# QUANTITATIVE ETHNOBOTANY AND PHYTOCHEMISTRY OF SELECTED PLANTS USED IN TRADITIONAL THERAPEUTICS BY ETHNIC TRIBES OF WAYANAD DISTRICT, KERALA.

Natural products have provided us, and continue to provide, essential materials for shelter, for furniture, for food, for clothing, for writing, for colouring materials, for weapons, for gifts, and for the treatment of numerous diseases (Balick and Cox, 1996). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. The first records on the use of natural products for medicine written on clay tablets in Cuneiform are from Mesopotamia and date back to about 2600 BC; among the substances that were used were oils of *Cedrus* species (Cedar) and *Cupressus sempervirens* (Cypress), *Glycyrrhiza glabra* (Licorice), *Commiphora* species (Myrrh) and *Papaver somniferum* (Poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation (Gurib, 2006). The World Health Organization estimates that approximately 80 percent of the world's population relies primarily on traditional medicines as sources for their primary health care. Many other frontiers also remain within the field of natural products that can provide opportunities to improve our quality of life. Furthermore, the continual emergence of new natural product chemical structure skeletons, with interesting biological activities along with the potential for chemical modification and synthesis bode well for the utility of natural products. Finally, the uses of natural products need to be by no means restricted to pharmaceuticals but can also be expanded to agrochemicals, nutraceuticals *etc*. (Duke *et al*., 2000).

Ethnobotany though dates back to the times of early explorers who listed the native uses of the indigenous tribes of the lands they discovered, until 1980's it was merely a list making exercise of useful plants. Since then quantitative methods in ethnobiology are proliferating (Garcia, 2006). This new era started with Trotter and Logan in 1986 when they used the informant consensus method for the first time to evaluate the relationship between the effectiveness of medicinal plants quoted and their biological activity.

Quantitative botany is recent in Indian context and hence only very few works had been done in this regard. Raghupathy *et al*. (2008) has done the quantitative ethnobotany of '*Malasars*' –an indigenous forest tribe residing at Vellangiri Hills, Tamil Nadu, India and has estimated the consensus factor for determining the homogeneity in the informants knowledge. Ragupathy and Newmaster (2009) have studied the ethnobotany of *Irulas in* Kodiakkarai Reserve Forest, India using quantitative techniques and found that a high consensus existed among them with regard to medicinal plant use.

Quantitative techniques in ethnobotanical data inventorying has never been attempted in the Wayanad district, Kerala earlier. Major focus in the early works done here were mere listing of data regarding edible plants used by different tribes and of medicinal plants used by some individual tribes seen in Wayanad district. No serious attempts have been done so far regarding the quantitative aspects of medicinal plant use among and across different socio cultural groups seen in the study area. Quantitative indices such as Informant Consensus, Use Value, Family Use

Value *etc.* were used to analyze the patterns and preferences regarding medicinal plant use in this study.

Traditionally, natural product research has primarily centered on the structural elucidation of compounds. However, in recent years, research has been directed towards the physiological and ecological significance of these chemicals. We have just begun to understand the role of naturally occurring chemicals in the biological interactions of organisms with their ecosystems. The study of natural products not only provides novel bioactive compounds, but also helps in the understanding of nature's way of tackling environmental problems. These processes, which may be called "Natural Technology", might provide us with totally new means and agents for combating diseases, controlling pests or improving agricultural productivity. The extension of research to marine natural products has paid rich dividends. Poly oxygenated compounds such as tetrodotoxins, palytoxins and halichondrins show novel bioelectric properties which influence the ionic permeability of biological membranes. Some of these compounds show remarkable anti tumor and immunomodulatory activities. So far, only a small proportion of the known flora has been subjected to chemical or biological investigations; the vast unexplored biotechnical potential of flora awaits discovery and exploitation (Banerji, 1992).

Ecdysteroids are a class of more than 300 polar steroid compounds which act as moulting hormones in insects but expressed in variable quantities inside plant kingdom. They are extremely non toxic to animals including humans and numerous therapeutic functions have been attributed to them by pharmacologists worldwide. Hundreds of nutraceutical and food supplement formulations containing ecdysteroids are available for sale in the internet (www.ecdybase.org). Kerala flora has not been screened before in this perspective. With regard to its high diversity and endemic nature, Kerala flora emerges as one among the best in this regard. The possibility of sourcing a large quantity of ecdysteroids from locally available plants can lead to more accurate and efficient bio activity studies inside the country.

