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SCREENING OF PLANTS FOR ECDYSONES (FAMILIES AMARANTHACEAE AND CHENOPODIACEAE)

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Forty two species representing ten genera of Amaranthaceae and six genera of Chenopodiaceae families have been screened for ecdysones, of which eleven species of Amaranthaceae and three of Chenopodiaceae were found positive for ecdysones. Screening of ecdysones was based on the use of TLC and UV of the plant extracts and their comparison with that of authentic ecdysterone.

Key words: Ecdysones, Amaranthaceae, Chenopodiaceae, Ecdysterone.

Introduction

Ecdysones are polyhydroxy steroids and are so named because the first compound of this group, isolated from insects called α-ecdysone caused ecdysis in insects. The ecdysones obtained from the animal kingdom are called as zooecdysones while those from plants are known as phyto-ecdysones.

Nakanishi *et al.* [1] were the first to isolate ecdysones from the plants while studying the chemical constituents of a Formosan folk drug prepared from dry leaves of *Podocarpus nakai* which was reported to possess anti-cancer activity.

Shunji Imai *et al.* [2] while screening a large variety of plant material for biological activity of ecdysones, reported that more than a thousand species belonging to Pteridophyta, Gymnospermae and Angiospermae gave positive results for ecdysones. This prompted the screening of a large range of the plant material for ecdysones. To begin with Amaranthaceae and Chenopodiaceae families have been selected for this purpose.

Experimental

Silica gel 60 R_f 254 + 366 was used for thin layer chromatography and silica gel Woelm, activity-1 for column chromatography. The UV were recorded on a Pye Unicam SP-1800 spectrophotometer.

Spray reagents used for TLC. (i) 10% Sulphuric acid. (ii) Vanillin - sulphuric acid:- 3 g vanillin dissolved in 100 ml of absolute alcohol and 0.5 ml conc. H_2SO_4 added to it. (iii) Anisaldehyde - sulphuric acid. 0.5 ml anisaldehyde was added to 50 ml glacial acetic acid and then 1 ml of conc. H_2SO_4 added to the mixture slowly.

Extraction of plant material for ecdysones. 20 Grams of the dry plant material (root, leaves, flowers or fruit) was powdered and extracted in a Soxhlet apparatus with methanol (150 ml) for 20 - 24 hrs. The extract was concentrated to about 30 ml under reduced pressure and distilled water added to get 30% methanolic solution in each case. It was partitioned with n-hexane to remove chlorophyll and the aq. solution was then repeatedly extracted with *n*-butanol, dried over anhydrous sod. sulphate, concentrated, mixed with Kiesulguhr and separated by column chromatography (5 x 60 cm.) on silica gel with $CHCl_3$: CH_3OH, 15:1, 9:1 and 5:1 as the solvent systems. Fractions of 50 ml each were collected, concentrated in vacuo at 60° and checked on TLC plates eluting with CH_2Cl_2 : C_2H_5OH (5:1). The TLC plates were visualised first under UV at 254 nm and then sprayed with various spray reagents and heated at 105 - 110° for 5 - 10 mins. An authentic sample of ecdysterone was also spotted alongwith it as a reference. The R_f values and colour of the spots with different spray reagents are given in Table-1. The extraction procedure is a modification of the one used by Matsuoka *et al.* [3].

Results and Discussion

Different parts of various species of Amaranthaceae and Chenopodiaceae were screened for the presence of ecdysones and the results are shown in Table 2. Use is made of thin layer chromatography and UV spectrophotometry for identification of ecdysones.

Since the ecdysones possess α-β unsaturated keto system, they absorb UV light at 254nm and show clear blue spots

TABLE 1. TLC RESULTS.

Material	Colour Obtained		R_f. Value	
	Vanillin H_2SO_4	Anisaldehyde H_2SO_4	CH_2Cl_2:C_2H_5OH 5 : 1	$CHCl_3$:CH_3OH:H_2O 3.5 : 2.5 : 1
Pure Ecdysterone	Yellowish green	Leafy green	0.16	0.51
A. albus	"	"	0.16	0.51
P. lappaceae	"	"	0.17	0.51
C. album	"	"	0.17	0.52

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on TLC plates. Characteristic colour reactions with various spray reagents have proved of immense use for preliminary identification. Sulphuric acid of various concentrations has been widely used for this purpose in TLC of steroids [4]. Matsuoke *et al.* [3] have used 10% H_2SO_4 for location of a number of ecdysone spots. Spraying with 20% H_2SO_4 and heating at 110° for 5 - 10 mins. showed brown spots for ecdysterone.

It was observed that when ecdysones from members of Amaranthaceae and Chenopodiaceae were sprayed with 10% H_2SO_4 and heated at 110° for 5 mins. an initial green colour, turning to permanent greenish brown on further heating, was visible. It was noted that when a TLC plate was sprayed with vanillin-sulphuric acid reagent, yellowish green spot of ecdysones alongwith authentic ecdysterone was observed. But apart from it many other spots similar in colour were also visible which were not ecdysones at all, as indicated by their UV absorption and R_f values. Anisaldehyde-sulphuric acid proved to be the most useful spray reagent than both H_2SO_4 and vanillin-sulphuric acid. It displayed very prominent leafy green spots of ecdysones and was therefore used in this work for ascertaining the location and detection of ecdysones.

During screening of the species various solvent systems were tried for TLC and out of them CH_2Cl_2 : C_2H_5OH (95%), 5:1 was found the most suitable for separation and identification of ecdysones.

