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Research Article

Isolation of phytoconstituent from fruits of *Gmelina arborea* roxb.

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Abstract

The plant *Gmelina arborea* Roxb. has been traditionally used in India for several medicinal purposes like anthelmintic, diuretic, antibacterial, antipyretic, antioxidant and antidiabetic. The aim of the present study is to isolate the phytoconstituent from ethanolic fruits extracts of *G. arborea*. The *G. arborea* plant materials are rich source of flavonoid compounds and are responsible for various pharmacological activities. The isolation of phytoconstituent was done from the ethanolic extract by the chromatographic method. The structure of the isolated compound was established on the basis of physical, chemical test and spectroscopic evidences such as IR, UV, H^1 NMR and C^{13} NMR and MS. A flavonoidal structure was isolated from the ethanolic extract of the plant in mobile phase of 5 % methanol in ethyl acetate (1:4). The yellow colored compound which solubilizes in methanol, chloroform and n-hexane and water having melting point was 177°C. It could be concluded that isolated compound may be quercetin (5,7-dihydroxy-4 methoxy flavone) which may be responsible for various pharmacological activities of the plants.

Key words: *Gmelina arborea,* Flavonoid, fruits, phytoconstituent, Spectroscopy.

INTRODUCTION

Gmelina arborea Roxb (Family *Verbenaceae*) fruits are oval in shape, ¾ inches in length and are yellow in color. The fruits are sweet in taste and sometimes astringent[1,2]. The plant, *G. arborea* was reported to have several medicinal properties such as aphrodisiac, astringent, analgesic, antipyretic, antidiabetic, diuretic, anti-inflammatory and tonic characteristics[3].

The literature survey reveals that fruits of *G. arborea* contain cardiac glycosides, flavonoids and steroids. The ethanol extract contains alkaloids, carbohydrates, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds and flavonoids[4].

MATERIALS AND METHODS

Chemicals used

The solvents, ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. Other solvents, ethyl acetate, chloroform, methanol and petroleum ether AR 40-60°C were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Silica gel G (Qualigen) was procured from Fisher Scientific, Mumbai, India.

Instruments used

The ultraviolet spectrophotometer (UV-1700 Schimadzu, Japan) used was procured from Thermo Laboratory Pvt. Ltd., Kolkata, West Bengal. The infrared (8400S Schimadzu IR spectrophotometer, Japan), nuclear magnetic resonance (Bruker AM-400 NMR) and mass spectrometer (Bruker APEX-III, APEX Technologies Inc.) were procured from Shimazu Pvt. Ltd., Japan.

Collection of plant materials, identification and size reduction

The fruits of *G. arborea* were collected from local area of Koraput district (India) and was authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M.S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Odisha (Letter no. MJ/DBT (13)/1067, dated 12.04.2013). The fruits were shade dried and were

pulverized to form coarse powder by using electrical grinder and stored in a closed air tight container for further use.

Preparation of solvent extracts

The coarse powder form of dried fruits was extracted by Soxhlation method by using ethanol as solvent. A total amount of 1500 g coarse powdered fruits was extracted with 1200 ml of each solvent. The crude extract was evaporated to dryness in a in a rotary flash evaporator, with the percentage yield being 2.2 %. Crude extract was kept in closed air tight containers under cool and dark place for further study.

Isolation of phytoconstituent[5-7]

About 25 g of sample (Ethanol extract) was weighed and dissolved in 100 ml of chloroform for 3 h by continuous stirring. The suspension was filtered by using Whatmann filter paper no. 1 and the supernatant liquid thus collected in the beaker was concentrated by evaporating the solvent by heating at 60 °C for 10 min. The concentrated extract was again redissolved in chloroform, loaded at the top of the column and kept for 3 h. Mobile phases used were: Ethyl acetate - n-hexane (25:75), ethyl acetate - n-hexane (50:50), ethyl acetate - n-hexane (25:75), ethyl acetate (100 %), methanol - ethyl acetate (5:95), methanol - ethyl acetate (10:90), methanol - ethyl acetate (20:80), methanol - ethyl acetate (50:50) and methanol (100 %). About 25 ml fraction was collected at each time. The eluates were collected in test tubes and each fraction was subjected to TLC study.

