SCREENING OF PLANTS FOR ECDYSONES (FAMILIES AMARANTHACEAE AND CHENOPODIACEAE)

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(Received July 31, 1991; revised November 11, 1992)

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 $60 \text{ R}_{f} 254 + 366$ 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

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(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 CHCl₂: CH₂OH, 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 CH₂Cl₂ : C₂H₂OH (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, 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 _t . Value | | |
|---------------------|--|--|--|---|--|
| | Vanillin H ₂ SO ₄ | Anisaldehyde H ₂ SO ₄ | CH ₂ Cl ₂ :C ₂ H ₅ OH 5 : 1 | CHCl ₃ :CH ₃ OH:H ₂ O 3.5 : 2.5 : 1 | |
| Pure Ecdysterone | Yellowish green | Leafy green | 0.16 | 0.51 | |
| A. albus | u | | 0.16 | 0.51 | |
| P. lappaceae | " | " | 0.17 | 0.51 | |
| C. album | . 66 | | 0.17 | 0.52 | |

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

| Name of species | Part used | TLC | U.V. | Results |
|-----------------------------|-------------|---------|-------|----------------|
| (A) AMARANTHACEA FAMILY | | | | |
| 1. Aerva tomentosa Forssl | k Flower | + | + | + |
| | Leaf | +1 | 1904 | + Dil |
| | Stem | ++ | 06+00 | CI+nop |
| | Root | + | diat- | este tor |
| | Whole plant | + | + | + |
| 2. A. pseudotomentosa | Root | + | + | + |
| Blatt and Hall | Flower | + | + | + |
| | Whole plant | 0+0 | + | 112 + 1 |
| 3. A. javanica Juss | Whole plant | 10+11 | ed+g | icre, the |
| 4. Achyranthus aspera L. | Whole plant | + | + | + |
| | Fruit | + | + | + |
| | Stem | + | + | + |
| | Root | | + | (++) |
| 5. Alternanthera | Whole plant | 04.0 | - | - |
| bittzickiana Nicholas | | | | |
| 6. A. repens L. | Whole plant | walls | - | em' |
| 7. A. sessilis L. | Whole plant | Terong. | - | 1000 4000 |
| 8. Amaranthus hybridus L | Whole plant | + | + | + + |
| 9. A. spinosus L. | Whole plant | + | + | puidly ? |

| 10. A. gangeticus L. | Whole plant | - | + | - |
|--|--|------------------|------------------|-----------------|
| 11. A. viridis L. | Wholeplant | สีราค | 2.50 | 35 |
| 12. A. acutilobus Uline et Bray | Whole plant | - <u>1</u> 4 - 1 | + | |
| 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 | 77 | - | - |
| of which cleven species of Ama | Flower | iones | - | - |
| | Root | (has) | 101 | - |
| | Stem | ធិម ៧ | 191 | |
| nes Amerailiscere Cherend | Leaf | S'M | • | - |
| 17. A. cripspus Terrac | Whole plant Whole plant | - | | - |
| 18. A. hypochondriacus L. 19. A. lividus L. | Whole plant | - | 1 | |
| 20. Celosia argentia L. | Root | 18 82 | idy son | B |
| 21. C. laxa. Schumm and | Whole plant | fing | e the | 0080 |
| Thoun Thouse boots | sodysone caus | | | |
| 22. C. trigyna L. | Root months | -int | in -he | -0-0- |
| an manager and transmission | Stem | - | | - |
| | Leaf | - | | - |
| 23. C. plumosa Hort | Whole plant | - | | 100 <u>-</u> |
| 24. C. cristata L. | Whole plant | | ein <u>a</u> nsi | |
| 25. Cyanthula | tritore prane | | ie piar | |
| prostrata Blume | | | san fo | |
| 26. Deeringia polysperma | Whole plant | 58W | which | |
| 27. Gomphrena celosioides | whole plant | stipm | il staa | 18 + |
| Mart 28. <i>G. globosa</i> L. | Whole plant | nol h | indian | a Ine |
| 29. Pandiaka involucrata | Whole plant | 14 m | ul por | im 4 |
| Benth and Hook | Leaf | | | nin+ |
| echine of a large mined of the | Stem | | | |
| | Root | | | + |
| 30. Rupalia lappaceae | Whole plant | + | + | + |
| Juss | i sommer one | | | |
| (B) CHENOPODIACEAE FAMILY | | | | |
| 1. Atriplex patula L. | Whole plant | - | 1211 | - |
| 2. A. hortensis L. | Whole plant | 1-CAL | here an | 12 - |
| 3. Beta vulgaris L. | Underground | -111- | ture un | |
| All and a set of the set of the set of the | portion | | | |
| 4. Kochia scoparis Schrad | Whole plant | e u e nete | ai e aiola | gep nos |
| 5. Spinacea oleracea L | Leaves | | - | - 50 |
| 6. Chenopodium | Leaves | + | + | · + |
| album L. | | | | + |
| S rpl anisaidebyde was added | Element | + | + | + |
| | Stem | + | + | + |
| | Root | + | + | + |
| 7. C. ambrosioides L. | Whole plant | OIS O | mixin | |
| 8. C. bonus henricum L | | | inerti | 3- |
| 9. C. ficifolium Sm. | | +1.3 | / ptan | nb + |
| 10. C. murale L. | Whole plant | the b | red an | obvi |
| 11. <i>C. viride</i> L. | Whole plant | - 10 | - | - |
| | State of the state | a marke | | |
| 12. Corispermum hyssopifolium L. | Whole plant Whole plant | 1 note | - | cienc Instit |

