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Isolation, quantification and chemical characterisation of ecdysterone from medicinal plants of Kerala, Western Ghats

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Abstract
Ecdysteroids are a group of compounds responsible for molting in insects and is variously expressed in plant kingdom, believed to be a means of deterring insects by influencing the metabolism and metamorphosis in these vectors. Kerala flora has not been screened for the presence of ecdysteroids before. This work is a follow up study based on a preliminary Bio prospection study on fifty medicinally important plants used by indigenous tribes of Kerala for the presence of ecdysterone. Four potential plant species which were found to have adequate amount of compound-*Diploclisia, Cyathula, Sesuvium and Coscinium*-were put to detailed extraction, isolation, quantification and chemical chaceterisation using HPLC, UV and IR spectroscopy. Literature survey suggested that soil and geographical regime has direct influence on the expression levels of ecdysterone. Some variations were observed in the expression levels of ecdysterone in our study too, in comparison with published literature but potential sources from indigenous plants were identified during this study. Availability in adequate quantity of this wonder molecule will increase its multi-faceted activity related studies in future.

Keywords: Ecdysterone-Kerala-medicinal plants- *Diploclisia-Cyathula-Sesuvium –Coscinium*

Introduction
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. So far, only a small proportion of the known flora has been subjected to chemical or biological investigations [1]. Ecdysteroid (EC) s were first recognised as steroidal hormones, controlling the moulting and metamorphosis in insects. Phytoecdysteroids are analogues of Ecdysteroids, which occur in 5–6 % of plant species [2] in relatively large concentrations and hence being a better source than arthropods. Galbraith *et al.* [3] showed that Ecdysterone derived from plants was identical with the hormone derived from insect sources. ECs are mainly C_{27}-C_{29} molecules derived from phytosterols, which have been modified to generate an A/B-*cis* ring junction, an α,β-unsaturated ketone in ring- B, and the incorporation of multiple hydroxyl groups, together with other substituent as well.

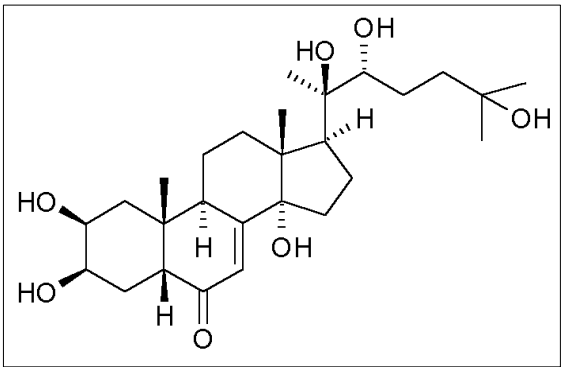

Fig 1: Structure of Ecdysterone

ECs are apparently non-toxic to mammals and a wide range of beneficial pharmacological-adaptogenic, anabolic, anti-diabetic, hepatoprotective, immunoprotective, wound healing, and perhaps even anti-tumour [4] - activities are claimed for them. In particular, this has led to a large and unregulated market for EC-containing neutrceutical preparations for bodybuilders, sportsmen, and pets, among others.

Kerala, being a part of the mighty Western Ghats range has a huge potential in exploiting its rich, unique and highly endemic biodiversity. This part of the world has not been screened for the presence of ECs earlier. Availability of large amount of ECs from native sources will certainly boost the activity oriented studies of the same within the country. As a part of this study, four species were selected for detailed bioprospection based on a preliminary survey from among fifty plants [5] for ecdysterone from Kerala flora (table 1).

Table 1: List of the four plants selected for detailed Bioprospection study.