Thin Layer Chromatography study

The slurry of adsorbent that is Silica gel-G (Gypsum i.e. Calcium Sulphate) as stationary phase was transferred to various TLC plates by spreading method. The prepared TLC plates were air dried at room temperature for 30 min. The prepared TLC plates were activated by heating the plates at 105°C for 1 h in a hot air oven (ACM-22066-1, ACMAS Technocracy (Pvt.) Ltd., New Delhi). The solvent systems used for development of chromatogram in TLC plates were chloroform and ethyl acetate in the ratio of 5:95. The chromatograms were detected by spraying the above reagents. The resultant bands were also visualized by charring with a reagent containing methanol and concentrated sulphuric acid (85:15). The R_f values were recorded. The column with the solvent system methanol and ethyl acetate (5: 95) gave least number of fractions. The larger band was collected as eluent and subjected to for column chromatography once again, using chloroform and ethyl acetate in ratio of 5: 95. The column and thin layer chromatography studies were continued until the purification of compounds achieved, which was ensured by obtaining single spot on TLC plate.

Identification of isolated phytoconstituent

Physical and chemical evaluations

The properties like appearance, color, taste, odor, solubility and melting point of the isolated constituents was determined. The isolated constituent was dissolved in ethanol and evaluated chemically for detection of flavonoid by using Shinoda, zinc hydrochloride reduction and alkaline reagent test[6,7].

Structural elucidation of isolated phytoconstituent

The compound was dissolved in methanol and ultra violet absorption spectrum was determined by using UV spectrophotometer by scanning in the range of 200 to 800 nm using methanol as blank. The λ_{max} was determined from UV spectral analysis by considering that the corresponding wavelength at which maximum absorbance took place[8,9]. The FT-IR was used for IR analysis in the frequency range between 4000 and 600 cm^{-1} and at 1 cm^{-1} resolution. The sample of pure isolated compound was prepared by palletization technique in KBr using IR press. The IR peaks of the sample were analyzed and interpretated to elucidate the structure of isolated compound[10]. Nuclear magnetic resonance spectra (H^1 and C^{13}) were recorded at 400 MHz for H^1, 100 MHz for C^{13} – nuclei respectively[11,12]. Electron impact (EI) mass spectra were recorded coupled with PDP 11/34 computer system. High resolution spectrometry (HR-MS) and field desorption mass spectrometry (FD-MS) were also performed on ApexIII mass spectrometer[13,14].

RESULTS AND DISCUSSIONS

All phytochemical tests for flavonoid such as shinoda (Magnesium turning), ferric chloride, lead acetate, zinc hydrochloric acid reduction, sodium hydroxide and sulphuric acid were found to be positive. The appearance of the compound was crystalline powder and yield was 2.4%. The color of the compound was yellowish white. It is soluble in methanol, chloroform and n-hexane. The melting point of compound was 176 to 178 °C and optical rotation was $^{30}[\alpha]_n$ – 32.5° (C = 0.84 in methanol)[6,7].

The infrared spectroscopy study (Fig 1) of isolated compound reveals that the major peaks were obtained at wave numbers (υ) of 3405.57, 3211.76, 2954.96, 2834.58, 2722.24, 1594.95, 1497.82, 1348.62, 1302.16, 1252.69, 1168.52, 1090, 1020.49, 831.94, 721.76 and 559.15 cm^{-1} respectively[15,16]. It signifies that compound has hydroxy (OH), methoxy (OCH_3), keto groups etc. The compound presented the characteristic intensities of C=O absorption band at υ of 1594.95 cm^{-1} and the OH stretching band at υ of 3405.57 cm^{-1}. The υ of 1497.82 and 13483.62 cm^{-1} shows the absorption band of C=C and C-OH. The wave number of frequency 1252.69 cm^{-1} shows the stretching band for C-O-C. The other wave number frequencies show characteristic bands for C-C and C=C[15,16].

