arbitrary shape
d) minimal requirements for domain knowledge to determine input parameters
e) ability to deal with noise and outliers
f) insensitivity to order of input records
g) high dimensionality
h) interpretability and usability

**Literature Review**

The following are the few research contributions done by researchers. Tung-Shou Chen et al. [2] introduced a new method, hierarchical k-means regulating divisive or agglomerative approach. They applied this method in two original microarray datasets. Finally, they concluded divisive hierarchical clustering on cluster quality is superior to k-means on clustering on computational speed.

F. Murtagh [3] discussed a general framework for hierarchical, agglomerative clustering algorithms, which opens up the prospect of much improvement on current, widely-used algorithms. The progress report details new algorithmic approaches in this area, and reviews recent results.

Ying Zhao and George Karypis [4] evaluated different partitional and agglomerative approaches for hierarchical clustering. Their experimental evaluation showed that partitional algorithms always lead to better clustering solutions than agglomerative algorithms, which suggests that partitional clustering algorithms are well-suited for clustering large document datasets due to not only their relatively low computational requirements, but also comparable or even better clustering performance. They also presented a new class of clustering algorithms called constrained agglomerative algorithms that combine the features of both partitional and agglomerative algorithms. The experimental results showed that they consistently lead to better hierarchical solutions than agglomerative or partitional algorithms alone.

P. A. Vijaya et al. [5] proposed an efficient hierarchical clustering algorithm, suitable for large data set. They also proposed effective clustering and prototype selection for pattern classification. As an example, a two level clustering algorithm—Leaders–Subleaders, an extension of the leader algorithm is presented. Classification accuracy (CA) obtained using the representatives generated by the Leaders–Subleaders method is found to be better than that of using leaders as representatives.

**Main Types of Clustering Algorithms**

Clustering algorithms are classified as [6]:

a) Partitioning methods
b) Hierarchical clustering
c) Fuzzy clustering
d) Density-based clustering
e) Model-based clustering

**Partitioning methods:** Divide the data set into various groups or partitions and evaluate them according to some criteria. E.g: k-means, k- Medoids, CLARANS

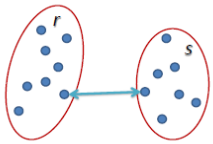
**Hierarchical Clustering:** It works by grouping data objects into a tree of clusters i.e. it performs Hierarchical decomposition, by some particular criteria. It uses several popular methods like Diana, Agnes, BIRCH, ROCK, CURE [7].

Hierarchical clustering techniques use various criteria to decide at each step which clusters should be joined

as well as where the cluster should be partitioned into different clusters. It is based on measure of cluster proximity.

The following methods differ in how the distance between each cluster is measured [8].

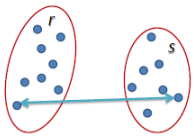
**Single link:** The distance between two clusters to be the smallest distance between two points such that one point is in each cluster.




$$L(r,s) = \min(D(x_{ri}, x_{sj}))$$

**Fig 2: Single Link**

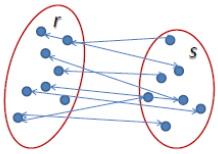
**Complete link:** The distance between two clusters to be the largest distance between two points such that one point is in each cluster.




$$L(r,s) = \max(D(x_{ri}, x_{sj}))$$

**Fig 3: Complete Link**

**Average link:** The distance between two clusters to be an average distance between two points such that one point is in each cluster.




$$L(r,s) = \frac{1}{n_r n_s} \sum_{i=1}^{n_r} \sum_{j=1}^{n_s} D(x_{ri}, x_{sj})$$

**Fig 4: Average Link**

**Fuzzy Clustering:**

Fuzzy clustering (also referred to as soft clustering or soft *k*-means) is a form of clustering in which each data point can belong to more than one cluster.

**Density-based Clustering:**

This method clusters based on a local criterion such as density-connected points. The major features of the clustering include the abilities to discover clusters of arbitrary shape and handle noise. The algorithm requires just one scan through the database. However, density parameters are needed for the termination condition. The algorithms in this category are DBSCAN], OPTICS, DENCLUE, CLIQUE, and so on.

**Model-based Clustering:**

These algorithms find good approximations of model parameters that best fit the data. They can be either partitional or hierarchical, depending on the structure or model they hypothesize about the data set and the way they refine this model to identify partitioning. They are closer to density-based algorithms, in that they grow particular clusters so that the preconceived model is improved. However, they sometimes start with a fixed number of clusters and they do not use the same concept of density.

**Hierarchical Clustering**

Hierarchical clustering involves creating clusters that have a predetermined ordering from top to bottom. For example, all files and folders on the hard disk are organized in a hierarchy.
There are two Strategies for hierarchical clustering. They are as follows:

a. Agglomerative
b. Divisive

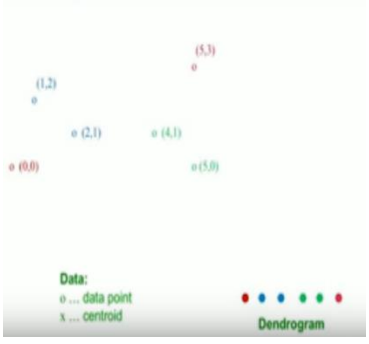
**Agglomerative:** It is a "bottom-up" approach: initially, each data point is a cluster; repeatedly combine the two “nearest” clusters into one.

**Key Operation:** Repeatedly combine the two nearest clusters into a largest cluster

**Terminologies:**

Data points - set of point

Centroid - The average of two points



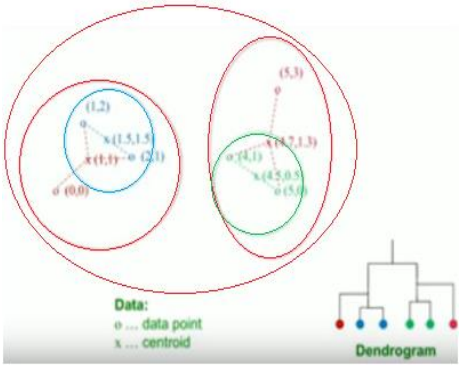
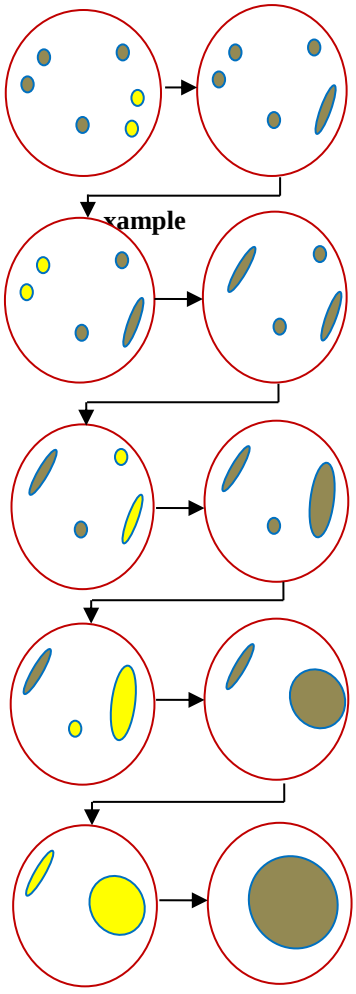



**Fig 5(A):**

**Agglomerative Clustering E**

| | |
|---|---|
| Identify the two clusters that are | Merge the two most similar |



**xample**

**Fig 5(B): Agglomerative Clustering Example**

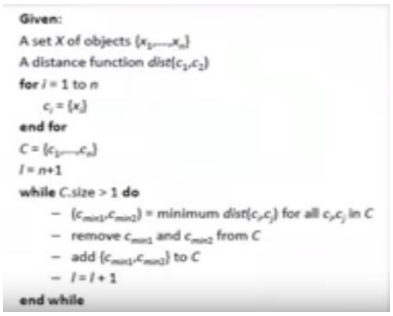



**Fig 6: Agglomerative Algorithm**

**DIVISIVE:** It is a "top-down" approach: all the data points are in a single cluster; recursively split it until there is one cluster.


**Fig 7: Example for Divisive Algorithm**

Step 1: Begin

Assign number of cluster=number of objects.

Step 2: Repeat:

When number of cluster = 1 or specify by User.

a) Find the minimum inter cluster

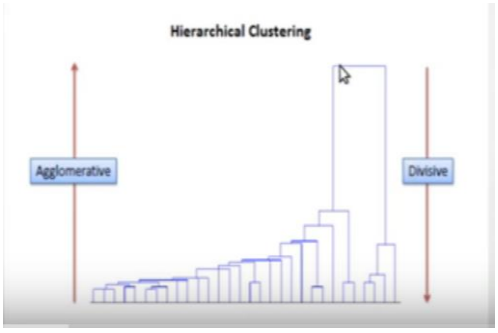



**Fig 9: Agglomerative vs. Divisive**

| Agglomerative Clustering | Divisive Clustering |
|---|---|
| Starts with a single data point | Starts with a big cluster |
| Add two or more clusters recursively | Divide into smaller clusters recursively |

**Table 1: Agglomerative vs. Divisive**

## Conclusion

In this paper, a study has been performed on Hierarchical Clustering Algorithm. The main points regarding this technique have been discussed in detail. This paper also provides a quick review of the different clustering techniques in data mining. The drawback is Hierarchical Clustering is not commonly used for big data sets that don't fit in memory. It is really used for small data sets that fit in memory. For handling large data sets other methods of clustering are used.

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# A Comparative Study of Identifying Frequent Item Set Using Apriori and FP-Growth Algorithm


***[1]A.Emima, [2]V.Amala Deepa,[3]M.Amutha Gracy Alexis***

*[1,2,3]Assistant Professors*
*Department of Computer Science,*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*



## Abstract

In recent years a burly, torrential deluge of data, everywhere. But not quite compartmentalized. While mining the frequent item set in massive database, it will lead to find the association among items in huge database. In this paper, two algorithms are comparing of finding Frequent itemsets are discoursed: Apriori algorithm finds the itemset which is repeated, at that point the majority of its subsets should likewise be repeated and generate promising candidate itemset and tests if they are repeated. FP growth strategy is done by constructing FP-Trees to mine the incessant data from expansive database and it does not generate candidate itemsets. This two algorithm comparison which guides the enterprise for decision making processes like mobile ecommerce, students performance, disease prediction etc.



## Keywords

Frequent item set, Apriori, Candidate, FP growth, FP tree.


## Introduction

In general, Data mining is the way toward breaking down information from alternate points of view and condensing it into helpful data [1]. Association mining is an essential strategy for imperative connections in extensive datasets. A few successive frequent itemsets mining strategies have been proposed utilizing a apriori, FP growth, Eclat algorithm [2]. Association rule mining can be

isolated into two sections: discover all frequent item sets, and generate consistent association rules from all frequent item sets. [3] For finding the possible best frequent item set apriori and FP growth algorithms are used. Assosiation among frequent item set will lead to identify the frequent pattern of data to be retrieved in future. Apriori algorithm has to do large number of Scans for deciding standards of association among items, it takes generally longer to finish the mining procedure. FP growth comparatively quicker than Apriori Algorithm as the as the scanning process is just carried out once [4]. In this paper Apriori algorithm and FP growth algorithm are compared in the sample dataset to identify which is fast, reliable to find frequent pattern. The rest of the paper is organized as follows: Section 2 describes the literature review, Section 3 illustrate dataset in Aprirori algorithm 4 depict dataset in FP algorithm. Section 5 explains the Comparitive study of Apriori and FP algorithm concepts and Section 6 concludes the paper with future work directions.

**Literature Review**

Finding frequent pattern in any dataset is quite tedious. Appling data mining algorithms make dataset to segregates as per the frequent pattern we achieved. The following are the few research contributions done by researchers to deal these challenges.

Pan Zhaopeng et al [5] Max-IFP maximum frequent patterns mining calculation, utilizing the new age of FP - tree uncovered all the greatest frequent thing sets. The exploratory outcomes demonstrate that the new

FP-tree involves a little space, and the calculation proposed in this paper is shorter and more successful than different calculations when mining the most extreme incessant thing The development technique for the new FP-tree proposed in this paper spares a considerable measure of space while developing the FP-tree but time consuming is questionable

Md.Mahamud Hasan et al [6] utilized Binomial Circulation (BD) to discover minimum support adaptively. It has been mined ideal with incessant itemsets. The difficulties of setting minimum threshold is reduced as well as the proper execution time of Apriori and FP growth algorithm is found but segregation of data is violated.

Chunhua Fu et al [7] explained the paralleled FP-Growth calculation executed precisely mine frequent item sets, with a superior execution and versatility but the modify of minimum support scale on the operation of the algorithm is more obvious.

Bekti Cahyo Hidayanto et al [8] identified the data retrieved from Intrusion Detection Systems sensor using Frequent Item Set Mining. In comparison, every pattern in FP-Max is equal with Apriori and they found that malware frequently occurred are SQL attack, Malware Virut DNS and DoS. Alhassan Bala et al [9] utilized Weka to look at two calculations (Apriori and FP-development) in light of execution time and database scan parameters but the number of occasions, confidence and support levels it is completely evident that FP-Growth calculation is superior to apriori calculation.

Arkan A. G. Al-Hamodi et al [9] discussed the strategy actualized the EFPGrowth in light of MapReduce structure utilizing Hadoop approach based on their technique, the carrying out time under different minimum supports is decreased.

Pamba Pravallika et al [10] Medical data is implemented on Data mining to make rules and patterns using Frequent Pattern (FP)-Growth algorithm they discussed how Medical data is applied in data mining.

**Association Rule Mining**

The problem of discovering all association rules from a set of transactions consists of generating the rules that have a support greater than a given threshold. These rules are called strong rules.

This association mining task can be broken into two steps [14]:

A. A stage for discovering all continuous k-item sets known for its outrageous I/O filter cost, and the monstrous computational expenses, and

B. A clear advance for creating strong rules.

The commonest indicators of association rule strength are support and confidence. sup(X) designate the frequency of X in information. the boldness of (X⇒Y) is outlined as,Confidence (X ⇒ Y) = sup (X ∪Y) / sup(X). Association rules mining formula aims to go looking a frequent itemsets convention user **specific** minimum support and confidence, then generate association rules required [11].

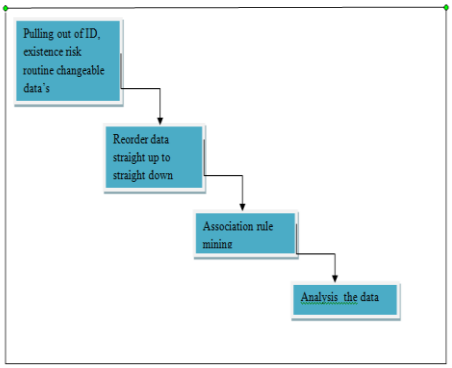



Fig. 1 Process of Association Rule Mining

**General Process of Apriori Algorithm**

The Apriori algorithmic rule is a prestigious algorithmic rule for the mining of frequent items. Apriori uses a " bottom up " approach wherever frequent subsets are extended one item at a time can be call it as a candidate generation. Basic algorithms is as follows:[1]

1. Generate candidate of length n from dataset.

2. Any (n-1) itemset that isn't always common can't be subset

Of frequent k-itemset.

3. Generate c_n : candidate object set of size n.

L_n: frequent itemset of size n

Four. Generate c_n+1 itemsets from l_n

5. Generate l_n+1 candidate with min_support

6. Return generated candidates in l_n+1.

A. Computation of Apriori Algorithm:

Find the frequent item sets in the database of Five transaction with Minimum support=60% minimum confidence=40% using Apriori algorithm

| Transaction ID | Items bought |
|---|---|
| T1 | {S,P,A,R,R,O,W} |
| T2 | {C,R,O,W} |
| T3 | {C,R,E,W} |
| T4 | {R,O,W} |
| T5 | {W,A,T,E,R} |

**Step 1:** Scan D for count of each candidate.

The Candidate list is {S, P,A,R,O,W,T,E,C}

and find the support

Generate C1:

| Items | No.of Transactions |
|---|---|
| S | 1 |
| P | 1 |
| A | 2 |
| R | 6 |
| O | 3 |
| W | 5 |
| T | 1 |
| E | 2 |
| C | 2 |

**Step 2:** Compare candidate support with minimum support count (60%)

$I_1$:

| Items | No.of Transactions |
|---|---|
| R | 6 |
| O | 3 |
| W | 5 |

**Step 3:** Generate candidate C2 from L1

$C_2$: Scan items for count of each candidate in C2 and find the support

| Items | No. of Transactions |
|---|---|
| R,O | 3 |
| O,W | 3 |
| W,R | 5 |

**Step 4:** Compare candidate with minimum support=3

$I_2$: All are satisfied with minimum support

Generate candidate C3 for {R, O, W}

| Items | No. of Transactions |
|---|---|
| R,O,W | 3 |

The database contain the frequent item set is{R,0, W}Therefore the association rule that can be created form $I_2$ can be shown below with support and confidence

| Association Rule | Support | Confidence | Confidence % |
|---|---|---|---|
| R->O | 3 | 3/6=1/2 | 50% |
| O->W | 3 | 3/3=1 | 100% |
| W->R | 3 | 3/6=1/2 | 50% |

Minimum confidence threshold is 40%. Since all the association rule satisfied confidence percentage so all can be frequent pattern

**General Process of FP-Growth Algorithm**

The Frequent Pattern (FP)- Growth technique is utilized with databases and not with streams. It is an elective method to discover visit itemsets without utilizing candidate generation, in this manner it enhancing the execution. By utilizing the FP-Growth technique, the quantity of sweeps of the whole database can be diminished to two.

The principle thought of the calculation is to utilize a divide and conquer methodology:

Horde the database which gives the successive sets; at that point isolate this compacted database into an arrangement of contingent databases, each related with an incessant set and apply data mining on every database.

- FP-Tree is constructed using 2 cycles over the data-set:

Cycle 1:

- Scan data and discover support for every items.
- Discard rare items.
- Sort visit things in diminishing request based on their support.

Nodes compare to items and have a counter

Cycle 2:

- FP-Growth peruses 1 exchange at once and maps it to a way
- Settled request is utilized, so ways can cover when exchanges share items.
- For this situation, counters are increased
- Pointers are kept up between nodes containing a similar thing, making singly linked list. (spotted lines).
- Visit itemsets extricated from the FP-Tree

**A. Computation of FP-Growth Algorithm:**

We have taken the same item set which is used in Apriori to find the frequent patterns in the database of Five transaction with Minimum support=3 and confidence=40% using FP-Growth algorithm

| Transaction ID | Items bought |
|---|---|
| T1 | {S,P,A,R,R,O,W} |
| T2 | {C,R,O,W} |
| T3 | {C,R,E,W} |
| T4 | {R,O,W} |
| T5 | {W,A,T,E,R} |

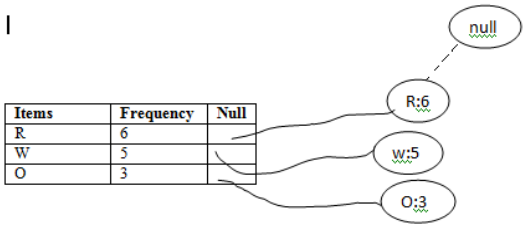
Step 1: Find the frequency of Occurrences

Generate C1:

| Items | Frequency |
|---|---|
| R | 6 |
| W | 5 |
| O | 3 |

Step 2: Prioritize the items

| Transaction ID | Items bought | Order Items |
|---|---|---|
| T1 | {S,P,A,R,R,O, W} | R,W,O |
| T2 | {C,R,O,W} | R,W,O |
| T3 | {C,R,E,W} | R,W |
| T4 | {R,O,W} | R,W,O |
| T5 | {W,A,T,E,R} | R,W, |




| Items | Frequency | Items | Conditional FP tree |
|---|---|---|---|
| R | 6 | O | O:3 |
| W | 5 | W | W:5 |
| O | 3 | R | R:6 |

| Association Rule | Support | Confidence | Confidence % |
|---|---|---|---|
| R->O | 3 | 3/6=1/2 | 50% |
| O->W | 3 | 3/3=1 | 100% |
| W->R | 3 | 3/6=1/2 | 50% |

All the association rule satisfied the minimum support for frequent pattern.

**Comparison of Apriori and FP-growth:**

In this section Apriori and FP-Growth were compared and analayzed through simple dataset. And the comparison with various criteria are discussed below:

**A) APRIORI**

**i)Methods:**

It creates singletons, pairs, triplets, etc.

**ii)Run time:**

Candidate creation is enormously time-consuming. Runtime enlarge exponentially depend upon the number of dissimilar items.

**iii)Memory Usage**

Preserve singletons, pairs, triplets, etc.

**iv)Parallelizability**

Candidate creation is very parallelize.

**B) FP- GROWTH:**

**i) Methods:**

It includes sorted items by regularity into a pattern tree.

**ii) Run time:**

Runtime elevate linearly, depends on the number of items and transactions

**iii) Memory Usage**: Stores a compacted description of the database.

**iv)Parallelizability**

Data are extremely burying reliant, each one node desires the root

Table 1: Comparison of Apriori and FP-Growth

| Uniqueness | Data sustain | Rapidity in initial phase | Rapidity in later phase | Accurateness |
|---|---|---|---|---|
| APRIORI | Restricted | Elevated | Sluggish | Fewer |
| FP-GROWTH | Very Hefty | Elevated | Elevated | More Accurate |

**Conclusion**

In this paper, we have got made a comparative look at on Apriori algorithm and FP increase in set of rules Both the calculations effectively mine the frequent pattern from database. Were, Apriori finds the regular itemsets with candidate itemset generation however FP Growth calculation finds the frequent itemsets without candidate itemset so requirement of time is less. Were, Apriori, it generating more candidate set hence it required more memory. In Apriori more scan is necessary but in FP Growth number of scan is less, since no candidate itemset is used. Here we conclude FP-Growth is best to mine the frequent pattern than Apriori algorithm.

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## A Study on K-NN and Naive Bayes Classification Algorithm in Data Mining

***[1]M.Amutha Gracy Alexis,***
***[2] A.Emima,[3] V.Amala Deepa***
*[1,2,3]Assistant Professors*
*Department of Computer Science,*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*

**Abstract**

Data mining is characterised as a procedure used to remove usable data from a bigger arrangement of any crude information. There are a substantial number of classification algorithms in data mining such as ANN algorithm, SVM algorithm, ID3 algorithm, Sence Clusters and so on. This paper concentrated on KNN (K- Nearest Neighbors) and Naive Bayes Algorithm. The k-Nearest-Neighbors (KNN) is a basic yet viable technique for classification. It is one of the primary ten data mining calculations, has been commonly associated in various fields. The Naive Bayes Classifier system depends on the Bayesian hypothesis. It is especially used when the dimensionality of the sources of info is high. The objective of this paper is to give a review of k-Nearest-Neighbors and Naive Bayes Algorithm.

**Keywords**

Data Mining, Algorithm, Classification, Techniques, KNN, Naive Bayes.

**Introduction**

A technique is a particular process of doing an activity, usually a method that involves practical skills. Data mining is the method of arrangement through large data sets to identify patterns and establish relationships to solve problems through data analysis.

Data mining strategies are utilized in many research zones, including arithmetic, artificial intelligence, hereditary qualities and retail. The seven most common data mining techniques are tracking patterns, classification, association, outline detection clustering, regression and prediction.

Classification techniques in data mining are capable of processing a large amount of data. It is used to forecast clear-cut class marks and arranges information dependent on preparing set and class names and it tends to be utilized for ordering recently accessible data [1].

There are several major sets of classification algorithms including Machine Learning Based

Approach, Statistical Procedure Based Approach, and Machine Learning Based Approach etc.

K - Nearest Neighbors is a powerful classification algorithm used in pattern recognition. KNN is a basic algorithm that stores every single accessible case and arranges new cases dependent on a comparability measure. The nearest neighbor rule recognizes the arrangement of an obscure information point. KNN is a calculation in view of machine learning, there are very few preparing parameters, the computational intricacy is not high, and the execution is agreeable, so we picked KNN as our framework system [2].

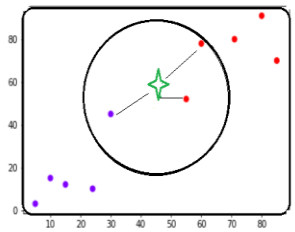


Fig1.1 K - Nearest Neighbors

Bayesian classifier depends on Bayes theorem. The Bayesian Classification speaks to an administered learning strategy and a measurable technique for arrangement. Naive Bayes has proven effective in many practical applications, including text classification, medical diagnosis, and systems performance management [3].

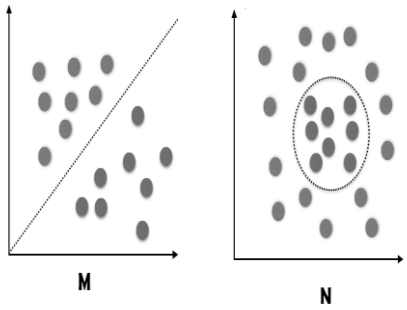


Fig1.1 Naive Bayes Algorithm

**Literature Review**

Data Mining is not only widely used in the sense of the sectors of industry where it has been applied but also in its applicability to a wide range of tasks [10]. The following are the few research contributions done by researchers to explain about the classification algorithm.

Badria Abaker Ibrahim [10] explained some data mining algorithms has been used to offer a solution to classification problems in databases. To explain this task, comparison between the k-nearest neighbor (K-NN)

Yun-lei Cai, Duo Ji ,Dong-feng Cai[2] presents KNN text categorization method based on shared nearest neighbor, effectively combining the BM25.