S. No	Botanical name	Family	Parts used	Collected from
1	*Diploclisia glaucescens* (Blume) Diels	Menispermaceae	Stem, Leaves	Kalpetta, Wayanad district
2	*Cyathula prostrata* (L.) Blume	Amaranthaceae	Flowering Shoot	Chalakudy, Thrissur district
3	*Sesuvium portulacastrum* (L.) L.	Aizoaceae.	Shoot	Edavanakkad, Ernakulam District
4	*Coscinium fenestratum* (Gaertn.) Colebr	Menispermaceae	Stem, Leaves	Mananthavady, Wayanad district

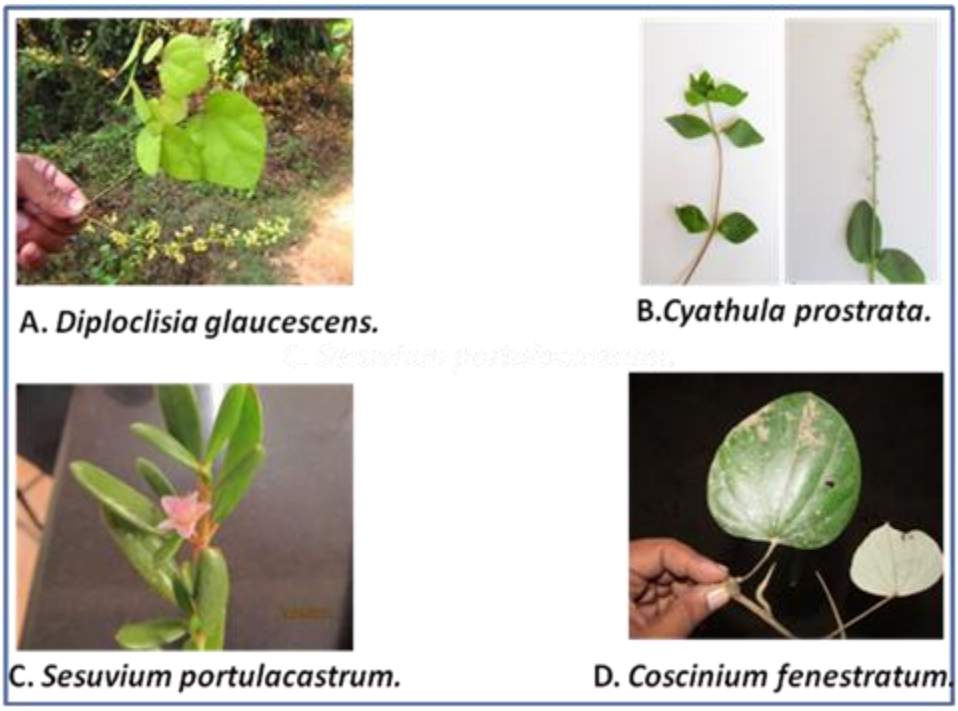

Fig 2: Plants selected for ecdysterone bioprospection

Diploclisia glaucescens is a liana growing in South India, Sri Lanka and the Southern part of China and the only species available for the genus in India. The leaves of the plant have been used in the treatment of biliousness and venereal diseases [6]. The highest percentage yield ever reported for EC (3.2%), from any plant material investigated so far, is from the stem of *Diploclisia* collected from Sri Lanka [7]. The specimen collected from India has not been screened for EC before.

The species *Cyathula prostrata* is a much branched slender prostrate or decumbent herbaceous annual, rooting profusely at the nodes. It is found throughout India, and its propagation is done by seeds and vegetative method. Shah and de Souza in 1971 [8] isolated EC for the first time from this species with a very low yield of 0.05%, from the specimen collected from Bombay, India. No work has been done in this species since then, with regard to EC so far.

The species *Sesuvium portulacastrum* grow in coastal saline soils or inland habitats. In India, it grows well in the eastern and western coastline as a mangrove associate. The plant has he remarkable ability to survive under different stress conditions. Banerji *et al*., in 1971 [9] isolated EC from this species with a yield of 3.5 g/kg.

The use of *Coscinium fenestratum* as a source of the important Ayurvedic raw drug '*daruharidra*' dates back to several centuries. Its stem has long been used in South India and Sri Lanka as a source for yellow dye and a bitter tonic and has found its way to Europe under the name False Calumba or Tree Turmeric. It is a dioecious, large, woody climber of the family Menispermaceae, a more or less primitive group, indigenous to the Indo-Malayan region [10]. Ecdysterone was isolated for the first time from this species by our team [11].

