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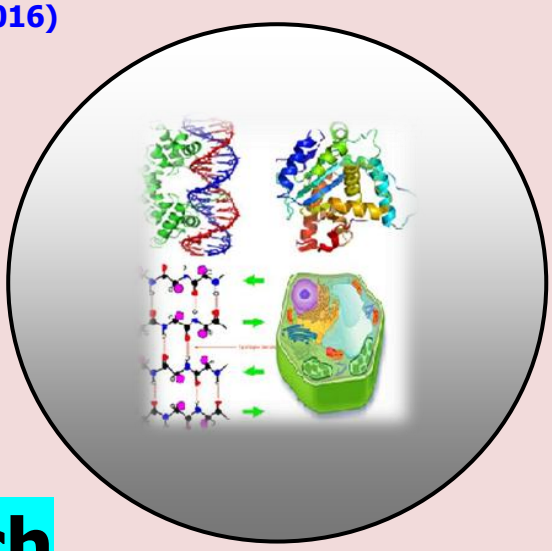

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RESEARCH PAPER

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Bioprospection of Kerala Flora for the Multipurpose Drug - Phytoecdysoids

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ABSTRACT

Phytoecdysoids are analogues of the arthropod steroid hormone ecdysterone, found in plants which are apparently non-toxic to mammals with a wide range of pharmacological - adaptogenic, anabolic, anti-diabetic, hepatoprotective, immunoprotective, wound healing, and perhaps even anti-tumor- activities. Though they have been reported from more than 100 terrestrial plant families, till date, only less than 2 % of the world's flora has been investigated for their presence. Considering its pharmacological activities and extremely non toxic nature, it can very well be the darling of pharmaceutical companies in future. Kerala, being a part of the mighty Western Ghats range has a huge potential in exploiting its rich, unique and highly endemic biodiversity. A bio prospection study for ecdysteroids has been done with regard to the Angiosperm flora of the state for the first time. Fifty plant species were screened from the study area. A simple protocol for screening ecdysteroids using very less amount of the plant source was developed using ultra sonication and Thin Layer Chromatography. Ten species were found positive for phytoecdysoids. Ecdysterone was reported for the first time ever from Coscinium fenestratum.
Key words: Kerala flora-ecdysterone-bioprospection-phytoecdysoids.

INTRODUCTION

The study of natural products not only provides novel bioactive compounds, but also helps in understanding the 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; the vast unexplored biotechnical potential of flora awaits discovery and exploitation (Banerji, 1992). Ecdysteroid (EC)s are steroidal hormones, controlling the moulting and metamorphosis in insects. The first EC, ecdysone, was isolated by Burtenandt and Karlson (1954) from silkworm pupae. Phytoecdysteroid (PE)s are analogs of EC, occurring in 5–6 % of plant species (Dinan,

1995), generally at far higher concentrations than those typically found in arthropods and hence are a far better source of ECs than arthropods. PEs 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.

The most common PE is the 20- hydoxyecdysone (ecdysterone) which is widely accepted to be the biologically active zooecdysteroid too. (Dinan, 2001). Over the past forty years, more than 300 structural analogues of PE have been isolated from plant sources (structural, biological and spectral data are collated on the web site "www.ecdybase.org"). 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-tumor) activities are claimed for them. In particular, this has led to a large (and unregulated) market for EC-containing preparations for body-builders, sportsmen, and pets, among others. ECs are also being considered as nutraceutical additives to food products (Dinan, 2009).

PEs have been reported from more than 100 terrestrial plant families representing Ferns, Gymnosperms and Angiosperms. However, till date, only less than 2% of the world's flora has been investigated for the presence of ECs (Dinan, 2001). Only a handful of species have been bio-prospected for the presence of ECs in India. Currently in India, ECs are not available in adequate quantities at affordable prices for active research. Kerala, being a part of the mighty Western Ghats range has a huge potential in exploiting its rich biodiversity which is highly endemic and has not been screened for the presence of ECs earlier. A bio prospecting study for detecting indigenous sources of EC has been done with regard to the Angiosperm flora of the state.

