Isolation and characterization of stigmasterol and β-sitosterol from
Acacia nilotica (L.) Delile ssp Indica (benth.)Brennan

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ABSTRACT

Phytosterols are group of steroidal alcohol play significant roles in structural component in the cell membrane and play a role in membrane stability. There are nearly 22 different sterols are found yet and the major phytosterols include β-sitosterol, campesterol and stigmasterol. The present study deals to characterize sterols from melacholic extract on leaves of Acacia nilotica ssp indica. (L.). Powdered leaves were subjected to sequential extraction using soxhlet apparatus. Methanol extract was fractionated with n-hexane: chloroform (5:5) using column chromatography and isolated sterols were further confirmed by co-thin layer chromatography using sterol standard. Structural elucidation of sterols was done by spectrum analysis such as, Fourier Transform Infrared Spectroscopy, 1H and 13C Nuclear Magnetic resonance and mass determined by Liquid Chromatography Mass spectroscopy. From the physical, chemical and spectral evidences, the compounds isolated from methanolic extract of Acacia nilotica ssp indica (L.) is confirmed as stigmasterol and β-sitosterol.

Key words: Stigmasterol, β- sitosterol, Nuclear Magnetic resources, Acacia nilotica

INTRODUCTION

The genus Acacia contains approximately 19 species that are widely distributed throughout tropics and sub tropics. Acacia nilotica is a spineless woody legume, belonging to the family Mimosaceae. Acacia nilotica commonly known as babul tree and in Tamil it is named as Karuvelam. The plant distributed in Deccan parts of the tropical Africa and native from Australia. Leaves are bipinnated with 7-24 pairs, with 2-5 mm long thin, straight, light grey spines present in auxiliary pairs, Acacia species have a long history of medicinal use in the treatment of diarrhea, urinary infections, throat inflammation, gastritis, tuberculosis and AIDS (Marquez, 1999). Tannin, gum and timber are the three major products of Acacia nilotica (Kirtikar et al., 1975). Few publications have been described the composition of Acacia species, and the most relevant results have reviewed (Seigler, 2003). Apart from Acacia species, phytosterols have been isolated and characterized from Phyllanthus columnarius (Jamil, 2009). Three stigmastane-skeleton sterols have been isolated from Eriocaulon sieboldianum and structures were elucidated using IR, NMR and MS (Song, 2008).

Plant sterols have important pharmacological activities, including cholesterol lowering and antimicrobial effects (Lee et al., 2004). β-sitosterol is used in the treatment of immune dysfunctions, inflammatory disorders and rheumatoid arthritis (Atif, 2000), breast cancer, colon cancer and benign prostatic hyper trophy (Bouic, 1996), Anti Oxidant, anti inflammatory, analgesic and anthelmintic activity. It has hypoglycemic and hypercholesterolemic property and also used as a precursor in the synthesis of steroidal drugs (Panda, 2009).

In light of the above applications this paper deals the isolation and characterization of sterols from Acacia nilotica ssp indica (L.).

MATERIALS AND METHODS

Plant material and processing

Leaves of Acacia nilotica indica (L.) were collected from the Potheri forest, Kancheepuram district, Tamil Nadu, India. The plant material was authenticated in the herbarium unit of Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore (Voucher Number BSISRC/ 5123/2010-11/ Tech 1344). The leaves were shade dried, powdered, sieved and used to isolate phytocatalysts.

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RESULTS AND DISCUSSION

The yield of the methanol extract was 32.6 g/kg of leaf sample. The extract was further subjected to column chromatography and verified using TLC in hexane-ethyl acetate 8:2 (v/v) and chloroform–methanol 4:4 (v/v) solvent systems, Rf value 0.7 in the former solvent system corresponded to stigmasterol while Rf value 0.56 in later corresponded to β-sitosterol. The compounds were further subjected to thin layer chromatography using n-hexane: ethyl acetate 8:2 (v/v) and chloroform: methanol (4:4 v/v). Rf value 0.7 in former solvent system corresponded to stigmasterol while Rf value 0.56 in later corresponded to β-sitosterol. The isolated fractions were confirmed using a Shimadzu 160A UV–visible spectrophotometer. The UV–visible spectrum of the isolated compounds in methanol was recorded and elucidation study of stigmasterol and β-sitosterol
Different spectroscopic methods including UV, FTIR, and 1H, 13C NMR and LC MS/MS were used to elucidate the structure of stigmasterol and β-sitosterol. The UV–visible spectrum of the isolated compounds in methanol was recorded using a Shimadzu 160A UV–visible spectrophotometer. The Fourier transform infrared spectrum was recorded using a Perkin-Elmer instrument with spectrum XR 1 software version 5.0.1 at room temperature. The compounds were dissolved in chloroform and scanned in the range of 4000–500 cm−1. 1H NMR spectra and 13C NMR spectra were recorded at 400 MHz and 100 MHz respectively in NMR spectrometer (BRUKER) at SAIF, Indian Institute of Technology Chennai, India. The 1H NMR and 13C NMR spectra were recorded in CDCI3 solvent and TMS initial standard using topshim software. Liquid chromatography coupled with tandem mass spectroscopy was recorded in 3200 Q TRAP fitted with an electron spray ionization source using software version 1.4.2, in which data has been acquired at SGS Laboratories, Chennai.