Materials and Methods

1. Plant collection

The *Diploclisia* specimen (stem and Leaves) for this study was collected wild from Kalpetta, Wayanad district (11^0 59'43" North, 76^0 09'43"East), *Cyathula* specimen (flowering shoot) was collected from Chalakudy, Thrissur district, (10^0 20'36" North, 76^0 22'47" East), *Sesuvium* specimen (vegetative shoot) was collected from Edavanakkad sea coast, Vypeen island, Ernakulam district, (10^0 04'22" North, 76^0 11'48" East) and *Coscinium* specimen (stem and Leaves) was collected from Boys Town, Manathavady, Wayanad district, Kerala (11^0 84'18" North, $75^0$92'09" East).

2. Extraction

The parts collected in the case of *Diploclisia*, *Cyathula* and *Coscinium* were shade dried, powdered and subjected to sequential hot extraction in a Soxhlet apparatus, starting with Petroleum ether, followed by Chloroform ($CHCl_3$), Ethyl Methyl Ketone (MEK), and finally with methanol (MeOH). The extracts were filtered and solvent removed under reduced

pressure in a flash evaporator. The Thin layer chromatography (TLC) of the extracts was performed with standard EC (Sigma) on pre-coated silica plates (Merck). The solvent systems used were 7:3 $CHCl_3$: MeOH and 50:2:3:6 Ethyl acetate: MeOH: Formic acid: Water. The plate was developed using acidic vanillin at 104 ^{0}C in an oven for 2-3 min. The standard EC developed an olive green colour with acidic vanillin spray and was UV positive. Extracts which developed an olive green spot with the same *Rf* that of standard 20E were considered positive, and others were considered negative. As a confirmation, ferric chloride was sprayed on another plate, which responds negative to EC. MEK and MeOH extacts were EC positive in all the three species.

In the case of *Sesuvium* fresh stem was minced to medium sized particles in a blender. This was put to cold extraction inside a shaker, with MeOH for 24 hours. The MeOH extract was filtered and the solvent was removed under reduced pressure using a rotary evaporator at 35 ^{0}C. The MeOH was completely removed, and the water portion remained. The water portion was sequentially partitioned with Petroleum ether, $CHCl_3$, MEK and Butanol solvents. In this case MEK and Butanol solvents were EC positive.

3. Column Chromatography

EC positive extracts were put to column chromatography on acidic alumina (Merck) columns. The elution was always started with 100% $CHCl_3$ and then moved on to $CHCl_3$/MeOH solvent mixtures. The EC positive fractions always came with 10% MeOH fractions. EC positive fractions were collected; solvent removed and was re-suspended in MeOH/Di ethyl ether mixture and left over night (10 ^{0}C). The EC crystals from the above mixture was collected, dried and exact weight was recorded.

4. Chemical characterization

In addition to TLC, EC isolated were subjected to HPLC, UV and IR spectral analysis. HPLC Analyses were performed on a Shimadzu-SPD-M20A HPLC, equipped with DAD (Diode Array detector) detector, with Phenomenex luna 5u C_{18} (2) 100A, size 250 × 4.60 nm column. All the compounds were detected at 254 nm at room temperature with an elutent flow rate of 1.2 ml/min and an injection volume of 10µL. The mobile phase consisted of MeOH (A) and water (B) in 1: 1 ratio. UV spectra were recorded on a Shimadzu UV spectrophotometer Model UV-1800 in MeOH. IR spectra were recorded on a Shimadzu IRAffinity-1 machine in Potassium bromide. 1 mg of EC standard (Sigma) and EC isolated from Diploclisia leaves were dissolved in 1 ml MeOH (HPLC Grade) each and 10 µL of the both solutions were injected into the HPLC column respectively for recording their individual profiles.

Results and Discussion

The amount of EC isolated from each part of the four species selected for study is shown in table 3.

