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OCCURRENCE OF INSECT MOULTING HORMONE (β-ECDYSONE) IN SOME LOCALLY AVAILABLE PLANTS

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ABSTRACT
Twenty locally available plants belonging to families where some of their members were reported to be having insect moulting hormone were taken for identifying the occurrence of this hormone i.e. β-ecdysone or 20-hydroxyecdysone in them. Leaves and soft shoots of these plants were collected, washed, dried, powdered and extracted with methanol. (Hot extraction by Soxhlet apparatus) The detection of insect moulting hormone (β-ecdysone or 20-hydroxyecdysone) was carried out in these extracts by thin layer chromatography (TLC) using chloroform: methanol @ 4:1 as mobile phase. Out of 20 plant extracts only 6 extracts showed the presence of 20-hydroxyecdysone under thin layer chromatography (TLC). After developing TLC plates these were sprayed with anisaldehyde for steroid visualization and their retention factor (R*f*) values were also calculated. The presence of moulting hormone was confirmed under high performance liquid chromatography (HPLC) by running the marker 20-hydroxyecdysone along with these six plant extracts with a mobile phase of methanol and water in the ratio of 55:45 respectively. Results showed reasonable concentration of 20-hydroxyecdysone (β-ecdysone) in 3 plant extracts of *Taxus wallichiana* Zucc. (Himalayan Yew), *Cupressus tularosa* Linn (Cupreous) and *Datura stramonium* Linn. (Datura). Maximum quantity of this moulting hormone was recorded in *Taxus wallichiana* Zucc. followed by *Cupressus tularosa* Linn and least was in *Datura stramonium* Linn. The effect of 20-hydroxyecdysone was later on tested on silkworm *Bombyx mori* L. for synchronization of maturation, and on other commercial cocoon characters.

KEY WORDS: Moulting hormone, 20-hydroxyecdysone, β-ecdysone, TLC, HPLC

INTRODUCTION

It is known that analogues of ecdysteroids also occur in certain proportions in plants. These plants synthesize these ecdysteroids as a defense mechanism and these occur in them in large quantities (Schmelz *et al*., 2000). The ecdysteroid derived from plant source is popularly known as Phytoecdysteroid. These phytoecdysteroids have been seen to be 20 times more active than Zoo-ecdysteroids (Nair *et al*., 2002). As a defense mechanism in plants it is also believed that they provide some degree of protection to the plant against non-adopted phytophagous insects (Bergamasco and Horn, 1983; Kubo and Hanke, 1986). Trivedy *et al*., (2006) reported that a plant belonging to *Caryophillaceae* i.e *Silene gallica* contains highest amount (0.1% by dry weight) of ecdysteroid both among the plants screened by her and among the plants so far reported from India. Ecdysteroid (ES) in the context of sericulture can be any phyto-sterol structurally closer to the original insect ecdysteroid, 20- hydroxyecdysone, which can induce a response in silkworm equal to that of the natural ecdysteroid and could be used for synchronizing the maturation activity. It can also advance the maturation activities and hasten the cocoon spinning process especially when a partial or complete crop loss is feared either due to leaf shortage or due to disease attack. In addition to this it has been reported that Phytoecdysteroid is also used commercially to increase productivity in sericulture (Chou and Lu, 1980; Zhuang *et al*., 1992) and reported that phytoecdysteroid administration at a particular time also increases cocoon weight, shell weight and shell percentage.

MATERIALS AND METHODS

20 different plant species were taken for detection of 20-hydroxyecdysone i.e. Insect moulting hormone (β-ecdysone) which will be later on used during silkworm rearing. These plant species are as under in table -1.

All these plant materials (leaf and tender branches) were taken and washed with distilled water first and later on shade dried till brisk. The dried plant material was powdered in a grinder in order to make a fine powder for getting plant extract. The hot extract of each plant material was prepared by boiling powdered plant material along with methanol and water in the ratio of 3:2 in a Soxhlet Apparatus continuously for 6 hours. The extract was separated from the residue by filtration and centrifugation. This extract was concentrated at a temperature of 60^0 C in a rotary evaporator and again dissolved in methanol and water solvent. The crude extracts were defatted in a solvent system by a partition between *n*-Hexane and 80% aqueous MeOH (Hoffman and Hetru, 1983; Bathori *et al.*, 2000).

The presence of 20-hydroxyecdysone- like substance was identified in the extracts by thin layer chromatography, keeping ecdysone (20-hydroxyecdysone) procured from M/S Sigma, St.Louis, M.O., USA as standard. The samples and standard (20E) were run on the Silica gel G coated plates for which mobile phase (thin-layer

chromatography solvent) used was chloroform: methanol in the ratio of 4:1. After developing these plates were initially placed in iodine chamber and later on sprayed with anisaldehyde reagent for steroid visualization. These plates were also viewed in a UV hood at UV 254 nm, where identical spots between the standard (20E) and plant samples were identified.