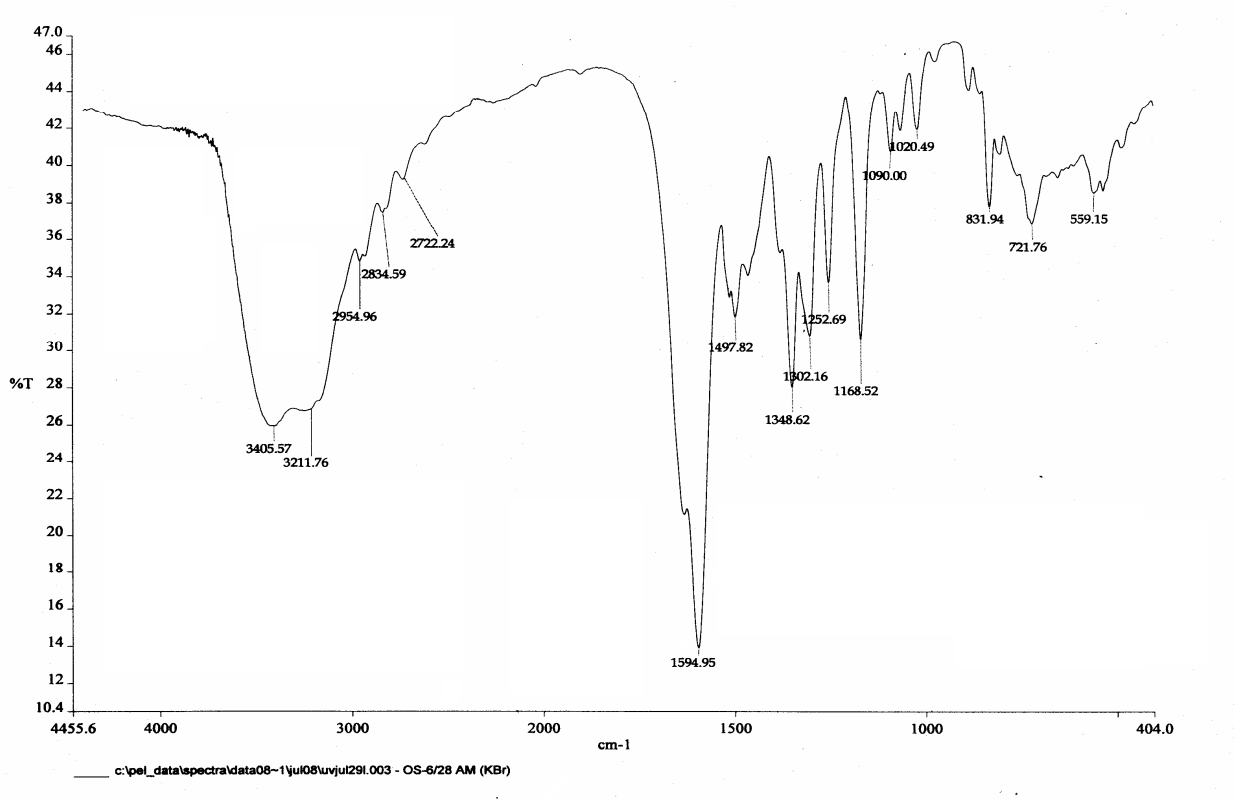

Fig 1. Infrared spectral data of isolated compound in the frequency range between 4000 and 600 cm^{-1}.