S. No	Scientific Name	Part Used	Yeild
1	*Diploclisia*	Stem	0.17%
2	*Diploclisia*	Leaves	1.40%
3	*Cyathula*	Flowering shoot	0.18%
4	*Sesuvium*	Vegetative shoot	0.12%
5	*Coscinium*	Stem	0.22%
6	*Coscinium*	Leaves	0.12%

In the case of *Diploclisia*, EC yields were 1.4% and 0.17% for leaves and stem respectively with respect to the dry weight of part used. However the stem collected from Indian sub-continent did not yield as much EC as reported from the specimen collected from Sri Lanka and contained only very low levels of EC (0.17%). But the leaves were identified as a good source for EC in this part of the country with a 1.4% yield. An approximate four time increase in yield (0.18%) of EC was reported for *Cyathula prostrata* collected from the study area in comparison to what was reported from the specimen collected from Bombay. *Sesuvium* collected from the study area yielded a lesser amount of EC (0.12%) as opposed to the higher yield (0.35%) reported from the specimen collected from Bombay. EC was reported for the first time ever from *Coscinium fenestratum* of the Menispermaceae family. EC yields for stem and leaves of *Coscinium* 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 EC in good amounts from the leaves hence acts as a value addition to the species.

Spectral data for chemical chacterisation of EC isolated from *Diploclisia* is compared with that of standard EC (Sigma) in figure 3. The HPLC profile recorded a major peak with retention time (Rt) at 6.3 min and an absorbance of 900 mAU for standard EC (Sigma). The HPLC profile for EC from *Diploclisia* recorded a major peak with Rt at 6.3 min and an absorbance of 700 mAU. The UV spectrum (UV) for the peak in HPLC with Rt at 6.3 min recorded maximum absorbance at 246 nm in both cases indicated the presence an α, β, unsaturated carbonyl group within them, characteristic of EC.