Sericulture is both an art and a science of raising silkworms for silk production. India is the second largest producer of raw silk after China and the biggest consumer of raw silk and silk fabrics. An analysis of trends in international silk production suggests that sericulture has better prospects for growth in the developing countries rather than in the advanced countries. A study on sericulture was done for testing the effect of pure ecdysterone - Moulting Hormone (MH) and backuchiol which is a Juvenile Hormone (JH) analogue for increase in yield and synchronisation of cocooning on *Bombyx mori* L. The aim of the study was to develop and transfer Indigenous Growth Regulator (IGR) technology using these two compounds for the benefit of the poor sericulture farmers of Kerala state. The experiment was designed to be conducted at field conditions to actually assess how the MH and JH performed in the Kerala sericulture field scenario.

The broad objectives of the study are outlined in three separate heads as follows:-

## A. Quantitative ethno botany

- Construct an inventory of traditional healers belonging to the four socio cultural groups-– *Kattunaikkan, Mulla kuruman, Adiyan* and *Kuruchiyar* from Wayanad District, Kerala.

- Record the perceptions, beliefs, conservation strategies, patterns of use and taboos on medicinal plant use.
- Systematically record the plant use data from each informant as separate user reports for further quantification.
- Use Quantitative indices such as Relative Frequency of Citation, Informant Consensus Factor, Use Value, Family Use Value, Shannon-Wiener Index, Simpson Index and Berger Parker Index to understand the preferences and consensus existing among the informants regarding medicinal plant use.
- Compare the knowledge transmission occurring among and across the different socio cultural groups studied.

## B. Bio prospecting study on Kerala flora with respect to ecdysterone.

- Bio prospecting Angiosperm flora of Kerala state with respect to Wayanad district for the wonder drug ecdysterone.
- Standardising protocols for easy and effective screening for the compound.
- Extraction, isolation and quantification of ecdysterone from ecdysterone rich plants identified during preliminary survey by column chromatography.
- Characterisation of ecdysterone isolated from plants using TLC, UV, FTIR, HPLC-DAD and LC-MS.
- Resource mapping of ecdysterone rich sources in the study area.

## C. Application of IGR s in Sericulture.

- Developing and standardisins an easily affordable Indigenous Growth Regulator (IGR) technology for the poor sericulture farmers of the country.
- Identifying potential indigenous plant sources for Juvenile Hormone (JH) Analogues so that import of the same can be reduced.
- Identifying indigenous plant sources rich in Moulting Hormone (MH) for use in sericulture.
- Use pure ecdysterone (MH) as well as water extracts of MH rich plants and compare the results.
- Assessing the change in economic parameters such as larvae weight, cocoon weight, shell weight, denier *etc*. by the application of JH analogues.
- Study the effect of MH on hastening and synchronising the maturation process in the last instar stage of *Bombyx mori*.
- Developing a model for using both JH and MH on the same batch of *Bombyx mori* Bivoltine double hybrid variety (FC1X FC2) which is widely reared in the state.

## Methodology.

The methods of data collection were ethno botanical interviews and transect walks. Data collection was done all the year round covering all seasons from November 2008 to December 2012. Fifteen informants mainly healers and elders representing all regions and tribes in focus were selected from the information collected from other individuals during pilot survey. A large number of interviews were made with these informants each one extending about half a day followed by a transect walk. The informants were asked to explain their therapies and to list the species they used.

Fifty plants were selected from Kerala flora for testing the presence of ecdysteroids. Selection was based mainly on three criteria, early reported species, related members of early reported species and plants used by indigenous tribes. Other than these a few species were selected on random basis also. The parts selected for screening were leaf, stem, root and seeds depending on the species.

Well maintained sericulture farms were selected for field trial experiments after assessing and satisfying with the infrastructure provided. Farmers, whose farms were selected, were having more than 15 years of experience in sericulture. Six individual experiments were done to assess the effect of Backuchiol and ecdysterone during 6 months time from July 2012 to December 2012 in their farms.

The major observations recorded during the study are concluded in three separate heads as follows:-

## 1. Quantitative ethno botany.

An inventory of fifteen knowledgeable tribal healers and helpers to healers from four socio cultural groups-– *Kattunaikkan, Mulla kuruman, Adiyan* and *Kuruchiyar* from Wayanad District Kerala were made. Five hundred and sixty five user reports regarding165 species were recorded from them. All the four groups had their own magico- religious rituals for getting rid of diseases and their own traditional healers entrusted with the power to do it for them. Herbaceous species were quoted the most with 197 user reports. Trees and shrubs recorded 128 and113 user reports respectively. The lianas were the least category in the list with 45 user reports. No bias in quoting a particular habit category was seen among the different socio cultural groups. Of the 165 species quoted, sixty six species were recorded in the herbs category and 26 species each for both shrubs and climber category respectively. There were 41species in the tree category and 6 species in the liana category. Leaves, bark, stem, seeds, root, fruits, rhizomes, flowers, nut, petals and phyllode were the different medicinally important plant parts quoted by the informants in their user reports. There was no bias among the different socio economic classes in using these parts.

