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Fatty Acids and Phytochemical Components of *Ipomoea* spp. Seeds

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Abstract – Twelve species of *Ipomoea* were investigated for allelopathic behavior. Seed extracts of *I. quamoclit, I. nil* and *I. pes-tigridis* showed significant allelopathy over germination of other seeds. Investigation on seed extracts of *I. quamoclit* revealed the presence of several phytochemical components *viz.*, *n*-triacontanol, sitosterol, stigmasterol, α-amyrin, taraxerone, taraxerol, erythrodiol, cucurbitacin-G. Seed fat of twelve species were also analyzed.

Key words – *Ipomoea* spp., allelopathy, seed extract, phytochemical components, seed fat.

Introduction

In continuation of our work on species relationship and allelopathy of *Ipomoea* seed extracts (Das and Mukherjee, 1995, 1996, 1997), seeds of *Ipomoea quamoclit* showing significant allelopathy were analyzed phytochemically to trace clues relating allelopathy. Seed fat of twelve species of *Ipomoea* were also analyzed.

Experimental

General experimental procedures – Silica gel column chromatography and TLC were conducted, IR spectra were obtained from Hitachi 260-infrared Spectrophotometer. HR-MS of isolated compounds and EI-MS of seed meal were carried out and methyl esters of fatty acids were analyzed by GLC using DEGS column.

Plant material – Seeds of twelve species of *Ipomoea* comprising nine wild species namely, *I. hispida* Roem and Schult., *I. hederifolia* L., *I. pes-caprae* (L.) Sweet., *I. fistulosa* Mart ex Choicy., *I. sepiaria* Koen., *I. obscura* (L.) Ker-Gawl., *I. chryseides* Ker-Gawl., *I. pes-tigridis* L., *I. triloba* L., two horticultural species namely, *I. quamoclit* L., *I. nil* (L.) Roth and one semicultivated *I. aquatica* Forsk. formed the material. *I. quamoclit, I. hederifolia, I. fistulosa* and *I. nil* are neotropical native species of India and *I. chryseides* is synonym of *Merremia hederacea* (Burm.f) Hall.f.

Allelopathy – Each 10 g seed meal of the twelve species was extracted with 100 ml ethanol, and ethanolic extracts were evaporated. Dried extracts were dissolved in 50 ml of water. Seeds of different crop plants namely, rice, jute, mustard were soaked in water extracts for 24 hours, placed on moist filter paper and germination percentage were recorded.

Extraction and Isolation of components – Air dried seeds of *I. quamoclit* (1 kg) was crushed to powder. Seed meal was first defatted with petroleum ether (60-80°C). Defatted seed meal was further extracted with chloroform for 72 hours. Petroleum ether extract was concentrated and chromatographed over Brockman alumina using petroleum ether, benzene, mixture of benzene:chloroform (1:1) and chloroform, successively. Eluents were collected in each fraction (70 ml). Residue left on evaporation was subjected to TLC to obtain homogeneity of components. Petroleum ether extract eluted with benzene and benzene:chloroform (1:1) yielded white solid compounds, which were named Compounds A and B, respectively.

Chloroform extract was concentrated and chromatographed over a silica gel column, eluted with petroleum ether, benzene and finally with chloroform to yield white waxy compound (Compound C), on evaporation with benzene, two different white solid compounds (Compounds D and E), with chloroform, a white solid mass (Compound F).

Seed fat analysis – Seed lipids were extracted according to the method of Kates (1986), isolated total seed lipids of each species were esterified and methyl esters of fatty acids were separated by GLC (Pye Unicam, Pye series 104 Chromatograph, Phillips) using DEGAS column. The percentage of total seed fat and individual fatty acid components were

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determined by comparing R_f value and peak area of standard fatty acid methyl esters with those of sample methyl esters with an integrator in the GLC.

Results and Discussion

Phytochemicals called secondary plant substances have been reported to participate in the interaction between plant species (Rice, 1984). Chemical investigation indicated indole alkaloids and resin glycosides as the most common constituents in the Convolvulaceae. Presence of chlorogenic acid in *Ipomoea* seed (Friedman *et al.*, 1989), pes-caproside E, a fatty acid from *I. pes-caprae* (Srivastava *et al.*, 1991), tricolorin-A, a major phytogrowth inhibitor from *I. tricolor* (Pereda-Miranda *et al.*, 1993) is well documented. Tubers of *I. digitate* revealed the presence of β-sitosterol and a neutral compound (m.p. 72°C) (Tewari *et al.*, 1964), a fixed oil containing palmitic, oleic, linoleic and linolenic acid (Mishra *et al.*, 1965). The petroleum ether extract of *I. fistulosa* leaves yielded β-sitosterol, an ester of *n*-triacontanol and hexacosanoic acid, besides triacontanol and *n*-triacontane (Sen *et al.*, 1979), *I. mauritiana* was found to yield taraxerol acetate (Sharma *et al.*, 1972),

In general crude seed extract of *I. quamoclit, I. nil, I. pes-tigridis* showed significant inhibition as well as the delayed germination of rice, jute and mustard seeds. Seeds of *I. quamoclit* were subjected to chemical analysis.

