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Research Article

ISOLATION AND IDENTIFICATION OF PHYTOCONSTITUENTS FROM DELONIX REGIA LEAVES

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ABSTRACT

Objective: Phytoconstituents can play an important role in traditional medicines and are under investigation for antineoplastic, antibacterial and other pharmaceutical functions. The present study is to explore phytoconstituents present in the ethyl acetate extract of Delonix regia (Hook) Raf leaves.

Methods: 95% ethanol crude extract was further partitioned with different solvents. The ethyl acetate soluble part was subjected to column chromatography and eluted with solvent mixtures of increasing polarity. The structures of these isolated compounds were identified on the basis of spectral analysis. i.e. FT-IR, ^{1}H NMR, ^{13}CNMR, EI/MS and ESI-MS/MS.

Results: Fractionation of the 95% ethanolic extract of the leaves of Delonix regia (Hook) Raf (led to the isolation of three sterols and its glucoside namely, Stigmasten-diol-3-o-glucoside, 12,15-Dihydroxy-chol-8-en-24-oic-acid-3-oxy-6'-acetyl-glucoside and sodium, potassium adduct of 12,15-Dihydroxy-5-chol-9-en-24-oic-acid-3-oxy-rhamnosyl-rhamnoside,one flavonol, namely, Kaempferol.

Conclusion: This is the first report on the isolation and identification of chemical constituents from Delonix regia (Hook) Raf leaves. These molecules would be useful in treating inflammations of cancer patients.

Keywords: Delonix regia (Hook) Raf, Kaempferol, Stigmasten-diol-3-o-glucoside, Sterol.

INTRODUCTION

Delonix regia (Hook) Raf is a species of small attractive tropical trees [1], it is commonly occurring flowering plant grown as an ornamental tree and given the name, flamboyant or flame tree, Gulmohar, peacock, Royal Poinciana [2]. Up till now even species were discovered in this genus, one occurs in Northeast Africa, nine species were found in endemic to Madagascar and the remaining species occurs from East and Northeast Africa to India [3]. Chemical constituents of different classes such as; flavonoid, terpenoids and its glycosides, phenolics, phytosterol [4-6], were reported from flowers and leaves of Delonix regia (Hook) Raf species. The flower of Delonix regia (Hook) Raf was used as natural color and as an acid-base indicator [7]. A number of published papers report the medicinal properties for Delonix regia (Hook) Raf [8-12]. The leaves are reported for its antimicrobial and antioxidant effect [13-14].

Many of distinct steroids are found in plants, animals and fungi [15]. All steroids are made in cells either from the sterols, lanosterol (animals and fungi) or from cyclo artenol (plants). Both lanosterol and cycloartenol are derived from the cyclization of the triterpene squalene [16]. A large of medicinal plants and its phytoconstituents have been beneficial therapeutic potentials and a majority of Indian medicinal plants are evaluated for such properties [17]. The present investigation reports the isolation, identification of phytoconstituents present in the Delonix regia leaves. Here, we isolated four compounds, compound-1 were already reported by researchers and the other compounds like, compound 2,3,4 were reported first time from Delonix regia (Hook) Raf leaves.

MATERIALS AND METHODS

Collection, identification and preparation of plant

The leaves of the plant Delonix regia (Hook) Raf collected from Thanjavur District in the month of May 2010 and authenticated by Dr.John Britto, Rapinet Herbarium, St.Joseph's College, Tiruchirappalli. The leaves were cleaned and dried in the shade and crushed into powder.

Extraction

The powdered sample was extracted with 95% ethanol by using cold method extraction in room temperature for one week. The 95% extract was further fractionated successively with petroleum ether, n-hexane, chloroform, ethyl acetate, ethanol, n-butanol and methanol. The solvents were recovered under reduced pressure.

Isolation

Ethyl acetate soluble part (5.8g) was subjected to silica gel (70-130 mesh) column chromatography (60cm x 4.5cm) for the isolation of phytoconstituents. The ethyl acetate soluble part was eluted gradient with Ethyl acetate, Ethyl acetate: Methanol mixtures 4.5:0.5, 4:1 and Methanol. The eluents were collected and the progress of separation was monitored by micro thin layer chromatography using Ethyl acetate: Methanol (4.75:0.25) solvent system and iodine vapor as detecting agent.

The solvent were recovered under reduced pressure from fractions eluted with ethyl acetate. Greenish yellow residue obtained which was treated with chloroform and recrystallized with methanol to give a yellow color compound with quantitative yield. The yellow color compound showed single spot on TLC, afforded the compound 1 (50 mg). Fractions eluted with ethyl acetate: Methanol (4.5:0.5), which was recrystallized from methanol, afforded a compound 2 (30 mg).