Hamid Parvin,Hoseinali Alizadeh,Behrouz Minati[4] improving the performance of KNN classifier is proposed which is called Modified K-Nearest Neighbor, MKNN. The proposed method, which considerably improves the performance of KNN method, employs a kind of pre-processing on train data.

(Gou, Jianping and Ou, Weihua) propose a generalized mean distance-based k-nearest neighbor classifier (GMDKNN) by introducing multi-generalized mean distances and the nested generalized mean distance that are based on the characteristic of the generalized mean. In the proposed method, multi-local mean vectors of the given query sample in each class are calculated by adopting its class-specific k nearest neighbors.

Haiyi Zhang ; Di Li[11] discussed the Naive Bayes is a classification algorithm which is based on Bayes theorem with strong and naïve independence assumptions. It simplifies learning by assuming that features are independent of given class. Naïve Bayes is a subset of Bayesian decision theory. It has called naive because the formulation makes some naïve assumptions.

[11] Naive Bayes classifier is widely used in machine learning for its simplicity and efficiency. However, most of the existing work on naïve Bayes focused on improving the Bayes model itself or whether the "naïve assumption" is satisfied.

Amit Ganatra [12] concentrated on data mining techniques have been used in clinical decision support systems for prediction and diagnosis of various diseases with excellent accuracy. The statistics show that heart diseases (or cardiovascular disease) are one of the leading causes of deaths all over the world. Many researchers are using statistical and data mining techniques for the diagnosis of heart disease. Naive Bayes is one of the simple data mining technique has shown better result and accuracy

**K – Nearest Neibors**

K-Nearest Neighbor (KNN) classification algorithm is one of the most central and basic order techniques. It

is the simplest classifier of all machine-learning algorithms.

It is more widely used in classification problems in the business. To assess any strategy, we for the most part take a gander at three essential viewpoints:

a. Simplicity to decipher yield

b. Computation time

c. Prophetic Power

**Process of K- Nearest Neighbors Algorithm:**

K-Nearest Neighbor algorithm is very simple. It is one of the basic classification algorithms in data mining. It has been used in statistical estimation and pattern recognition. The following steps despite how to compute K-Nearest Neighbor.

i. Determine parameter K = number of nearest neighbors.

ii. Take the k nearest neighbors of the new data point

iii. Calculate the distance between the query-instance and all the training samples according to the Euclidean function.

$D = \sum_{i=1}^{n}$ $(X_i - Y_i)^2$

iv. Sort the distance arest neighbors based on the K-th minimum distance.

v. Group the category of nearest neighbor

vi. Return the discrete value.

**Benefits:**

- Extremely basic and perceptive
- Great grouping if the quantity of tests is sufficiently vast
- It can be applied to the data from any distribution
- Provides good generalisation accuracy on many domains
- It is very simple technique that is easily implemented
- Versatile—useful for classification or regression

**Drawbacks:**

- Computationally exclusive - because the algorithm stores all of the working out data
- Classification time is long
- Difficult to find optimal value of K
- It has substantial capacity necessities since it needs to store every one of the data
- Calculation stage might be slow
- High memory requirement

**Naïve Bayes Algorithm**

Naïve Bayes is a subset of Bayesian decision theory. It has called naive because the formulation makes some naïve assumptions [6].

Naive Bayes classifiers are a assortment of classification algorithm dependent on Bayes' Theorem. It is anything but a solitary calculation yet a group of algorithms where every one of them shares a typical guideline, i.e. each combine of highlights being arranged is autonomous of one another.

**Process of Naïve Bayes Algorithm:**

The Naive Bayes classification algorithm is a probabilistic classifier. It depends on prospect models that consolidate solid autonomy presumptions. A Naive Bayes model consists of a large cube that includes the following magnitudes:

a. Input field name

b. Input field value for discrete or continuous fields.

c. Target field value

To comprehend the guileless Bayes classifier, we have to comprehend the Bayes hypothesis. So allows first talk about the Bayes Theorem.

Bayes theorem named after Rev. Thomas Bayes. It takes a shot at contingent probability. Restrictive probability is the probability that something will occur, given that something unique has just happened. Utilizing the contingent probability, we can ascertain the probability of an occasion utilizing its earlier information.

The following formula is for calculating the conditional probability. Utilizing the conditional probability, we can ascertain the probability of an occasion utilizing its earlier information.

$$P(R/S) = \frac{P(S|R) * P(R)}{P(S)}$$

P(R/S) - Probability of instance S being in class R.

P(S|R) - Probability of generating instance S given class R.

P(R) - Probability of occurrence of class R

P(S) - Probability of instance of class R

**Benefits:**

- It is relatively simple to understand and build
- It is easily implement and very fast
- Great outcomes got in a large portion of the cases
- Handle missing qualities by disregarding the instance during probability estimate calculations.

**Drawbacks:**

- Assumption: It expects each component is independent, which is not generally the situation.
- Class conditional independence, which ay cause loss of accuracy
- The primary impediment is that the Naive Bayes classifier makes an exceptionally solid presumption on the state of your information dissemination, i.e. any two highlights are free given the yield class. Because of this, the outcome can be conceivably terrible - henceforth, a "guileless" classifier.

**Conclusion**

This paper presents the study of K – Nearest Neighbor and Naive Bayes Algorithm. In data mining, when using Naive Bayes classification technique, it is necessary to overcome the problem of how to deal with continuous attributes. The Bayesian Classifier is capable of calculating the possible output. KNN (k-nearest neighbor) is an extensively used classification algorithm owing to its simplicity, ease of implementation and effectiveness. It is one of the top ten data mining algorithms, has been widely applied in various fields. We can also implement the KNN algorithm using ball tree, k-d tree, and orthogonal search tree.

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## Smart Health Tracking System

***[1]Dr. D. Roselin Selvarani,***
***[2]Ms. J. Vinnarasi***
***[3]Ms. S. Amirthavalli,***
***[4]Ms. L. Liciya Jenifer***
*[1,2]Assistant Professors*
*[3,4]MCA Students*
*Department of Computer Science,*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*

**Abstract**

The recent development of Smart technologies has brought changes in Healthcare arena significantly. Healthcare has become one of the application domain of Internet of Things (IoT). Due to the emergence of mobile technology and IoT, a reliable Smart Health Care system is made possible with promising improvement in economic, social and technological aspects. Nowadays, many people who work full time are facing a problem of tracking their loved ones when they face health issues. They need someone who can take care of the sick person at home in their absence and in case, if any illness happened suddenly, they should be informed immediately so as to take action soon. In order to overcome this problem, a reliable Smart Health Tracking System is designed using IoT. This system is used effectively to monitor and detect the health conditions using sensors such as temperature sensor, pressure sensor, heartbeat sensor and drip sensor and compute the value using the microcontroller and display the result on LCD. If any deviation from the normal range is identified by the system, immediately alert or call will be sent to the person in-charge (care taker) or doctor of the sick person immediately. Thus, this system is used to find the current status of the health condition and if any deterioration of health is identified, it will help the care taker to get the information immediately so that they can take the necessary action soon. Timely detection and proper treatment at an early stage of the sickness is the main advantage of this system.

**Introduction**

The recent development of Smart technologies has brought dramatic changes in every walk of our life Today Internet assumes a noteworthy tool in our day by day life and has changed how individuals live, work, play and learn. It tends to be used for some reasons, for example, Business, Educations, Entertainment, Finance, Industries, Social Networking, E-Commerce and so forth. The present pattern of Internet will be Internet of Things (IOT). Visualizing an existence where a few objects can detect, impart and share data over a Private Internet Protocol (IP) or Public Networks. The IOT is commonly considered as connecting things to the Internet and utilizing for control of those things or remote monitoring. Yet, this definition was alluded uniquely to part of IOT development considering the machine to machine showcase today. However, real meaning of IOT is making a splendid, undetectable system which can be detected, Controlled and modified.

One of the global challenge for humanity is Good Health. According to the constitutions of World Health Organization (WHO) the highest attainable standard of health is a fundamental right for an individual. In traditional method, doctors play an important role in health checkup. For this process requires a lot of time for registration, appointment and then checkup. Also reports are generated later. Due to this lengthy process working people tend to ignore the checkups or postpone it.

When people get severe health issue, their heartbeat, temperature and pressure are some of the parameters to be continuously monitored. It is possible in hospital, where the monitoring facility is available. But once they returned from the hospital, there is no provision to check the parameters. And hence there is a chance that the disease may return again. Therefore, there is a need for developing a monitoring system that can be used anywhere. Due to this requirement, a secure reliable, cost effective health monitoring system is developed.

**Review of Literature**

In paper [1], the authors propose another strategy for ECG observing dependent on light weight Message Queuing Telemetry Transport convention. ECG information is assembled utilizing an ECG checking sensor by Texas Instruments and are transmitted through ADS1115 16-piece ADC interfaced with Raspberry Pi utilizing I2C convention. The computerized ECG sensor information got from ADC is distributed to a CloudMQTT agent utilizing MQTT mosquitto customer utilizing IEEE 802.11 (WLAN) in-buit in Raspberry Pi 3. MQTT buy in is utilized to envision the ECG utilizing a GUI at any remote social insurance place, emergency clinic or treating specialist.

In paper [2], the authors intend to monitor Heart beat with android application which is used to measure the heart beat of different persons. It is built using Android Studio. It consists of various based on the system requirement, software specification, hardware requirement and disk space.

In paper [3], the authors present a new method for continuously measuring and monitoring patient's health conditions using sensors, web server and android application. The doctor can continuously monitor the patient's condition on his smart phone using an Android application and the patient history will be stored on the web server and doctor can access the information whenever needed from anywhere.

In paper [4], the authors survey advances in IoT-based health care technologies and reviews the state-of-the-art network architectures/platforms, applications, and industrial trends in IoT-based health care solutions. They also propose an intelligent collaborative security model to minimize security risk and discuss how different innovations such as big data, ambient intelligence, and wearables can be leveraged in a health care context. They also provide some avenues for future research on IoT-based health care systems and a set of open issues and challenges.

In paper [5], the authors provide an algorithm that controls the load in Wi-Fi networks to guarantee the delay requirement for physiological signals, especially for emergency messages, in environments with coexistence of ZigBee-based WBAN and Wi-Fi. Since Wi-Fi applications generate traffic with different delay requirements, they focused only on WiFi traffic that does not have stringent timing requirements. An adaptive load control algorithm was proposed for ZigBee-based WBAN/Wi-Fi coexistence environments, with the aim of guaranteeing that the delay experienced by ZigBee sensors does not exceed a maximally tolerable period of time. Simulation results showed that their proposed algorithm guarantees the delay performance of ZigBee-based WBANs by mitigating the effects of Wi-Fi interference in various scenarios.

In paper [6], the author gives a brilliant wellbeing observing framework that utilizes biomedical sensors to check patient's condition and uses web to advise the concerned individuals. The biomedical sensors are associated with Arduino UNO controller to peruse the information which is thusly interfaced to a LCD show/sequential screen to see the yield. Information is transferred to the server to store and changed over it into JSON connect for picturing it on a Smartphone. An android application has been structured so as to effectively observe the patient's data by their primary care physicians and relatives.

**Design and Experiment**

Smart Health Tracking system is developed using Software-Hardware co-design**.** The hardware components of the system are PIC16f877a microcontroller, Temperature sensor, Pressure Sensor, Heart beat sensor and Drips Sensor, GSM and LCD display. The software is developed using Embedded C.

**Components used in the Kit**

**Microcontroller**

Microcontroller is a general purpose gadget, which incorporates some of the segments of a microchip framework on to single chip. It has inbuilt CPU, memory and peripherals to make it as a small PC.

**PIC16F877A**

PIC represents Peripheral Interface Controller begat by Microchip Technology to recognize its single chip microcontrollers. These gadgets have been wonderfully effective in 8-piece microcontroller showcase. The primary explanation is that Microchip Technology has continually updated

**Heart Beat Sensor**

An individual's pulse is the sound of the valves in his/her's heart contracting or extending as the power blood starting with one district then onto the next. The occasions the heart thumps every moment (BPM), is the heart beat rate and the beat of the heart that can be felt in any corridor that falsehoods near the skin is the beat.

**Pressure Sensor**

The interest for pressure estimating instruments expanded during the steam age. At the point when weight detecting advancements were first produced they were mechanical and utilized Bourdon tube checks to move a needle and give a visual sign of weight. These days we measure pressure electronically utilizing pressure transducers and weight switches.

**Temperature Sensor**

The interest for pressure estimating instruments expanded during the steam age. At the point when weight detecting advancements were first produced they were mechanical and utilized Bourdon tube checks to move a needle and give a visual sign of weight. These days we measure pressure electronically utilizing pressure transducers and weight switches.

**Drips Sensor**

A drips sensor is an electronic gadget, that discharges so as to detect a few parts of the environment. A sensor can quantify the warmth of an article just as identifies the movement. These sorts of sensors gauges just infrared radiation, instead of discharging it that is called as a drips sensor.

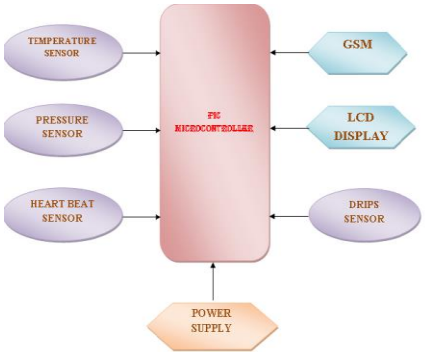


**Fig.1 Block diagram of Health Tracking System**

The heartbeat of the patients is measured using heartbeat sensor and check against normal value (60-100bpm). If the measured level is lower or higher than that of the required level and alert (sms / call) is given to caretaker/doctor. The temperature of the patients is measured using heartbeat sensor and check against normal value (97°F to 99°F). If the measured level is lower or higher than that of the required level and alert (sms / call) is given to caretaker/doctor. The pressure of the patients is measured using pressure sensor and check against normal value 20/80 mmHg from 120/80mmHg and up to 139/89mmHg are in the normal to high range. If the measured level is lower or higher than that of the required level and alert (sms/call) is given to caretaker/doctor. The drips level of the patients is measured using and check against normal value. .If the measured level is lower or higher than that of the required level and alert (sms/call) is given to caretaker/doctor.

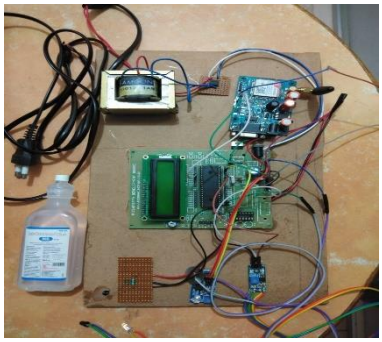

**Fig.2 Health Tracking Kit**

Fig. 3 Fig. 4 Fig. 5

Fig.3-5 Initializing the Sensors

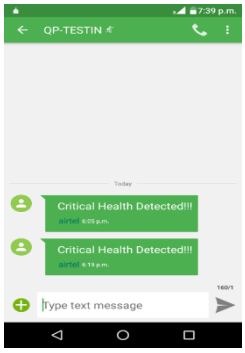


**Fig.6 Screenshot for sending alert message**

**Conclusion**

The Kit for Smart Health Tracking System has been successfully designed and tested. It has been developed based on IoT to monitor the patients, collect the data from the sensors, calculate, diagnose and alert the Care takers when there is a deviation found in their health issues related to temperature, heart beat and pressure. It also identifies the level of the drip and send the message if it goes beyond the level.

In future, the system can be enhanced by incorporating more sensors to measure the other required parameters related to health issues as the new issues are increased day by day. Once the kit is developed and tested, it can be converted to wearable device so that even the very sick patient can be easily watched by the care takers from anywhere else.

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## A Survey on Software Frameworks in Parallel Execution of Serial Code to Achieve High Performance

***[1]J. Arockia Mary, [2]P. Mercy and [3]P. Xavier Jeba***
*[1,2,3]Assistant Professors*
*Department of Computer Science,*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*

**Abstract**

A software framework is a concrete platform consist of generic functions with common code. These generic functions can be modified by the user but the framework only control the flow of execution and not by the user of the framework. A software framework in High performance computing applications is doing different jobs. Different software frameworks are developed to easily execute High Performance Computing applications in many core architectures and also in distributed systems by user. Today's executing environment consists of cluster and cloud computing with distributed processors and with many core architectures because of hardware enhancements and distributed storage spaces to store vast amount of data like big data. So, there is a need for parallel execution of applications to achieve high performance and high degree of parallelism. This paper describes various software frameworks that uses different methods to convert serial code applications into parallel code and also to decompose data and arrange them in different processes in order to execute the applications in HPC environment and also time critical applications.

**Keywords**

high performance computing, Many core architecture, Parallel computing, Task-based parallel programming, Scientific computing

**Introduction**

Today the execution environment is changed. The execution environment is changed from single core architecture to multicore architectures. Many applications are written already to solve scientific and time critical problems are in serial and executed in a single core architecture. Now these applications are to be executed in multicore architectures in parallel to achieve high performance. These applications are not rewritten in parallel format instead software frameworks are converting these serial applications into parallel applications. When converting serial into parallel tasks, the input is also partitioned for parallel tasks. These type of frameworks are working on large data sets such as big data. It uses shared memory. When tasks are partitioned, then the input data is also partitioned. Identification of data dependency between parallel tasks are done by these frameworks. Some frameworks find using of python language for parallel conversions has lot of advantages. Any new ideas in research can be easily prototyped in python because of high quality numerical libraries in python. Most of the HPC applications are written in python language is CPython[1]. Applications are written in CPython using MPI wrapper to execute computations in parallel otherwise user include infrastructure details in the application to run application parallel. The user parallelize python applications based on the platform such as symmetric multiprocessing, cluster computing and grid computing. Some frameworks use Lustre which is a synchronous data-flow language and its industrial version, Scade, is used for time-critical applications, such as in avionics. Tasks are time triggered. Some framework concentrates on Scientific applications which are executed multiple times typically with different parameter configurations, running concurrently, composing parallel workflows. These tasks have multiple processes and the degree of parallelism often varies when different numbers of tasks are running with the different numbers of processes. This framework increase degree of parallelism and use shared

memory. Scheduling processes to tasks and decomposition of data into processes are written manually. Some framework studies the program and arrange number of tasks and decompose data automatically to increase parallelism for today's architecture and avoid hand written code. It also parallelize the sequential code by using transformations [2, 3] in which some sequential code is parallelized. Some other works analyze the code to find independent tasks so that they are executed in parallel [4]. Genetic Programming is applied to parallelize code [5, 6] but same semantics cannot be preserved in parallel programs. The Virtual Savant [7] framework works on observations and does not use the original source code. It uses Machine Learning to observe and generate new parallel program. It is suitable to non-deterministic programs. Task based parallel programming models [8] are developed to meet the challenges of parallel programming for multicore hardware. In this programming paradigm, the programmer work is reduced to write an algorithm into tasks and to access the shared data, at the same time scheduling and execution of tasks in parallel are managed by DuctTeip [9] framework.

This paper shows how different software frameworks are used to implement parallel implementation of sequential code. The remainder of the paper is structured as follows. Section 2 classifies five different frameworks based on data accessing methods, partitioning of codes, computing environments and framework models. Section 3 describes the current use of these frameworks in scientific environment. Finally, section 4 concludes this paper.

**Classification of Frameworks**

Number of frameworks are developed to convert serial applications into parallel applications to be executed in heterogeneous architectures. These frameworks are automatically generating parallel codes as well as partition the data. Table 1summarizes the features supported by these frameworks described below.

**PyCOMPSs framework:**

In this framework, the user detects the functions to be executed as asynchronous parallel tasks and cover them with a standard Python decorator. It automatically finds the concurrency of the script, finds the data dependencies between tasks and send these tasks to the available resources, which can be nodes either in a cluster, cloud or grid. Python decorators are written as function calls with additional properties such as input data type, number of inputs and output data type for the functions. Python scripts are wrapped by python decorators are executed by PyCOMPSs [10] and draw the dependency graph. This graph shows the parallel tasks. This graph consists of nodes and edges. Nodes are tasks and edges are data dependencies. PyCOMPSs uses java run time system to parallelize the tasks and executes in different environment such as cloud, cluster and grid computing. The number of available resources are represented in XML. The java runtime system allocates tasks in the available resources and execute these tasks in parallel. Scheduling a task in a particular resource based upon resource with highest score. The score is based on number of needed inputs that are already present in that resource for a scheduled task. So that inputs are not needed to transfer to this particular resource. Tasks execution speed is decreased. PyCOMPSs framework use large datasets as big data objects storing in persistent storage system like file. Data are stored as python objects. Objects are stored in persistent memory. Python object is stored in persistent dictionary like key-value pair. These keys are stored in resources in which it will be used by PyCOMPSs runtime. So that these data become local to the task. The StorageAPI helps the PyCOMPSs runtime system to locate the resources where data is stored, in which tasks are using those data. It uses HECUBA interface to interact with non-relational databases. This interface is used with Apache Cassandra database. It is a non-centralized architecture and peer to peer communication. So each node can contact with every other node and send and receive the queries from other nodes in a cluster.

**Parallel Code Generation of Synchronous Programs for a Many-core Architecture [11]**

Parallel code is generated for many core architectures for Lustre programs using shared memory or NoC (Network on Chip). The tasks are time triggered and reduced interference and WCTT (Worst Case Traversal Time). WCTT are bounded by existing framework and also find the release dates of tasks. A Lustre program is called a node. It consists of sub nodes connected by data flow network. It produces output during first reaction and then

produce the output of the sub node in the previous reaction. Lustre sequential program is called step which involves data dependency. When it is converted into parallel, data independency is preserved. This paper generates parallel code for Kalray MPPA256[12] many core architectures. Cores are arranged as clusters with NoC. In this architecture, memory is multibanked i.e. One memory bank is accessed without affecting other memory banks and memory is shared. The Multicore Response Time Analysis (MRTA) [13] is a generic framework to analyze response time by considering memory bus, preemption and DRAM interference. MRTA is used in MPPA architecture. The parallel code is generated by taking only sub nodes into parallel tasks and not a main node. Main node does not consist of any code but control the communication between sub nodes. Functional code of each sub node is generated by Lustre compiler. Communication channels between communicating sub nodes are identified by syntax analysis. The communicating channel consists of data, delay(pre) and an initial value. Tasks are scheduled by Nguyen et al. [14]'s proposal. It uses an Integer Linear Programming-based mapping and scheduling algorithm for multi-core. Two types of tasks either Data Triggered(DT) or Time Triggered (TT) are executed. When all data received, the tasks execution starts is Data Trigggered (DT). When tasks start execution at release date, then it is Time Triggered(TT). Rihani et al. [15] provide a framework to compute the release date considering memory interference. Tasks that are waiting for release date are Time Triggered. Tasks are waiting for inputs are Data Triggered. This method uses both Time Triggered and Data Triggered. Delayed communication makes the task run in parallel. Local timers are used among cores for synchronization.

**A Scalable Multi-Granular Data Model for Data Parallel Workflows [16]**

To execute serial tasks in multicore architecture, the user has to manually rewrite the code. The user has to perform 3 steps. The user has (1) to allocate enough number of processes to each task, (2) divide the input data and send them to each processes and (3) execute the task. It is time consuming and more errors are generated in this way of manual writing of code and it can be replaced by automatic generation of parallel code by this framework. This framework uses the Multi view data model allows the user to specify the rules for data decomposition for arranging input data into parallel processes of a task. The input of parallel task is treated as multi-dimensional arrays which is decomposed and sent to parallel processes. It also increases degree of parallelism to achieve high performance i.e. Number of parallel processes are increased. Before this method, decomposition rule of input was specified by Global Array [17] Model and PGAS [18] (Partitioned Global Address space). Data parallel scheduling is impossible in these methods and also MapRreduce [19] model do not support MPI parallel programs. Because they do not decompose data. So there is no proper solution to divide input data. But Multi View data model is used in this framework. The application developer specifies the data decomposition rules for the input and arrange the partitioned input data into parallel processes. Multidimensional array is called as Data store. Decompose the Data store based on number of processes. First DS is divided into blocks and send to tasks. Then these tasks are executed. Remote access of memory is not supported. It provides enough number of processes into tasks at run time and increasing degree of parallel processing and provides the formula to compare resource utilization with load balance. It schedules the tasks as well as rearrange the data into processes and using MPI.

**The Virtual Savant: Automatic Generation of Parallel Solvers**

This VS framework [20,21] uses machine learning technique to learn the rules of sequential algorithm that generate the solutions from the input and then construct parallel algorithm, then execute this parallel algorithm in parallel architecture. It does not modify the sequential existing algorithm into parallel algorithm but to produce the new parallel algorithms. Other methods use transformations [22,23] and finding independent code to execute in parallel [24], and Genetic programming [25,26]. But It finds accurate solutions. It does not require sequential program to convert into parallel rather requires only draft work. It is based on virtual savant syndrome [27,28]. People with this syndrome execute sequential tasks by unknown methods in short term. Example. Calendar computations. Finding the day of same date for several years in short time. From these observations, parallel program is built without need of source code. It observes so many problems and their solutions by applying Machine Learning and produce new parallel algorithm. It consists of two steps. The first step is the prediction step. It uses pattern recognition in each predictor. Each predictor

assigns one input to variable. All predictors are running in parallel by assigning each input values to each variable. Number of parallel processes correspond to number of variables. The second step is the refinement step. The initial solutions are found in the prediction step. The best solution is found in the second step. The number of solutions for a given problem are stored in array. The length of array is number of parallel processes or predictors. Next the input problem is to be partitioned based on number of predictors. To accurately partition the problem, small number of features are given to predictors. Each predictor is running independently. To train the predictor, number of information are given to one predictor not to all predictors. The output of the prediction step is vector probabilities. The refiner chooses the best probability value as solution.