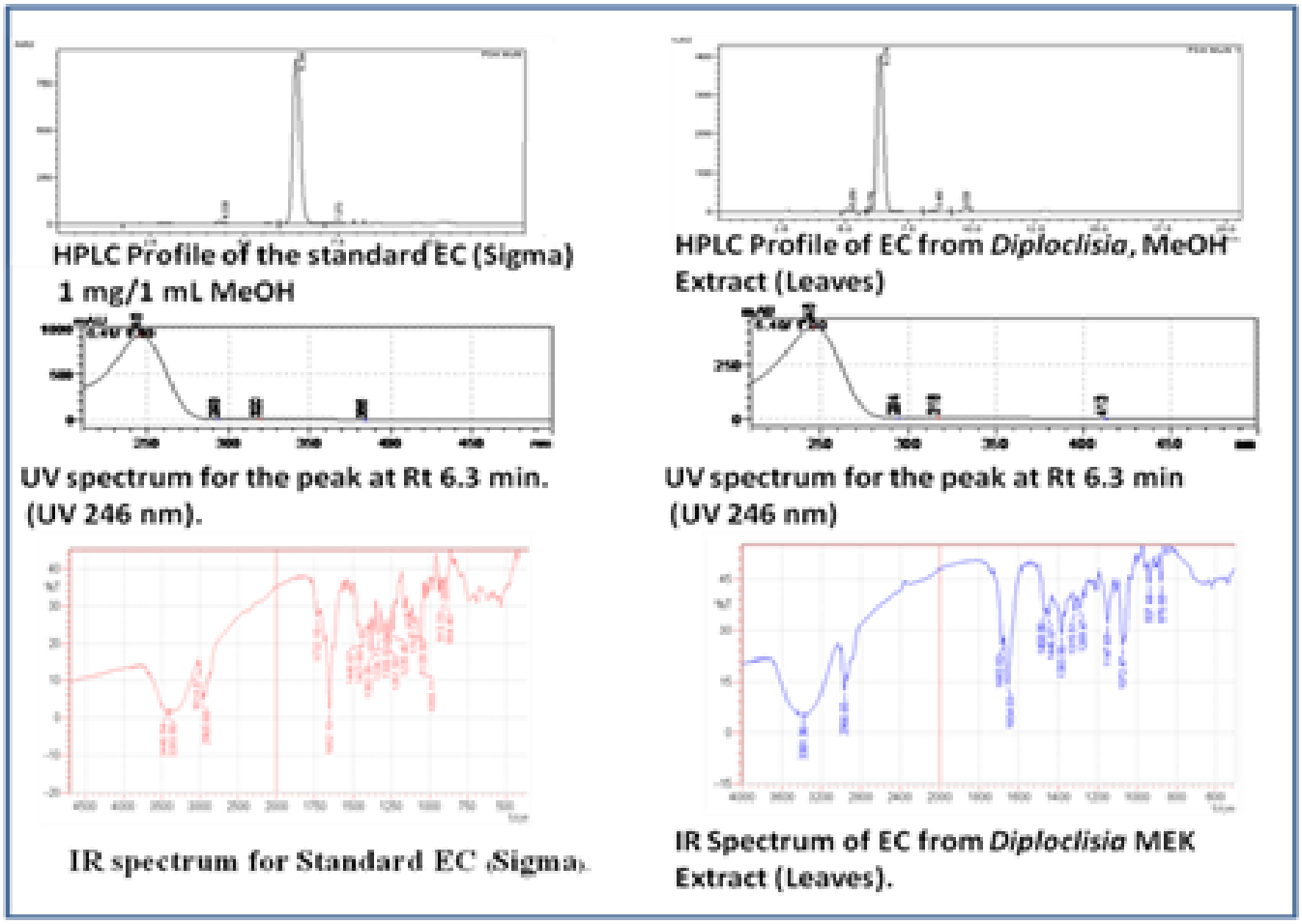

Fig 3: Comparison of HPLC profile, UV and FTIR spectra of Std EC (sigma) and that EC isolated from *Diploclisia* Leaves.

The FTIR spectrum of Standard EC (Sigma) revealed a broad hydroxyl absorption (3393, 3446 cm^{-1}) and strong conjugated carbonyl absorptions (1652 cm^{-1}) characteristic of an α, β unsaturated keto group and also corresponding to the 7-en-6-one of ecdysteroids. The IR spectrum of EC isolated from *Diploclisia* also revealed a broad hydroxyl absorption (3381 cm^{-1}) and strong conjugated carbonyl absorptions (1654 cm^{-1}) characteristic of an α, β unsaturated keto group as in the case of standard EC.

Hence the EC isolated all the four plants were of sigma standard in its purity on chemical analysis. However, only the spectral data regarding *Diploclisia* leaves were discussed here to avoid monotony of repetition. Isolation of large quantities of EC from indigenous plants will increase the activity studies for this wonder drug molecule in future.

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References

1. Banerji A. Biotechnical potential of natural products. Bioelectrochem Bioenerg. 1992; 27(2):105-113.
2. Dinan L. A strategy for the identification of ecdysteroid receptor agonists and antagonists from plants. Eur J Entomol. 1995; 92:271-283.
3. Galbraith MN, Horn DHS, Hocks P, Schulz G, Hoffmeister H. The identity of 20-hydroxy-ecdysones from various sources. Naturwissenschaften. 1967; 54:471-472
4. Dinan L. Phytoecdysteroids: Biological aspects. Phytochemistry 2001; 57:325-339.
5. Sreejit CM, Chinchu Bose, Asoke Banerji, Thomas Mathew P. Bioprospection of Kerala Flora for the Multipurpose Drug-Phytoecdysoids. J Biol. Chem. Research. 2018; 35(1):115-123.
6. Chopra RN, Chopra IC. Indigenous Drugs of India. Second ed., Academic Publishers, Kolkata, 1958, 391-394.
7. Bandara BMR, Jayasinghe L, Karunaratne V, Wannigama GP, Bokel M, Kraus W et al. Ecdysterone from stem of *Diploclisia glaucescens*. Phytochemistry. 1989; 28:1073-75.
8. Shah VC, De Souza NJ. Ecdysterone from *Cyathula prostrata*. Phytochemistry. 1971; 10:1398-1399.
9. Banerji A, Chintalwar GJ, Joshi NK, Chadha MS. Isolation of ecdysterone from Indian plants. Phytochemistry. 1971; 10:2225-2226.
10. Tushar KV, George S, Remashree AB, Balachandran I. *Coscinium fenestratum* (Gaertn.) Colebr.-A review on this rare, critically endangered and highly-traded medicinal species. Journal of Plant Sciences. 2008; 3:133-145.
11. Sreejit CM, Chinchu Bose, Asoke Banerji, Thomas Mathew P. Indian medicinal plant, *Cosciniunt fenestratum*- A new bio source for the multifunctional bio active molecule ecdysterone. International journal of herbal medicine. 2014; 2(5):05-09.