TABLE 1. Plant Botanical Name, family and their local name

	Botanical Name	Family	Local Name
1	*Amaranthus hybridus* Linn.	Amaranthaceae	Lissa
2	*Artemisia abrinthium* Linn.	Asteraceae (Compositae)	Tethwen
3	*Conyza canadensis* (L) Cronquist	Asteraceae (Compositae)	Shali luth
4	*Datura stramonium* Linn.	Solanaceae	Datur
5	*Cannabis sativa* Linn.	Cannabinaceae	Bhang
6	*Cupressus tularosa* Linn	Cupressaceae	Cupreous
7	*Mentha arvensis* (L) Hudson	Lamiaceae	Vena
8	*Ajuga bracteosa* Wall.	Labiatae	Jane-adam
9	*Taxus wallichiana* Zucc.	Taxaceae	Posthal
10	*Urtica dioica* Linn.	Urticaceae	Soi
11	*Portulaca oleracea* Linn	Portulacaeae	Nunar
12	*Salix alba* Linn.	Salicaceae	Veer
13	*Lavandula angustifolia.* Mill	Lamiaceae	Lavander
14	*Dioscorea deltoidea* Wall. ex Griseb	Dioscoreaceae	Kreench
15	*Viola odorata* Linn.	Violaceae	Banafsha
16	*Taraxicum officinale* Linn.	Asteraceae (Compositae)	Handh
17	*Cedrus deodara* (Roxb.) G.Don.	Pinaceae	Deodar
18	*Pinus wallichiana* –A.B.Jacks	Pinaceae	Kairoo
19	*Acerr pictum* Linn.	Coniferaceae	Kanzul
20	*Malva parviflora Linn.*	Malvaceae	*Sonchal*

RESULTS & DISCUSSION

Out of 20 plant samples only six showed 20-hydroxyecdysone under TLC. These were Himalayan Yew (*Taxus wallichiana* Zucc), Cupreous (*Cupressus tularosa Linn*), Datura (*Datura stramonium* Linn), Portulaca (*Portulaca oleracea*) Canabis (*Cannabis sativa* Linn.) and Conyza (*Conyza canadensis* (L) *Cronquist*)and their retention factor (R_f) values were also calculated by using the following formula:-

$$R_f = \frac{\text{Distance the substance travels from the origin}}{\text{Distance the solvent front travels from the origin}}$$

TABLE 2: R_f values of 20-hydroxyecdysone-like substance from 6 plant species on Silica-TLC eluted with Chloroform: methanol (4:1)

No	Plant species	Plant part	R_f
1	*Cupressus Tularosa*	Leaf and tender branches	0.68
2	*Taxus wallichiana*	Leaf and tender branches	0.69
3	*Conyza Canadensis*	Leaf and tender branches	0.75
4	*Portulaca oleracea*	Leaf	0.74
5	*Datura stramonium*	Leaf and tender branches	0.71
6	*Cannabis sativa*	Leaf	0.74

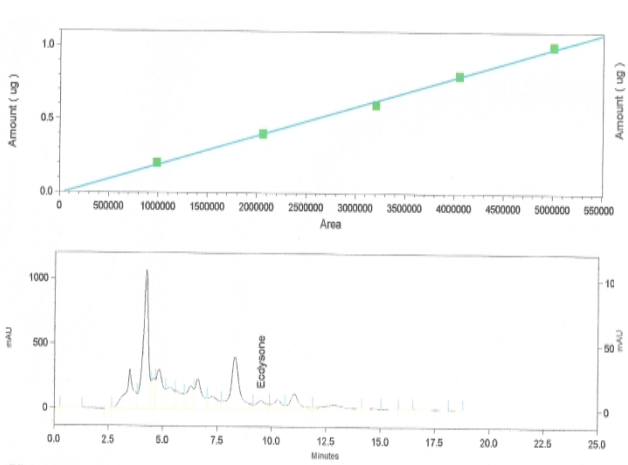
R_f of 20-hydroxyecdysone = 0.72

High performance liquid chromatography (HPLC)