A residue obtained from soluble part of ethanol, and butanol, Which was treated by methanol and recrystallization with chloroform:methnol mixture to give white crystalline powder and white amorphous powder afforded the compound 3(150 mg) and compound 4 (75mg). 10 mg of compound 3 and 4 subjected to slow evaporation method of crystallization [18] using methanol as a solvent. After a few days, three types of crystals were obtained (sodium, potassium and sugar), which was subjected to crystallography. The isolated compounds were characterized by ^{1}HNMR, 13CNMR, IR, LC-MS, ESI-MS/MS, EI/MS and crystallographic studies (compound-3).

General experimental procedures

FT-IR (Fourier Transform- Infra red) spectra were obtained using Perkin Elmer FT-IR 450-4000 in KBr disc and absorption peaks in terms of wave numbers (cm^{-1}). EI-MS (electron impact mass spectrum) were recorded on Jeol instrument ESI-MS (electron spray ionization mass spectrum) in positive mode, were recorded on Thermo LCQ instrument. NMR (Nuclear magnetic resonance) was acquired on Brucker at 400 MHZ (^{1}H) and 100 MHZ (^{13}C). Chemical shifts were recorded as δ Value (ppm), chloroform and DMSO as an inert solvent.

Screening test for flavonoids

Compound 1 was subjected to micro thin layer chromatography with suitable solvent system [19]. The TLC plate was visualized by ammonia vapor (ammonia + sulphuric acid) a yellow color appeared and after a few minutes yellow color disappeared. Presence of flavonoids.

Screening test for steroids

Compounds 2, 3, 4 were subjected to micro thin layer chromatography with suitable solvent system [19]. The TLC plates were placed in iodine chamber, there is no change in iodine vapor, after a minutes, Vanilin sulphuric acid was sprayed by TLC plates and put in a Oven at 110 ^{0}C. A violet color appeared. Presence of steroids.

RESULTS AND DISCUSSION

The results of characterized crystal data were given in Table 1. ICP-OES analysis of compound 3 was given in table -2. The structure of isolated compounds as shown in Fig 1.

Identification of isolated compounds

The identification of the component was based on direct comparison of computer library matching and literature survey.

Compound-1

The product 1 is a yellow amorphous powder solid. From the positive test for flavonoid and EI-MS shows the m/z value- 286 via it was assumed to be a compound containing flavonol nucleus. In IR spectral analysis, the observed absorption frequency at 3393 cm^{-1} shows characteristic of OH stretching and absorption at 1682 cm^{-1}, 1526 cm^{-1} indicating the presence of C=O group, other absorption frequency 1605 cm^{-1} due to C-H group, frequency at 1473 cm^{-1}, 1354 is a C-H bending frequency for cyclic. The O-H group of environment is tertiary because the IR frequency appeared at 1152 cm^{-1}. The proton NMR (DMSO) showed the characteristic signals at 7.696 ppm, 7.688ppm assigned to the H-6 & H-8 protons and a pair of A_2B_2 aromatic system of protons at 7.866 ppm & 6.838 ppm assigned to H-2', H-6' and H-3,H-5 respectively. The ^{13}C NMR (DMSO) signal at 161.20 ppm, 167.79 ppm, 131.25 indicating the presence of C-6, C-8 & C-10 and C-2. The carbon signals obtained at 114.66 assigned the carbon at position C-3' & 5'. This signal was also supported by ^{1}H NMR spectra. The EI/MS shows the molecular ion peak at m/z-286 and fragmented peaks at 217, 202 and 94. It was in good agreement with literature data [20,21]. On the basis of spectral evidence, the structural of compound-1 was decided to be Kaempferol ($C_{15}H_{10}O_6$ - 286).

Compound-2

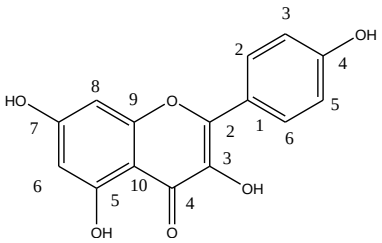
It appeared as white amorphous powder which gave positive test for steroids [19]. The molecular ion peak for compound - 2 was observed at m/z - 608 corresponding to the molecular formula $C_{35}H_{60}O_8$. The IR spectra shows absorption at 1646 cm^{-1} indicates the presence of olefin group. Absorption at 1480 cm^{-1}, 1391 cm^{-1} belongs to CH_3,CH_2 group and frequency at 1236 cm^{-1},1197 cm^{-1}, due to presence of secondary alcoholic group of environment. Absorption at 1141 cm^{-1}, assigned to be glycosidic linkage. It supports the ^{1}H NMR (DMSO) signal at 2.898 ppm, and signal at 5.338 ppm, due to olefin proton. Signals at 5.032 assigned to be anomeric proton corresponding signals at 3.148 ppm to 4.445 due to glycosidic proton. The EI/MS peak at m/z - 447 belongs to aglycone of stigmastien triol and at m/z – 608 confirmed the glucose molecule with stigmasteintriol. Based on the above characterization and by comparing with other similar compounds [8, 21], the proposed structure of the isolated compound is stigmastien-diol-3-o-glucoside.