**DuctTeip: An efficient programming model for distributed task based parallel computing:**

To extend parallel programming to distributed memory systems, distributed task parallel framework DuctTeip [9] is constructed. It uses hybrid parallel implementation with MPI for distribution of data. This is built on shared memory library SuperGlue [29]. This does not require any task graph. Tasks are generated or submitted by other tasks dynamically in sequential order. Data can be in any format which is managed by data handles. Shared data or shared resource are protected by handle with version counter. This version counter allows the run time system to access the data. The version counter is initialized at task submission time. After task accessing the data handles, its version number is incremented. So the run time system determines whether the task is ready or not, based on required version number compared with existing version number of a data handle. If task got all arguments, it is ready and stored in ready queue. Ready tasks are executed where the data is stored locally in cache memory which eliminates scheduling rules. This framework executes a task in hierarchical manner for hierarchical architecture which is a distributed memory. Two types of data block are used. Large data block for communication to avoid communication complexity on small data blocks. Small data blocks for computation. Initially large data block is transferred then it is divided into small data blocks and stored in local cache memory. In this hierarchical model, level concepts are used. Level 0 is root. Level L is last level i.e. leaves. Un partitioned data is at Level 0. Task at Level L work data at level L+1. This framework uses only 3 levels. This framework is working at level 0 and using data at level 1. SuperGlue shared library at level 1 using data at level 2. After data partitioning, data is stored in contiguous memory blocks. The ownership of the task is determined based on node of the output data. If the task is owned by local node, then it is submitted to level 0. The tasks at level L and L+1 have parent child relationships. The tasks at last level is having actual computations and working in kernels. Memory pool is created in every computational node by this framework before the task is submitted to reduce the memory allocation and deallocation time. Each data is stored in continuous memory block. Memory block consists of two parts. First is header part to hold metadata including data handle version number and the second is content part. This content part of data is used by local task directly. When it is required for remote task both header and content part is sent to remote system in which data will be partitioned into small parts and used by tasks at lower levels. To manage the communication at distributed levels listener object is used. MPI thread mechanism is working along with SuperGlue library. So, computations and communications are managed.

**Table 1. Properties Addressed by Software Frameworks.**

| Framework/ Properties | PyCOMPSs | Parallel Code Generation | A Scalable Multi-Granular Data Model | Virtual Savant | DuctTeip |
|---|---|---|---|---|---|
| Concurrent execution | ✓ | ✓ | ✓ | ✓ | ✓ |
| Local disk Access | ✓ | ✓ | ✓ | ✓ | ✓ |
| Shared file system Access | ✓ | ✓ | ✓ | ✓ | ✓ |
| Remote access of data | ✓ | ✓ | X | ✓ | ✓ |

**Conclusion**

In this paper, some of frameworks used to convert serial applications into parallel applications with less human interference is discussed. It surveyed mechanisms of these frameworks working in multi core architectures and in distributed memory and nodes. It has given some ideas on how data and code is divided to work in distributed environment. No separate scheduling policy is required in all these five frameworks. Since scientific applications are increasing in size, so, we need to develop better

framework to achieve high performance in today's distributed environment.

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# Anti Arthritic Activity of Leaves of *Hibiscus Rosasinensis* by Inhibition of Protein Denaturation and Human Cell Membrane Stabilization (Hrbc) *in-Vitro* Methods

***[1]S. Jenny Angel & [2]D. Prema***
*[1,2]PG Department of Biochemistry,*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*

**Abstract**

Rheumatoid arthritis (RA) is an immune mediated inflammatory disease that mainly affects the tissue near the joint system. This RA is seen mainly in women above 50 years of age and the rate of incidence is increasing every year. A large number of drugs are available in the market but as this medicine has to be taken for prolonged period of time it may lead to various side effects. Currently more attention isgiven to traditional folklore medicines as there are no side effects. In the present study ethanolic extract of leaves of *Hibiscus rosasinensis*was investigated for anti-arthritic activity. This was carried out by using inhibition of protein denaturation and Human red blood cell membrane stabilization (HRBC) *invitro* methods. In inhibition of protein denaturation method various concentrations of 20, 40, 60, 80 and 100µg/ml of leaf extract was used. The percentage of inhibition for HRBC was 86.50% and egg albumin was 83.80% at a concentration 100µg/ml. This showed that the leaves of *Hibiscus rosasinensis*had potent anti-arthritic activity and henceforth can be used for the treatment of rheumatoid arthritis.

**Keywords**

Rheumatoid arthritis; *Hibiscus rosasinensis;* HRBC; protein denaturation; inflammatory disease

## Introduction

Rhematoid arthritis is a chronic inflammatory condition which affects the bones, cartilage and joints. The prevalence of RA is higher in developed countries which ranges from 0.3% to 1% and mainly affects women than men in the ratio 3:1. It affects 0.5-1.0% of adultsbetween 30 and 55 years, and in India this illness has a prevalence rate of approximately 0.75% in India**.**RA can be classified into many types such as Palindromic rheumatoid arthritis, Juvenile rheumatoid arthritis, Rheumatoid spondylitis, Primary osteoarthritis, Secondary osteoarthritis, Infectious arthritis, Gout and Gout arthritis (**Kumar and Cortan, 2005)**. The exact etiology of RA is still unclear and various factors such

as environmental factors, hormones and genetic factors are known to influence it. The clinical form of RA may lead to mild forms such as joints to severe deformity, loss of function and severe damage to cartilage. This condition is acquired due to presence of inflammatory mediators such as cytokines, eicosanoids, interleukins (IL1), tumor necrosis factor (TNF-α), and reactive oxygen species cells (**Kore and Shete2011**) which are released from macrophages during immune reaction.

RA initially affects the synovium, the membrane sac which surrounds the joint. This sac contains synovial fluid which lubricates and cushions the joints; it also supplies nutrients and oxygen to cartilage which coats the end of bones. In rheumatoid arthritis, the immune system produces a series of inflammatory mediators that in turn leads to inflammation of synovium. In addition, the collagen present in the bones is also destroyed thus leading to narrowing of joint space and finally damages the bone. In case of progressive rheumatoid arthritis, it becomes more chronic and further leads to pannus formation (thickened synovial tissue) that occurs due to the accumulation of fluid and immune cells in the synovium. The pannus produces more enzymes which destroy nearby cartilage, damaging the area and attracting more inflammatory white cells (**Firestein*et.al.*,2005; Lemke and Williams.,2008**).

The main strategy used for the treatment of RA is mainly focused on reducing the joint pain, bone damage and inflammation. Hence drugs that subsides these symptoms are mostly available such as - steroidal anti-inflammatory agents (Non-Steroidal Anti-inflammatory Drugs-NSAIDs), corticosteroids, disease modifying anti-rheumatic drugs (DMARDs) and biological agents. Decreasing the disease activity or decreasing the inflammation condition with some remission if possible, along with a minimization of joint destruction and finally improving the physical condition and quality of life can be also followed. NSAIDS have lost their historical role as first line treatment because of concern about their limited effectiveness, inability to modify the longterm course of disease **(Chen and Jobanputra .,2008**).One of the most common toxicity observed in case of regular use of these drugs is gastrointestinal disturbances with further leading to the development of gastric ulcers followed by bleeding **(Schaffer 2006).**

Some of the commonly used glucocorticoids in disease remission are prednisone, methyl prednisolone etc. that reduce synovitis and joint damage, but develops various infections and osteoporosis (**Ravindran.,2009**). Methotrexate is dominant DMARD, sulfasalazine and Leflunomide are widely used but limited due to their toxic effects. Adverse effects of DMARDs include those that are minor e.g. nausea and serious e.g. hepatotoxicity, blood dyscrasias, interstitial lung disease (**Scott *et.al.*,2010**).

Although several modern drugs are used to treat these types of disorders, their prolonged use may cause severe adverse side effects on chronic administration, the most common being gastrointestinal bleeding and peptic ulcers. Consequently, there is a need to develop new anti–inflammatory agents with minimum side effects. It is worthwhile to note that most of the present day analgesic drugs also exert a wide range of side effects. In recent years' interest in herbal medicines has increased considerably both at home and abroad as they are believed to be comparatively less toxic than the synthetics.

*Hibiscus rosasinensis* belong to the family Malvaceae and it is grown as an ornamental plant in gardens throughout India and often planted as a hedge or fence plant (**Sharma *et.al.*,2001**). The common name of *Hibiscus rosasinensis* is are china rose, shoe black plant, Sembaruthi, Rose mallow. The leaves contain carotene, fatty acids, fatty alcohols, hydrocarbons hence in the present study the leaves extract of *Hibiscus rosasinensis*was used to evaluate the anti-arthritic property by *in vitro* method.

**Materials and Method**

**Collection of plant material**

The leaves of *Hibiscus rosasinensis* were collected in the month of May from purathakudi, Thiruchirapalli, Tamil Nadu, India. The plant was identified and leaves of *Hibiscus rosasinensis*and were authenticated and confirmed from Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, Tamilnadu for identifying the plants.

**Preparation of ethanol extracts:**

The leaves of *Hibiscus rosasinensis* were washed in running water, cut into small pieces and then shade dried for a week, after which it was grinded to a uniform powder of 40 mesh size. The ethanol extracts were prepared by soaking 100g each of the dried powder plant materials in 1L of ethanol using a soxhlet extractor continuously for 10 hours. The extracts were filtered through whatmann filter paper

No.42 (125mm) to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labelled sterile bottles and kept at -20ºC. The filtrate obtained was used as sample solution for the further isolation.

**Phytochemical screening**

The leaves of *Hibiscus rosasinens is* was screened qualitatively for the presence of various phytochemical constituents such as tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, leucoanthocyanin, anthocyanins, anthroquinone, xanthoproteins, coumarins, glycosides, phenols, alkaloids, emodin, carbohydrates as per standard protocol. The quantitative estimation for flavonoid, tannin, saponin, alkaloid, phenol and terpenoid was also carried out as per standard protocol.

**Evaluation of anti-arthritic activity**

**Inhibition of protein denaturation model**

To 2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer (PBS, pH 6.4) and 2 ml distilled water was added and used as control solution. To 0.2 ml of egg albumin, 2.8 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) (20, 40, 60, 80 and 100 µg/ml) was added which served as standard drug. To 0.2 ml of egg albumin, 2.8 ml of phosphate buffer and various concentrations of leaves of *Hibiscus rosasinensis* (20, 40, 60, 80 and 100 µg/ml) were added and it was taken as test solution (**Chandra *et al*.,2012). All** of the above solutions were adjusted to pH, 6.4 and were incubated at 37°C for 15 minutes later heated at 70°C for 5 minutes. After cooling, the absorbance was read using UV-Visible spectrophotometer at 660nm. The percentage inhibition of protein denaturation was calculated using the following formula-

Percentage inhibition = (Vt/Vc-1) x 100

Where, Vt = absorbance of test sample, Vc = absorbance of control.

**Human red blood cell (HRBC) membrane stabilization model**

**Preparation of suspension (10% v/v) of human red blood cell (HRBC*)***

The blood was collected from healthy human volunteer who had not taken any NSAID'S for 2 weeks prior to the experiment and was mixed with equal volume of sterilized Alsevers solution (**Saleem *et al*.,2011 and Kumar *et al*.,2012**). This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline solution. This HRBC suspension was used for the study.

**Assay of membrane stabilizing activity**

The assay mixtures contain 1ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension & 0.5 ml different concentrations of extract, reference sample and control were separately mixed. 1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of leaves of *Hibiscus rosasinensis* of various concentrations (20, 40, 60, 80 and 100 µg/ml) and 0.5ml of 10% w/v human red blood cells were used as test solution. 1ml of phosphate buffer and 2ml of water and 0.5ml of 10%w/v human red blood cells in isotonic saline were served as test control.1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of standard drug (Diclofenac sodium) of various concentration (20, 40, 60, 80 and 100 µg/ml) and 0.5ml of 10% w/v human red blood cells were taken as standard solution. All the assay mixtures were incubated at 37ºC for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in control as 100% (**Azeem *et al*.,2010; Chippada and Meena.,2011**). The percentage of HRBC membrane stabilization or protection was calculated by using the following formula-

**Percentage protection**

100- [(optical density of sample/optical density of control) × 100].

**Result and discussion**

**Phytochemical screening**

Presence of different phytochemical compounds were analysed in ethanol extract of leaves of *Hibiscus rosasinensis* and the results were tabulated in Table 1. It was evident that phytochemical constituents such as phlobatannin, saponin, flavonoids, tannin, steroids, terpenoids, cardiac glycosides, leucoanthocyanin, protein, coumarin, glycosides, phenol, alkaloids, and carbohydrate were present whereas anthoquione, xanthoprotein and emodine were absent. There are many reports on the presence of phytochemical constituents in the leaves of *Hibiscus rosasinensis*

like steroids, carbohydrates, glycosides: flavonoid, fats and alkaloids.

**Table:1- Qualitative analysis of ethanol extract of leaves of *Hibiscus rosasinensis***

| S.No | Phytochemical Constituents | *Hibiscus Rosasinensis* |
|---|---|---|
| 1 | Tannin | +++ |
| 2 | Phlobatannin | ++ |
| 3 | Saphonin | +++ |
| 4 | Flavonoids | +++ |
| 5 | Steroids | ++ |
| 6 | Terpenoids | ++ |
| 7 | Cardiac glycosides | +++ |
| 8 | Leuco anthocyanin | +++ |
| 9 | Anthocyanine | +++ |
| 10 | Anthoquione | - |
| 11 | Protein | + |
| 12 | Coumarin | ++ |
| 13 | Glycosidase | +++ |
| 14 | Phenol | +++ |
| 15 | Alkaloids | +++ |
| 16 | Xanthoprotein | - |
| 17 | Emodine | - |
| 18 | Carbohydrate | +++ |

(Key: + presence; - absence)

**Quantitative analysis**:

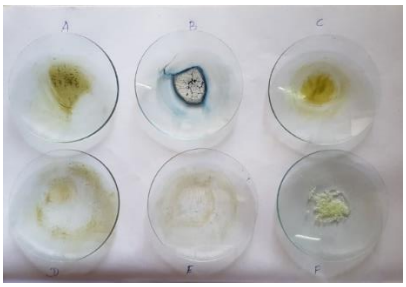
The results of quantitative analysis of important phytochemicals in the medicinal plant of *Hibiscus rosasinensis* suggested alkaloids (0.02mg/g) to be present in highest quantity followed by saponin, flavonoids, phenol, tannin and terpenoids respectively, as shown in (Table 2; Fig:1).

**Federico ferrered *et. al.*, 2005** were used HPLC-ESI/MS method to derive the phenolic compounds from the leaf and roof of *Hibiscus rosasinensis.* The root and leaves of *Hibiscus rosasinensis* contain high level of flavonoids. Flavonoids used to prevent the oxidative cell damage, have strong anticancer activity. *Hibiscus rosasinensis* contain high concentration of flavonoids followed by alkaloids, glycosides and sterols have been reported to be present in the alcoholic root extracts. The antistress activity of *Hibiscus rosasinensis* that contain antioxidant activity. The flowers also contains thiamine [ 0.031mg % ], riboflavin [ 0.048 mg % ], niacin[ 0.61 mg %] and ascorbic acid [ 4.16 mg % ], apigenidin, citric acid, fructose, glucose, oxalic acid, pelargonidin, quercetin.Leaves and stems gave teraxeryl acetate, ß-sitosterol and the cyclicacidssterculic and malvalic acids .

**TABLE.2: Quantitative analysis of ethanolic extract of leaves of *Hibiscus rosasinensis***

| S.No | Phytochemical Constituents | Hibiscus Rosasinensis (mg/g) |
|---|---|---|
| 1. | Flavonoids | 0.015 |
| 2. | Tannin | 0.03 |
| 3. | Alkaloids | 0.02 |
| 4. | Saponin | 0.07 |
| 5. | Terpenoids | 0.045 |
| 6. | Phenol | 0.005 |

**Fig 1: Quantitative analysis of methanolic extract of leaves of *Hibiscus rosasinensis* (A.Flavonoids ,B.Tannin , C.Terpenoid, D.Alkaloids , E.Phenol , F.Saponin)**



**Inhibition of protein denaturation model**

Diclofenac sodium was used as standard drug which at different concentrations (20, 40, 60, 80 and 100 µg/ml) showed inhibition of protein denaturation. The methanolic extract of leaves of *Hibiscus rosasinensis* at different concentrations (20, 40, 60, 80 and 100 µg/ml) also showed inhibition of protein (egg albumin) denaturation. The results are summarized in Table 3 and Fig 2. **Chandra *et.al.*,2012** suggested that the Inhibition of protein denaturation model has been used for the study of Ashwagandha, *Mikaniascandens* and Coffee

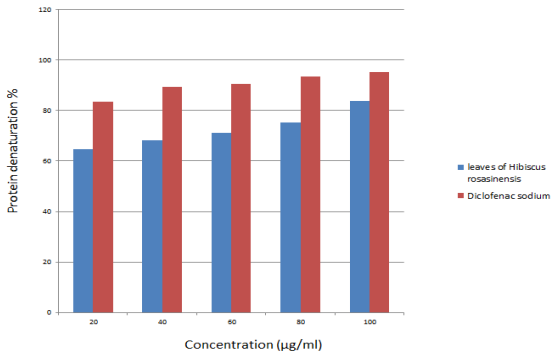
**Table 3. Anti-arthritic activity of the leaves of *Hibiscus rosasinensis*using protein denaturation method and comparison with standard drug diclofenac sodium.**

| S.No | Concentrations | Protein denaturation (%) |
|---|---|---|

| | | Leaves of *Hibiscus rosasinensis* | Diclofenac sodium |
|---|---|---|---|
| 1 | 20 (µg/ml) | 64.79±0.35 | 83.65±0.54 |
| 2 | 40 (µg/ml) | 68.31±1.64 | 89.35±1.77 |
| 3 | 60 (µg/ml) | 71.13±1.75 | 90.49±1.62 |
| 4 | 80 (µg/ml) | 75.35±1.52 | 93.53±1.57 |
| 5 | 100 (µg/ml) | 83.80±0.37 | 95.43±0.27 |

Each value was obtained by calculating the average of three experiments and data are presented as mean± SEM

**Fig 2: The graph represents the *in vitro* anti-arthritic activity of the leaves of *Hibiscus rosasinensis* using protein denaturation method and comparison with standard drug diclofenac sodium.**




**Human red blood cell (HRBC) membrane stabilization model**

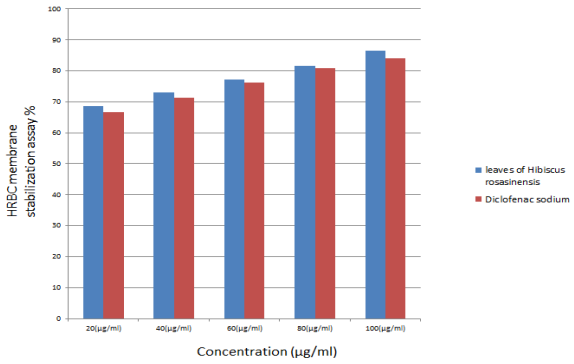
Diclofenac sodium was used as standard drug which at different concentrations (20, 40, 60, 80 and 100 µg/ml) exhibited stabilization towards HRBC membrane. The methanolic extract of leaves of *Hibiscus rosasinensis at* different concentrations (20, 40, 60, 80 and 100 µg/ml) also exhibited stabilization towards HRBC membrane. The effect of stem extracts was found to be more as well as diclofenac sodium. The results are summarized in Table 4 and Fig 3.

**Table 4. *In vitro* anti-arthritic activity of the leaves of *Hibiscus rosasinensis* using HRBC membrane stabilization method and comparison with standard drug diclofenac sodium.**

| S.No | Concentrations | HRBCmembrane stabilization assay (%) | |
|---|---|---|---|
| | | Leaves of *Hibiscus rosasinensis* | Diclofenac sodium |
| 1 | 20 (µg/ml) | 68.71±0.87 | 66.66±0.36 |
| 2 | 40 (µg/ml) | 73.00±1.68 | 71.42±1.23 |
| 3 | 60 (µg/ml) | 77.30±1.79 | 76.19±1.22 |
| 4 | 80 (µg/ml) | 81.59±1.82 | 80.95±1.45 |
| 5 | 100 (µg/ml) | 86.50±0.47 | 84.12±0.27 |

Each value was obtained by calculating the average of three experiments and data are presented as mean± SEM

**Fig 3. The graph represents the *in vitro* anti-arthritic activity of the leaves of *Hibiscus rosasinensis* using HRBC membrane stabilization method and comparison with standard drug diclofenac sodium.**




HRBC method was selected for the *in vitro* evaluation because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (**Azeem *et al.*,2010**). Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes,

which may stimulate or enhance the efflux of these intracellular components (**Kumar *et al*.,2012**). HRBC membrane stabilization has been used for the study of *Skimmiaanquetilia*, *Gendarussa vulgaris*, *Thunnusalalunga* by **Kumar *et al*., 2012; Saleem *et al*., 2011; Azeem *et al*., 2010**, respectively.

**Conclusion**

Rheumatoid arthritis is an autoimmune inflammatory disorder affecting almost 1-3% of the world population. The word Arthritis means inflammation of the joint ("artho" means the joint and "itis" meaning inflammation of the joint). The ethanolic extract of Leaves of *Hibiscus rosasinensis* at different concentrations (20, 40, 60, 80 and 100 µg/ml) also showed inhibition of protein (egg albumin) denaturation. Diclofenac sodium was used as standard drug which at different concentrations (20, 40, 60, 80 and 100 µg/ml) exhibited stabilization towards HRBC membrane. The ethanolic extract of leaves of *Hibiscus rosasinensis* at different concentrations (20, 40, 60, 80 and 100 µg/ml) also exhibited stabilization towards HRBC membrane. The percentage of inhibition for HRBC is 86.50% and egg albumin is 83.80% at a concentration 100µg/ml. The effect of leaves extract was found to be more than diclofenac sodium. However further research on detailed isolation of another active phytoconstituents possessing the therapeutic activity and clinical study for the evaluation of safety and efficacy of the drug needs assessed.

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## Study on Minerals, UV-Visible and Ftir Spectroscopy from srirangam Temple Flower Waste manure

***[1]Ramya. B, [2]Josephinol .S and [3]Rexida Janthark Mary .S***

*[1, 2 &3]PG Department of Biochemistry*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*

**Abstract**

The trend of using inorganic fertilizers is on a boom amongst agricultural society, farmers are magnetized towards the short term advantages privileged by inorganic fertilizers, but they are unable to understand the ill effects of these fertilizers on human health and soil fertility. The availability of nutrition in the soil is influenced by the availability of organic matter in the soil. Resulting in increase of essential elements in organic compost makes it an important source for control of acidic soil $P^H$ and soil nutrient replenished. Flower waste collected from Srirangam Temple has proven to be significant economic and ecological solution in organic manure preparation. Flower waste was composted and used as organic manure for plants. The minerals present in flower waste are iron, manganese, calcium, potassium, zinc, magnesium, nitrogen and copper. The results signify that use of flower waste as the organic manure enriches the soil quality and increases the crop yield. The bonds and functional groups present in sample prove that it can be used as best manure and this Organic manure is very cheap and effective as a good source of nitrogen for sustainable crop production.

**KeyWords**:

Minerals, UV VISIBLE, FTIR

**Introduction**

Herbicides and Pesticide are chemicals used to kill or damage unwanted plants or parts of them.Chemical pesticides may negatively influence the structure and composition of soil micro-flora (Abbasi et *al*., 2015[1]) .Due to soil fertility problems, crop returns often decrease and the crops are more susceptible to pests and diseases because they are in bad condition. In order to increase soil fertility, nutrients have to be added to the soil. Manure is a good fertilizer because it contains nutrients as well as organic matter (Mark Farrell*et al*., 2010[2]). Recycling of green waste through composting could not only reduce environmental problems caused by landfills and incineration but also decrease the cost of green waste disposal. Manure is a well-known and widely used method for converting organic manure into a nutrient enriched soil product. Composting is the biological decomposition and stabilization of organic substrates that allows development of thermophilic temperatures as a result of biologically produced heat. The end product produced is stable. It is free of pathogens and can be beneficially applied to land. These organic fertilizers are eco-friendly and do not damage natural resources. Instead they improve the fertility of the soil resulting in healthy plants. Additionally, the soil organic matter, soil microbial biomass and activities are enhanced by using organic fertilizers (Dayanand*et al*., 2017[3]). Manure was the first concept for using effective microorganisms (EM) in environment.

