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Original Research Paper

PHYTOCHEMICAL STUDIES ON THE AERIAL PARTS OF *INDIGOFERA LINNAEI*, Ali

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ABSTRACT

Indigofera linnaei, Ali, belonging to the family Fabaceae, is a medicinal plant growing wild in tropical countries. The phytochemical screening and spectral analysis revealed that the two unknown compounds, 5-[(E)-2-(4-hydroxyphenyl] benzene-1,3-diol, and Gitoxin belonging to tannols and steroidal glycoside have been isolated from the aerial parts of *Indigofera linnaei*. The structures of these compounds have been established by spectral data. Both these compounds are reported in this plant for the first time.

Keywords: *Indigofera linnaei,* Fabaceae, 5-[(E)-2-(4-hydroxyphenyl] benzene-1,3-diol, Gitoxin.

INTRODUCTION

Indigofera linnaei, Ali, (Fabaceae) is shrublet 20-90 cm tall with a long tap root and ascending branches. This plant is commonly known as 'Birdville Indigo'. *Indigofera* is distributed throughout India, commonly found in Asia temperate, grows in deciduous vine thicket, various types of woodland, shrub land and wood grassland. In traditional system of medicine this plant is claimed to be useful in treating ulcer, solid tumours, insect stings and snake bites, epilepsy (Kritikar KB,2001; Ananymous, 1997; Mail, 2006) etc. They are also reported to have anti-nociceptive, anti-inflammatory (Raju *et al*, 2013), antimicrobial (Esimone *et al*, 1999) and antidyslipidemic (Narender *et al*, 2006) activities. The recent surge of interest in chemistry of this plant has led us to the isolation of the following components with varied biological activity. Some of the reported compounds include Flavones such as 4'-hydroxy-3'5,7-trimethoxy flavones, eupatorin (Rehman, 2005); Flavonols: kaempferol 3-O-α-L-rhamnopyranoside, : kaempferol 7-O-α-L-rhamnopyranoside, quercitin 7-O-β-D-glucopyranoside, quercitin 3-O-[β-D-xylo pyrano -syl-(1→2)-β-D-galactopranoside] (Hasan, 1996), monoterpene glycosides such as 3,7-dimethyl-2(E), 6-octadien-5-one-1-O-β-D-6-O-acetylgluco pyranoside, 3,7-dimethyl-2E, 6-octadiene-5-one-O-[-β-D-6'-O-acetylglucopyra-nosyl(1'→6')-β-D-glucopyranoside]; indigo dye: Indigo, indigotin, indirubin, isoindigo, isoindurubin .Xanthene: 3-isopropyl-9a-methyl-1,2,4a,9a-tetrahydroxanthene (Thangadurai *et al*, 2001), COX inhibitory pterocarpan: indigocarpan, mucronulatol (Selvam, 2004). The present work deals with the isolation, structural elucidation and identification of the tannols, 5-[(E)-2-(4-hydroxyphenyl] benzene-1,3-diol and steroidal glycoside, Gitoxin from the methanol extract of the aerial portion of *Indigofera linnaei.*

MATERIALS AND METHODS

IR spectra were taken on a Shimadzu FTIR 8400s spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHZ, respectively, with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X 32 control. Mass spectra were recorded on a Shimadzu LCMS-2010A spectrometer. Silica gel 60-120 mesh and

TLC was performed with kieselgel 60F_{254} (Merk aluminium support plates) and spots were viewed under UV at 254 and 366 nm.

Indigofera linnaei was collected in November 2011 from their natural habitats in Tirupati (Andhra pradesh). The fresh plant was identified and authenticated by Dr. Madavachetty, Professor, Botany department, Sri Venkateshwara University, Tirupati.

Extraction (Khandelwal, 2009; Kokate, 2008; Agarwal, 2007)

Dried leaves of the plant (2.5 Kg) were milled into powder and then successive solvent extraction was carried out with petroleum ether, chloroform and methanol by distillation method. All the extracts obtained were subjected to lyophilisation to get 1.6, 1.9 and 2.4% extract residue respectively. The extracts so obtained were subjected to preliminary phytochemical tests as per the standard procedures available (Harbone, 1973; Mukherjee, 2003). The alcohol extract on evaporation at reduced pressure furnished a residue (60 g) which was chromatographed over silica gel (200g) using n-hexane with increasing amounts of chloroform, methanol followed by distilled water. 15ml of each fraction was collected. Totally 352 fractions were collected which were monitored by TLC. Fractions 172- 205 exhibited two spots on TLC (compound 1 and compound 2). These fractions after repeated purification by using the solvents hexane, petroleum ether, ethyl acetate, acetone, chloroform, methanol yielded 100 mg of compound 1 and 54 mg of compound 2.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the three extracts (Pet. Ether, Chloroform, and Methanol) revealed the presence of alkaloids, saponins, flavonoids, cardiac glycoside, steroids and tannins.