Table 1: Crystal data for Compound-3

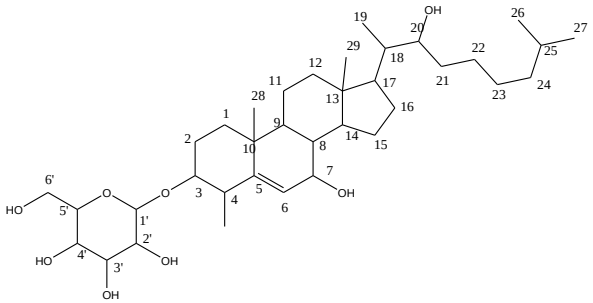
Crystal data	Result
Temperature	273(2) K
Wavelength	0.71073 A
Crystal system, space group	CUBIC P
Unit cell dimensions	Unit cell dimensions b = 6.31 Å, β= 90.00 deg. c = 6.31 Å, γ= 90.00 deg.
Volume	250.73 Å3
Instrument	BRUKER KAPPA APEX II

Table 2: ICP-OES analysis of minerals in the crystal

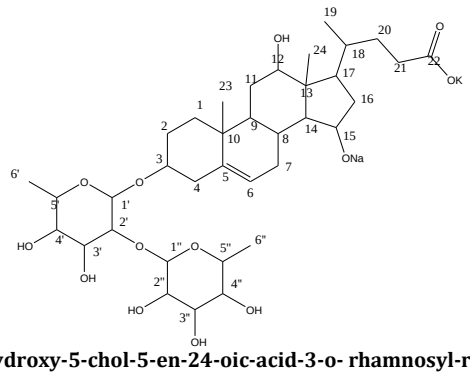
Minerals	Wave Length (nm)	Result (mg/kg)
Sodium	589.592	358.10
Potassium	766.490	1068.0