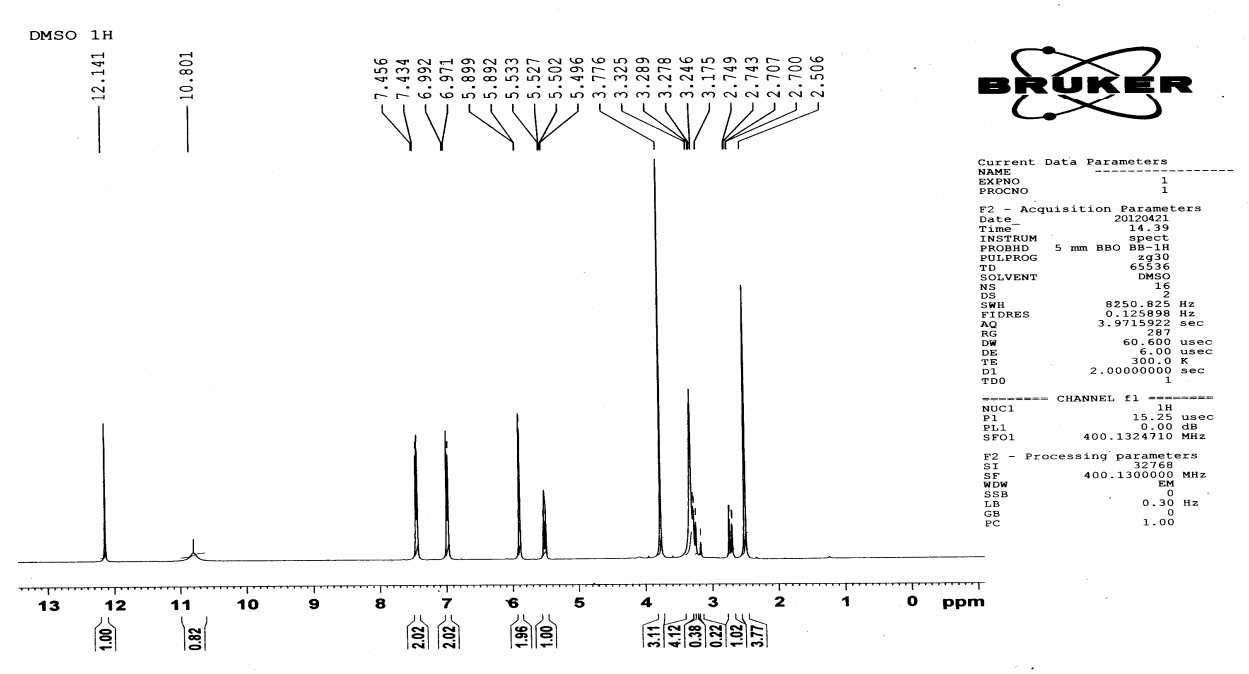

Fig 2. H[1] (Proton) NMR data of isolated compound at 400 Hz.