One such option is conversion of waste biomass into organic fertilizer through composting. When a substrate is composted, it converts the latter into fine

peat like material and transforms some of its nutrients into more bioavailable forms (Hussain and Abbasi 2015[4]). The compost acquires several species of microflora, besides hormones and enzymes. The recycled material when applied to soil, improves soil fertility. The compost serves as an excellent source of nutrient in organic farming and migrates the ill effects due to usage of chemical fertilizers (Subramanium Thiyageshwari*et al*., 2018[5]). Increases the nutrient level of the soil or improve the soil's physical condition by improving soil structure and aeration. Increases the infiltration capacity of the soil, thus reducing surface runoff and helps to retain plant nutrients and moisture. Well decomposed compost buffers soil reaction and controls soil temperature. It also increases soil microbial activity which helps mineralization. Organic manures are beneficial in the cultivation of crops. They are natural products used by farmers to provide food for the crop plants. Organic manures enable a soil to hold more water and also help to improve the drainage in clay soils. They even provide organic acids that help to dissolve soil nutrients and make them available for plants. The compost serves as an excellent source of nutrient in organic farming and migrates the ill effects due to usage of chemical fertilizers (Subramanium Thiyageshwari*et al*., 2018[5]). Manure is an organic fertilizer that can be made at very low cost. The most important input is the farmer's labour. However, now there is a modern approach to convert the flower wastes into value-added products viz., compost, biofuels, bioethanol, organic acids, and pigment (Mishra 2013 [6]).

Organic manure is being increasingly popular for organic farming. Composting was the first concept for using effective microorganisms in environmental management (Lokman and Joseph 2013[7]). Conventional farming and its basic common practices still guarantees a sufficient crop production to match the present demand for human food. The inevitable raise on the cost of these fertilizers calls for the development of innovative low- cost and eco sustainable crop nutrition practices. The capacity of soil microorganisms to affect plant growth depends on sophisticated nutritional and chemical signalling, but also on soil conditions and climate factors.

Flower waste gets accumulated at religious sites like Temples, Mosques and churches due to a number of religious practices and is also generated in places like residential areas, community centers. Flower waste (FW) falls under the category of municipal solid waste. India has a variety of cultural heritage and uses flowers for decoration purposes as well as for worship in holy places and as an offering to deities. Later these flowers are thrown away as waste material. Most of the time their flowers are mixed with municipal solid waste or allowed to decay naturally. They are also at times dumped into nearby water bodies thereby leading to water as well as environmental pollution. The Flower waste thrown into water bodies, affects the aquatic life.

In land treatment final state of the waste is disposed by making intimate contact with the soil. The land treatment exploits the natural capacity of the soil to return substances to a condition forthcoming the unique state from which they were won by a process of extraction and purification. Volatilization method is also used for the treatment and disposal of wastes. Dried and decayed flowers are considered waste material and thus, dumped in landfills, various waterbodies. These flowers are thrown into water or dumped into landside causing water pollution as well as environmental pollution.

Srirangam temple is the world's largest temple complex. It is the highest temple tower in the world. Large numbers of garlands are used for god and goddess in temple. Minimum 3-6 times garlands are changed in normal days and dried garlands are thrower as waste. In festival season flower showers are increased and flower waste also increased. Planning made to convert the flower waste to organic manure. The aim of this study is to analysis the minerals, bonds through UV VISIBLE and functional groups using FTIR analysis present in Flower waste manure of Srirangam temple.

**Experimental Materials and Methods**

**Collection of Flower Waste and Preparation af Manure**

The flower garlands were collected from Srirangam temple in Tiruchirappalli. The threads and fibers from the garland were removed, cutted into small pieces and let it allowed to dry for few days in shade. After that it was dumped in the composter. The plant and the Saw dust are sandwiched between the soils upon three to four layers. Then added dry leaves of the same quantity as the waste and semi-composted material, buttermilk or cow dung to start with the decomposition process. To that Efficient microbes (EM) solution was added which is available in the market. The composition of efficient microbes

(EM) solution and water in the ratio of 1/2l: 2l was added to the flower waste. This EM solution was sprinkled once in a 2 to 3 days to the flower waste which was dumped in to the composter. Turn the pile around every other day. Keep the pile at the right level of dampness. If it is too wet, add dry leaves and stir and if it is too dry add water and stir. Once it is full, leave the pot open for 30-45 days for the composition to happen. Then move the semi composted matter into a larger container or. Water was sprinkled after every layer in order to maintain moisture content. Continued this procedure for 2 months then it becomes manure and the manure was taken for analysis.

Qualitative Analysis of Minerals in Manure: As per the standard Protocol (Ramya et al., 2015[8])

UV-Visible and FTIR Spectroscopic Analysis for Identification of Bonds in flower wasteManure (Mlarvili et al., 2015[9])

The extracts were examined under visible and UV light for proximate analysis.For UV spectrophotometer analysis, the extracts were centrifuged at 3000rpm for 10 minutes and filtered through Whitman No.1 filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elemer Spectrophotometer system, which was used to detect the characteristic peaks ranging from 400-4000 $cm^{-1}$and their functional groups. The peak values were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

**Results and Discussion**

**Qualitative and Quantitative analysis of minerals in flower *manure* extract**

In this present study, the flower manure extract of Srirangam templewas subjected to mineral analysis and it is represented in (Table 3.1a and 3.1b). The result shows that manure contains Iron in rich amount , Manganese, Calcium, Zinc, Potassium, Magnesium are in abundant level, Nitrogen, Copper in moderate amount and Phosphorus, Sodium, Cobalt, Sulphur were present in trace amount.

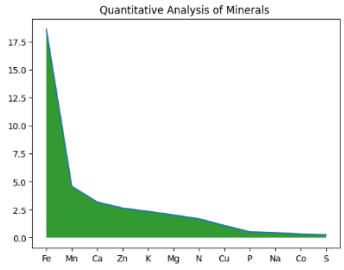
**Table 3.1a –Qualitative analysis of Minerals**

| S.No | Mineral | Observations |
|---|---|---|
| 1. | Iron | +++ |
| 2. | Manganese | ++ |
| 3. | Calcium | ++ |
| 4. | Potassium | ++ |
| 5. | Zinc | ++ |
| 6. | Magnesium | ++ |
| 7. | Nitrogen | + |
| 9 | Copper | + |
| 10 | Sodium | + |
| 11 | Phosphorus | + |
| 12 | Cobalt | + |
| 13 | Sulphur | + |

+++ = High Concentration, ++ Moderate, + Trace

**Table 3.1b Quantitative analysis of minerals**

| S.No. | Mineral | Quantity |
|---|---|---|
| 1. | Total Iron (ppm) | 18.64 |
| 2. | Total Manganese (ppm) | 4.59 |
| 3. | Total Calcium (%) | 3.19 |
| 4. | Total Zinc (ppm) | 2.64 |
| 5. | Total Potassium (%) | 2.35 |
| 6. | Total Magnesium (%) | 2.03 |
| 7. | Total Nitrogen (%) | 1.69 |
| 8. | Total Copper (ppm) | 1.09 |
| 9 | Total Phosphorus (%) | 0.52 |
| 10 | Total Sodium (%) | 0.45 |
| 11 | Total Cobalt (%) | 0.32 |
| 12 | Total Sulphur (%) | 0.26 |




Graph: 3.1- Quantitative estimation of minerals

Plants require macro and micronutrients for normal growth. Minerals are called a "spark plugs of life". Iron is the component of metallo flavoprotein and iron porphyrin proteins such as cytochrome, peroxidase and catalase. It is also an important part of ferrodoxin and nitrite reductase. It plays critical role in metabolic process such as DNA synthesis, respiration and photosynthesis and plays a significant role in various physiological and biochemical pathways in plants (Gyanna*et al*., 2015[10]). Manganese is used in plants as a major contributor to various biological systems including photosynthesis, respiration, and nitrogen assimilation. Manganese is also involved in pollen germination, pollen tube growth, root cell elongation and resistance to root pathogens (Frans 2014[11]). Calcium is an essential plant nutrient that absorbed from the soil in the form of calcium nitrate or calcium sulphate which is essential for the formation of cell membrane and lipid structures. Calcium involves in metabolic processes of other nutrients uptake and also helps in protecting the plants against diseases. Zinc is one of the essential micronutrients that helps the plants to produce chlorophyll and plays a key role in physical growth and development (Hafeez et *al*., [12]). Potassium occurs mainly as soluble inorganic salt or salts of organic acid in the cells and is highly mobile in plants. In photosynthesis potassium regulates the opening and closing of stomata, and therefore regulates $co_2$ uptake. Potassium plays a major role in the regulation of water in plants (wakeel*et al*., 2011[13]). Magnesium plays significant role in photosynthesis, carbohydrate metabolism and important role in respiratory mechanism by regulating phosphate metabolism in plant (Bose *et al*., 2011[14]).

Nitrogen being a major food for plants which is an essential constituent of protein and chlorophyll present in many major portions of the plant body. It is an essential component of proteins, protoplasm, enzymes and also chlorophyll. It also a constituent of purines, pyrimidines porphyrins and coenzymes (Legahari*et al*., 2016 [15]).Copper activates some enzymes in plants which are involved in lignin synthesis. It is also required in the process of photosynthesis, essential in plant respiration and assist in plant metabolism of carbohydrates and proteins. Copper also serves to intensify flavour and colour in vegetables and colouring flowers. Phosphorus is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next.Sodium has the benefit to the growth of algae and cyanobacteria .Sodium used in small quantities similar to micronutrients to aid in metabolism and synthesis of chlorophyll. Cobalt is a trace element in plants which is a component of a number of enzymes and increases the drought resistance of seeds (Legahari*et al*., 2016[16]). Sulphur is essential for the synthesis and helps to form important enzymes and assists in the formation of plant protein.In Mineral analysis, based on the light of current literature, we conclude that manure extract has a high concentration of nutritionally important minerals .It enriches the soil fertility and enhances the nutrition supply to the plants.

**UV Visible Spectroscopic Analysis for Identification of Bonds in flower wasteManure**

The UV-Visible spectra were performed to identify the compounds containing σ- bonds, π bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed the peaks at 275 and 328 nm with the absorption 10.32 and 1.82 respectively (Table 3.2a and figure 3.2b).The occurrence of peaks at 244-1064 nm reveals that the absorption bands are due to the presence of flavonoids, phenol and its derivatives in the Manure. Through this we came to conclusion that this manure enriches the soil fertility and enhances the plant growth with no side effects.

**Table 3. 2a UV-VISIBLE Spectra Wave Length and Absorption Peak of Flower waste *Manure***

| S. No. | Wave length (nm) | Absorption peak |
|---|---|---|
| 1 | 275 | 10.32 |
| 2 | 328 | 1.81 |

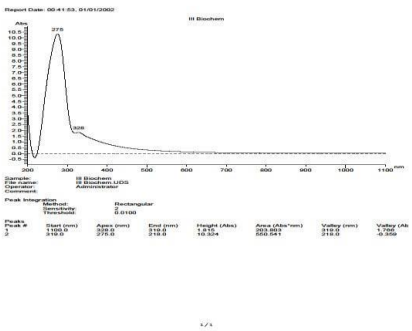


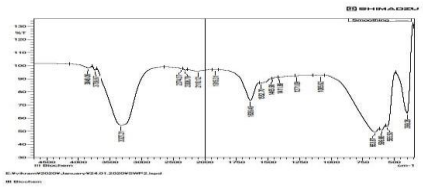
Figure 4.2b - UV-VISIBLE Spectra Peak Values of flower waste manure *Manure*

**FTIR Spectroscopic Analysis for Identification of Functional Groups in Flower waste Manure**

FTIR Spectrum identified the functional group of the active chemical components present in the flower waste manure based on the peak value in the region of infra-red radiation. When the manure was passed in to the FTIR, the functional group of the components was separated based on its ratio. The peak values and the functional groups were represented in (Table 3.3a and Figure 3.3b).The identified functional groups depicts that the manure extract contains rich effective compounds for plant growth and also it enriches the soil fertility (Sandosh et al., 2013 [17],Karpagasundari and S.Kulothungan 2018 [18], Lata,N.and Veenapani,D 2011 [19], Adrian*et al*., 2019 [20], Hussain*et al*., 2018 [21] )

**Table 3. 3a FTIR Spectra Peak and functional group of flower waste *Manure***

| S. No | Peak Values | Functional groups |
|---|---|---|
| 1 | 3846.o6 | Unknown |
| 2 | 3739.97 | Unknown |
| 3 | 3327.21 | Alcohol |
| 4 | 2374.37 | Phosphorous Compounds |
| 5 | 2308.79 | Carbon dioxide |
| 6 | 2110.12 | Alliphatic hydrocarbon |
| 7 | 1915.31 | Aromatic Compounds |
| 8 | 1639.49 | Primary amide NH2 bending |
| 9 | 1552.70 | Aromatic Compounds C=C Stretching |
| 10 | 1465.90 | Aromatic Compounds C=C Stretching |
| 11 | 1411.89 | Azo compounds N=N stretching |
| 12 | 1271.09 | Silicon Compounds |
| 13 | 1085.92 | P-OH groups |
| 14 | 653.87 | Halo Compound |
| 15 | 599.86 | Halo Compound |
| 16 | 555.50 | Halo Compound |
| 17 | 399.26 | Phosphorous Compounds |



**Graph 3. 3b FTIR Spectra Peak and functional group of *Manure***

**Summary and Conclusion**

Manure is a well-known and widely used method for converting organic manure into a nutrient enriched soil product. Composting is the biological decomposition and stabilization of organic substrates that allows development of thermophilic temperatures as a result of biologically produced heat. The end product produced is stable. It is free of pathogens, and plant seeds, and can be beneficially applied to land. These organic fertilizers are eco-friendly and do not damage natural resources. Instead they improve the fertility of the soil resulting in healthy plants. Additionally, the levels of soil organic matter, soil microbial biomass and activities are enhanced by using organic fertilizers. Organic manure material has sufficient nutrient content for plants growth and development. Elements present in organic manure in the form of macronutrients and micronutrients. Macronutrients generally have an important role for the growth of plants, strengthen rooting, simulate translocation, storing and

transferring energy from photosynthetic and used in metabolic processes. Resulting in increase of essential elements in organic compost makes it an important source for control of acidic soil $P^H$ and soil nutrient replenished.Srirangam flower waste is valuable green compost used for plant. The minerals present in water hyacinth are iron, manganese, calcium, potassium, zinc, magnesium, nitrogen and copper.The results signify the use of flower waste as the organic manure.The bonds and functional groups present in sample proves that it can be used as best manure and this Organic manure is very cheap and effective as a good source of nitrogen for sustainable crop production. The end product produced is stable. It is free of pathogens, and plant seeds, and can be beneficially applied to land. These organic fertilizers are eco-friendly and do not damage natural resources. Instead they improve the fertility of the soil resulting in healthy plants. Additionally, the levels of soil organic matter, soil microbial biomass and activities are enhanced by using organic fertilizers.

**Acknowledgement**

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## Antioxidant and Antidiabetic Activity of Synthesized Silver Nanoparticles using Flower and Root of *Euphorpia Hirta*

***[1]S.Josephinol, [2]B.Ramya, [3]S.Rexida jandhark mary***
*[1, 2 &3]PG Department of Biochemistry*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*

**Abstract**

In the present study, synthesis of silver nanoparticles (AgNPs) using flower and root of *Euphorbia hirta* were compared by studied for alpha amylase, alpha glucosidase inhibition assay and also examined for its antioxidant activities by using free radical 1,1 diphenyl-2 picrycl hydrozyl(DPPH) scavenging method under *in vitro* model separately. The colour variation of synthesized silver nanoparticles was checked and confirmed by UV-vis spectral analysis. The synthesized nanoparticles morphology was analysed using SEM. The crystalline structure of compound was determined by XRD method. FTIR measurements are carried out to analyse the possible biomolecule responsible for capping and efficient stabilization of the silver NPs synthesized using *Euphorbia hirta.* The result reports that the flower-AgNPs exhibits significant inhibitory effect on alpha amylase (78.01%) and for the root-AgNPs (48.96%). Alpha glucosidase showed inhibitory effect in comparison with flower -AgNPs (78.70%) and root –AgNps (38.67%) by using standard acarbose drug. The antioxidant activities by DPPH method exposed activity for flower-AgNPs (80.46%) and for the root-AgNPs (51.25%) were compared with standard ascorbic acid by measuring the percentage inhibitory effect at the concentration of 500µg/ ml reciprocally. Therefore, it is suggested that the synthesis silver nanoparticles using flower and root of *Euphorbia hirta* is a possible source for natural antidiabetic and antioxidant compounds and could have potential use in the management of diabetes mellitus.

**Keywords**

Flower-AgNPs and Root-AgNPs of *Euphorbia hirta,* alpha amylase and alpha glucosidase inhibitory activity, antioxidant (DPPH) activities.

**Introduction**

Nanotechnology and nanoscience grew rapidly because all fields of technical endeavor could participate: chemistry, physics, engineering, biology and medicine (Buyuktiryaki *et al*., 2020). Synthesis of nanoparticles involves various methods such as physical method (mechanical method), chemical method (sol-gel method), biological method (synthesis using plant extracts) (KannanBalan *et al.*, 2016). Green synthesis method of nanoparticles is an easy, efficient and eco-friendly method. It consumes low energy and hence produce safer products and by products (Behera *et al*., 2011). Synthesis of nanoparticles by biological method provides an environmentally friendly way without using any harmful and toxic chemicals (Hayrunnisa Nadaroglu *et al*., 2017; Jonathan Boutell *et al*., 2007). A Green substance which presents in green synthesis nanoparticles reduces toxicity (Chakravarthy *et al*., 1980). Silver nanoparticles are widely used in various fields such as Antibacterial effects, optical property, Anti-inflammatory, Anti-viral, Anti-diabetic activity (Bansal *et al*., 2014).

In both developed and developing countries the universal endocrine metabolic disorder that is the diabetes mellitus affects people. The prolong period

of the presence of high blood sugar level cause diabetes mellitus due to this there is metabolic changes of carbohydrates, proteins, fats and lipids (Gurupriya *et al*., 2018). It is a disorder where blood glucose levels are high due to the lack of insulin or body fails to produce insulin (Sincy Joseph *et al*., 2016). Type-1 diabetes mellitus caused by the insufficiency of an insulin secretion which is produced by the βcells of pancreas. Type-2 diabetes mellitus occurs due to no response of body cells to insulin (Maria *et al*., 2018). Insulin injection and drug therapy is the treatment for this disorder. The breakdown of long chain carbohydrates involves two important digestive enzymes α-amylase and α-glucosidase. The breakdown of long chain carbohydrates is done by α-amylase which results in breakdown of starch and disaccharides into glucose and it also helps in intestinal absorption (Hamden *et al*., 2010). These two digestive enzymes inhibitors are useful for the process of lowering the glucose absorption and glucose level in blood (Baron, 1998). General treatment involves diet, exercise, weight loss, insulin injection and drugs by oral (Makarim Wibisono *et al*., 2012). Diabetes can be prevented lifestyle changes (Guanguan Li *et al*., 2012).

*Euphorbia hirta* belongs to the family Euphorbiaceae, popularly known as Amman patcharasi (Probin Phanjom *et al*., 2012). It is widely distributed throughout the hotter parts of India and Australia (Williamson EM *et al*., 2002). it shows the pharmacological activity such as antibacterial, antimalarial, anti-inflammatory, anti-asthmatic and strong anti-oxidant activity (Prajapati ND *et al*., 2003). Therefore, this study was designed to synthesize AgNPs using flower and root of *Euphorbia hirta* and to evaluate for in vitro antioxidant and antidiabetic activity separately.

**Materials and methods:**

**Chemicals and reagents:**

Silver nitrate, Alpha(α)-Glucosidase, Porcine pancreas alpha (α)-amylase, p-nitrophenyl- α-D-glucopyranose(p-NPG), 3,5-dinitrosalicylic acid (DNS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Acarbose, Soluble starch, Sodium potassiumtartarate, Sodium dihydrogen phosphate (NaH2PO4), Disodium hydrogen phosphate (NaH2PO4) sodium chloride, Sodium hydroxide, Potassium ferricyanide and Ferric chloride (FeCl3). All the chemicals used for the study including the solvents, were of analytical grade.

**Collection and preparation of plant extract:**

*Euphorbia hirta* flower and root sample were collected from Manachanallur in Trichy. The *Euphorbia hirta* flower and root sample were washed by using fresh water and dried in shade for about 30 days and then made into powder. Absolute amount of the plant sample was taken in a dry beaker and adding few amounts of Ethanol. This solution is transferred into beaker and added appropriate amount of ethanal, stirred with glass rod and closed the solution with watch glass, the solution was boiled for 20-30 minutes by occasional stirring. The solution color changes from slight green to dark green color. Then this solution was kept cooled at room temperature for one hour, the solution was then filtered using Whatman No.41 filter paper through funnel into clean beaker, the whole solution was filtered for about 3hours 50minutes. The stock solution obtained was transferred into the brown bottle and stored in cool place (Shikuo Li et al., 2007).

**Synthesis of silver Nanoparticles**

The stock solution was prepared by extracting the sample from *Euphorbia hirta flower and root* using Ethanol as the solvent. Absolute amount of silver nitrate was weighed and transferred into 50ml standard flask and dissolved using de-ionized water. Aluminium foil was used to cover the prepared silver nitrate solution to prevent photochemical reaction. 1ml of the silver nitrate solution was taken and added 50µl of the stock solution gradually without any contamination for the synthesis of silver nanoparticles. The solution was kept at room temperature for the formation of silver nanoparticles, the color changes from colorless to reddish brown color shows that the silver nanoparticles are thus formed without any agglomeration. This shows that the formed silver nanoparticles are highly stable (Manish Dubey et al., 2009).

**Characterization Techniques (**Jae Yong Song and Beom Soo Kim**,** 2009**;** Singh et al., 2010)

**UV- Vis spectroscopy**

The optical properties of silver nanoparticles were characterized using UV-Vis spectrophotometer. Silver nitrate was added to the plant extract; UV was taken after 24hours of addition. The absorbance was recorded between 350-500nm

**FT-IR**

The functional group of the synthesized silver nanoparticles was studied using FT-IR spectrometer. Using KBr pellet method dried powder sample was characterized in the range between 4000-400cm$^{-1.}$

**XRD:**

Crystalline nature and grain size of synthesized silver nanoparticles was characterized using X-ray diffraction spectroscopy.

**Scanning electron microscopy (SEM) and EDX analysis**

The size and morphology of synthesized silver nanoparticles were evaluated using SEM analysis.SEM image confirmed the development of silver nanoparticles. The composition and elements of the green synthesized silver nanoparticles was investigated using EDX (Shital Bonde, 2011; Joyita Banerjee *et al*., 2011).

**Comparative analysis of synthesized silver nano praticles using *Euphorbia hirta* flower and root:**

The antioxidant activity of the aqueous extract was examined on the basis of the scavenging effect (Amit Kumar Mittal *et al*., 2012). Ethanolic solution of DPPH (0.05 mM) (300µl) was added to 40µl of aqueous nano extract with different concentrations (20 - 100µg/ml). The freshly prepared DPPH solution was kept in the dark at 4°C. 96% (2.7 ml) of ethanol was added and shaken vigorously. The mixture was kept constant for 5 min and absorbance was measured at 517nm spectrophotometrically. Ethanol was used to set the absorbance at zero. A blank sample was also prepared which contains the same amount of ethanol and DPPH. All the determinations were performed in triplicate. The radical scavenging activities of the tested samples expressed are calculated as percentage of inhibition according to the equation,

DPPH activity (% inhibition) = [(A – B) / A] x 100

Where A and B are the absorbance value for the test and blank sample respectively.

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions.

**Alpha-amylase Inhibitory Assay**

The alpha-amylase inhibitory assay was carried out by using the synthesized silver nanoparticles (Anshuman Bhattacharya *et al*., 2016). A total of 250µL of aqueous extract (20-100 µg/ml) was placed in a tube and 250µL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was added. This solution was pre-incubated at 25oC for 10 min, after which 250µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at regular time intervals and then further incubated at for 25oC for 10min. The reaction gets terminated by addition of 500µL of dinitro salicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled at room temperature. The reaction mixture was diluted by adding 5mL distilled water and the absorbance was measured at 540nm using spectrophotometer. A control was prepared during the same procedure by replacement of the extract with distilled water. The α-amylase inhibitory activity was calculated in terms percentage inhibition.

%Inhibition = [(Abs control – Abs aqueous extract) / Abs control] x 100

Graphical method was used to determine the concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50).

**Alpha-Glucosidase Inhibitory Assay:**

The activity of aqueous extract on α-glucosidase was determined by using α-glucosidase from *Saccharomyces cerevisiae (*Charushila *et al*., 2015). P-nitro phenyl glucopyranoside (p-NPG) was prepared in 20mM phosphate buffer as a substrate solution and pH 6.9. 100µL of α- glucosidase (1.0 U/mL) was pre-incubated with 50µL of the different concentrations (20-100µg/ml) of the aqueous extract for 10min. Then 50µL of 3.0mM (pNPG) substrate was dissolved in 20mM phosphate buffer (pH 6.9) were added to start the reaction. The reaction mixture was incubated at 37oC for 20min and stopped by adding 2mL of 0.1M Na2CO3.The α-glucosidase activity was determined by measuring the yellow-colored p-nitro phenol released from pNPG at 405nm.

The results were expressed in percentage of the blank control. The α-glucosidase inhibitory activity was calculated by percentage inhibition.

%Inhibition = [(Abs control – Abs aqueous) / Abs control] x 100

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined by using graphical method.