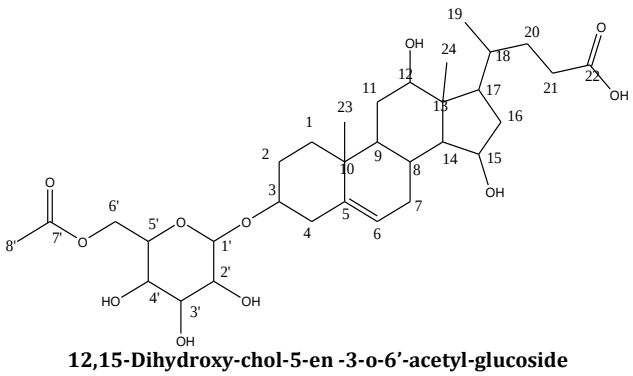
Kaempferol



Stigmasten-diol-3-o-glucoside



12,15-Dihydroxy-5-chol-5-en-24-oic-acid-3-o- rhamnosyl-rhamnoside



12,15-Dihydroxy-chol-5-en -3-o-6'-acetyl-glucoside

Fig. 1: Structure of isolated compounds

Compound-3

Compound-3 was obtained as white crystalline powder. According to crystal data, the volume of crystal shows 250 A^o3 indicates, the compound having salt nature. According to unitcell dimensions, the compound may be sodium, potassium adduct of cholic acid rhamnoside. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) report shows the compound have sodium and potassium. This was supported by mass spectra. The m/z value appeared at $(M + 22 + 39)^+$. The structure of the compound mainly deduces on the basis of LC-MS and ESI-MS-MS. These reports were strengthening by ^{1}HNMR ($CDCl_3$), ^{13}CNMR ($CDCl_3$) and IR spectral data's. The IR spectrum showed an absorption band at 3436 cm^{-1} for a OH group and the other vibration frequencies such as 2932 cm^{-1}, 2858 cm^{-1} and 1630 cm^{-1} assigned to the presence of CH_3, CH_2 group and olefin group respectively. The peak noticed at 1022 cm^{-1} due to presence of glycosidic linkage in the molecule. The aliphatic region of the ^{1}HNMR spectrum of the compound has the characteristic set of signals of steroid moiety. ^{13}CNMR spectrum of the compound showed signals which suggested the presence of a steroid moiety and a sugar moiety. The sugar moiety was determined to be rhamnosyl-rhamnoside from analyses of ^{1}HNMR signals at 2.79 ppm, 5.3 ppm, 3.49 ppm,to 4.2 ppm and 13CNMR signals at 70.74 ppm to 76.98 ppm. In LC-MS spectrum, the signal at m/z 713-718 and also ESI-MS/MS spectrum gave the signal at 718. So, we focus on ms- 2 of 718 ($C_{37}H_{59}NaO_{12}$), the m/z value of mass peak was 699 [$C_{36}H_{58}O_{13}$, $(M+H)^+$], 675 [$C_{35}H_{55}NaO_{11}$, $(M+H+Na-COOK)^+$], 634 [$C_{35}H_{54}O_{10}$, $(M-COOK-NaOH)^+$], 489[$C_{29}H_{44}O_6$, $(M+H-COOK-NaOH-Rhamnoside)^+$], 361 [$C_{23}H_{36}O_3$, $(M+H-Rhamnosyl – Rhamnoside-Na-COOK)^+$]. These mass peaks were confirmed by EI/MS peaks. i.e. The present compound exhibited a pseudo molecular ion peak at m/z 758 $[M+Na+K]^+$, other fragmentation peaks at m/z-613 [$C_{30}H_{46}KNaO_9$, $(M+Na+K-Rhamnoside)^+$], 653 [$C_{35}H_{56}O_{11}$, $(M+H-COOH)^+$], 466 [$C_{24}H_{36}KNaO_5$ $(M+Na+K)^+$]. On the above deliberations, isolate was deduced as sodium, potassium adduct of 12,15-Dihydroxy-5-chol-9-en-24-oic-acid-3-o-Rhamnosyl-Rhamnoside.

Compound-4

Compound-4 appeared as white amorphous powder. In IR frequency at 2925 cm^{-1}, suggested cyclic nature, absorption at 1706 cm^{-1} and 1678 cm^{-1} due to presence of ester and acid group. The IR frequency observed at 1431 cm^{-1}, 1363 cm^{-1} belongs to CH_3,CH_2 group, which was supported by 'HNMR spectra. The signal at 1.03 ppm to 1.57 ppm. IR frequency at 1259 cm^{-1}, 1225 cm^{-1} assigned to be secondary alcoholic group of environment and the ^{1}HNMR (DMSO) spectra showed signal at 3.38 & 4.2 ppm. The IR absorption band at 1088 cm^{-1}, 1015 cm^{-1} corresponds to glycosidic linkage. This glycoside linkage was also shown in 'HNMR signals at 2.6 ppm, 5.32 ppm (anomeric proton) and 3.59 ppm to 3.24 ppm. In Mass spectrum, the molecular ion peak showed at m/z - 611 in ESI-MS/MS mode and m/z - 610 [$C_{32}H_{50}O_{11}$] in EI/MS mode. The ESI-MS/MS and EI/MS fragmentation pattern confirms the removal of glucose moiety (204) m/z – 406 ($C_{24}H_{38}O_5$) followed by side chain (100) m/z - 306 and the aglycone (304) ($C19H_{28}O_3$). On the basis of above spectral characterization the proposed structure of compound4 was 12,15-Dihydroxy-chol-8-en-3-o-6'-acetyl-glucoside.

CONCLUSION

Delonix regia (Hook) Raf possesses several medicinal characters apart from the early stated uses. All these therapeutic applications of this extract is absolutely due to the phytoconstituents like flavonoids, sterols, saponins, glycosides, alkaloids and tannins present in the plant. The flavonoids isolated, are widely dissipated in plant kingdom and all have been reported to have biological activities. Kaempferol was also reported to have hepatoprotective, antioxidant and antibacterial activities [9-15]. Our studies show that Delonix regia (Hook) Raf leaves contain Stigmastentriol 3-o-glucoside, Cholan-12,15-diol-3-oxy-3',4',5'-trimethoxy glucoside and sodium, potassium adduct of Dihydroxy-5-chol-8-en-24-oic acid-3-oxy-6-acetyl-3',4',5'-trimethoxy glucoside, Kaempferol; which may account at least in part for their application in treating inflammations of cancer patients. Since the secondary metabolite content may vary as a function of multiple factors, such as harvest period, environmental conditions, reproduction of this analysis over a long period of time is needed before the effectiveness of our method is totally demonstrated. The pharmacological studies of above compounds were work-in-progress. In future, the analysis by the changing the solvent system one could congregate various other compounds present in the plant.

CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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