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_t 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

other with 0.1% potassium metabisulphite. Pectolypiq enzym (0.1%) was added to both the lots and mixed well. The pulp were then kept at room temperature, 30° \pm 4° for 16 hrs. The treated samples were pressed in a cloth and the juice filtere through a collite bcd. The clear juice obtained was pareautize at 75-80° for 2 min. The enzymatically clarified juicer of cact variety were concomrated separately in a rotary evaporator un der vacuum at 50-55° to 77°. Brix and scored at 2-4° ma cooking incubator over night. Fresh clarified pear juice was added as at back to the concentrate and final "Bitx adjusted to 75°.

by Abbe refractometer, moisture, titratable acidity as maliacid, ascorbic acid and sugars were determined by standar AOAC methods [21]. Non-enzymatic browning was meas used by extracting a 2 g sample with 60% alcohol filters through Whatutan filter paper No. 41 and reading at oplica density (OD) in a spectrophotometer (Erma Modal 1.57) a 440 nm. All the analytical measurements were made in replicates. Organoloptic quality was evaluated by a parel of 22 parelists for colour, flavour and taste of the ready to serve drinks on a 5 point Medonic scale [22].

Results and Discussion

Average frait weight and recovery of juice from the different varieties of peace is given in Table 1. The chemics

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 - β^6 .

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is yellow with some reducts plush near maturity. The fruit is ready for picking in the first week of October. Batang is a round sweet poar with dots on the skin. Batang is ready for harvesting in September [1-5]. Total production of pears in wursh (routing) une 31012 production of pears in

Enzyme proparations are playing an important role in modera fixed processing. Pectolytic enzymes have been employed in the extraction and clarification of pieces [7,8]. In the preparation of juice and concentrates, colour deterioration axidase (PPO) [9-14]. SO, pessesses bactericidal properties and inhibits enzymatic and non enzymatic darkening [15,16]. Embe and Markakis [15] found that the mechanism by which subsistate compounds such as the ancentration with subsistate compounds such as the enzymatically produced organizes from the existing polyphenols. There is also dreet enzyme mactivation by the SO, Addition of ascorbic acid has databiliting PPO action in fanits [17-19].

Concentration of any fruit or pulp is of great economic advantage from the point of view of packaging, storage and transportation. It is a method for milication of excess produce during peak reason. Studies on the preparation of apple, crange, pincapple, banana, guava and mango concentrates have been reported [20]. Little information is available on the preparation and concentration of enzymatically classified pear