## Results and Discussion

### Visual color change and UV-Vis spectroscopy

In this experiment, addition of ethanol extract of plant sample in to the glass vial containing $AgNO_3$ led to the change in color from colorless to reddish brown indicates the presence of silver nanoparticles. Plasma resonance band was observed by UV spectra at the range of 438 nm for the ethanol flower samples and at the range of 446 nm for the ethanol root samples related to literature reported (fig. 1).

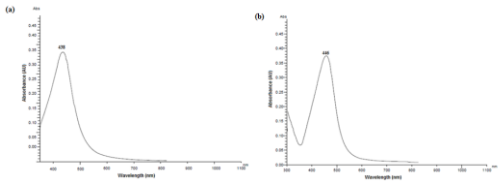


**Figure 1: UV-VIS spectrum of synthesized silver nanoparticles using *Euphorbia hirta* (a) flower-Agnps (b) root-Agnps**

### Functional group determination using FT-IR spectroscopy

The FT-IR spectrum of ethanol extract gives details about the functional group involved in the silver ions reduction. The FT-IR spectra of synthesized silver nanoparticles by using *Euphorbia hirta* flower and root extract are shown in (tables 1 and 2) and (fig. 2).

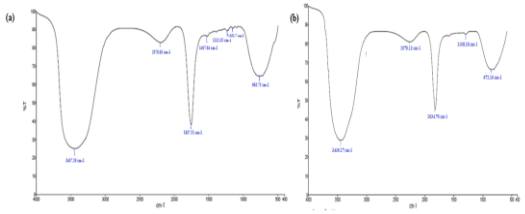
**Table 1: FTIR band values of synthesized silver nanoparticles using of flower of *Euphorbia hirta***

| **Functional group** | **Band** | **Frequency. $Cm^{-1}$** |
|---|---|---|
| Primary amine | Strong band | 3437.26 $cm^{-1}$ corresponds to broad O-H stretching alcohol |
| Isothiocyanate | Strong band | 2078.63 $cm^{-1}$ corresponds to N=C=S stretching vibrations |
| Alkene | Medium band | 1637.51 $cm^{-1}$ corresponds to C=C stretching conjugated alkene |
| Amine | Medium band | 1407.64 $cm^{-1}$ corresponds to O-H bending alcohol |
| Alcohol | Strong band | 1112.35 $cm^{-1}$ corresponds to C=O secondary alcohol |
| Anhydride | Strong band | 1031.70 $cm^{-1}$ corresponds to CO-O-CO anhydride |
| Halo compound | Strong band | 663.75 $cm^{-1}$ corresponds to C-Br stretching vibrations |

**Table 2: FTIR band values of synthesized silver nanoparticles using of root of *Euphorbia hirta***

| **Functional group** | **Band** | **Frequency. $Cm^{-1}$** |
|---|---|---|
| Primary amine | Medium band | 3436.17 $cm^{-1}$ corresponds to N-H stretching vibrations |
| Isothiocyanate | Strong band | 2075.13 $cm^{-1}$ corresponds to N=C=S stretching vibrations |
| Alkene | Medium band | 1634.76 $cm^{-1}$ corresponds to C=C stretching vibrations |
| Amine | Medium band | 1108.18 $cm^{-1}$ corresponds to C-N stretching vibrations |
| Halo compound | Strong band | 672.16 $cm^{-1}$ corresponds to C-Br stretching vibrations |

**Figure 2: FTIR spectrum of synthesized silver nanoparticles using *Euphorbia hirta* (a) flower-AgNPs (b) root-AgNPs**

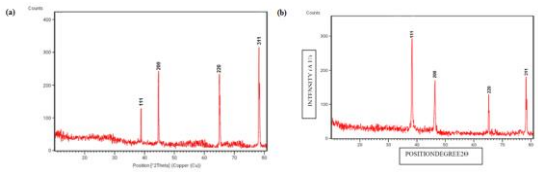


### X-ray diffraction (XRD)

The crystalline nature of silver nanoparticles was confirmed using X-ray crystallography. The X-ray diffractogram pattern of synthesized silver nanoparticles was represented in **Fig 3**. The diffraction peaks were obtained by flower-AgNPs is observed at 39.78, 47.73, 68.07 and 78.224 in the 2Ɵ range **(fig.3a)**. The obtained XRD pattern for silver nanoparticles synthesized using *Euphorbia hirta* root extract showed the characteristic peaks 38.43, 46.37,

65.16 and 78.59 in the 2Ɵ range **(fig. 3b)**. X-Ray Diffraction analysis confirms the crystalline nature of silver nanoparticles. The peak corresponds to 38.0824, 44.7077 following diffraction facets are (111), (200) respectively. This pattern (111),(200),(220) and (311) reflection of the face centered cubic structure for silver according to (JCPDS, File No. 04-0783). According to (JCPDS, File n04-0783) the pattern shows the face centered cubic structure for silver.

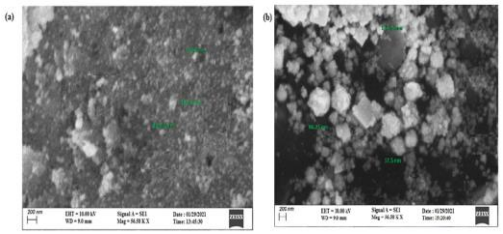
**Figure 3: XRD analysis of synthesized silver nanoparticles using *Euphorbia hirta* (a) flower-Agnps (b) root-Agnps**



**Sem Image**

The SEM image is employed to predict the size and morphology of resultant silver nanoparticles. The size (diameter) of the nanoparticles lie between 33-45.9 nm region in case of flower-Agnps sample and 37.9-124.5 nm in case of root- Agnps sample, the average size of the nanoparticles is ~ 200 nm, whereas the shapes were spherical and cubic.

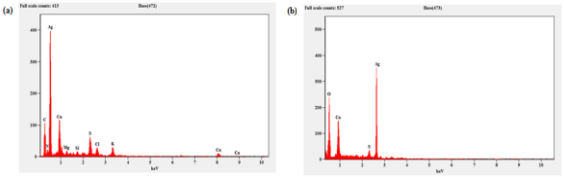
**Figure 4: SEM photograph of synthesized silver nanoparticles using *Euphorbia hirta* (a) flower-Agnps (b) root-Agnps**



**EDX analysis**

Energy dispersive X-ray (EDX) spectrometer analysis confirmed the elemental signal of silver nanoparticles. The Y-axis (vertical) represents the number of X-ray counts while X-axis (horizontal) shows the energy in KeV. EDX spectrum recorded for the silver nanoparticles was shown in Fig. 5 with additional peak of oxygen because of biomolecules attached to the silver nanoparticles surface. From EDX spectra, it is found that silver nanoparticles are reduced by *sample* have the silver weight percentage as 58.44% for root and for flower 68.73 %.

**Figure 5 EDX spectrum of synthesized silver nanoparticles of *Euphorbia hirta* (a) flower-Agnps (b) root-Agnps.**



The previous study suggested that the Green synthesized silver nanoparticles using switch grass (*Panicum virgatum*) extract (Cynthia Mason *et.al*.,2012), ethyl acetate extract of *Ulva fasciata* (Delile) (Rajesh *et al*., 2012) and the characterization carried for the study was UV, XRD and TEM, found the synthesized silver nanoparticles.

**Antioxidant activity of synthesized silver nanoparticles by DPPH assay:**

The result showed that the silver nanoparticles synthesized using *Euphorbia hirta* flower extract showed maximum potent antioxidant activities at high concentrations when compared with ascorbic acid **Table 3.** The synthesized silver nanoparticle showed the maximum inhibition for flower-Agnps (80.46%) and for root-Agnps (51.25%) at concentration 500 µg/ml while ascorbic acid gave 84.37 % at the same concentration **Fig 6**.

**Table-3: *In vitro* antioxidant activity of the synthesized silver nanoparticles using DPPH method and comparison with standard drug ascorbic acid.**

| S.No | Concentration | Alpha glucosidase | | |
|---|---|---|---|---|
| | | E. Hirta Flower | E. Hirta Root | Ascorbic Acid |
| 1 | 100 (µg/ml) | 57.82 | 66.34 | 72.65 |
| 2 | 200 (µg/ml) | 64.07 | 62.16 | 77.34 |
| 3 | 300 (µg/ml) | 69.54 | 59.73 | 79.68 |
| 4 | 400 (µg/ml) | 78.22 | 54.84 | 82.03 |
| 5 | 500 (µg/ml) | 80.46 | 51.25 | 84.37 |

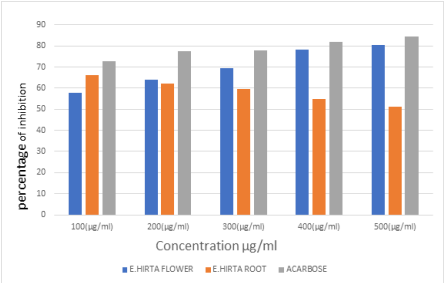



**Fig. 6. Antioxidant activity of synthesized silver nanoparticles vs Acarbose using a *euphorbia hirta* flower and root extract**

***In vitro* alpha amylase inhibitory assay:**

In this study the *in vitro* alpha amylase inhibitory activities of the silver nanoparticles synthesized using a *euphorbia hirta* extract was investigated. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against alpha amylase enzyme. The synthesized silver nanoparticles (100-500 µg/ml) of the various concentrations exhibited potent α-amylase inhibitory activity in a dose dependent manner. The synthesized silver nanoparticles showed inhibitory activity from 55.31% to 78.01% for flower and 50.34%to 48.96% for root at concentration 500 µg/ml (Table 4). Acarbose is a standard drug for α-amylase inhibitor. Acarbose at a concentration of (100-500 µg/ml) showed α-amylase inhibitory activity from 67.37% to 85.81%at the same concentrations 500 µg/ml. A comparison of α–amylase inhibitory activity between the standard drug has been depicted in fig. 7.

**Table-2: *In vitro* antidiabetic activity of the silver nanoparticles synthesized using a *Euphorbia hirta* extract using alpha amylase method and comparison with standard drug acarbose.**

| S.No | Concentration | Alpha Amylase (%) | | |
|---|---|---|---|---|
| | | E.hirta Flower | E.hirta Root | Acarbose |
| 1 | 100(µg/ml) | 55.31 | 50.34 | 67.37 |
| 2 | 200 (µg/ml) | 60.28 | 56.62 | 73.04 |
| 3 | 300 (µg/ml) | 66.66 | 51.73 | 78.01 |
| 4 | 400 (µg/ml) | 73.04 | 49.81 | 82.26 |
| 5 | 500 (µg/ml) | 78.01 | 48.96 | 85.81 |

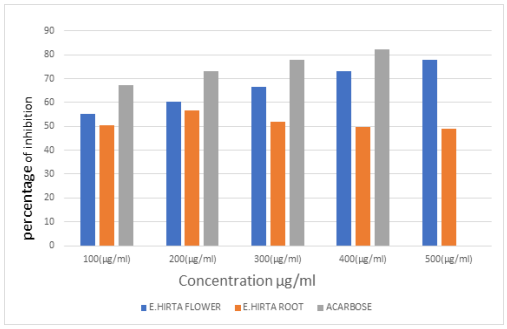



**Fig. 7. α-Amylase inhibitory activity of Acarbose vs silver nanoparticles synthesized using a *Alpinia calcarata* rhizomes extract**

**In Vitro α-glucosidase inhibitory assay:**

The results of antidiabetic activity using α- glucosidase inhibitory assay of the silver nanoparticles synthesized using a *euphorbia hirta* extract. The extracts revealed a significant inhibitory action of α-glucosidase enzyme. The percentage inhibition at 100-500 µg/ ml concentrations of extracts showed a dose dependent increase in percentage inhibition.

The synthesized silver nanoparticles showed inhibitory activity from 52.77% to 78.70% for flower and 49.78%to 38.67% for root at concentration 500 µg/ml (Table 5). Acarbose is a standard drug for α-amylase inhibitor. Acarbose at a concentration of (100-500 µg/ml) showed α-amylase inhibitory activity from 74.07% to 86.11%at the same concentrations 500 µg/ml. A comparison of α–amylase inhibitory activity between the standard drug has been depicted in fig.8.

**Table-5: *In vitro* antidiabetic activity of the synthesized silver nanoparticles using alpha glucosidase method and comparison with standard drug acarbose.**

| S.No | Concentration | Alpha glucosidase | | |
|---|---|---|---|---|
| | | E.hirta Flower | E.hirta Root | Acarbose |
| 1 | 100 (µg/ml) | 52.77 | 49.78 | 74.07 |
| 2 | 200 (µg/ml) | 61.11 | 46.64 | 77.77 |
| 3 | 300 (µg/ml) | 71.29 | 44.83 | 80.55 |
| 4 | 400 (µg/ml) | 74.04 | 39.16 | 83.33 |
| 5 | 500 (µg/ml) | 78.70 | 38.67 | 86.11 |

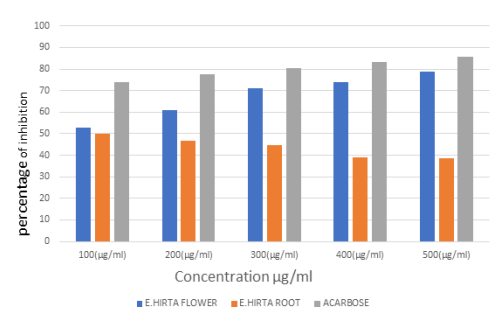



**Fig. 8. α- glucosidase inhibitory activity of Acarbose vssilver nanoparticles synthesized using a *Euphorbia hirta* flower extract**

## Conclusion

The silver nanoparticles synthesis was successfully carried out by green method using silver nitrate as the precursor and *euphorbia hirta flower and root e*xtract as the reducing agent. The method does not require any chemicals. The method used is a simple, safe, inexpensive, eco-friendly and non-toxic. The aqueous extract of *euphorbia hirta showed* great capability to synthesize the silver nanoparticles. The Surface Plasmon Resonance band in the UV-Visible spectrum shows absorption peak at 433.5nm clearly indicates the formation of silver nanoparticles. The FTIR analysis showed the biological material and functional groups present in the flower-AgNps and root- AgNps of *euphorbia hirta.* The study revealed that the synthesized nanoparticles of flower-AgNps and root-AgNps of *Euphorbia hirta* have antioxidant and antidiabetic activities more effectively.

## Acknowledgement

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## Hptlc Finger Print Profile and Evaluation of *in Vitro* Anti-Oxidant and Anti-Microbial Activity from The Rhizome of *Zingiber Officinale*

***[1]Ms.Prudence Elizabeth Savielle, [2]A.K. Umera Begum and [3]R. Gomathi***

*[1, 2 &3]PG Department of Biochemistry*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002.*

**Abstract**

The present study has been designed to carry out the phytochemical screening, HPTLC finger print profile and *in vitro* anti-oxidant and anti-microbial activity of ethanolic extract of Rhizome of *Zingiber officinale*. Preliminary phytochemical screening confirmed the presence of terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, phlebotomine, protein, coumarin, emodin, anthraquinone, anthocyanin, carbohydrates, cardiac glycosides,

xanthoprotein and phenol. In HPTLC analysis, luteolin bands where identified in sample chromatogram (100 µg/ml) which was confirmed by the chromatogram obtained from the standard luteolin and by comparing the retention factors of luteolin (rf = 0.58) value is from sample and standard solution. *In vitro* anti- oxidant and anti-microbial activity of *Zingiber officinale*rhizomes was also performed by using disc fusion method against the pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*and *E. coli*, pathogenic fungi such as *Candida albicans* and *Aspergillus flavus*. 100 µg of ethanolic extract of rhizome was active against both the bacterial and fungal species. The results of our study indicated that flavonoid luteolin is present in the *Zingiber officinale*rhizomes which may be responsible for potent anti-oxidant and anti-microbial activity against pathogenic microorganism.

**Keywords**

Anti-Microbial Activity, Anti-Oxidant

**Introduction**

Quality assessment includes one main tool which is the phytochemical evaluation which includes primary phytochemical screening, chemo- profiling and marker compounds analysis using modern analytical techniques. The WHO (World Health Organization) introduced the use of chromatography for standardization of plant products. It became a strategy for assessing the Quality (identification and evaluation) of plant medicines. (Dhalwal K *et al*., 2010). Phytochemical evaluation has many efficient tools like HPLC and HPTLC. HPTLC has high accuracy, precision and reproductivity of results they are widely used across the world. It also has many advantages like easy sample preparation, analytical assurance and high sample throughput at low operating cost. (Di X *et al*., 2003; Larsen T *et al*., 2004; Suthar AC *et al*., 2001)

Secondary metabolite is natural products which play an important role in ecology in regulating interactions between the environment and plants. (Hanson JR., 2003) the secondary metabolite of plants in medicine has much important significance in agriculture and industry, which has led to enormous studies on the synthesis and biosynthesis and biological activity of the substances. (Gershenzon J et al., 1999) Luteolinwhich is a yellow crystalline is a type of flavonoids. (Secondary Metabolism (2nd ed.). Oxford, UK: Oxford University Press. pp. 279–280. ISBN 978-0-19-855529-2.). Flavonoidsalso known as bioflavonoids; which are yellow color in nature are a class of polyphenolic secondary metabolites found in plants, and thus commonly consumed in diets. (Delage, PhD, Barbara (November 2015). "Flavonoids". Linus Pauling Institute, Oregon State University, Corvallis, Oregon. Retrieved 2021-01-26.)

Bioactive compounds present in plants *Zingiber officinale*contain variety of antioxidant capabilities including sterols, alkaloids, flavonoids, phenolics, carotenoids and glycosylates (Gershenzon J et al., 1999). The primary activities of*Zingiber officinale*rhizomes include anti-inflammatory, antioxidant, analgesic effect, antiproliferative and hepatoprotective activity. (Islam M.S et al., 2015;Wang J et al., 2017; Karthik et al., 2011; Kumar G et al., 2011; Rihmat A et al., 2010; Bhargava S et al., 2012). Anti-oxidant activity is defined as a limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions. (Karthik et al., 2011In fork medicine and cooking the frequently used ingredients is *Zingiber officinale* (commonly called ginger). Strong anti-oxidant, anti-bacterial, anti-fungal, anti- cancer and anti-inflammatory activities are shown in ginger extract. (Islam M.S et al., 2015). To inhibit the broad range of pathogens and to treat throat infections, ginger has been traditionally used. (Malu SP *et al*., 2009) Countries like china, India, US, Bangladesh, Jamaica as it cures many digestive disorders such as Indigestion, Constipation, Flatulence. (Lindmark L *et al*., 2005) (Kumar G *et al*., 2011) The Anti-microbial activity differs from place to, usage of plants parts, extraction type and protocols employed. (Soares., 2013) The main aim and objective of the present study was to investigate the anti-oxidant and Anti-microbial activity of Rhizome of *Zingiber officinal*

**Materials and Methods**

**Collection of Plant Material**

The Rhizome of *Zingiber officinale* was collected in the month of February from the local market, Tiruchirappalli, Tamilnadu, India. The plant was identified and confirmed by Dr. S. John Britto, Director, Rapinat Herbarium, St. Joseph College, Tiruchirappalli, Tamilnadu. The voucher specimen number PP001 dated 11.08.2020.

**Preparation of Ethanol Extracts**

The Rhizome of *Zingiber officinale* were washed in running water, cut into small pieces and then shade dried for a week at 35-40˚C, after which it was grinded to a uniform powder of 40 mesh size. The ethanol extracts were prepared by soaking 10 g of the dried powder plant materials in 100 ml of ethanol using a hot percolation method for 10 hr. The extracts were filtered through Whatman filter paper No. 42 (125mm) to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labelled sterile bottles and kept at -20˚C. The filtrate obtained was used as sample solution for the further isolation. (Al-Amin*et al*., 2006)

**Preliminary Phytochemical Screening:**

The ethanolic extract of rhizome of *Zingiber officinale* was subjected to preliminary phytochemical investigations to determine the different phytoconstituents using standard procedures. (Harborne., 1975) Preliminary phytochemical screening confirmed the presence of terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, phlobatannin, protein, coumarin, emodin, anthraquinone, anthocyanin, carbohydrates, cardiac glycosides, xanthoprotein and phenol.

**Quantification and validation of phytochemical compounds from the rhizome of *zingiber officinale* by hptlc (akhani*et al*., 2004; malu*et al*., 2009)**

**Sample Preparation**

500 mg of *Zingiber officinale*rhizomes was extracted with 10 ml of methanol. The solution was vortexed for 5 mins and kept overnight for extraction. Then it was filtered through Whatman filter paper no.41 and filtrate were subjected to HPTLC for simultaneous quantification of luteolin.

**Standard Solutions Preparation**

Stock samples were prepared by weighing standard luteolin(10 mg) separately. Each standard weighed powder was accurately transferred to a volumetric flask of 100 ml and diluted to the mark with methanol and chloroform to obtain standard stock solutions of concentration (100 µg/ml).

**HPTLC Instrumentation**

The sample solutions were spotted in the form of bands of width 8 mm wide with a constant application rate of 150 NI s-1, with an automatic TLC scanner (ATS4) under a flow of N2 gas, 15 mm from the bottom, 15 mm from the side, and the space between two spots was 6 mm. The plate with a Camagmicroliter syringe on precoated silica gel aluminum plate 60F254 (20 cm x 10 cm with 200 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologist, Mumbai) using a CamagLinomat V (Switzerland). The linear ascending development was carried out in a camag twin through chamber (20 cm x 10 cm), which was pre-saturated with a 25 ml mobile phase such as n-hexane: ethyl acetate (80:20 v/v) for a luteolin mobile phase toluene: ethyl acetate (70:30) for 30 min, at room temperature (25ºC±2ºC) and 50±5% relative humidity. The length of chromatogram run was 9 cm. Subsequent to the scanning, TLC plates were dried in a current of air with the help of an air dryer. The plates were pre-washed by methanol and activated at 60 ºC for 5 min prior to chromatography. The HPTLC plate was performed in the absorption- reflection mode at 538 nm, using a slit width 6.00 x 0.45 mm, with data resolution 100 µm/step and scanning speed 20 mm/sec. The source of radiation utilized was a tungsten lamp emitting continuous visible spectra of 366 nm and operated by Win CATS software (1.3.0 Camag). Concentration of the compound chromatographed was determined from the intensity of diffusely reflected light. Evaluation was carried out by comparing peak areas with linear regression.

**Method Validation**

The proposed HPTLC method was validated according to the International Conference on Harmonization guideline in 1994, 1996 and 2003. All measurements were performed in triplicates.

**Calibration Curve and Linearity**

Linearity method was constructed by calibration curves at six concentration levels. Calibration curves were plotted over a concentration range of (5000-10000 ng/spot) for luteolin, (50-250 ng/spot). Aliquots of standard working solution of luteolin, were applied to the plate (1- 6 µL/spot). The calibration curves were developed by plotting peak area versus concentration (n=6) with the help of the win CATS software.

**Recovery**

In order to evaluate the validity of the proposed method, accuracy was evaluated through the percentage recoveries of known amounts of mixture of standards were added to prenasalizes sample of leaves. The spiked samples were then analysed by the proposed HPTLC method and the analysis was carried out in triplicate.

**Precision**

Precision was studied for the Intra-day and Inter-day precisions. Each level of precision was investigated by three sequential replicates of injections of luteolin, at concentration of 1000, 2000 and 3000 ng/spot were analysed on the same day. The Inter-day precision was studied by comparing assays performed on three different days.

**Specificity**

The specificity method was performed by analyzing standard compounds luteolin, separately and those bioactive compounds were present in rhizome of *Zingiberofficinale*.

**Antimicrobial activity of isolated compounds from the Rhizome of *Zingiber officinale* (**Gao*et al*., 2010)

**Collection of test Pathogens**

The antibacterial and antifungal activity of isolated compounds were exhibited against *Escherichia coli* (MTCC 25922), *Enterococcus aerogenes* (MTCC 29212), *Staphylococcus aureus* (MTCC 25923) and for fungal culture used in the study are *Candida albicans* (MTCC 282). All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India and the fungal strains from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

**Antibacterial studies of Ethanolic extract of Rhizomes of *Zingiber officinale***

The disc diffusion method is used to determine the antibacterial activity of each isolated compounds. The isolated compounds (10 mg) were re-dissolved in 1 ml of methanol, sterilized through Millipore filter (0.22 µm) then loaded over sterile filter paper disc (8 mm in diameter to obtain final concentration of 10 mg/disc. Ten ml of Mueller-Hilton agar medium was poured into sterile petri dishes (diameter 60 mm) and inoculated with test organism. Sterile filter paper disc loaded with various concentrations of isolated compounds of 20, 60 and 80 µg/ml were placed on the top of Mueller-Hilton agar plates. Filter paper disc loaded with 5 µg of amoxicillin was used as positive control. Negative control was prepared using the respective solvent. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was recorded in millimeter and the experiment was repeated twice.

**Determination of Antifungal Activity by Disc Diffusion Method**

Disc diffusion method in order to test the antifungal activity of isolated compounds against test pathogens was carried out. In petri dishes (60 mm) filled with Sabouraud's dextrose agar (SDA) and seeded with a 0.3 ml of test organism, a sterile filter paper disc (diameter 6 mm, Whatman paper no.3) was placed. The sterile disc was impregnated with 10 µl of isolated compounds each at varying concentration of 20, 60 and 80 µg/ml respectively. The zones of growth inhibition around the disc were measured after 24 h of incubation at 37ºC. Each microorganism tested in triplicate and the solvent methanol was used as a negative control, while Fluconazole was used as a positive control.

