PHYTOCHEMICAL INVESTIGATIONS OF INDIGOFERA TINCTORIA LINN LEAVES

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Received: 14.03.2001 Accepted: 10.02.2002

ABSTRACT: Studies of Indigofera tinctoria Linn has shows that it possesses low toxicity. Phytochemical evaluation of leaf extract of Indigofera tinctoria Linn has been carried out to characterize some constituents present therein. Qualitative analysis of the extracts showed the presence of flavonoids, alkaloids, glycosides, terpenoids. Five compounds have been isolated from petroleum ether extract and methanolic extract and have been characterized by U.V.IR and $H^1$ NMR data, petroleum ether extract were also characterized by HPTLC.

INTRODUCTION

The genera of indigofera (Family Fabaceae) are distributed throughtout India and are medicinally useful. Indigofera tinctoria Linn (Fabaceae) has been extensively used in various folklore and traditional medicines, Studies on the plant reveal high LD$_{50}$ thus low toxicity. The plant possesses antitoxic, hemostatic, sedative properties and are useful in the treatment of piles, healing of ulcers, dropsy. The roots stems and leaves are useful for promoting growth of hair, in gastropathy, splenomeghaly, cepholagia, cardiopathy, chronic bronchitis, asthma and ulcers (1, 2).

Extracts of I. tinctoria (whole plant) contains glycoside ‘Indan”, about 2.5% alkaloids, about 0.5% stimulant deobstruent, antiseptic and astringent (3). A galactomannan composed of galactose and mannose in the molar ratio of 1:1.52 was isolated from seeds of I. tinctoria and partially characterized (4). Rotenoids are isolated from I. tinctoria and their bioefficiency seen against Cyclops, the carrier of dracunculiasis (5). On preliminary chemical examination, various species of Indigofera showed the presence of terpenes, alkaloids, β –sitosterol and flavanoids (2, 6, 7).

EXPERIMENTAL

Materials and Methods

The fresh leaves of I. tinctoria collected in Tamilnadu Medicinal Plant Farms, Chennai. An herbarium was prepared and deposited in Department of Pharmaceutical Sciences, BIT, mesra, Ranchi.

EXTRACTION

The fresh leaves of Indigofera tinctoria were air dried, pulverized and extracted exhaustively with petroleum ether, Chloroform, Ethyl acetate and methanol. The extracts were dired under reduced pressure to obtain a dry extract. Various extracts were subjected to qualitative analysis to detect the phytoconstituents present (8, 9).
From the qualitative analysis of various extracts, it was found that the leaf extract contained various phytoconstituents like alkaloids, terpenoids, flavonoids, glycosides, sugars and tannins (Table – 1).

**Processing of Petroleum ether extract by column chromatography**

The dried petroleum ether extract was subjected silica gel column chromatography after formation of slurry. The column was eluted with chloroform: water (49:1) to isolate the compounds A, B and C. One fraction was allowed to evaporate to give a white residue to which acetone was added and refrigerated overnight. Acetone insoluble portion was separated as white flakes and dried to give compound a, acetone soluble portion upon cooling gave a pale yellow amorphous solid named as compound B. To another fraction methanol was added and a white amorphous compound precipitated our. This compound was collected, washed with methanol and dried to give compound C.

**Processing of Methanolic extract by Partition chromatography**

The dried methanolic extract was subjected to partition chromatography after mixing it with water and shaking with different organic solvents like petroleum ether, chloroform, benzene, acetone and pyridine which gave the compounds D and E.

Methanolic extract of the dried leaf material was homogenized for 5 min with methanol: water (4:1) and filtered. The filtrate was evaporated (<40°C), acidified and extracted with 3 volumes of chloroform. The aqueous layer was basified to pH 10 with ammonium hydroxide and extracted with chloroform: methanol (3:1). Aqueous basic layer was evaporated and extracted with methanol. Methanolic extract was evaporated to dryness and purified by repeated extraction with organic solvents of varying polarities. The aqueous pyridine and pyridine fraction evaporated slowly gave two crystalline compounds which were named as compound D and compound E. The compound E was further purified by repeated recrystallization with water.

**HPTLC Analysis of Petroleum extract.**

From the HPTLC Analysis of petroleum ether extract, the developed plates on scanning in CAMAG TLC scanner and by using the software CAMAG CATS4 version, the following data was obtained (Table2) (10).

| SPOT | Rf  | λmax | Peak area |
|------|-----|------|-----------|
| 1    | 0.30| 400  | 724.6     |
| 2    | 0.37| 400  | 832.5     |
| 3    | 0.62| 400  | 991.0     |
| 4    | 0.82| 200  | 648.9     |
| 5    | 0.90| 207  | 3076.9    |

**RESULTS AND DISCUSSION**
**Compound A:** Pale white amorphous powder. M.pt: 190-192°C. Rf 0.65 (Chloroform: water, 49:1), its IR spectrum exhibited following characteristic absorption bands 3452 cm⁻¹ (OH stretching) 2917.5 (CH stretching (alkane), Strong), 2848.5 (CH stretching (alkane), weak), 1736 cm⁻¹ (C=O Stretching, saturated aliphatic), 1473/1 (CH bending (alkane), 1463.3 (CH bending) 1629.6 (C-H multiple bond stretching, variable), 1414.9 (alkane, monosubstituted), 1377.4 (alkane, CH3), 1330.2 (C-O stretching (phenols), O-H bending 1175 (alcohol, O-H deformation), 806.1 (Substituted aromatics, C-O-C), 729.7 (C-H bending, aromatics), H1 NMR: δ 7.265 (H aromatic, phenols), 4.05 δ (rhamnoglucosyl), 1.61 δ rhymnosyl methyyl), 1.25 δ (CH3 protons of alcohol), 0.89.0.88.0.86 δ (saturated alcohols). The melting point, IR and H1 NMR data were comparable to Rutin (11-13).