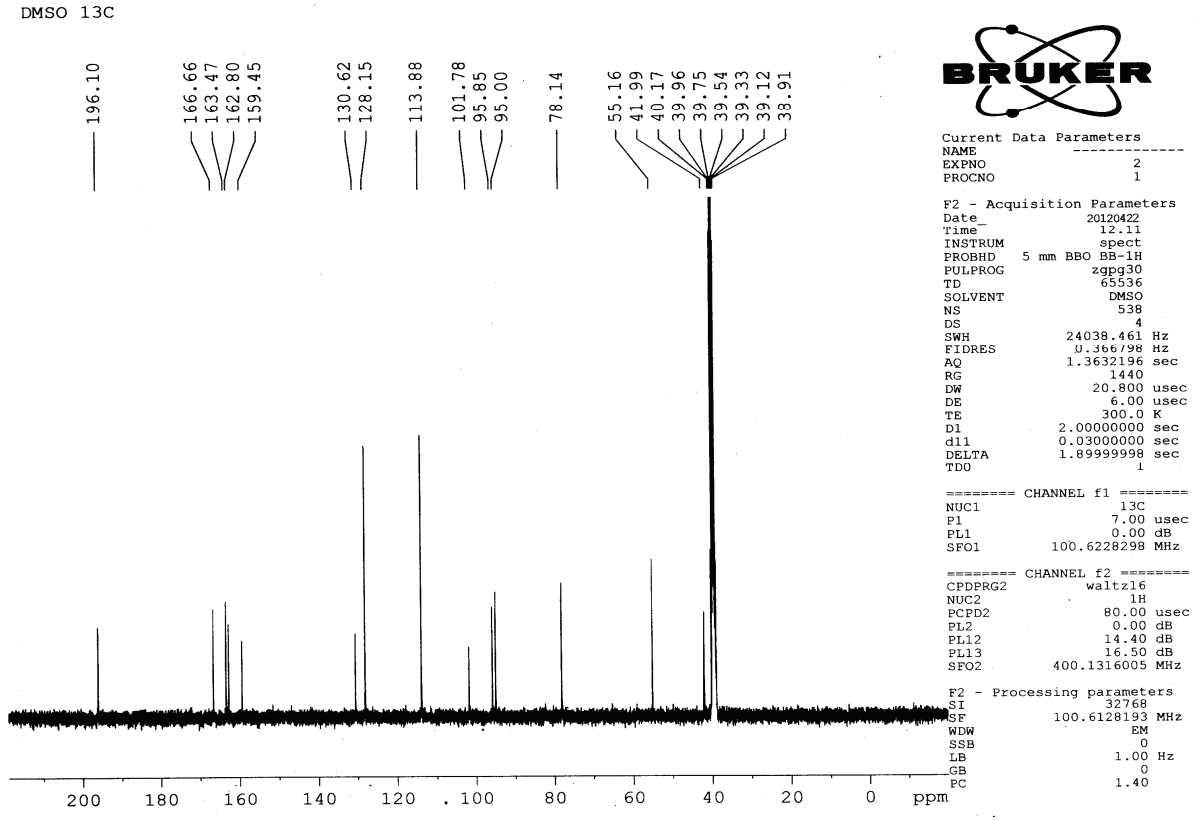

Fig 3. C[13] NMR data of isolated compound at 400 Hz.

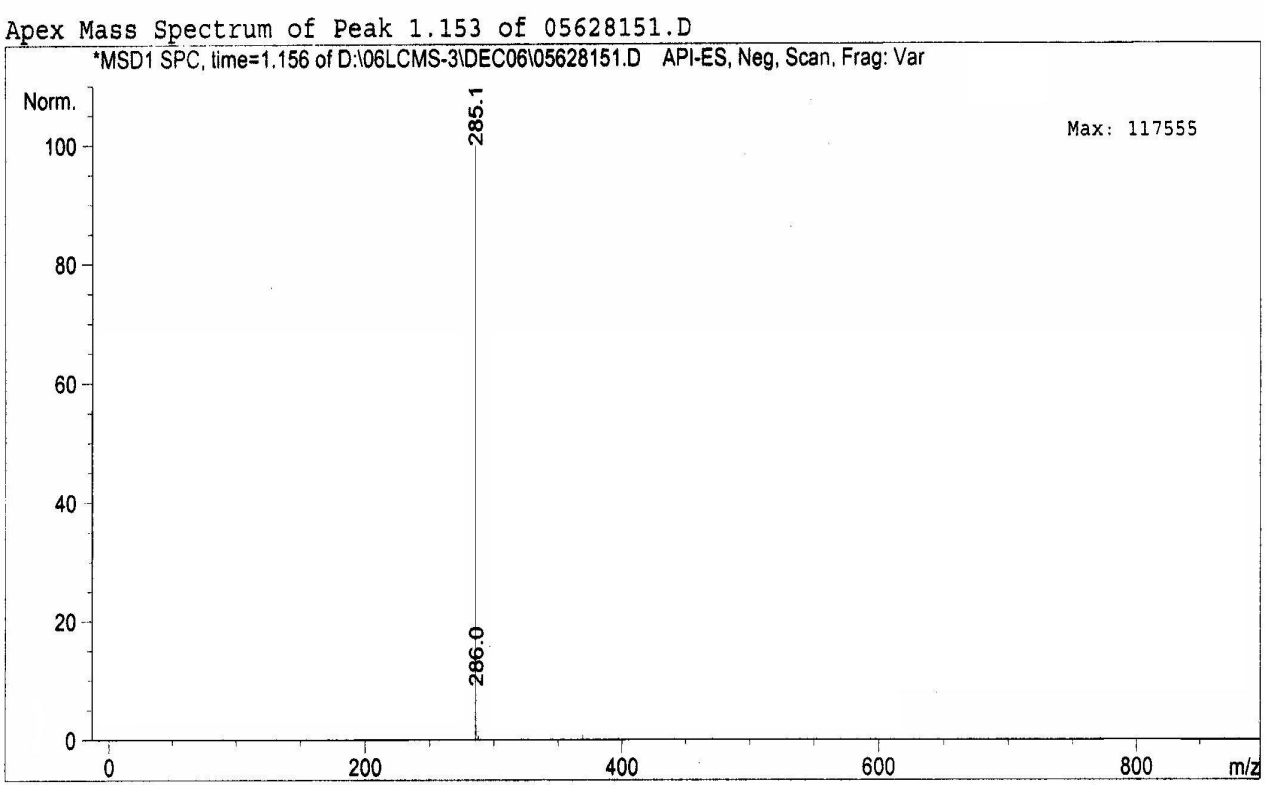

Fig 4. Mass spectral data of isolated compound of ethanol fraction.