**Statistical Analysis**

Statistical analyses were performed in triplicates and the results were expressed as the mean values ± SD with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at $P < 0.05$ were considered to be significant.

**Results and Discussions**

**Optimization of HPTLC Chromatographic Conditions**

HPTLC fingerprint patterns have been therefore evolved for ethanolic extracts of rhizomes of *Zingiber officinale*. Luteolin standard was quantitated accurately using silica gel $F_{254}$ HPTLC pre-coated plates with the mobile phase toluene: ethyl acetate (7.0: 3:0 v/v), the Rf value was about 0.58. The chromatographs of luteolin and ethanolic extract of rhizomes of *Zingiber officinale* are shown in (Fig. 1). The Rf value of luteolin was matched with the Rf value of extract of *Zingiber officinale* was about 0.58 was shown in peak (Fig. 2 (a) and (b)).

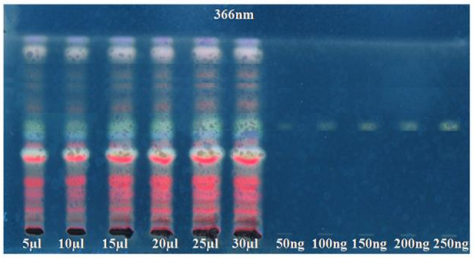


**Fig. 1: Quantitative estimation of luteolin in rhizomes of *Zingiber officinale***

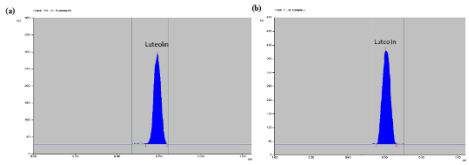


Fig. 2 (a) HPTLC chromatogram of standard luteolin; (b) HPTLC chromatogram of luteolin in rhizomes of *Zingiber officinale*.

### Calibration Curve and Linearity

The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot (Fig. 3). The regression equation and correlation curves for luteolin in *Zingiber officinale* were regression via height y= 6.234+0.101X and r= 0.99315 sdv= 6.52 Fig. 3 (a) and regression via area y=132.296+2.085 X and r=0.99977 sdv=1.17 Fig. 3(b).

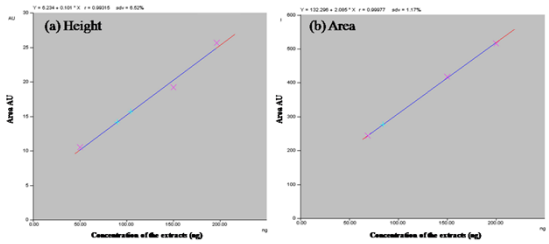


Fig. 3 Linear graph for luteolin in *Zingiber officinale* in all tracks (concentration vs. area).

### Accuracy and Recovery

The results showed that the percentage recoveries after sample processing and application were in the range of 99.77 % to 100.11 % (luteolin) (Table 1). The percentage of luteolin in rhizome of *Zingiber officinale* (Table 2).

**Table 1: Recovery study of luteolin by HPTLC (*n*=3)**

| Compound | Amount of compound present in the plant material (mean, µg/100 mg) | Amount of standard added (mg) | Amount of standard found in mixture (mg) | Recovery (%) |
|---|---|---|---|---|
| Luteolin | 1186 | 1186<br>2290 | 2293.17<br>4590.23 | 99.85 ±1.44<br>99.87 ± 0.97 |

n is number of determinations

**Table 2: Amount of luteolin in *Zingiberofficinale*rhizomes (n=3)**

| Compound | Quantity (mean) (mg/100 mg) | Mean ± SE | CV (%) |
|---|---|---|---|
| Luteolin | 1.186 | 1.178 ± 0.007 | 0.57 |

n is number of determinations, SE is standard error, CV is cumulative value

### Precision

The developed method was found to be precise as indicated by percent RSD (Relative Standard Deviation) not more than 1.5 (Tables 3 and 4).

**Table 3: Intra-day and inter-day precision of the method (n = 6)**

| Compound | Amount (ng/spot) | Intra-day precision | | | Inter-day precision | | |
|---|---|---|---|---|---|---|---|
| | | Mean area | SD | %RSD | Mean area | SD | %RSD |
| Luteolin | 1000 | 1384.38 | 1.432 | 0.059 | 1396.43 | 1.592 | 0.106 |
| | 2000 | 3473.47 | 1.742 | 0.044 | 3473.64 | 1.649 | 0.054 |
| | 3000 | 4988.75 | 1.478 | 0.036 | 4695.79 | 1.468 | 0.039 |

n is number of determinations, SD is standard deviation, RSD is relative standard deviation

**Table 4: Summary of Validation parameter**

| Parameters | Luteolin |
|---|---|

| | |
|---|---|
| Linearity | |
| i. Range | 200-1200 ng |
| i. Correlation coefficient | 0.99956 |
| a. Height | 0.99998 |
| b. Area | 0.55 |
| ii. Rf value | |
| Precision (%RSD) | |
| i. Instrument precision (CV%, n=6) | 1.86 |
| i. Method precision (CV%, n=6) | 2.79 |
| LOD (ng/spot) | 80 |
| LOQ (ng/spot) | 450 |
| Specificity | Specific |
| Robustness | Robust |
| Ruggedness (%RSD) | 0.8935 |

n is number of determinations, RSD is relative standard deviation, CV is cumulative value, LOD is Limit of detection, LOQ is Limit of quantification, Rf is retention factor.

**Specificity**

It was observed that the other herbal constituents present in the formulations did not interfere with the peak of luteolin. Therefore, the method was specific. The spectrum of standard compound luteolin and the corresponding spot present in *Zingiber officinale* matched exactly, indicating no interference by the other plant constituents and excipients. The peak purity of luteolin was assessed by comparing the spectra at three different levels like peak start (S), peak apex (M) and peak end (E) positions of the spot. Good correlation r = 0.99977 and SD = 1.17 for luteolin were obtained between the standard and sample overlain spectra of luteolin (Figures 4).

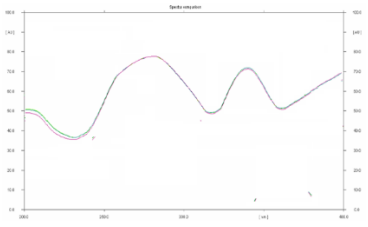

**Fig. 4: Spectral comparison of standard luteolin (green colour) and luteolin quantified from rhizomes of *Zingiber officinale* (pink colour).**

**Limit of detection and limit of Quantification**

The limit of detection was found to be 80 ng/spot for luteolin while the limit of quantification was found to be 450 ng/spot for luteolin (Table 4).

**Robustness**

Robustness tests examine the effect of the operational parameters on the analysis results. By introducing small changes in mobile phase composition, the results indicated that the method was robust (Table 5).

**Table 5: Robustness of the method(n=6)**

| Compound | Amount (ng/spot) | Mobile phase | %RSD |
|---|---|---|---|
| Luteolin | 1000 | Toluene: Ethyl a66<br>cetate (70:30 v/v)<br>Toluene: Ethyl acetate (60:40 v/v) | 0.83<br>1.35 |

n is number of determinations, RSD is relative standard deviation

**Ruggedness of the method**

It expresses the precision within laboratories variations like different days, different analyst, and different equipment. Ruggedness of the method was assessed by spiking the standard 6 times in two different days with different analyst (Table 4).

**Antibacterial activity of ethanolic extract of *zingiber officinale***

The results of the antibacterial activity of crude extracts were tested against pathogens by disk diffusion method are shown in (Table 6). The crude extracts showed growth inhibitory activity against and Escherichia coli (9 mm) at concentration 100 µg/ml. At concentration 80 µg/ml, the crude extracts exhibited the antibacterial activity but was more susceptible against *Escherichia coli*. As the concentration of extracts increased from 20-60 µg/ml, the inhibitory actions of the plant extracts increased towards all the strains used in this study (fig.5).

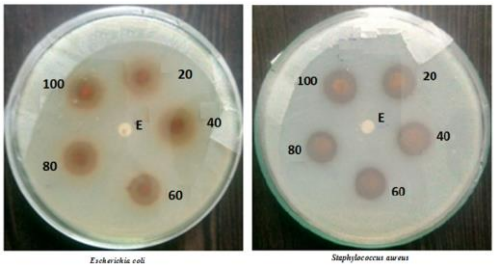

**Figure 5: Antibacterial activity of ethanolic extract of *Zingiber officinale***

**Table 6: Antibacterial activity of ethanolic extract of *Zingiber officinale***

| Samples | Concentrations (µg/ml) | Organisms/Zone of inhibition (mm) | |
|---|---|---|---|
| | | Ethanolic extract of *Zingiber officinale* | |
| | | *Escherichia coli* | *Staphylococcus aureus* |
| Ethanolic extract | 20<br>40<br>60<br>80<br>100 | 5<br>6<br>7<br>8<br>9 | 4<br>5<br>6<br>7<br>8 |
| Ethanol | 10 µl/disc | 0 | 0 |

**Antifungal activity of ethanolic activity of *zingiber officinale***

Table 7 shows results of the antifungal susceptibility test of the different plant extracts and against the test organisms. From the result, the ethanolic extracts were the most effective and the highest activity was demonstrated against *Aspergillus flavus* (2 mm zone of inhibition) at 100µg\ml, followed by the highest activity against *Candida albicans* (7 mm zone of inhibition) at 100µg\ml) and at concentration 80µg/mlAs the concentration of extracts increased from 20-60µg/ml, the inhibitory actions of the plant extracts increased towards all the strains used in this study (fig. 6).

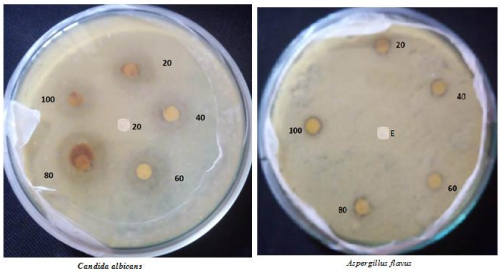


**Figure. 7: Antifungal activity of ethanolic activity of *Zingiber officinale***

**Table 2: Antifungal activity of ethanolic extract of Zingiberofficinale**

| **Samples** | **Concentrations (µg/ml)** | **Organisms/Zone of inhibition (mm)** | |
|---|---|---|---|
| | | **Ethanolic extract of zingiberofficinale** | |
| | | ***Candida albicans*** | ***Aspergillusflavus*** |
| Ethanolic extract | 20<br>40<br>60<br>80<br>100 | 3<br>4<br>5<br>6<br>7 | 1<br>1<br>1<br>1<br>2 |
| Ethanol | 10 µl/disc | 0 | 0 |

**Conclusion**

Zingiber officinale rhizomes in ethanolic extract has shown the presence of Tannin, Steroids, Saponin, AFlavonoids, Alkaloids, Terpenoids, Phlobatannin, Phenol, Leucoanthocyanin, Cardiac glycosides, Anthocyanin, Anthraquinones, Glycosides, Coumarin, Emodine, Xanthoprotein, Carbohydrates and shows the absence of Protein content. In conclusion, an HPTLC method has been developed with some modifications and it can be used for the quantitative determination of luteolin in ethanolic extract of rhizomes of *Zingiber officinale*; its main advantages are its simplicity, accuracy and selectivity. The average recovery values of luteolin were found to be about 99.94%, which showed the reliability and suitability of the method. The ethanolic extracts of *Zingiber officinale* was showed growth inhibitory activity against *Escherichia coli* (10 mm) and *Staphylococcus aureus* (9 mm) at concentration 100 µg/ml. The highest activity was demonstrated against *Candida albicans* (10 mm zone of inhibition) at 100 µg/ml, followed by the highest activity against *Candida albicans* (9 mm zone of inhibition) at 100 µg/ml). However further research on detailed isolation of another active phytoconstituents possessing the therapeutic activity and clinical study for the evaluation of safety and efficacy of the drug needs to be assessed.

**Acknowledgement**

S.r. acknowledges Dr. K. Manjula, chief executive officer, BioTechno Solutions training and Research Institute, Ponmalaipatti, Trichy for her constant support for this research.

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## Anti-ageing Activity and Identification of Bio Active Compound from the Seed of Linum Usitatissimum by GC-MS Techniques

***[1]S.Rexcida Janthark Mary, [2]S.Josephinol, and [3]B. Ramya***

*[1, 2 &3]PG Department of Biochemistry*
*Holy Cross College (Autonomous),*
*Tiruchirappalli – 620 002*

**Abstract**

**Objective:** Aging occurs in all living organisms and it is an inevitable process. Aging is caused due to both intrinsic and extrinsic factors. Intrinsic aging is an inevitable physiological process. Extrinsic aging occurs in addition to intrinsic aging as a result of sun and environmental damage. Activation of hyaluronidase, collagenase and elastase, leads to skin aging. By using the medicinal crop *Linumusitatissimum*were investigated to assess their skin aging properties.

**Methods:** The activity of anti- elastase, anti-collagenase and anti-hyaluronidase in ethanol extract from the seed of *Linumusitatissimum*was determined by using spectrophotometric methods. The bio-active compounds of *Lainumusitatissimum*was analyzed using GC-MS method.

**Result:** The anti-elastase assay in ethanol extract of *Linumusitatissimum*shows 70% inhibition, for anti-collagenase assay shows 73% inhibition and for anti-hyaluronidase assay shows 61% inhibition. The ethanol extracts of *Linumusitatissimum*contained the highest anti- collagenase activity.

**Conclusion:** The enzymes which are responsible for aging is inhibited by ethanol extract of *Linumusitatissimum,* suggests that it can help to restore skin elasticity and slows down the process of aging.

**Introduction**

Aging occurs in all living organisms and it is an inevitable process. The skin protects us from microbes and the elements, helps to regulate body temperature, and permits the sensation of touch, heat and cold. Chronological aging and photoaging are the two types of skin aging [Mukheijee*et al*., 2011]. The extrinsic factor causes photoaging which includes symptoms like leathery appearance, dark or light pigmentation and deep furrows [Fisher GJ *et al*., 2002; Maity N *et al*.,2011].

The epidermis, dermis and subcutaneous tissue are the three layers in skin [Ritte L *et al*., 2002]. The outermost part of the skin is the extracellular matrix (ECM) which is made up of fibroblasts and proteins that include collagens and elastin [Fulop T *et al*., 2012]. The ECM plays an important role in the maintenance of physiological functions of the body because it is essential for growth and elasticity of the skin [Fulop T *et al*., 2012;Kurtz A Oh S.,2012]. Skin aging is directly linked with degradation of ECM and it is correlated with an increase in activity of some enzymes, which is involved in skin aging including hyaluronidase, collagenase and elastase [Maity N *et al*.,2011; Wary KK Thakkar GD.,2003; Losses JN.,2004; Maity N *et al*.,2011].

The main component of connective tissue, hair and nails is collagen and is the building blocks of skin [Mukheijee*et al*.,2011]. The elasticity, strength and flexibility of the skin is maintained by collagen. The moisture of the skin is retained by hyaluronic acid and it is also responsible for its structure and elasticity of the skin. It is involved in rapid tissue proliferation, regeneration and repair, it also facilitates the exchange of nutrients and base products [Manuskiatti W *et al*.,1996; Hsu M-F *et al*.,2009]. Hyaluronic acid is required for the organization and structural maintenance of the ECM. When collagen, elastin and hyaluronic acid level decreases it leads to loss of strength and flexibility in the skin, which further results in visible wrinkles.

*Linumusitatissimum* Linn, commonly known as Alsi belongs to the family Linaceae and it is cultivated in cooler regions throughout the world [ShwetaGokhale*et al*.,2016]. It has anticancer[Yan L *et al*.,1998; Kuijsten A *et al*.,2008], antidiabetic[Ghule AE et al.,2012], antimicrobial[Amin T *et al*.,2014] and it helps to reduce cardiovascular diseases[Gambus H *et al*.,2004;Cintra DEC *et al*.,2006]. Its oil is known as linseed oil. It is used for various medicinal purposes.

Textiles made from flax are known as linen and are used for bed sheets, underclothes and table linen [ShwetaGokhale*et al*.,2016]. This flax seed protein helps to improve immune function, lowers cholesterol, prevents tumor, has antifungal activity and it has been proved by animal studies.[Xu Y.2007; J. Agric.2010;Rabetafika, H.N *et al*.,2011]

**Material and Methods**

**Collection of plant materials and preparation of ethanolic extracts:**

The *Linumusitatissimum* seed is collected from the nearby villages in trichy and conformed its presence with the help of guide.

In a uniform powdered manner of 40 mesh size the *Linumusitatissimum*seed are grinded, before grinding the *Linumusitatissimum*seed is washed in water and dried it for a week and place it in the room temperature. The 20g of dried seed powder of*Linumusitatissimum* is dissolved in a prepared ethanolicextract(40ml) and keep it in a hot perculation for 1 hour of duration. After cooling the extract, it is filtered by using Whatmann filter paper No.42 (125mm). By using this method the undissolved products are removed which contain cellular material and some other materials. The solution which is filtered is used for the further analysis of qualitative and quantitative method.

**Chemicals and reagents:**

FALGPA [N-[3-(2-furyl)acrylolyl]-leu-Gly-Pro-Ala, TES[tris(hydroxymethyl)-methyl-2-aminoethane sulponate (TES), The type I collagenase from clostridium, histolytium, ethanol, isopropanol, ninhydrine solution , cirate buffer, 4-dimethylamino benzaldehyde (DMAB), The HEPES at pH 7.5 , 4-(2-hydroxyethyl)-1-pipirzine ethanesulfonic acid, Potassium metaborate ($KBO_2$) , Hyaluronic acid , Hyaluronidase acid ,calcium chloride dihydrate , human leukocyanteelastase, N-methoxysuccinyl – Ala-Ala-Pro-Chloro.

**Anti-elastase activity determination:**

For the anti-elastase activity determination, the preparation for bulk needs various concentration of *Linumusitatissimum*seed extract (20,40,60,80,100 µg/ml) was taken in the test tube and labelled it; the 100µg/ml of extract is added with 900 µg/ml of ethanol and 200 µg/ml extract is added with 800 µg/ml of ethanol and 300 µg/ml extract added with 700 µg/ml of ethanol, 400 µg/ml of extract is added with 600 µg/ml of ethanol, and 500 µg/ml of extract is added with 500 µg/ml of ethanol. For the control solution ethanol (1000µg/ml) is taken. The elastase of 500µg/ml is taken and added with 500µg/ml hepes buffer and then add 1000µg/ml of various concentration of plant sample extract and keep it in room temperature for 20 minutes then add 500µg/ml N-methoxysuccinyl-ala-ala-pro-chloro in test tubes which are incubated for further 20 minutes then check the absorbance at 540nm. The percentage inhibition was calculated as follows

Inhibition (%) = [(A control – A sample) / A control] x 100

**Anti-hyaluronidase activity determination:**

The method of finding out hyaluronidase activity is based on flurometric Morgan - Elson assay

Method. 500µg/ml calcium chloride which was taken and added it with 1000µg/ml of various

Concentration from the bulk solution and then add 500µg/ml hyaluronidase acid keep it for 20 minutes in a room temperature. Then add 250µg/ml of hyaluronic acid they are all kept in it for 10 minutes. The 250µg/ml of $KBO_2$ is added in a test tube. They are heated and cooled for 5 minutes then 500µg/ml DNAB is added in test tube keep it for 10 minutes incubation in room temprature check for the absorbance at 540 nm.

**Anti- collagenase activity determination:**

The method for finding out anti-collagenase activity is based on the Moore stein method which was modified by Mandal et.al. The 500µg/ml of collagenase is taken in a test tube with that 500µg/ml of TRIS buffer at the various concentration of the extract 1000 µg/ml is taken. They are all kept for 10 minutes incubation in a room temperature and then add 500µg/ml FALGPA enzyme which has been keep it in room temperature for 10 minutes then 500 µg/ml of citrate buffer solution is added and incubate it for 5 minutes in room temperature. 500µg/ml ninhydrin solution is added heat and cool it for 5 minutes and then 500µg/ml isopropanol which is added and incubate it for 5 minutes and check for the absorbance at 540nm

**.GC-MS Analysis of Flax Seed of ethanolicextrct of *Linumusitatissimum*:**

GC/MS analysis was performed at Heber Analytical Instrumentation Facility, Thiruchirapalli, for MS identification of the GC components. The column

used was DB-5 (J & W Scientific, Folosm, CA) cross–linked fused silica capillary column (30 m long, 0.25 mm internal diameter) coated with polydimethylsiloxane (0.5µm film thickness). The oven temperature was programmed from 50oC for 2 min., at isothermal, then heating by 7oC/ min. to 250oC and isothermally for 10 min., at 250oC. Injector temperature was 250oC and the volume injected was 0.5 µl. Transition line and ion source temperature were 250oC and 200oC respectively. The mass spectrometer had a delay of 2 min. to avoid the solvent peak and then scanned from m/z 50 to m/z 500. Ionization energy was set at 70 eV (Fatma Mohamed El-Feky*etal*., 2016).

**Result and Discussion**

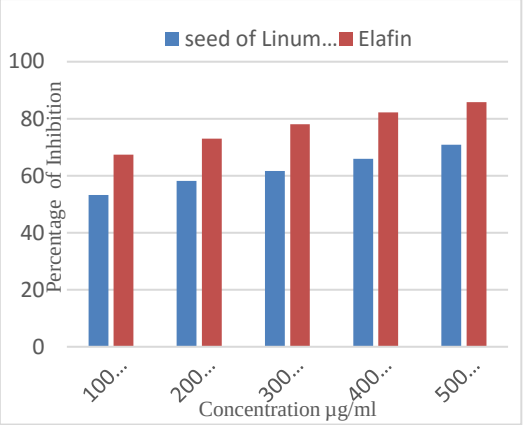
**Anti-elastase activity determination:**

In the place of connective tissues elastin is found. The elastin provides the elasticity to our skin and lungs. It is a kind of protein and it is monitorized by the enzyme called elastase. If the elastin content in our body is decreased or by exposure to UV radiation causes skin aging. Our crop *Linumusitatissimum*has provided a good firm to our skin and improve the elasticity of our skin. The ethanolic extract of seed of *Linumusitatissimum* at different varying concentrations (100µg/ml,200µg/ml,300µg/ml,400µg/ml,500µg/ml) (table 1) shows the various % inhibition concentration and the maximum inhibition concentration is showed at 100µg/ml =53.19% which is compared with the standard Elafin which show the % concentration at 100µg/ml=48.23% (Figure 1).

**TABLE 1: Determination of anti-elastase activity of seed of *Linumusitatissimum* with the standard Elafin:**

| **Concentration** | **Anti - elastase % inhibition concentration** | |
|---|---|---|
| | **Seed of *linumusitatissimum*** | **Elafin** |
| 100µg/ml | 53.19 | 67.37 |
| 200µg/ml | 58.16 | 73.04 |
| 300µg/ml | 61.70 | 78.01 |
| 400µg/ml | 65.96 | 82.26 |
| 500µg/ml | 70.92 | 85.81 |

**Figure 1: Comparitive graph of seed *linumusitatissimum*with the standard elafin**



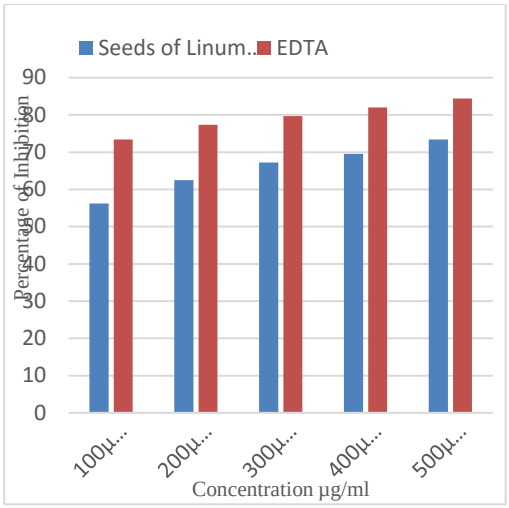

**Anti collagenase activity determination:**

The skin is the most important layer in our body and it consist of some important components like collagen with it. The collagen is declined by the presence of enzyme collagenase. If the collagenase is hindered means the aging process slowly occurs and the formation of pre-collagen fibres is delayed. The ethanolic extract of seed of Linumusitatissimum with different varying concentration (109µg/ml,200µg/ml,300µg/ml,4000µg/ml,500µg/ml) (table 2) shows the different % inhibition concentration and the maximum % inhibition concentration in the anti-collagenase activity shows at 100µg/ml=56.25% and it is compared to the standard EDTA which show the anti-collagenase (Figure 2).