Cited plant knowledge were categorised on the basis of Relative Citation Frequency (*RCF*) to understand the relative importance of plants among and across socio cultural boundaries. Consensus (*Fic*) among the different informants with respect to the degree of agreement on the use of a particular species for a particular disease category was calculated. *Fic* value was high when the knowledge dissemination regarding the use of a particular plant for a specific treatment was effective among the informants. *Fic* value was low when knowledge dissemination between informants was low as they used different plants for the same disease category.

Plant species quoted were ranked based on their individual use value (UV). Hence preferences among informants regarding species were prioritised. Family Use Value was calculated among the quoted species and the most widely used plant families by the informants were identified. Knowledge regarding a plant shared among the informants can be used as a measure of biodiversity richness for a particular area. Based on the above assumption the

Shannon Weiner index for the collected information was calculated. Simpson's index was calculated for the data collected which gave an idea on the evenness of knowledge distribution regarding medicinal plants among the informants.

*Diploclisia glaucescens* (Blume) Diels in Engl. and *Coscinium fenestratum* (Gaertn.) Colebr. from Menispermaceae family, *Pterospermum rubiginosum* Heyne ex Wight & Arn. from Sterculiaceae family, *Thottea siliquose* (Lam.) Ding Hou. from Aristolochiaceae family, *Selaginella lepidophylla* from Sellaginellaceae family, *Pittosporum neelgherrense* Wight & Arn. from Pittosporaceae family, *Musa acuminata* Colla. and *Musa paradisiaca* L.(Local variety '*Vettan*') from Musaceae family, *Lepianthes umbellata* (L.) Rafin. from Piperaceae family, *Hydrocotyle javanica* Thunb. from Apiaceae family, *Entada rheedei* Spreng. from Fabaceae family, *Briedelia stipularis* (L.) Blume. and *Croton persimilis* Muell.-Arg. from Euphorbiaceae family, *Naringi crenulata* (Roxb.) Nicolson in Saldanha & Nicolson. from Rutaceae family are some of the species which were quoted frequently. Activity guided phytochemical fractionation can be done on these species as future work which may result in lead molecules for drug discovery. A digital online inventory regarding the data collected can be made as future work.

## 2. Bio prospecting study on Kerala flora with respect to ecdysterone

Kerala flora has been put to bio prospecting study for the wonder molecule ecdysterone for the first time. The criteria chosen for selecting the plants were early reports of ecdysterone occurrence, immediate relatives of early reported species used in ethno medicine, other relatives available in the area and a few randomly selected plants. A simple protocol for screening ecdysterone using very less amount of the plant source was developed and standardised in this study using ultra sound sonication, thin layer chromatography and HPLC –DAD. Fifty plants belonging to twenty one families were selected for screening from the study area. Twenty two percentage of the plant species selected were found positive for ecdysterone in this study. Normally ecdysterone is expressed only in 5-6% of species in the whole plant kingdom and hence it can be assumed that the selection criteria worked in favour. Eleven species *Achyranthes aspera* L., *Achyranthes bidentata* Blume., *Pupalia lappacea* (L.) Juss. var. *lappacea*; Hook., *Sida rhomboidea* Roxb. ex Fleming., *Tinospora cordifolia* (Willd.) Miers., *Alternanthera brasiliana* (L.) Kuntze., *Gomphrena celosioides* Mart., *Diploclisia glaucescens* (Blume) Diels., *Sesuvium portulacastrum* (L.) L., *Cyathula prostrata* (L.) Blume. and *Coscinium fenestratum* (Gaertn.) Colebr were found positive for ecdysterone in this study.

New negative reports on thirty one species with regard to this compound were recorded during the study and this data can be used during further bio prospecting ventures to avoid duplication. Ecdysterone was reported for the first time ever from *Coscinium fenestratum* of Menispermaceae family. Four species, *Diploclisia glaucescens* and *Coscinium fenestratum* of Menispermaceae family, *Sesuvium portulacastrum* of Aizoaceae Family, *Cyathula prostrata* of Amarathaceae family were identified as rich sources for ecdysterone from this geographical regime.