From the above results, evidence for presence of ecdysones has been found for ten out of thirty species representing ten

TABLE 2. SCREENING RESULTS.

Name of species	Part used	TLC	U.V.	Results
(A) AMARANTHACEA FAMILY				
1. *Aerva tomentosa* Forssk	Flower	+	+	+
	Leaf	+	+	+
	Stem	+	+	+
	Root	+	+	+
	Whole plant	+	+	+
2. *A. pseudotomentosa* Blatt and Hall	Root	+	+	+
	Flower	+	+	+
	Whole plant	+	+	+
3. *A. javanica* Juss	Whole plant	+	+	+
4. *Achyranthus aspera* L.	Whole plant	+	+	+
	Fruit	+	+	+
	Stem	+	+	+
	Root	+	+	+
5. *Alternanthera bittzickiana* Nicholas	Whole plant	-	-	-
6. *A. repens* L.	Whole plant	-	-	-
7. *A. sessilis* L.	Whole plant	-	-	-
8. *Amaranthus hybridus* L	Whole plant	+	+	+
9. *A. spinosus* L.	Whole plant	+	+	+
10. *A. gangeticus* L.	Whole plant	-	+	-
11. *A. viridis* L.	Wholeplant	-	-	-
12. *A. acutilobus* Uline et Bray	Whole plant	-	+	-
13. *A. adulturnus* Thellung	Whole plant	-	+	-
14. *A. gracizans* L.	Whole plant	-	+	-
15. *A. albus* L.	Seeds	+	+	+
	Whole plant	+	+	+
16. *A. caudatus* L.	Whole plant	-	-	-
	Flower	-	-	-
	Root	-	-	-
	Stem	-	-	-
	Leaf	-	-	-
17. *A. cripspus* Terrac	Whole plant	-	-	-
18. *A. hypochondriacus* L.	Whole plant	-	-	-
19. *A. lividus* L.	Whole plant	-	+	-
20. *Celosia argentia* L.	Root	-	-	-
21. *C. laxa.* Schumm and Thoun	Whole plant	-	-	-
22. *C. trigyna* L.	Root	-	-	-
	Stem	-	-	-
	Leaf	-	-	-
23. *C. plumosa* Hort	Whole plant	-	-	-
24. *C. cristata* L.	Whole plant	-	-	-
25. *Cyanthula prostrata* Blume	Whole plant	-	-	-
26. *Deeringia polysperma*	Whole plant	-	-	-
27. *Gomphrena celosioides* Mart	Whole plant	+	+	+
28. *G. globosa* L.	Whole plant	-	-	-
29. *Pandiaka involucrata* Benth and Hook	Whole plant	+	+	+
	Leaf	+	+	+
	Stem	+	+	+
	Root	+	+	+
30. *Rupalia lappaceae* Juss	Whole plant	+	+	+
(B) CHENOPODIACEAE FAMILY				
1. *Atriplex patula* L.	Whole plant	-	-	-
2. *A. hortensis* L.	Whole plant	-	-	-
3. *Beta vulgaris* L.	Underground portion	-	-	-
4. *Kochia scoparis* Schrad	Whole plant	-	-	-
5. *Spinacea oleracea* L.	Leaves	-	-	-
6. *Chenopodium album* L.	Leaves	+	+	+
	Seeds	+	+	+
	Flowers	+	+	+
	Stem	+	+	+
	Root	+	+	+
7. *C. ambrosioides* L.	Whole plant	-	-	-
8. *C. bonus henricum* L.	Whole plant	-	-	-
9. *C. ficifolium* Sm.	Whole plant	+	+	+
10. *C. murale* L.	Whole plant	-	-	-
11. *C. viride* L.	Whole plant	-	-	-
12. *Corispermum hyssopifolium* L.	Whole plant	-	-	-

(Contd....)

genera of the family Amaranthaceae.

In the genus Amaranthus, only three spp. i.e. *A. mangostanus., A. spinosus* and *A. viridis* are reported positive [5]. It was interesting to note that *A. viridis* which is reported positive for ecdysone in the literature, was found negative in this work, which might be due to different climatic and soil conditions. *A. spinosus* also reported to contain ecdysone, has been confirmed ecdysone positive during this work. *A. albus* and *A. hybridus*, not reported so far, gave positive results, which was corroborated by their R_f values. All the other species i.e. *A. acutilobus, A. adulturinus, A. gracizans, A. caudatus, A. crispus, A. hypochondriacus* and *A. lividus* were found negative for ecdysones.

From the genus Gompharaena, only one species i.e. *G. celosioides* Mart has been found positive. No work on the other genera e.g. *Deeringia, Aerva, Alternanthera, Celosia, Pandiaka* and *Pupalia* is reported in the literature. In these studies *Deeringla polysperma, Alternanthera bittzikiana, A. repens, A. sessilis* and five species of *Celosia* namely *C. argentia, C. cristata, C. laxa, C. plumosa* and *C. trigyna* were shown to be negative. While one species each from genus *Pandiaka* and *Pupalia* i.e. *P. involucrata* and *P. lappaceae* gave positive tests for ecdysones.

Only two species of Chenopodiaceae i.e. *Chenopodium album* and *C. ficifolium* responded to ecdysone tests. No work on these plants has been reported in the literature except the roots of the former which contained β-ecdysone and polypodine - B^6.

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