**TABLE 2: Anti-collagenase activity of seed of *Linumusitatissimum* with the standard EDTA*:***

| **Concentration** | **Anti-collagenase % inhibition** | |
|---|---|---|
| | **Seed of *usitatissimuum*** | **EDTA** |
| 100µg/ml | 56.25 | 73.43 |
| 200µg/ml | 62.5 | 77.34 |
| 300µg/ml | 67.18 | 79.68 |
| 400µg/ml | 69.53 | 82.03 |
| 500µg/ml | 73.43 | 84.37 |

**Figure 2: Comparitive graph of *linumusitatissimuum* seed with the standard edta:**



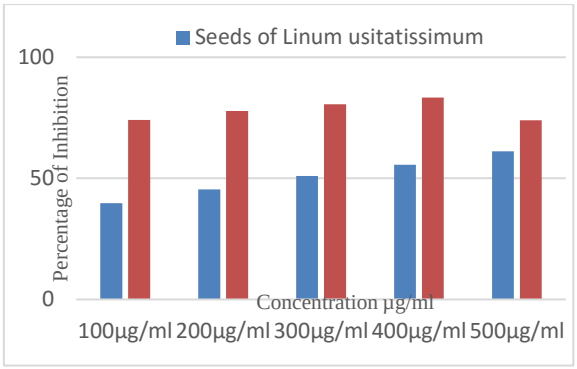

**Anti-hyaluronidase activity**

The hyaluronidase activity is more important for our skin because it reduces the fine lines in our skin and it consists of some healing property with it. The ethanolic extract of the seed of *usitatissimuum* in the different concentrations (100µg/ml,200µg/ml,300µg/ml,400µg/ml,500µg/ml) (table ) show the different % inhibition concentration and the maximum % inhibition concentration is shown at the range of 100µg/ml=39.81% which is compared to the standard Sodium aurothiomalate. To find out the anti-hyaluronidase activity in the standard the maximum % inhibition concentration is shown at 100µg/ml= 74.07% (Figure 3).

**TABLE 3: 3Anti-hyaluronidase activity of seed of *Linus usitatissimum*compared with sodium aurothiomalate:**

| Concentration | Anti-hyaluronidase activity % inhibition concentration | |
|---|---|---|
| | Seed of *Linumusitatissimum* | Sodium aurothiomalate |
| 100µg/ml | 39.81 | 74.07 |
| 200µ/ml | 45.37 | 77.77 |
| 300µg/ml | 50.92 | 80.55 |
| 400µg/ml | 55.55 | 83.33 |
| 500µg/ml | 61.11 | 86.11 |

**Figure 3: Comparative graph on seed of *Linumusitatissimum* with the standard Sodium aurothiomalate:**

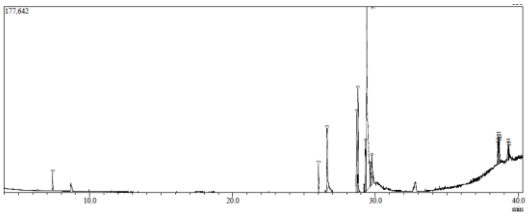



**Gas chromatography- mass spectrometry of flax seedof *linumusitatissimum*:**

BICYCLO[4.1.0]HEPT-3-ENE-2-THIOL,3,7,7-TRIMETHYL-,[1S-(1.ALPHA.,2.ALPHA.,6.ALPHA.)]-; HEPTADECANOIC ACID, METHYL ESTER; HEXADECANOIC ACID; METHYL OCTADECA-9,12-DIENOATE; (Z,Z)-6,9-CIS-3,4-EPOXY-NONADECADIENE; 9,12-OCTADECADIENOIC ACID, METHYL ESTER, (E,E)-; 7-Tetradecenal, (Z)-; Bicyclo[3.3.1]nonan-1-ol; 9-OCTADECENOIC ACID (Z)-; 1-NAPHTHALENEMETHANOL, 1,4,4A,5,6,7,8,8A-OCTAHYDRO-2,5,5,8A-TETRAMETHYL-; 1-UNDECENE-5,9-DIYNE;

1,3,5-TRIPHENYL-1,5-PENTANEDIONE; Silane, methyldiisopropoxyethoxy-; (+)-2,3-o-Benzylidene-D-threitol; Chromium, (.eta.-5-cyclopentadienyl)-(.eta.-6-toluene) (Figure 4) (Table 4).

**Figure 4: Identification of Bio active compounds from the flax seeed of *Linumusitatissimum*:**



The previous studies suggested that the aging process mainly on skin wrinkling and identified that the skin wrinkling causes due to the body mass destruction (Kumar *et al*., 1980). Middelkoop TB *et al*., 1985 studied about theplant *Saracaasoca* are treated against menorrhagia, anti-estrogenic activity, utertonic, anti-bacterial, anti-tumour and anticancer activity. Verma A et al., 2010 studied that the natural herbs contains phytochemicals such as terpenoids polyphenols, carotenoids and some herbs which are treated against

antiaging are jatamansi, alover, gensing, cucumber and honey etc.,Dhawan BN et al., 1977 studied about the anti-aging and sunscreens and they investigated the biological process of the skin aging and what are the factors responsible for aging and how to prevent the process of aging by applying the sunscreen products.

**Conclusion**

The present study concluded that the *Linumusitatissimuum* has the presence of the antiaging property and it is resulted under different methods. From the qualitative test it is confirmed that the seed of *Linumusitatissimuum*inethanolic extract has shown the presence of Tannin, Steroids, Saponin, Flavonoids, Alkaloids, Terpenoids, Phlobatannin, Phenol, Glucoanthocyanin, Cardiac glycosides, Anthocyanin, Antraquinones, Glycosides, Coumarine, Emodin, Xanthoprotein, Carbohydrates and Protein content..The anti- elastase activity in the ethanolic extract of seed of *Linumusitatissimuum* showed the maximum inhibition 5.19% which is compared to the standard elafin48.23 % at 100µg/ml. The anti-collagenase activity showed the maximum 56.25 % compared to the standard EDTA 73.44 % at 100µg/ml. The anti-hyaluronidase activity showed the maximum inhibition 39.81% compared to the standard sodium aurothiomalate74.07 % at 100µg/ml. so, it is confirmed that the seed of *Linumusitatissimuum*is helpful for the treatment anti-aging due to the presence of bioactive compounds confirmed by GCMS spectra.

**Acknowledgement**

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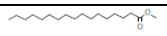
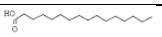
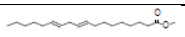
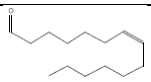
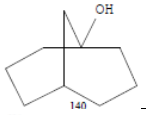
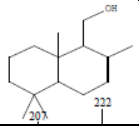
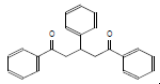
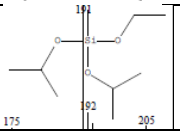
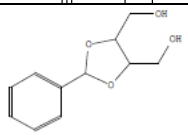
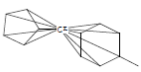
**Table 4: Identification of Bio active compounds from the flax seeed of *Linumusitatissimum*:**

| COMPOUND NAME | RETENTION | AREA% | MOLECULAR FORMULAE | MOLECULAR STRUCTURE |
|---|---|---|---|---|
| BICYCLO[4.1.0]HEPT-3-ENE-2-THIOL, 3,7,7-TRIMETHYL-, [1S-(1.ALPHA.,2.ALPHA.,6.ALPHA.)]- | 7.401 | 1.33 | $C_{10}H_{16}S$ | |
| HEPTADECANOIC ACID, METHYL ESTER | 26.012 | 2.54 | $C_{18}H_{36}O_2$ |  |
| HEXADECANOIC ACID | 26.613 | 8.14 | $C_{16}H_{32}O_2$ |  |
| METHYL OCTADECA-9,12-DIENOATE | 28.683 | 6.66 | $C_{19}H_{34}O_2$ |  |
| (Z,Z)-6,9-CIS-3,4-EPOXY-NONADECADIENE | 28.779 | 11.02 | $C_{19}H_{34}O$ | |
| 9,12-OCTADECADIENOIC ACID, METHYL ESTER, (E,E)- | 29.301 | 6.25 | $C_{19}H_{34}O_2$ |  |
| 7-Tetradecenal, (Z)- | 29.392 | 43.71 | $C_{14}H_{26}O$ |  |
| Bicyclo[3.3.1]nonan-1-ol | 29.62 | 2.76 | $C_9H_{16}O$ |  |
| 9-OCTADECENOIC ACID (Z)- | 29.759 | 4.73 | $C_{18}H_{34}O_2$ |  |
| 1-NAPHTHALENEMETHANOL, 1,4,4A,5,6,7,8,8A-OCTAHYDRO-2,5,5,8A-TETRAMETHYL- | 38.565 | 3.1 | $C_{15}H_{26}O$ |  |
| 1-UNDECENE-5,9-DIYNE | 38.614 | 1.81 | $C_{11}H_{14}$ | |
| 1,3,5-TRIPHENYL-1,5-PENTANEDIONE | 38.65 | 2 | $C_{23}H_{20}O_2$ |  |
| Silane, methyldiisopropoxyethoxy- | 38.675 | 2.5 | $C_9H_{22}O_3Si$ |  |
| (+)-2,3-o-Benzylidene-D-threitol | 39.29 | 1.72 | $C_{11}H_{14}O_4$ |  |
| Chromium, (.eta.-5-cyclopentadienyl)-(.eta.-6-toluene) | 39.35 | 1.75 | $C_{12}H_{13}Cr$ |  |

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## Phytochemical and antimicrobial activity of *Solanum torvum* against respiratory tract pathogens

***[1]M.Rajathi D Modilal ,[1]R. Cecily Rosemary Latha , [1]B. Jeyanthi , [2]R.Sindhu and [3]R Anandan***

*[1] P.G and Research Department of Zoology, Holy Cross College (Autonomous), Tiruchirappalli – 620 002*

*[2]Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chinna Kolambakkam, Kanchipuram-603308.*

*[3] Department of Computer Science and Engineering, School of Engineering, Vels Institute of Science, Technology and Advanced Studies, Chennai-600 117.*

**Abstract**

Medicinal plants are extensively used to cure various infectious diseases in human beings, hence *S. torvum* was investigated for its activity against the isolated pathogens. The study was designed to screen and characterize the bacteria isolated from the respiratory tract infected patients. Phytochemical analysis of the plant showed the presence of alkaloids, terpenoids, flavonoids, saponins, steroids and phenols. Aqueous, ethanol and diethyl ether extract of *S. torvum* was prepared; four different concentrations of each extract was taken to determine the antibacterial activity against the isolated bacteria. The ethanolic extract of *S. torvum* showed highest antimicrobial activity in comparison to aqueous and diethyl ether extracts. Thus *S. torvum* has antimicrobial activity and can be used clinically to find novel antibacterial compounds for respiratory tract pathogens.

**Keywords**: Respiratory tract infections, antibacterial activity, phytochemical activity, *S. torvum*

### Introduction

Respiratory tract infections (RTIs), which involve the upper or lower respiratory tract, frequently occurs after birth [1]. RTIs, such as sore throat, earaches, laryngitis, common cold, sinusitis, and mastoiditis, are the most frequently-occurred infections of all human diseases and have been frequently documented [2&3]. RTIs are amongst the most wide

spread and serious infections, accounting for over 50 million deaths globally each year. Each year approximately seven million people die as direct consequences of acute and chronic respiratory infection. Bronchitis and pneumonia are the most common infection. Respiratory pathogens like *Klebsiella pneumoniae, Pseudomonas aeroginosa* and *Staphylococcus aureus* are some of the causative agents responsible for bronchitis and pneumonia [4.]

Most respiratory tract infections are caused by viral and bacterial pathogens responsible for higher morbidity and mortality [5].The leading causes of non-communicable disease deaths in 2008 due to respiratory diseases were 3.9% and 4.2 million deaths were reported due to asthma and Chronic Obstructive Pulmonary Disease globally [6.] Today, nearly 88% of the global population turn to plant derived medicines as their first line of defense for maintaining health and combating diseases. Currently, people of Asia especially India are utilizing plants as part of their routine health management [7]. Medicinal properties of plants are hinged on the presence of bioactive principles such as alkaloids, phenols, tannins, glycosides and essential oils amongst others [8]. The primary benefits of using plant derived medicines are that they are relatively cheaper than synthetic drugs, offering profound therapeutic benefits and more affordable treatments. Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional system of medicine relatively cheaper than modern medicine. As a result, many potent drugs have been purified from plants, including emetin, quinine, artemisin and introduced to modern medical practice.

*Solanum torvum* of the family Solanaceae is commonly known as Turkey berry and cultivated in Africa and West Indies. The plant is sedative and diuretic and the leaves are used as a haemostatic. The ripened fruits are used in the preparation of tonic and haemopoietic agents and also for the treatment of pain. It has antioxidant properties. It is intensively used worldwide in the traditional medicine as poison anti-dote and for the treatment of fever, wounds, tooth decay, reproductive problems and arterial hypertension. *S. torvum* also possesses antimicrobial, antiviral, immuno-secretory, antioxidant, analgesic and anti-inflammatory, anti-ulcerogenic activities, cardiovascular, nephron protective, antidiabetic, angiotensin and serotonin receptor blocking activities[9.]

Therefore, in the current investigation, bacteria responsible for respiratory tract infections were isolated from sputum samples of RTI patients and later on characterized. *Solanum torvum* leaves were subjected to extraction with solvents like ethanol, petroleum ether and water. The antimicrobial effect of the prepared extracts were evaluated against isolated respiratory tract pathogens.

## Materials and Methods

### Collection and authentication of Plant Material

The plant material *S. torvum*(Fig.1) was collected from in and around Chennai, Tamilnadu, India. The plant was authenticated by Prof. Dr. D. Aravind, Department of Medicinal Botany, National Institute of Siddha, Chennai, Tami Nadu, India.

Figure 1: Photograph of the plant

*Solanum torvum*

### Preparation of Extracts

Fresh leaves of *S. torvum* was washed thoroughly in tap water and with distilled water and airdried in the shade at room temperature for five days. Shade dried leaves were powdered .The plant powders (100 g) were successively extracted using ethanol and petroleum ether in soxhlet apparatus and crude extraction was done with water. The extracts were dried in vacuum desiccator and were stored in a sterile container for further use.

### Collection of Samples

The sputum samples were collected from patient's aseptically in well-labelled sterile, wide mouthed glass bottles with screw cap from Karpaga Vinayaga Institute of Medicinal Science and Government Hospital. Samples were then taken to the laboratory immediately for analysis [10.] On the labels were marked the name, age, sex of the patients and the time of sample collection.

**Isolation and identification of pathogens in sputum samples**

The collected samples were processed as per the standard procedure. For isolation and characterization of bacterial flora, the samples were inoculated into Blood Agar (BA) media and incubated at 37°C for 24 hours[11]. Characteristic colonies from the plates were isolated and then sub cultured to obtain pure culture. All the bacteria were isolated and identified using morphological and biochemical tests adopting standard procedures[12]. Stock culture was maintained in both Agar slant and 20% sterile buffered glycerol.

**Phytochemical Screening**

The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolic, alkaloids, tannins, flavonoids, saponin and steroids under qualitative analysis; the screening was carried for all the extracts of the plant [13].

**Antibacterial activity of various extracts:**

Muller-Hinton Agar (MHA) plates were seeded with 24 hours old culture of the isolates. The organic fractions were dissolved in dimethyl sulfoxide (DMSO). Wells were bored using sterilized syringe of pore size 8 mm. Various concentrations of the extracts (250µl, 500µl, 750µl and 1000µl) were added into the sterile 8mm diameter well. Incubation was made at $37^0$C for 24 hrs. Antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well using standard (Hi-Media) scale. The experiment was repeated four times and the average values were calculated for antibacterial activity [14].

**Results and Discussion**

**Phytochemical activity**

The phytochemicals present in *Solanum torvum* were alkaloids, flavonoids, steroids, terpenoids, Saponins and phenols(table 1). this is in accordance with an earlier investigation which showed the presence of various phytochemicals such as alkaloids, carbohydrates, reducing sugars, flavanoids, gums and mucilage and proteins[15].Preliminary phytochemical screening of *S.torvum* indicates the presence of These compounds are known to be biologically active because they protect the plants against infection. Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents. Many reports are available regarding anti-viral, anti-bacterial, anti-fungal, anti-helminthic and anti-inflammatory properties of plants[16].

**Table 1. Phytochemical Constituents of *Solanum torvum***

| Sl.No | Phytochemical | Ethanolic Extract | Aqueous Extract | Petroleum Ether extract |
|---|---|---|---|---|
| 1 | Alkaloids | + | + | + |
| 2 | Terpnoides | + | + | + |
| 3 | Saponins | + | + | + |
| 4 | Tannins | - | - | - |
| 5 | Flavonoids | + | + | + |
| 6 | Phlobatannins | - | - | - |
| 7 | Steroid | + | - | - |
| 8 | Phenols | + | + | + |

Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, anti-inflammatory, anti- microbial and anti-cancer activity[17 & 18]. Tannins are known to possess general antimicrobial and antioxidant activities[19.]Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents[20]. Other compounds like saponins also have anti-fungal properties. Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins[21.]In medicine, it is used in hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti inflammatory and weight loss, etc. It is also known to have anti-fungal properties [22].Saponins have been implicated as bioactive antibacterial agents of plants . Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and anti- microbial properties[23 &24] .Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammtory[25.]

The bacteria were isolated and identified using morphological and biochemical tests following standard procedures. Out of the 50 Sputum samples, 23 bacterial isolates were recovered and the biochemical tests revealed that, these isolates belong to 5 species (Table 2). The bacteria isolated from the samples were *Klebsiella pneumoniae*, *Streptococcus pyrogens*, *Escherichia coli*, *Staphylococcus aureus*

and *Pseudomonas aeruginosa were* isolated from the sputum samples. In an earlier study following bacteria were isolated from the sputum sample of RTI patients *S. aureus, E. coli, P. aeruginosa, S. pneumoniae* and *K. pneumonia*[26]; this is in accordance with the present investigation except *S. pneumoniae.*

**Table 2: Biochemical characterization of isolated bacteria from RTI patients**

| S.No | Characteristics | *E.coli* | *K. pneumoniae* | *S. aureus* | *P. Aeruginosa* | *S. pyrogens* |
|---|---|---|---|---|---|---|
| 1 | Shape | Rods | Rods | Cocci | Rods | Cocci |
| 2 | Gram Test | - | - | + | - | + |
| 3 | Coagulase | - | - | + | - | + |
| 4 | Catalase | + | + | + | - | + |
| 5 | Citrate | - | + | + | + | + |
| 6 | Oxidase | - | - | - | + | - |
| 7 | Indole | + | - | - | - | - |
| 8 | MR | + | - | - | - | - |
| 9 | VP | - | + | + | - | + |
| 10 | Motility | Motile | Motile | Non- Motile | Motile | Non- Motile |

**Antibacterial activity**

The results of the antibacterial activities( Table 3) showed that the plant extracts exhibit remarkable activity against the test organisms isolated (*E.coli, K. pneumonia, P. aeruginosa, S.aureus,* and *S.pyrogens*) with zone of inhibition ranging from 5 to 25 mm. From the table it is evident that for the four concentrations taken for each extract; the highest concentration 100 mg/ml showed the maximum zone of inhibition for the five bacterial species. Ethanolic extract of *Solanum torvum shows* maximum zone of inhibition for *S.aureus* followed by the *S.pyrogens.* Aqueous extract of *Solanum torvum shows* maximum zone of inhibition for *S.aureus* followed by *S.pyrogens.* Petroleum ether extract of *Solanum torvum shows* maximum zone of inhibition for *S.aureus* followed by *S.pyrogens*. Among the three extracts, ethanolic extract exhibited maximum antibacterial activity.

**Table 3: Antibacterial activity of leaf extracts of *Solanum nigrum***
(Zone of diameter of three replicates)

| | | Zone of inhibition (mm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S.No | Microorganism | Ethanolic Extract | | | | Aqueous Extract | | | | Petroleum ether extract | | | | Streptomycin (10 mg/ml) |
| | | A | B | C | D | A | B | C | D | A | B | C | D | |
| 1 | *E.coli* | 14 | 10 | 7 | 5 | 15 | 13 | 11 | 8 | 10 | 9 | 7 | 5 | 17 |
| 2 | *K.pneumonia* | 16 | 11 | 9 | 7 | 16 | 11 | 9 | 7 | 12 | 9 | 10 | 7 | 18 |
| 3 | *S.aureus* | 23 | 19 | 15 | 11 | 21 | 17 | 9 | 8 | 15 | 11 | 9 | 5 | 21 |
| 4 | *P.aeruginosa* | 15 | 13 | 9 | 7 | 17 | 14 | 11 | 7 | 12 | 13 | 11 | 7 | 16 |
| 5 | *S.pyrogens* | 19 | 15 | 11 | 9 | 21 | 17 | 8 | 5 | 13 | 9 | 7 | 5 | 20 |

**A, B, C, D indicates 100, 75, 50 and 25 mg/ml concentrations**

**Conclusion**

This study shows that *S.torvum* possess antimicrobial activity against bacteria associated with respiratory tract infectious. The plant can be used as a source of oral drug against respiratory tract infections; however, further studies are required to isolate the active principle from the crude extract for proper drug development.

**Acknowledgment**

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## *Invivo,* and *in silico* Analysis of Anti-inflammatory efficacy of *Curculigoorchioides* rhizome

***[1]Dr. Cecily Rosemary Latha, [2]Dr. Rajathi Modilal, [3] Monicka J, [4]Dr. Jayanthi***

*1,2,3 &4 P.G and Research Department of Zoology, Holy Cross College (Autonomous), Tiruchirappalli – 620 002*

**Abstract**

*Curculigo orchioides* of Amaryllidaceae, known as Golden eye grass is used inseveral disorders such as anti-inflammatory, adoptogenic, sedative, and anticonvulsant, androgenic and immune-promoting activity. In this study the anti-inflammatory and immune modulatory efficacy of ethanol extract of *C.orchioides* rhizome was studied. In hydrocortisone

induced immune- suppressed mice model, decrease in spleen weight and cell yield/spleen is brought back to the normal level by the administration of *Curculigo orchioides* rhizome extract. Anti-inflammatory effect was studied in the formalin induced paw oedema Mice Model. Percentage of inhibition of oedema by the bioactive compounds of plant extract after 1st, 2nd, 3rd and 4th hours were found to be 17%, 30%, 50% and 60% respectively, while the reference standard drug paracetamol (100mg/kg) revealed a less percentage of inhibition of paw oedema with 23% after 4 hours.

**KeyWords**

*Curculigo orchioides*, anti-inflammation, 6cox protein.

**Introduction**

In China rhizome of *C.orchioides* is called as "Xiamao". *C.orchioides* is used in several disorders such as anti-inflammatory, adoptogenic, sedative, and anticonvulsant, androgenic and immune-promoting activity (Joy *et al.*, 2004; Liu, 2001). *Curculigo orchioides* root/rhizome possess, anticancer, antioxidant, antiasthmatic, immunostimulatory, hypoglycemic, antibacterial and estrogenic activities without any side effects (Umang*et al.*, 2012). Among all these medicinal properties, the anti- inflammatory activity of the active compounds in the rhizome of *C.orchioides* was assessed in the present study. The anti-inflammatory effect of the ethanol extract of the rhizome of *Curculigo orchioides* Gaertn. (Hypoxidaceae) was investigated using carrageenan induced paw oedema in rats at different dose level.

**Materials and Methods Plant Extract Preparation**

In the present investigation, the ethanol extract of rhizome powder of *Curculigo orchioides* was prepared by sequential soxhalation extraction method. The extraction was done by following the method of SreenivasaRao and Parekh (1981). Rhizome powder of the plant *Curculigo orchioides* was extracted in Soxhlet apparatus using ethanol as solvent for 8 hours at 65°C. The content was filtered through No.1 Whatman filterpaper and the extract was stored in air tight bottle. The *curculigo archioides* rhizome power was obtained from authenticated ayurvedic dealer.

**Animal Treatment for Immunomodulatory Study**

Male albino mice weighing about 28±5g were used for this study. The experimental protocol was approved by the Institutional Animal's Ethics Committee and by the regulatory body of the government (Reg. No. 585/ 05/ A/ CPCSEA). The mice were kept in clean polypropylene cages and maintained at the local animal house conditions of temperature 24±2C, fed with a standard pellet diet and water ad libitum. After randomization into various groups, the mice were acclimatized to the laboratory conditions of temperature and photoperiod for a period of 1–2 weeks before initiation of the experiments. Mice were equally divided into three groups with six animals in each. Initial body weights of all the mice were recorded.

**GROUP I: Vehicle Treated Control Group**

1ml of distilled water equal to plant extract was administered orally two times daily by intragastric gavage needle for seven days, and physiological saline (0.9% NaCl) was administered intra peritoneal in similar dose of hydrocortisone after 72 hours from the beginning of the experiment.

**GROUP II: Hydrocortisone Induced Treated Group:**

The distilled water was administered orally two times daily for seven days, and the animals received intra peritoneal injection of Hydrocortisone (1mg) after 72 hours from the beginning of the experiment.

**GROUP III: Plant Extract and Hydrocortisone Induced Group**

The plant extract (1ml) at the dose of 100mg/kg b.wt was administered orally two times daily by intragastric gavage needle for seven days, and the animals received intra peritoneal injection of Hydrocortisone (1mg) after 72 hour from the beginning of the experiment. After completion of the treatment final body weights of all the rats were taken and the rats were anaesthetized one after another with chloroform and blood was collected directly from hepatic portal vein and allowed to coagulate, clear serum was collected and stored at -20°C for enzyme assay. Splenocytes were counted using standard Immunology Protocol. Triglycerides were estimated using Diagnostic kit from Bio Systems, Costa Brava, Spain. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) levels were estimated in all the

control and experimental animals by the method of Kind and King, 1954.