With regard to *Diploclisia,* stem collected from Indian sub continent was put for extraction and isolation for the first time. However it did not yield as much ecdysterone as reported from the specimen collected from Sri Lanka. But the leaves were identified as a good source for ecdysterone in this part of the country with a 1.05% yield.

Ecdysterone was isolated and characterised for the first time from *Coscinium* using UV, FTIR, TLC, HPLC-DAD and LC-MS. Ecdysterone yield for stem and leaves were 0.22 and 0.12 % respectively. Leaves of *Coscinium* are usually discarded as a waste while stem is over exploited by Ayurvedic industries. Extraction of ecdysterone in good amounts from the leaves thus act as a value addition to the species.

An approximate four time increase in yield (0.18%) of ecdysterone was reported for *Cyathula prostrata* collected from the study area in comparison to what was reported by Shah and De Souza in 1971 (0.05%) from the specimen collected from Bombay. However in *Cyathula prostrata* it was interesting to note that ecdysterone was present only in the flowering stage and the vegetative phase totally lacked its expression. *Sesuvium* collected from the study area yielded a lesser amount of ecdysterone (0.08%) as opposed to the higher yield (0.35%) reported by Banerji *et al*. (1971) from the specimen collected from Bombay.

Though *Coscinium* has been reported to be anti diabetic by ethno botanical and activity studies its active principle has not been isolated so far. Berberine, its major active compound can only be partially held responsible for the anti hyperglycemic activity. From several studies such as Sundaram *et al*. (2012), Foucault *et al*. (2012) *etc*. ecdysterone has been found to possess strong anti hyperglycemic activity. Ecdysterone has been reported and isolated in good quantities for the first time in our study and hence it can be rightly postulated that ecdysterone may be the right candidate for the anti diabetic principle present in the species.

Ecdysterone sources available in the study area have been resource mapped and easy protocols for isolation have been standardised during the study. Large amounts of easily available ecdysterone may flush open new vistas for ecdysterone research in India in future. Only a small amount of species available in the study area have been screened in this study and from the positive results obtained so far, further bio prospecting studies can be initiated in future for identifying more ecdysterone rich plant sources.

## 3. Application of IGR s in Sericulture.

Use of IGR technology is very recent to Indian sericulture scenario and hence this study has a large potential in the domestic sericulture market. In Kerala this technology is seldom used and hence the results of this study can be utilised for popularising the use of IGRs among sericulture farmers in our state. Indigenous and easily available sustainable sources for both Juvenile Hormone analogue (JH) and moulting hormone (MH) were identified during the study. Isolation protocols for JH and MH were standardised during this study. This work was done on the bivoltine double hybrid variety FC1X FC2of *Bombyx mori* L. which is the major variety under cultivation in Kerala now and also the breeding station for the same is situated in the state. No IGR work has been reported in this variety so far. *Diploclisia glaucescens* and *Coscinium fenestratum* collected from Wayanad District, Kerala were found to be promising sources for MH and were used for sericulture experiments for the first time ever. *Cullen corylifolium* (L.) Medik. was identified as a good source for JH.

Currently, JH analogues are imported by the industry and use of our own sources will reduce the cost of imported JH based products and cut short our current account deficit. Few MH based products available in the market at present are Karnataka based and they are not easily available to the poor farmers of Kerala due to logistic problems. Hence there is a potential for industrial utilisation of our indigenous sources of MH identified during the study so that low cost products could be made available to farmers in time and logistic problems could be solved. A suitable method for utilising both backuchiol and ecdysterone together on the same batch of silkworm has been developed during the study and this can be used as a model for popularising the use of IGRs among farmers as it incorporates both the benefits of JH and MH.

JH was found to increase the economic parameters of *Bombyx mori* L. Bivoltine double hybrids (FC1X FC2) by 15-20% in this geographical regime at 1-3 ppm concentration levels.MH at 10-30 ppm concentration levels was found to fasten the process of cocooning by about 12 hours and synchronise the event so that labour involved in mechanical seriposition can be reduced.

This can be used as a method for saving crop loss during the times when farmers run short of mulberry leaves which often occurs during the final stages of the crop. Water extracts of the leaves of *Diploclisia glaucescens* and *Coscinium fenestratum* were found to be as effective as ecdysterone itself. Hence the costly procedure of ecdysterone isolation could be avoided so that on industry level application this reduces the making cost of MH based products. In the case of *Cosinium,* leaves are usually discarded where it is normally used in Ayurvedic formulations and hence this finding has a potential on utilising the discarded waste product of Ayurvedic industry. This adds to the value addition to Western Ghats flora. As a concluding note it can be said that both these compounds can be recommended for use by sericulture farmers.

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