This technique is used to identify a particular compound from a mixture. Therefore this technique was used for confirmation of presence of phytoecdysteroid (20-hydroxyecdysone) from plant extracts. Those plant extracts which showed identical spots coinciding with standard under TLC thereby giving a preliminary clue regarding the presence of 20-hydroxyecdysone were taken for final identification and confirmation under High Performance Liquid Chromatography. This was done at Indian Institute of Integrative Medicine (Regional Research Laboratory) Srinagar. Samples (6 plant extracts) and standard (20-E) were run for identification and quantification of 20-hydroxyecdysone in a High performance liquid chromatography (HPLC). About 400 µl of each sample was taken in HPLC viol for identification and quantification. The mobile phase used for this purpose was MeOH: H_2O (Methanol: Water) in the ratio of 55:45 and flow rate of HPLC was maintained at 0.4ml/min. About 3-5 µl of each sample were run for identification of 20-hydroxyecdysone. After running the plant extracts along with standard 20-hydroxyecdysone, three plant extracts were identified for possessing 20-hydroxyecdysone as their retention time coincided with that of the standard which was run along with plant extracts. The three plants containing moulting hormone as identified under HPLC were as follows:

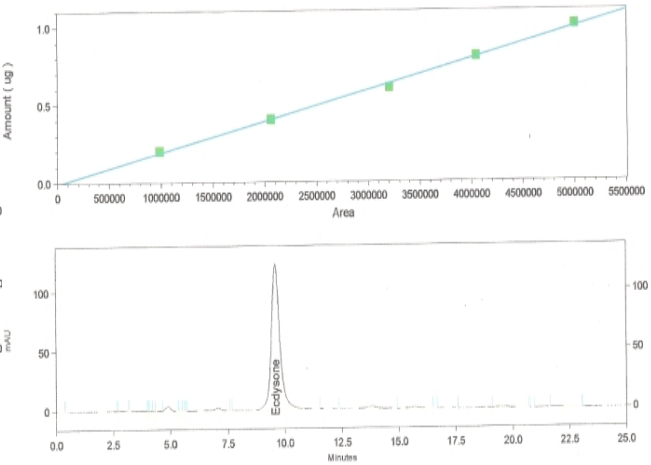
1. *Taxus wallichiana* Zucc.
2. *Cupressus tularosa* Linn and
3. *Datura stramonium* Linn.

As such it was confirmed by HPLC studies that these plants contain phytoecdysteroid in the form of 20-hydroxyecdysone. However out of these three plants identified *D. stramonium* was weakly positive in the presence of ecdysone as compared to first two plants



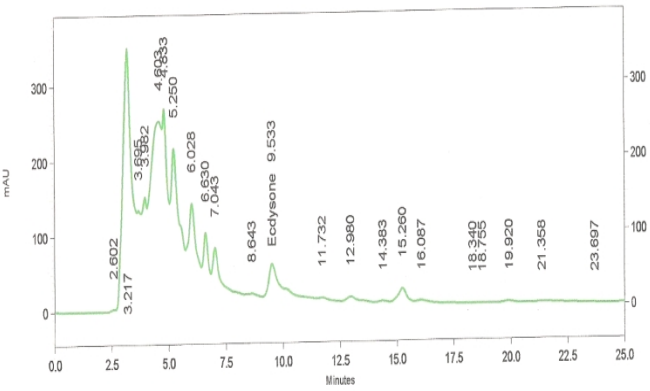
PDA-242nm Results (System (2/16/2009 2:08:29 PM) (Reprocessed) (Aborted Run))

Pk #	Name	Retention Time	Area	ESTD concentration	Units
14	Ecdysone	9.498	2067663	0.401	ug



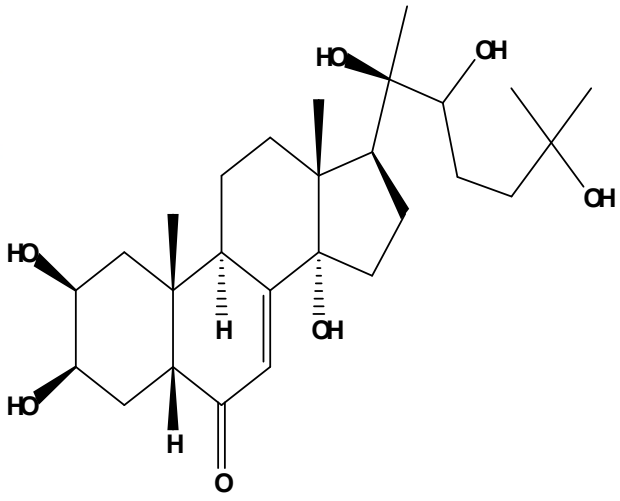
PDA-242nm Results (System (2/16/2009 2:08:18 PM) (Reprocessed))

Pk #	Name	Retention Time	Area	ESTD concentration	Units
15	Ecdysone	9.592	3209312	0.600 CAL	ug



PDA-242nm Results (System (2/16/2009 2:08:27 PM) (Reprocessed))

Pk #	Name	Retention Time	Area	ESTD concentration	Units
12	Ecdysone	9.533	3589220	0.704	ug



20-hydroxy ecdysone

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