MATERIALS AND METHODS

After detailed literature survey, fifty plants were selected from Kerala flora for testing the presence of EC (Table.1). Selection was based mainly on three criteria, *viz*., previously reported species, related members of the previously reported species and the plants used by indigenous tribes. Other than these, a few species were selected on random basis also. Leaf, stem, root and seeds were selected for screening, depending on the species (Table.1). Plants were collected from available regions throughout Kerala state.

A preliminary screening was done for the presence of EC using a small amount of the plant material. The collected plant part (fresh) was accurately weighed (15 g) and was cut into small pieces, and pounded into a medium sized paste in a blender. This was subjected to ultra sonication with 50 ml Methanol (MeOH) in a conical flask for 60 minutes. The extract was collected and filtered. Another 50 ml MeOH was added to the flask and the same procedure was followed. First and second extracts were combined together and the solvent was removed under reduced pressure in a rotary evaporator. Thin Layer Chromatography (TLC) of the MeOH extract was done with standard EC on pre-coated silica plates (Merck). The solvent systems used were 7:3 Chloroform ($CHCl_3$): MeOH and 50: 2: 3: 6 Ethyl acetate: MeOH: Formic acid: Water. The plate was developed using acidic vanillin at 104^0C in an oven for 2-3 min. The standard EC developed an olive green colour with acidic vanillin spray and was Ultra Violet (UV) light positive. Species which developed an olive green spot with the same Resolution front *(Rf)* that of standard EC were considered positive for the presence of EC, and others were considered negative (Table.1).

RESULTS AND DISCUSSION

Among the 50 species screened, 10 species *i.e*., *Achyranthes aspera, Achyranthes bidentata*, *Pupalia lappacea var. lappacea*, *Sida rhomboidea*, *Tinospora cordifolia*, *Gomphrena celosiodes*, *Diploclisia glaucescens*, *Sesuvium portulacastrum*, *Cyathula prostrata* and *Coscinium fenestratum* were identified as positive sources for EC from the study area (Table 2).

More members from Amaranthaceae and Menispermaceae families were selected for massive screening, as EC positive species were reported previously from these families. Thirteen species from Amaranthaceae and eleven species from Menispermaceae family, present abundantly in the study area were screened, hoping to get a more potential source for the same. This strategy worked, and of the 10 positive species, eight were from these two families, and of the total 50 plants screened almost 20% responded positive to EC. Usually the percentage of plants reported positive for EC from random surveys, comes between 5-6% (Dinan, 2001). The Amaranthaceae family reported the most number of positive species with five species. Even among the species selected from a family, ethno botanically used plants must be given priority, in future surveys, as the chances of hitting bio active molecules are more, from them.

Among the eleven species screened from Menispermaceae family, all the three species; *Coscinium*, *Diploclisia* and *Tinospora* reported positive for EC, were highly used by ethnic tribes. *Coscinium,* a high valued medicinal plant in ethno as well as traditional systems of medicine, turned out to be a new potentially good source for EC from the Menispermaceae family. EC was isolated and characterised for the first time from this species (Sreejit *et. al*., 2015). EC isolation and charecterisation from other sources is currently going on. Of the 50 plants screened for the presence of ECs, 31species were not been explored for this compound earlier and though the results were negative for most species, the information could be added to the database www.ecdybase.com after publication, as new negative reports and duplication can be avoided while exploring the area in future for the same purpose. A simple screening protocol for rapid survey of EC has been developed during this study. A massive screening study should be initiated in future for identifying more indigenous potential sources for EC.

Table 1. List of plants screened from I flora for the presence of Ecdysteroids.