**Compound B:** Pale yellow amorphous solid, M.Pt: 217-219°C, Rf 0.86 (Chloroform: Water, 49:1): its IR spectrum exhibited following characteristic absorption bands 3436.6 cm⁻¹ (alcohols), 2921.8 (C-H Stretching, alkane), 1726.5 (carbonyl stretching vibrations), 1463.1 (- CH2-alkane, medium), 1378.1 (quartenary carbon, gem dimethyl), 1278.6 (C-O stretching, O-H bending, O group). 1039.8, 1125.7 (aliphatc ethers, C-O-C stretching), 758.6 (aromatic substituted), 667.2 (C-H bending, alkenes), H1NMR: δ 8.0 (H aromatic), 4.05 δ (aliphatc proton), 0.86 δ (H alcoholic), 2.58 δ (titiary alcoholic), 2.58 δ (methylene oxy). The IR and H1 NMR data of the compound was comparable to that of louisfieserone (6,7,11,12)

**Compound C:** White amorphous powder, Rf0.88 (Chloroform-Water, 49:1),IR_vmax (KBr): 3428.6 cm⁻¹ (OH stretching) 2917.4 (CH stretching (alkene), strong), 2848.6 (CH stretching (alkene), weak), 1737.7 (carbonyl stretching), 1170.3 (= CH, in plane bend), 107.5 (carbonyl stretching, secondary alcohols), 795.2 (C, aromatic)729.3,719.3 cm⁻¹ (CH out of plane deformation) H1NMR: δ 0.88 (CH3-C, saturated), δ 1.2 (CH2 saturated), 1.5 δ (C-H saturated, δ 1.2 (CH2 saturated). 1.5 δ (C-H saturated, δ 2.17 δ (CH3-C+0), 7.26 δ (H,aromatic). The spectral data was not comparable with the available literature. So it was considered as a new compound (12,14).

**Compound D:** Colourless crystalline compound, m M.pt: 202-2040°C, Rf 0.74 (chloroform acetic acid, 198:1:IR Vmax (KBr): 169.5 (OH stretching (broad), alcohols), 1619.9 (carbonyl stretching, Ketone), 1401.3 (Phenols), 1111.2 (akanes) 979.3 (C=C disubstituted), 760 (aromatic substituted), 616.2, 655.4 (C-H bending, alkenes), H1NMR: 5.06 δ (=CH2), 3.99 δ (CH3), 2.47 δ (CH3group), 3.99 δ (-OCH3 – CHOH). The melting point, Rf and H1 NMR data of compound D was comparable to that of a rotenoid compound (7,12,13).

**Compound E:** Colourless crystalline compound, M.Pt: 285 – 287°C, UV λ_max : 255nm :IR_vmax (KBr): 3192.1cm⁻¹ (carboxylic acids), 1768.4 (carbonyl stretching), 1612.2(C=C stretching), 1402 (in plane C-O-H bending), 1098.7 (secondary alcohols), 751.3 (bonded O-H group), 654.7 (C-H bending), 614.8 (O-H out of plane deformation), H1 NMR:4.8 δ(aliphatic cyclohexane ring size), 2.47 δ (CH3group), 3.99 δ (-OCH3 – CHO). The melting point, H1NMR data of compound E were comparable to that of a tetracyclic acid (12,13).
HPTLC was carried out on CAMAG TLC scanner. The Rf 0.62, 0.82, 0.90 of 3 spots from HPTLC analysis of petroleum ether extract are comparable to that of compound A, Compo and B and Compound Cm isolated from petroleum ether extract and their corresponding peak areas has been investigated. From the peak areas, it was found that compound C is present in higher concentration in the petroleum ether extract. Work may be furthered to investigate the pharmacologically active principles present in the leaves as leaves are the renewal sources thus economically viable.

ACKNOWLEDGEMENT

K.B. Suresh gratefully acknowledges financial support in the form of junior Research fellowship provided during the period of study by university Grant Commission, New Delhi. Also authors are thankful to R.S.I.C., IIT, Chennai for IR and NMR spectra of our samples.

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TABLE -1
Results of Qualitative analysis of leaf extracts of Indigofera tinctoria

| TEST          | EXTRACTS |
|---------------|----------|
|               | Petroleum ether | Chlorofrom | Ethylacetate | Methanol |
| Alkaloid      | -        | -          | -           | +++      |
| Steroid       | -        | -          | -           | -        |
| Terpenoid     | -        | -          | -           | ++       |
| Flavonoid     | +++      | +          | +           | ++       |
| Glycoside     | -        | +          | +           | +        |
| Sugars        | +        | +          | +           | +++      |
| Saponin       | -        | -          | -           | -        |
| Amino acid    | -        | -          | -           | -        |
| Tannins       | -        | -          | -           | +++      |

+++ Intense      ++moderate     +slight       -absent