5-[(E)-2-(4-hydroxyphenyl] benzene-1,3-diol (Compound 1)

$C_{14}H_{12}O_3$, amorphous crystalline powder, m.p 261°C;

IR (KBr, υ_{max} ,cm^{-1}): 3398 (O-H St), 2939, 2908, 2750 (C-H St) 1647, 1510, 1454, 1383 (C=C St), 1278, 1193, 1128, 1072 (C-O St), 900, 862, 750, 667, 576, 511 (δ) out of plane bending.

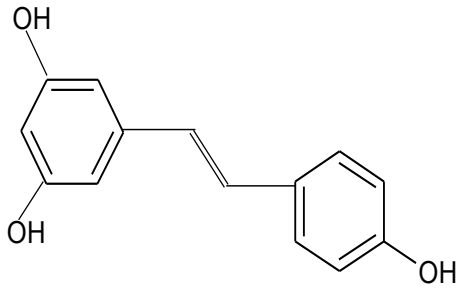
In ^{1}H-NMR spectrum, at 2.507 one shift corresponds to –CH_3- alkane, fr(om 3.089 to 4.513 twenty six shifts corresponding to OH/-H, at 7.861 and 7.864 two shifts corresponds to aromatic hydrogen.

In ^{13}C-NMR spectrum at 39.447 to 40.450 seven shifts corresponding to C-H saturated alkanes, at 56.623 to 78.913 thrity three shifts corresponds to C-OH/C-OR, at 82.377 to 98.108 five shifts corresponding to C=C (aliphatic) and at 102.301 to 104.936 three shifts supported the presence of aromatic carbon.

Gitoxin (Compound 2)

$C_{14}H_{12}O_3$, amorphous crystalline powder, m.p 265°C; IR (KBr, υ_{max} ,cm^{-1}): 3404, 3319 (O-H), 2947, 2906, 2731, 2578 (CH St), 1608, 1510, 1454, 1383 (C=C St), 1383, 1340, 1278, 1132 (C-O St), 1070, 960, 860, 748, 692, 669,572, 513(=C-Hδ). In ^{1}H-NMR spectrum, at 0.1944, 1.234, 1.2676, 2.085, 2.3020, 2.3362 seven shifts corresponding to CH_3/OH, at 5.9568, 6.0642, 6.7034, 6.7132, 6.8059, 6.8205, 6.8498, 6.8644, 7.4012, 7.4549, 7.4988 ten shifts corresponding to OH/M^+ or M^- sub alkene were identified.

In ^{13}C-NMR spectrum four shifts at 38.9723, 39.3968, 39.8213, 40.2458 four shifts corresponding to C-H saturated alkanes, at 77.8140, 78.4810, 78.6630 three shifts corresponding to C-OH/ C-OR and at 116.0189 to 160.7126 nine shifts corresponding to aromatic carbon were evident.



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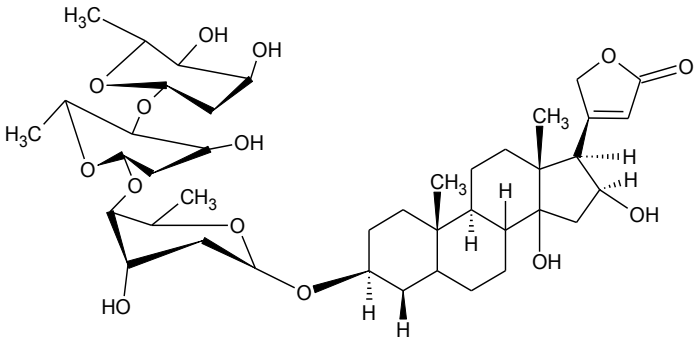

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CONCLUSION

The alcohol extract of aerial parts of *Indigofera linnaei*, Ali showed the presence of alkaloids, saponins, flavonoids, cardiac glycoside, steroids and tannins in the preliminary phytochemical investigation. Two compounds namely, 5-[(E)-2-(4-hydroxyphenyl] benzene-1,3-diol, and Gitoxin belonging to the class of tannols and steroidal glycoside have been isolated and the structures of these compounds have been established by IR, ^{1}H and ^{13}C NMR and Mass spectra. These compounds are reported in this genus, *Indigofera* for the first time.

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