Sl. No.	Name of the Species	Family	Early reports from www.ecdybase.org (+= EC positive,-=EC negative ; name of author; year of publication) EC= Ecdysteroid	Part used L=leaf; St=stem, Sh=shoot, B=Bark, Rh=Rhizome,I= Inflorescence, F=Fruit, G=Gall R =Root	Criteria used for selection A=previous report, B=ethno botanical importance C=related species of previously reported species R=Random	Ecdysteron epresence during this study + = present - = absent
1	***Diploclisia glaucescens*** (Blume) Diels.	Menispermaceae	+ Miller *et al.* (1985) + Bandara *et al.* (1989a) + Jayasinghe *et al.* (2002) + Jayasinghe *et al.* (2003a) + Jayasinghe *et al.* (2003b) + Jayasinghe *et al.* (2005)	L	A,B	+
				St	A,B	+
2	***Sesuvium portulacastrum*** (L.) L.	Aizoaceae	+ Banerji *et al.* (1971) + Sipahimalani *et al.* (1972 +Wong *et al.* (1979) + Bergamasco and Horn (1983) + Shivakumar et al. (1995) + Rele *et al.* (2003)	St	A	+
3	***Cyathula prostrata*** (L.) Blume.	Amaranthaceae	+ Shah and de Souza (1971)	Sh+I	A,B	+
4	***Coscinium fenestratum*** (Gaertn.) Colebr.	Menispermaceae	No early report	St	B,C	+
				L	B,C	+

5	***Achyranthes aspera*** L.	Amarantha ceae	+ Banerji *et al.* (1971) + Banerji and Chadha (1971) + Ikan *et al.* (1971) + Ogawa *et al.* (1971) + Chow and Lu (1980) + Kunert *et al.* (2000)	Sh+I	A,B	+
6	***Achyranthes bidentata*** Blume.	Amarantha ceae	+ Imai *et al.* (1969) + Ogawa *et al.* (1971) + Wong *et al.* (1979) + Chow and Lu (1980) + Yao and Hu (1989) + Meng *et al.* (2005)	Sh+I	A	+
7	***Pupalia lappacea (L.) Juss. Var. lappacea***; Hook.	Amarantha ceae	+Felix and Domingo (2008)	Sh+I	C	+
8	***Sida rhomboidea*** Roxb. Ex Fleming.	Malvaceae	+ Prakash and Ghosal (1979) + Dinan *et al.* (2001) + Jadhav *et al* (2007)	R	A	+
9	***Tinospora cordifolia*** (Willd.) Miers.	Menisperm aceae	+ Pathak *et al.* (1995) + Gangan *et al.* (1997) + Pradhan *et al.* (1997)	L	A,B	+
				St	A,B	+
10	***Gomphrena celosioides*** Mart.	Amarantha ceae	+ Banerji *et al.* (1971)	Sh +I	C	+
11	***Alternanthera brasiliana*** (L.) Kuntze.	Amarantha ceae	+ Banerji *et al.* (1971)	Sh +I	A	-
12	***Justicia gendarussa*** Burm.	Acanthacea e	No early Report	L	B	-
13	***Ipomoea alba*** L.	Convolvula ceae	No early Report	L	C	-
14	***Ipomoea obscura*** (L.) Ker-Gawl.	Convolvula ceae	No early report	L	C	-
15	***Ipomoea pes-caprae*** (L.) R. Br., ssp. ***pes-caprae.***	Convolvula ceae	No early report	L	C	-
16	***Vitex negundo*** L.	Verbenace ae	+ Kubo *et al.* (1985)	Sh	C	-
17	***Pilea microphylla*** (L.) Liebm.	Urticaceae	No early report	Sh	R	-
18	***Peperomia***	Piperaceae	No early report	Sh	R	-

	pellucida (L.) Kunth.					
19	***Pterospermum rubiginosum*** Heyne ex Wight & Arn.	Sterculiaceae	No early report	B	B	-
20	***Briedelia stipularis*** (L.) Blume.	Euphorbiaceae	No early report	B	B	-
21	***Tiliacora acuminata*** (Poir.) Miers ex Hook.	Menispermaceae	No early report	L	C	-
22	***Trianthema portulacastrum*** L.	Aizoaceae	+ Bergamasco and Horn (1983) + Banerji *et al.* (1971) + Ravishankar and Mehta (1979)	Sh	A	-
23	***Allmania nodiflora*** (L.) R.	Amaranthaceae	No early report	Sh	C	-
24	***Drymaria cordata*** (L.) Willd.	Caryophyllaceae	No early report	Sh	B	-
25	***Cyanotis arachnoidea*** Clarke.	Commelinaceae	+ Chow and Lu (1980) + Nien *et al.* (1978) + Nie and Qiu (1987) + Tan *et al.* (2002)	Sh	A	-
				R	A	-
26	***Cyanotis cristata*** (L.) D. Don.	Commelinaceae	No early report	Sh	C	-
27	***Moringa pterygosperma*** Gaertn.	Moringaceae	No early report	L	B	-
28	***Nelumbo nucifera*** Gaertn.	Nelumbonaceae	No early report	L	R	-
29	***Senna occidentalis*** (L.) Link.	Caesalpiniaceae	No early report	Sh	C	-
30	***Cleome viscosa*** L.	Capparidaceae	No early report	Sh	R	-
31	***Cleome burmannii*** Wight & Arn.	Capparidaceae	No early report	Sh	R	-
32	***Cyclea peltata*** (Lam.) Hook.	Menispermaceae	No early report	Rh	B,C	-
33	***Tinospora sinensis*** (Lour.) Merr.	Menispermaceae	No early report	L	C	-
34	***Cissampelos***	Menisperm	No early report	L	C	-