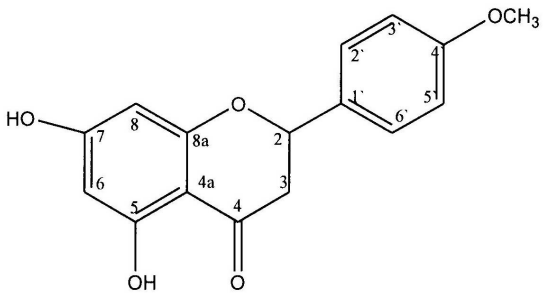

Fig 5. Chemical structure of the isolated flavonoid (Derivative) compound.

^{1}H NMR and ^{13}C NMR (DMSO, 400 Hz) is shown in Fig 2 and 3. 1H NMR (DMSO, 400 Hz): δ 2.514 (1H.ddJ=12.4.2.4Hz, H-2), 2.676 (1H.ddJ=16.8.2.4Hz, H-3a), 3.247 (1H.ddJ =17.0.4.4Hz, H-3b), 5.895 (1H,dJ=8.2Hz, H-6), 5.895 (1H,dJ =2.8Hz, H-8), 7.445 (1H,dJ =8.8Hz, H-2′), 6.981 (1H,dJ =8.4Hz, H-3′), 6.981 (1H,dJ =8.4Hz, H-5′), 7.445 (1H,dJ =8.8Hz, H-6′), 12.141(1H,brs, OH-5), 10.801 (1H,brs,OH-7) and 3.776 (3H,s, OMe-4′). ^{1}H NMR spectra showed the presence of thee protons attached to C-4′ bearing methoxy group appeared at δ 55.16. The proton attached to C-5 and C-7 carbon bearing hydroxyl groups appeared at δ 12.141 and 10.801 as multiplet. The ^{1}H NMR signals at 12.141 ppm should be attributed to hydroxyl protons of isolated constituent, which participated in a strong intramolecular hydrogen bond, between the hydrogen atom of the hydroxyl group OH (C-5) and the oxygen atom of the carbonyl group CO (C-4)[15,16].

^{13}C NMR (DMSO, 400 Hz): δ 78.14 (CH-2), 41.99 (CH_2-3a), 196.10 (C-4), 163.47 (C-5), 95.85 (CH-6), 166.66 (C-7), 95.00 (CH-8), 130.62 (C-1′), 128.15

(CH-2′), 113.88 (CH-3′), 159.45 (C-4′), 113.88 (CH-5′), 128.15 (CH-6′), 101.78 (C-4a), 162.80 (C-8a), 55.16 (CH_3-OMe-4′). ^{13}C NMR showed the presence of one quartet, one triplet, seven doublets and seven singlets corresponds to one methyl, one methylene, seven methine groups and seven tertiary carbon atoms. The spectrum of isolated constituent showed product on aromatic groups ranging from 6 to 8 ppm, and a strong intramolecular hydrogen bonding at 12.141 ppm[15,16].

From the mass spectroscopy study (Fig 4), the result showed a peak at 286 (molecular peak ion - m/z value) which corresponds to molecular weight of isolated compound was found to be 286 (M+) with molecular formula of $C_{16}H_{15}O_5$[15,16].

CONCLUSION

From the analytical studies such as ultraviolet, infrared, nuclear magnetic resonance and mass spectroscopy study, it can be concluded from their interpretation of their spectral data, the isolated compound is a derivative of flavonoid with a chemical structure "5, 7-dihydroxy-4 methoxy flavone" and the chemical structure is depicted in Fig 5.

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