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PHYTOCHEMICAL INVESTIVATIONS OF INDIGOFERA TINCTORIA LINN LEAVES

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***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 H1 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 tinactoria* Linn (Fabaceae) has been extensively used I 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 tincoria* (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 dired, 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 chromatorgraphy

The dired 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 dired 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 Dired 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 dired leaf material was homogenized for 5 min with menthanol: water (4:1) and filtered. The filtrate was evarporated (<40^{o}C), acidified and extracted with 3 volumes of chloroform. The aqueous acid 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 polartities. 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 CAMAGTLC scanner and by using the software CAMAG CATS4 version, the following data was obtained (Table2) (10).

Table-2 Results of HPTLC analysis of Petroleum ether extract.

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^{o}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), 1736cm-1 (C=0 Stretching, saturated aliphatic), 1473/1 (CH bending (alkane), 1463.3 (CH bending) 1629.6 (c-C multiple bond stretching, variable), 1414.9 (alkane, monosubstitued), 1377.4 (alkane, CH3), 1330.2 (C-O stretching (phenols), 0-H bending 1175 (alcohol, O-H deformation), 806.1 (Sudstituted aromatics, C-O-C), 729.7 (C-H bending , aromatics), H1 NMR: 7.265 (H aromatic, phenols), 4.05δ (rhamnoglucosyl), 1.61 δ rhymnosy1 methy1), 1.25 δ (CH3 protons of alcohol), 0.89.0.88.0.86 δ (saturated alcohols). The melting point, IR and H1NMR data were comparable to Rutin (11-13).

Compound B: Pale yellow amorphous solid, M.Pt: 217-2190C, Rf o.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 (carbony1 stretching vibrations), 1463.1 (- CH2- alkane), medium), 1378.1 (quartenary carbon, gem dimethy1), 1278.6 (C-O stretching, O-H bending, O group). 1039.8, 1125.7 (aliphatic ethers, C-O-C stretching), 758.6 (aromatic substituted), 667.2 (C-H bending, alkenes), H1NMR: δ 8.0 (H aromatic), 0.95 δ (aliphatic proton), 0.86 δ (H alcoholic), 4.05 δ (H, alcoholic), 2.58 δ (tertiary 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, Rf.0.88 (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 (carbny1 stretching), 1170.3 (= CH, in plane bend), 107.5 (carbony1 stretching, secondary alcohols), 795.2 (CH, 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: 202-2040C, Rf o.74 (chloroform acetic acid, 198:1:IR $_{Vmax}$ $^{(KBr)}$: 169.5 (OH stretching (broad), alcohols), 1619.9 (carbony1 stretching, Ketone), 1401.3 (Phenols), 1111.2 (akanes) 979.3 (C=C disbstituted), 760 (aromatic substituted), 616.2, 655.4 (C-H bending, alkenes), H1NMR: 5.06 δ (=CH2), 3.99 δ ()CH3), 4.79 δ (CH2=C, conjugated), 1.85 δ (CH3), 2.90 δ (methyleneoxy), 4.61 δ (OH, aromatic), 1.28 δ (menthylene). 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^{0}C, UV $_{\lambda max}$: 255nm :IR_{Vmax} $^{(KBr)}$: 3192.1cm-1 (carboxylic acids), 1768.4 (carbony1 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 – CHOH). 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 Rf0.62,0.82,0.90 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.

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

TEST	EXTRACTS			
	Petroleum ether	**Chlorofrom**	**Ethy1acetate**	**Methanol**
Alkaloid	-	-	-	+++
Steroid	-	-	-	-
Terpenoid	-	-	-	++
Flavonoid	+++	+	+	++
Glycoside	-	+	+	+
Sugars	+	+	+	+++
Saponin	-	-	-	-
Amino acid	-	-	-	-
Tannins	-	-	-	+++

+++ Intense ++moderate +slight -absent