	pareira L. var. ***hirsuta*** (Ham. Ex DC.) Forman.	aceae				
35	***Cocculus hirsutus*** (L.) Diels.	Menispermaceae	No early report	L	C	-
36	***Stephania japonica*** (Thunb.) Miers, var. ***japonica.***	Menispermaceae	No early report	L	C	-
37	***Stephania wightii*** (Arn.) Dunn.	Menispermaceae	No early report	L	C	-
38	***Chenopodium ambrosioides*** L.	Chenopodiaceae	+ Wong *et al.* (1979) +/- Báthory *et al.* (1984) - Dinan *et al.* (1998)	L	A	-
				St	A	-
39	***Cocos nucifera*** L.	Arecaceae	No early report	I	R	-
40	***Pistacia integrrima*** Stew. Ex Brindis.	Anacardiaceae	No early report	G	R	-
41	***Saraca asoca*** (Roxb.) de Wilde.	Caesalpiniaceae	No early report	B	R	-
42	***Anamirta cocculus*** (L.) Wight & Arn.	Menispermaceae	No early report	St	B,C	-
				L	B,C	-
				F	B,C	-
43	***Cassia fistula*** L.	Caesalpiniaceae	No early report	B	B,C	-
44	***Senna tora*** (L.) Roxb.	Caesalpiniaceae	+Wong *et al.* (1979) + Chow and Lu (1980) + Shivakumar *et al.* (1995)	Sh	A	-
45	***Aerva lanata*** (L.) Juss. Ex Schult.	Amarantaceae	+ Baltaev *et al.* (1992)	Sh+I	A,B	-
46	***Alternanthera sessilis*** (L.) R. Br. Ex. DC.	Amarantaceae	+ Takemoto *et al.* (1967)	Sh+I	A	-
47	***Alternanthera philoxeroides*** (Mart.) Grisb.	Amarantaceae	+Wong *et al.* (1979)	Sh	A	-
48	***Alternanthera tenella*** Colla, var. ***tenella.***	Amarantaceae	No early report	Sh+I	C	-
49	***Amaranthus***	Amarantha	+ Takemoto *et al.*	Sh+I	A	-

	spinosus L.	ceae	(1967) +Wong *et al.* (1979)			
50	***Boerhavia diffusa*** L.	Nyctaginaceae	+ Suri *et al*.(1982)	Sh	A,B	-

Table 2. The species which gave positive response to EC in the preliminary survey.

Sl No.	Name of the species	Family	Part used
1	***Achyranthes aspera***	Amaranthaceae	Flowering Shoot
2	***Achyranthes bidentata***	Amaranthaceae	Flowering Shoot
3	***Coscinium fenestratum***	Menispermaceae	Stem, Leaf
4	***Cyathula prostrata***	Amaranthaceae	Flowering Shoot
5	***Diploclisia glaucescens***	Menispermaceae	Stem, leaf
6	***Gomphrena celosioides***	Amaranthaceae	Flowering Shoot
7	***Pupalia lappacea var. lappacea***	Amaranthaceae	Flowering Shoot
8	***Sida rhomboidea***	Malvaceae	Root,
9	***Sesuvium portulacastrum***	Aizoaceae	Stem
10	***Tinospora cordifolia***	Menispermaceae	Stem, Leaf

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