Various phytochemicals isolated from seeds of *I. quamoclit* are as follows:

Compound A (m.p. 85°C). It showed a peak in its IR spectrum at 3,350 cm^{-1}, characteristic for hydroxyl group. Presence of this functional group was further substantiated by the preparation of *O*-acetate. A mass spectrometric examination of the sample registered a molecular ion peak at *m/z* 438 for the compound, revealed a fragmentation pattern in accordance with the normal straight chain structure. These data were compared in good accordance with those reported for *n*-triacontanol ($CH_3(CH_2)_{28}CH_2OH$).

Compound B (m.p. 153-155°C). It gave blue coloration with the acetic anhydride and sulfuric acid, a color characteristic of sterols. On repeated chromatography (twice) over Brockman alumina, it was separated into fractions (A), m.p. 137°C, (B), m.p. 143°C, and (C) m.p. 162°C. Fractions (A) and (C) were identified as β-sitosterol ($C_{29}H_{50}O$) and stigmasterol ($C_{29}H_{48}O$), respectively, by direct comparison of physical and spectroscopic data with authentic samples. Fraction (B) was identified as a mixture of dimethyl derivative of stigmasterol and β-sitosterol (Campesterol). The mass spectrum of crude sterol mixture not only showed molecular ion peak at *m/z* 414 (*β*-sitosterol) and 412 (stigmasterol) but also revealed presence of two other components having molecular weights 400 and 398. Later two compounds were recognized to be the dimethyl derivatives of β-sitosterol (campesterol) and stigmasterol.

Compound C (m.p. 199°C), crystallized from benzene-petroleum, a triterpene, showed a molecular ion peak at *m/z* 426. The compound was identified as α-amyrin ($C_{30}H_{56}O$) by direct comparison of physical and spectroscopic data with those of the authentic sample.

Compound D (m.p. 240°C). Crystallized from benzene, a triterpene, showed a molecular ion peak at *m/z* 428 indicating its molecular formula ($C_{30}H_{48}O$). The compound was identified as taraxerone by direct comparison of physical and spectroscopic data with those of authentic sample.

Compound E (m.p. 280°C). Crystallized from benzene-chloroform (1:1), showed a peak at 3460 cm^{-1}, characteristic for OH group in its IR spectrum. Mass spectrum showed a molecular ion peak at *m/z* 426. The compound was found to be identical with taraxerol ($C_{30}H_{50}O$) by direct comparison of physical and spectroscopic data with those of authentic sample.

Compound F. By further chromatography over silica gel, two white solids designated as I and II were obtained. The compound I crystallized from chloroform-methanol, m.p. 235° showed a molecular ion peak at *m/z* 442. The compound was identified as erythrodiol ($C_{30}H_{50}O$) by direct comparison with authentic sample. Compound II, crystallized from methanol, m.p. 149°C, gave red coloration with acetic anhydride and sulfuric acid, a color characteristic for tetracyclic triterpene. It showed a molecular ion peak at *m/z* 534. The compound was identified as cucurbitacin-G ($C_{30}H_{46}O_8$).

Seed fat analysis – Percentage based fatty acid composition in different species of *Ipomoea* are given in Table 1. Seeds of *I. hederifolia* have the highest quantity of fat (20%) and *I. obscura* the least quantity (5%). Myristic acid was found to be present only in *I. fistulosa* (0.54%), and palmitic, stearic, oleic, α-linoleic and α-linolenic acid were found to be present in all the species in varying proportions.

Table 1. Percent seed fat and fatty acid composition of *Ipomoea* spp.

Species	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	α-Linoleic acid	α-Lino-lenic acid	Arachidic acid	Behenic acid	Conjugated fatty acid	Total fat
I. hispida	–	46.15	4.05	19.02	26.11	4.66	–	–	–	5.20
I. quamoclit	–	34.55	10.20	21.68	30.15	3.42	–	–	–	10.00
I. hederifolia	–	32.30	19.35	13.46	30.62	4.26	–	–	–	20.00
I. pes-caprae	–	11.30	14.24	48.48	23.73	32.25	–	–	–	7.50
I. fistulosa	0.54	22.04	7.88	40.06	23.00	3.53	–	0.92	8.11	9.20
I. aquatica	–	25.17	9.11	26.27	36.76	2.68	–	–	11.20	8.00
I. sepiaria	–	26.38	9.63	31.91	28.66	3.42	–	–	8.55	7.00
I. nil	–	31.46	5.00	20.80	37.18	5.18	–	–	–	10.00
I. obscura	–	20.76	43.74	43.74	27.56	6.41	1.31	–	–	5.00
I. chryseides	–	21.57	3.44	14.29	51.34	2.51	6.10	0.41	–	9.40
I. pes-tigridis	–	27.13	9.08	14.93	38.27	8.71	–	1.49	–	10.00
I. triloba	–	25.07	4.91	32.41	31.43	6.18	–	–	–	6.00

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