**Anti-Inflammatory Study**

**Formalin- Induced Paw Oedema in Mice**

The plant extract was evaluated for its anti-inflammatory activity, based on the method of Winter *et al.*, 1962. Adult albino mice (23–27 g) of either sex randomized into three groups of 6 mice each were used for the experiment. The mice were fasted for 24 h before being used but allowed access to water. Formalin (0.1 ml of 1% formalin for inductionof paw edema) was injected into the sub-planter tissue of right hind paw to induce edema. The animals in group 1 (negative control) received 0.5% carboxymethyl cellulose solution,group 2 received plant extract at the dose of 100 mg/kg while group 3 received acetylsalicylic acid (Paraacetamol) at the dose of 100 mg/kg, 30 min before being challenged with buffered formalin. The responses were measured after formalin injection for four hours. The linear paw circumference was measured. The percentage of anti-inflammatory activity calculated by

= (vc –vt / vc) x 100 (Vc = control, Vt– test sample)

***Insilico* Molecular Docking Studies**

**Protein Data Bank (PDB)**

The 3D structure of of PDB ID 2R1A was obtained from PDB. 3D structure is visualized through Pymol.

**Pubchem**

The 2D structure format of Decane 2, 3, 5, 8-tetramethyl, Dodecane 2, 6, 11-trimethyl, Benzonic acid, 4 –ethoxyethylester, Docosanic acid 1, 2, 3-propanetriyl ester and Ethyl iso-allocolate were obtained from NCBI Pubchem compound database.

**Online Smiles Translator**

The canonical SMILES format of the 2D structures of the compounds Decane 2,3,5,8-tetramethyl, Dodecane 2,6,11-trimethyl, Benzonic acid, 4 –ethoxyethylester, Docosanic acid 1,2,3-propanetriyl ester and Ethyl iso-allocolate were converted to 3D structures and viewed through Pymol.

**Docking Using Hex**

The individual binding pose of each Ligand was observed and their binding affinity with the target proteins was analyzed and the best binding pose and the total energy of each Ligand was recorded. The details of binding poses and the total energy values were saved in a output folder. The docked complex is then viewed in Pymol. By using the SMILES of the antibiotics, the properties of the drugs such as molecular volume, number of hydrogen bond donors and acceptors, log P and rotatable bonds were predicted. The acceptability of the analogs is evaluated based on Lipinski's rule of five which is essential for structure based drug design. The structure of the model protein was visualized using Pymol.

**Results and Discussion**

**Table 1: Effect of ethanolic extract of *C. orchioides* rhizome in hydrocortisone induced immune suppressed Mice Model**

| S.No | Groups | Spleen weight (g) | Cell yield/Spleen | Level of serum biomarkers | | Serum Triglycerides mgs/dl |
|---|---|---|---|---|---|---|
| | | | | SGOT U/L | SGPT U/L | |
| 1. | Control | 0.18±0.03 | $5.2\pm0.1\times10^7$ | 55.3 U/L | 45.2 U/L | 51.1 mgs/dl |
| 2. | Hydrocortisone (1mg) | 0.15±0.03 | $3.8\pm1.7\times10^7$ | 65.8 U/L | 40.0 U/L | 56.0 mgs/dl |
| 3. | Hydrocortisone (1mg) +Plantextract (100mg/kg b.wt) | 0.17±0.02 | $5.9\pm0.8\times10^7$ | 61.3 U/L | 42.5 U/L | 50.2 mgs/dl |

**Table 2: Anti-inflammatory activity of ethanolic extract of rhizome of *C. orchioides in* mice model**

| S.No | Treatment of animal | Before formalin induced paw size | After formalin induced paw size | Percentage of inhibition (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1h | 2h | 3h | 4h |
| 1 | Control | 1 cm | 3 cm | - | - | - | - |
| 2 | Test Plant extract (100mg/kg bw) | 1.4 cm | 2.5 cm | 17% | 30% | 50% | 60% |
| 3 | Standard Paracetamol (100mg/kg bw) | 1.6 cm | 2.9 cm | 3% | 10% | 16% | 23% |

**Table 3: Energy value for the docked complex of the Compounds of ethanol extract of *C.orchioides* with 6cox protein**

| S.No. | Compounds | Energy value |
|---|---|---|
| 1 | Decane 2,3,5,8-tetramethyl | -129.58 |
| 2 | Dodecane 2,6,11-trimethyl | -129.22 |
| 3 | Benzonic acid,4 –ethoxyethylester | -147.37 |
| 4 | Docosanic acid 1,2,3-propanetriyl ester | -173.73 |
| 5 | Ethyl iso-allocolate | -167.54 |

### *Invivo* Immunomodulatory Activy

A treatment of hydrocortisone led to a significant immunosuppression expressed in decrease in the spleen weight and the total number of cells per spleen. The cell yield/spleen for normal control, hydrocortisone injected and plant extract treated groups were found to be 5.2±0.1×107 , 3.8±1.7×107 and 5.9±0.8×107 respectively, which clearly indicates the immunorestorative effect of *Curculigo orchioides* rhizome extract (Table 1). In normal control mice, the levels of serum biomarkers SGOT and SGPT was found to be 55.3U/L 45.2U/L respectively (Table 1). Mice treated with hydrocortisone revealed a slight increase in SGOT (65.8U/L) and no significant increase in SGPT (40.0U/L). In *Curculigo orchioides* extract treated immunosuppressed mice serum SGOT and SGPT levels were 61.3U/L and 42.5U/L respectively indicating reduction in inflammation. Total triglycerides were found to be 51.1mgs/dl, 56.0mgs/dl and 50.2mgs/dl for normal control, hydrocortisone injected and plant extract treated groups respectively. During the acute phase response and also in chronic inflammatory conditions, triglycerides (TG) metabolism is altered and plasma triglycerides level are elevated (Khovidhunkit *et al.,* 2004). The results obtained in our study showed that the triglycerides level elevated by hydrocortisone injection is brought back to the normal level by the administration of *Curculigo orchioides* rhizome extract.

Vane and Bolting (1987) tested the anti-inflammatory activity of the active compounds present in the rhizome of the *C.orchioides* using albino rats. The results obtained in the study on the effects of thymomimetic drugs and zinc supplementation on the cellular immune response in hydrocortisone-suppressed mice, showed that hydrocortisone injection drastically decreases the number of thymocytes and splenocytes, which is also accompanied by a decreasing weight ratio of the thymus and spleen. The results obtained in our study also showed the immunorestorative effect of *Curculigo orchioides* rhizome extract and was able to ameliorate the suppressive effect of hydrocortisone on the percentage of splenocytes.

### Anti-Inflammatory Activity of Plant Extract in Mice Model

The ethanolic extract of *Curculigo orchioides* was tested for their anti-inflammatory activity using formalin induced paw oedema method. Plant extract exhibited promising anti- inflammatory activity by oral administration at a dose level of 100 mg/kg compared to the reference standard drug paracetamol (100mg/kg). The results for percentage of inhibition of paw oedema were depicted in Table 2. The results revealed percentage of inhibition of oedema by the bioactive compounds of plant extract recorded after 1st, 2nd, 3rd and 4th hours with 17%, 30%, 50% and 60% respectively. While the reference standard drug paracetamol (100mg/kg) revealed a less percentage of inhibition of paw oedema with 23%, after 4 hours. The above results indicated that the plant extract (100mg/kg) have a significant anti-inflammatory effect on the formalin induced paw oedema in mice when compared with standard drug paracetamol.

There are several herbal drugs used for the anti-inflammatory activity. Plants exhibiting anti-inflammatory activity reveal that, species of 96 genera belonging to 56 families have anti-inflammatory activity, keeping in view the growing significance of anti- inflammatory related herbal medicines (Handa *et al.,* 1992). Oedema, which develops after carrageenan inflammation, is a biphasic event. The initial phase is attributed to the release of histamine and Serotonin. The oedema maintained between the first and second phase is due to kinin like substances (Vinegar *et al*., 1969). The second phase is said to be promoted by prostaglandin like substances. The second phase of oedema is sensitive to drugs like hydrocortisone, phenylbutazone and Indomethacin (Winter *et al*., 1962). The results of carragennan induced paw oedema model indicated dose dependent anti-inflammatory activity. The efficacy of ethanolic extract of rhizome of *Curculigo orchioides* used as an efficient therapeutic agent in acute anti-inflammatory conditions. The results of the study supported the traditional use of this plant in some inflammation and increased weight of spleen which confirms the presence of active chemical compounds related to these activities. Phytochemicals such as flavonoids, terpenoids, steroids and phenolic compounds expressed their anti-inflammatory activity at least in part by modulation of pro inflammatory gene expression such as cyclooxygenase-2, inducible nitric oxide synthase and several pivotal cytokines.

### *Insilico* Analysis

Methods developed to facilitate and speedup the drug designing process are Rational Drug Design (RDD).

RDD uses a variety of computational methods to identify novel compounds. One of those methods is docking of drug molecules with receptors. The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor. Protein 6cox is a common receptor for the anti-inflammatory studies.

Complexes were obtained via docking and from this docking studies, it was clear that the five compounds Decane 2,3,5,8-tetramethyl, Dodecane 2,6,11-trimethyl, Benzonicacid 4 ethoxy ethyl ester, Docosanic acid 1,2,3-propanetriyl ester and Ethyl iso-allocolateshowed good binding affinity. Energy value obtained as a result of docking with 6cox is -129.58, - 129.22,-147.37, -173.73, -167.54 for Decane 2,3,5,8-tetramethyl, Dodecane 2,6,11-trimethyl, Benzonic acid 4 ethoxy ethyl ester, Docosanic acid 1,2,3-propanetriyl ester and Ethyl iso- allocolate respectively (Table 3). Among these five compounds, Decane 2, 3, 5, 8-tetramethyl (-129.58) and Dodecane 2,6,11-trimethyl (-129.22) possesses the highest energy value. These two compounds could be further subjected for *invitro* and *invivo* anti-inflammatory activity. Hence isolation of these two individual phytochemical constituents and subjecting them to biological activity will definitely give fruitful results.

From this anti-inflammatory study we conclude that the decrease in spleen weight andcell yield/spleen by hydrocortisone injection in mice is brought back to the normal level by the administration of *Curculigo orchioides* rhizome extract, which clearly indicates the immunorestorative effect of *Curculigo orchioides* rhizome extract. Triglycerides level elevated by hydrocortisone injection is brought back to the normal level by the plant extract and significant changes were not seen in serum SGOT and SGPT level indicating the anti-inflammatory effect of hydrocortisone and plant extract. Percentage of inhibition of oedema by the bioactive compounds of plant extract after 1st, 2nd, 3rd and 4th hours were found to be17%, 30%, 50% and 60% respectively, while the reference standard drug paracetamol (100mg/kg) revealed a less percentage of inhibition of paw oedema with 23% after 4 hours, indicating significant anti-inflammatory effect on the formalin induced paw oedema in mice. Energy value obtained as a result of docking with 6cox revealed Decane 2, 3, 5, 8-tetramethyl(-129.58) and Dodecane 2,6,11-trimethyl (-129.22) possesses the highest energy value. Thesetwo compounds could be further subjected for *invitro* and *invivo* anti-inflammatory activity. Hence isolation of these individual phytochemical constituents and subjecting them to biological activity will definitely give fruitful results.

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# Studies on *Oryzaephilus surinamensis* infestation and management in the date fruit

***[1]Dr. Cecily Rosemary Latha R, [2]Dr. Rajathi Modilal, [3]Dr. Jayanthi, [5]Nivetha Selvam S, [6]Dr.Loganathan***

[1,2,3,4 &5] *PG and Research Department of Zoology, Holy Cross College (Autonomous), Tiruchirappalli – 620 002*

[6]*Indian Institute of Food Processing Technology, Thanjavur* [2]

**Abstract**

The date palm tree (*Phoenix dactylifera* L.) is the most important fruit tree and the date processing industry has been growing steadily during the past few years. Storage pest infestation is one of the most important deterioration factors in dried dates and the most common insect pest that infects dates is *Oryzaephilus surinamensis* (Silvanidae). The utilisation of elevated temperature is found to be effective in grain disinfestation. The results of thermal disinfestation of dates in hot air oven showed hundred per cent mortality of adults and larva of *O. surinamensis* when infested dates were exposed to temperature of 65ºC in hot air oven for 15 minutes, whereas the mortality of adult was only 23.33 per cent at 10 min. The pupa required 20 min duration at 65ºC for complete mortality. The exposure of dates to 65ºC in hot air oven for 15 min showed slight reduction in moisture content. Moisture contentof the dates was reduced from 3.63 to 2.67 per cent when the dates were exposed tomicrowave treatment for 5, 10 and 15 seconds. The microwave treatment of adults of *O. surinamensis* for 10 and 15 seconds gave significant mortality of 66.67 and 100 per cent respectively. The $CO_2$ fumigation study revealed that the concentration of 60 per cent gave significantly highest larval mortality (100%) whereas low concentration of $CO_2$ (45%) killed only 55 per cent of larva. Hence from the result of the present study, we infer that the date infested fruits should be disinfested in the initial stage itself by exposing to high temperatureof 65ºC for 15 min or exposing to microwave for 15 seconds or by 60 per cent $CO_2$ fumigation for 7 days. These treatments will help to store the fruits safely without insect infestation.

**keywords**

*Oryzaephilus surinamensis*, life cycle, thermal disinfestations, microwavetreatment, $CO_2$ fumigation

## Introduction

Dates are energy rich, highly nutritious fruit and important part of food for people of all ages all over the world. Dates are taken either in fresh, dehydrated or in processed form. Storage pest infestation is one of the most important deterioration factors in dried dates and the most common insect pest that infects and infests dates is *Oryzaephilus surinamensis* (Silvanidae). Beside fruit damage, insects leave residuals in fruits and decrease their commercial value. Stored-products insects such as *Oryzaephilus smercator*, *Cryptolestes ferrugineus*, *Ephestia cautella*, *Tribolium confusum* and *Ectomylosis ceratoniae* also have a role in infesting dried and partially-dried dates (Taylor, 1994; Lindegren, 1992).

The insecticides are used for the control of insects in date fruit storage. When dates become infested with any stored grain insect pests, fumigation is recommended with methyl bromide or phosphine. The saw toothed grain beetle is very small insect which has the ability to hide in many places in storage facility making it difficult to be controlled by insecticides, and it has built up resistance to several insecticides (Greening *et al*., 1974; Heather and Wilson 1983; Wallbank and Collins 2003). The use of insecticides in dates storage may leavethe residues and affect the quality of the dates. Alternative methods of insect control are required to maintain the quality of the date fruits till reaches the consumer. The utilisation of elevated temperature found to be effective in grain disinfestation. Hence, the present study is planned to analyse the effect to temperature, Microwave oven, Carbon dioxide Fumigation onthe disinfestations of dates and to study the effect of these treatment methods on the quality of dates.

## Methodology

### Thermal disinfestations of dates using hot air oven

The dates were collected from the market. The infested dates were identified using theStereozoom Trinocular Microscope (Make: Leica; Model S8APO with digital camera - DFC295). The infested dates were taken in the petriplates (20 Nos) and kept in the hot air oven (Make:ILTC; Model:2960) which already reached the temperature of 65◦C. The exposed petridishes (5 Nos) were taken out at different time interval of 10 mins, 15 mins, 20 mins, and 30 mins. Then the observation was made on the live and dead insect stages of larva, pupa, and adult in

the treated dates.

**Disinfestations of dates using Microwave Oven**

The infested dates were taken in the glass petriplates (12Nos) and exposed to microwave (1 Kw) using the microwave oven (Make: IFB; Model-30SC2). The exposed petridishes (4 Nos) were taken out at different time interval of 5 sec, 10 sec, 15 sec, and 20 sec. Then the observations were made on the live and dead insect stages of larva, pupa, and adult in the treated dates.

**Disinfestations of dates using Carbon dioxide fumigation**

The infested dates with different stages of insect, saw toothed grain beetle (*Oryzaephilus surinamensis*) were taken into the acrylic chambers for $CO_2$ fumigation studiesat different concentration of $CO_2$ level*viz.*, 60, 75, and 90 per cent separately. The chambers were fumigated with the required concentrations of $CO_2$ in each chamber from a $CO_2$ cylinderusing gas regulator and kept for seven days. The gas concentrations in the chambers were monitored using gas monitor (Make:PBIDansensor; Model:Checkmate) regularly for seven days. The dates were removed from all the chambers on seventh day. The observations were made for the presence of dead and live insect stages *viz*., larva and adults in the dates from each chamber separately**.**

**Results**

**Thermal disinfestations of dates in hot air oven**

The results showed that the cent per cent mortality of adults of *O. surinamensis* when infested dates were exposed to temperature of 65ºC in hot air oven for 15 min. The mortality of adult was only 23.33 per cent at 10 min and reached 100 per cent at 15 min. (Table 1). It was also observed that the there was no colour change in dates up to 30 min. of exposure to 65ºC

**Table 1: Mortality of adult of *O. surinamensis* in various exposure durations at 65ºC in Hot Air Oven**

| Temperature 65ºC (mins.) | Adult Mortality (%) |
|---|---|
| 10 | 23.33 (18.25) |
| 15 | 100.00 (90.00) |
| 20 | 100.00 (90.00) |
| 30 | 100.00 (90.00) |
| CD at 0.05% | 31.15 |
| S/NS | S |

S - Significant; NS – Non-significant

The values in the parentheses are arcsine transformed values

**Table 2: Moisture content of dates in various exposure durations at 65ºC in hot air oven**

| Treatment time at Temperature 65ºC (min.) | Moisture content (%) |
|---|---|
| 10 | 4.15 |
| 15 | 4.13 |
| 20 | 3.60 |
| 30 | 2.30 |
| CD at 0.05% | 2.53 |

**Table 3: Mortality of larva of *O. surinamensis* in various durations at 65ºC in Hot Air Oven**

| Temperature 65ºC (mins) | Larval Mortality (%) |
|---|---|
| 10 | 4.00 (0.00) |
| 15 | 100.00 (90.00) |
| 20 | 100.00 (90.00) |
| 30 | 100.00 (90.00) |
| CD at 0.05% | 33.96 |
| S/NS | S |

S - Significant; NS – Non-significant

The values in the parentheses are arcsine transformed values

The exposure of the dates to higher temperature may reduce the moisture content of the dates. The results of the experiment on the exposure of dates to 65◦C in hot air oven for 15 min showed slight reduction but no significant variation between 10 and 15 min exposure (Table 2). The larva came out during the heat treatment and gets killed outside the fruits. The results showed that hundred per cent mortality of the larvae was achieved within 15min of exposure for 65◦C in hot air oven. The exposure for 15min gave significant mortality when compared to 10min. exposure (Table3).

**Table 4: Mortality of pupa of *O. surinamensis* in various durations at 65ºC in hot air oven**

| Temperature 65ºC (mins) | Pupa Mortality (%) |
|---|---|
| 10 | 0.00 (0) |
| 15 | 34.29 (30.00) |
| 20 | 100.00 (90.00) |

| | |
|---|---|
| 30 | 100.00 (90.00) |
| CD at 0.05% | 33.09 |
| S/NS | S |

S - Significant; NS – Non-significant

The values in the parentheses are arcsine transformed values

The exposure of infested dates to elevated temperature also kills the pupa present in the dried fruit. The results of the thermal disinfestation study at 65ºC in hot air oven showed 100 per cent mortality at 20 min which is significantly higher than 34.29 per cent mortality at 15 min of exposure (Table 4). The results revealed that the pupa required 20 min duration at 65ºC in hot air oven for complete mortality.

**Disinfestation of dates in microwave oven**

The microwave will act on the each and every particle at a time and get heated very quickly. Hence, the infested dates were exposed to microwave treatment for 5, 10 and 15 seconds to kill the life stages of the saw toothed grain beetle. The result showed that the moisture content of the dates was reduced from 3.63 to 2.67 per cent when the dates were exposed to microwave treatment for 5, 10 and 15 seconds (Table 5). The microwave treatment for 10 and 15 second gave significant adult mortality of 66.67 and 100 per cent mortality at 10 and 15 seconds respectively when compared to 5 second exposure which should only 16.67 % mortality (Table 6).

**Table 5: Effect of microwave treatment on the moisture content of the dates**

| Microwave treatment Time (Seconds) | Moisture Content (%) |
|---|---|
| 5 | 3.63 |
| 10 | 3.33 |
| 15 | 2.67 |
| CD at 0.05% | 1.55 |
| S/NS | S |

S - Significant; NS – Non-significant

The values in the parentheses are arcsine transformed values

**Table 6: Effect of microwave on the mortality of the adult saw toothed grain beetle**

| Microwave treatment Time (Seconds) | Adult Mortality (%) |
|---|---|
| 5 | 16.67 (15.00) |
| 10 | 66.67 (60.00) |
| 15 | 100.00 (90.00) |
| CD at 0.05% | 86.51 |
| S/NS | S |

S - Significant; NS – Non-significant

The values in the parentheses are arcsine transformed values

**Disinfestation of dates with $CO_2$ fumigation**

The insects are sensitive to $CO_2$. The results of the $CO_2$ fumigation for disinfestation of dates showed that increase in concentration of $CO_2$ increases the mortality of adults (Table 7). The adult mortality of 16.53 and 79.75 per cent was achieved with 30 and 45 per cent $CO_2$ whereas 60 per cent $CO_2$ gave significant mortality of 100 %.

**Table 7: Effect of $CO_2$ fumigation on the mortality of the adult saw toothed grain beetle**

| Concentration of $CO_2$ (%) | Adult Mortality (%) |
|---|---|
| 30 | 16.53 (23.96) |
| 45 | 79.75 (63.38) |
| 60 | 100.00 (90.00) |
| 75 | 100.00 (90.00) |
| CD at 0.05% | 6.15 |
| S/NS | S |

S - Significant; NS – Non-significant

The values in the parentheses are arcsine transformed values

**Table 8: Effect of $CO_2$ fumigation on the larval mortality of saw toothed grain beetle**

| Concentration of $CO_2$ (%) | Adult Mortality (%) |
|---|---|
| 30 | 0 (0) |
| 45 | 55 (44.15) |
| 60 | 100 (90.00) |
| 75 | 100 (90.00) |
| CD at 0.05% | 48.50 |
| S/NS | S |

S - Significant; NS – Non-significant

The values in the parentheses are arcsine transformed values

Also 60 per cent $CO_2$ gave significantly highest larval mortality (100%) whereas 45 per cent$CO_2$ killed only 55 per cent of larva (Table 8). The lowest concentration of 30 per cent didnot have any effect on the larva of saw toothed grain beetle.

**Discussion**

**Thermal disinfestations of dates in hot air oven**

The effect of conventional and electrical thermal stresses on the destruction of *O. surinamensis* inside the fruits of some date varieties has been studied. The elevated temperature is used for killing the insects in the dates. The result of the present study is in concordant to the finding of Assiry (Assiry *et al.*, 2003) who indicates that the insect is very sensitive to temperature and can be destroyed instantaneously by heat exposure to 64°C.The results showed that the cent per cent mortality of adults of *O. surinamensis* when infested dates were exposed to temperature of 65ºC in hot air oven for 15 min. The mortality of adult was only 23.33 per cent at 10 min and reached 100 per cent at 15 min. It was also observed that the there was no colour change in dates up to 30 min. of exposure to 65ºC.

The larva of the saw toothed grain beetle is feeding in the dry part between the fruit flesh and the seed. The larva comes out during the heat treatment and gets killed outside the fruits. The results showed that the cent per cent mortality of larvae was achieved with in 15 min of exposure for 65ºC in hot air oven. The exposure for 15min gave significant mortality of larvae when compared to 10min. exposure. The exposure of infested dates to elevated temperature also kills the pupa present in the dried fruit. The results revealed that the pupa required 20 min duration at 65ºC in hot air oven for complete 100% mortality.

**Disinfestations of dates in microwave oven**

The microwave energy can be used for disinfestation of dates. When, the infested dates were exposed to microwave treatment for 5, 10 and 15 seconds, the moisture content of the dates was reduced from 3.63 to 2.67 per cent. The microwave treatment for 10 and 15 second gave significant adult mortality of 66.67 and 100 per cent mortality at 10 and 15 seconds respectively when compare to 5 second exposure. Similar studies were carried out by Zouba, *et al*. (2009) the infested dates to 1 Kw for 55 and 70s soft dates and 90 s for dry dates.

**Disinfestations of dates with $CO_2$ fumigation**

The Controlled Atmospheric Storage (CAS) is one of the nonchemical methods of storage and environmentally safe. Kader (Kader *et al.*, 2009) reported that Yellow Khalal Barhee dates can be stored in 20 per cent $CO_2$ enriched air at 0°C and 90-95 per cent relative humidity for up to 26 weeks but the shelf life of air- stored dates is only 7 weeks. Thus carbon dioxide at higher concentration is able to prevent the fungal growth.

The results of the present study on $CO_2$ fumigation for disinfestation of dates at room temperature also showed that increase in concentration increases the mortality of adults. The adult mortality of 16.53 and 79.75 per cent was achieved with 30 and 45 per cent $CO_2$ whereas 60 per cent $CO_2$ gave significant mortality of cent per cent. The results of the $CO_2$ fumigation study for dates revealed that the concentration of 60 per cent gave significantly highest larval mortality (100%) whereas low concentration of $CO_2$ (45%) killed only 55 per cent of larva. The lowest concentration of 30 per cent did not have any effect on the larva of saw toothed grain beetle. Hence from the result of the present study, we infer that the date infested fruits should be disinfested in the initial stage itself by exposing to high temperature of 65ºC for 15 min or exposing to microwave for 15 seconds or by 60 per cent $CO_2$ fumigation for 7 days. These treatments will help to store the fruits safely without insect infestation.

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