Structural elucidation study of stigmasterol and β-sitosterol

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EXTRACTION AND PURIFICATION

One kg of powdered leaf sample of Acacia nilotica was subjected to sequential extraction using soxhlet apparatus from non polar to polar solvents such as n-hexane < chloroform < ethyl acetate < ethanol < methanol. The solvent was recovered under reduced pressure using rotary evaporator under vacuum condition and the residue was stored in the refrigerator. The methanolic extract was dissolved in n-hexane and chloroform (1:1). The residue was refluxed for two to three hours. The filtrate was collected and stored at −20°C.

CHROMATOGRAPHIC SEPARATION

The filtrate was subjected to column chromatography using silica gel (70–240 mesh) as stationary phase. The solvent system consisted of n-hexane in combination with increasing percentage of chloroform. About 25 ml fraction were collected using hexane, chloroform and methanol by gradient elution technique and elutes were monitored using thin layer chromatography. The fractions showing similar Rf value were pooled and further re-chromatography. Isolated sterols were verified thin layer spray with Anisaldehyde - sulphuric acid and the plate was incubated at 120°C for ten minutes, the purple color spots were confirmed as sterols. Fraction corresponding to Rf value were pooled, washed with acetone and subjected to TLC with hexane–ethyl acetate 8:2 (v/v) and chloroform: methanol 4:4 (v/v) as mobile phase, Rf value 0.72 yielded a powdery substance while fraction corresponding to Rf value 0.56 yielded needles like crystals. These were confirmed using Salkowski reaction and Liebermann–Burchard reaction as described by Harborne (1998).

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was confirmed as stigmasterol and R value of 0.56 in the later was confirmed as β-sitosterol using standards. Salkowski and Lieberman–Burchard reaction confirms the two compounds as sterols. The yield of stigmasterol and β-sitosterol was 85 and 40 mg, respectively. The nature of stigmasterol was powdery and that of β-sitosterol was crystalline. The melting point of the compounds was 144–146°C and 138–139°C, respectively.

**Compound 1: Stigmasterol**

Stigmasterol was isolated as white powder. White powder (25 mg) UV-λ max 257 nm. The mass spectral data of this compound gave the molecular formula C_{30}H_{50}O_2. [m/z: 413.3 (M+)].

The IR absorption spectrum showed absorption peaks at 3474.19, 3414.07, 2944, 1646, 1370, 1214, 1168, 1114, 1062, 894 cm\(^{-1}\). The IR absorption spectrum showed absorption peaks at 3476, 2944, 1646, 1556, 1370, 1214, 1168, 1114, 1062, 894 cm\(^{-1}\).

The mass spectral data of this compound gave the molecular formula C_{30}H_{50}O. [m/z: 414.7 (M+)].

The IR absorption spectrum showed absorption peaks at 3474.19, 3414.07, 2935.73, 2867.38, 2027.73, 1637.63, 1617.28, 1465.5, 1377.14, 1063.34, 1056.31 cm\(^{-1}\). The IR absorption spectrum showed absorption peaks at 3476, 2944, 1646, 1556, 1370, 1214, 1168, 1114, 1062, 894 cm\(^{-1}\).

**Compound 2: β-Sitosterol**

The mass spectral data of the compound 2 gave the molecular formula C_{29}H_{48}O_2 [m/z: 414.7 (M+)]. Colorless needles (40 mg), UV-λ max 2950 nm. The mass spectral data of this compound gave the molecular formula C_{29}H_{48}O_2.

H NMR (400 MHz, CDCl_3): δ 1.29 (6H, t, H-6), 5.15 (1H, s, H-22), 5.01 (1H, d, J = 6.5 Hz, H-21), 0.83 (6H, s, H-19), 0.92 (3H, d, J = 6.5 Hz, H-21), 0.84 (3H, t, J = 7.2 Hz, H-29), 0.83 (3H, d, J = 6.5 Hz, H-26), 0.80 (3H, d, J = 6.6 Hz, H-27), 0.68 (3H, s, H-18).

**REFERENCES**


**CONCLUSION**

From the above physical, chemical and spectral evidences, the compounds isolated from methanolic extract of Acacia nilotica indica (L.) is confirmed as stigmasterol and β-sitosterol. This is the first ever report of these steroidal compounds from this plant.

**ACKNOWLEDGEMENT**

The authors are thankful to SRM Management for funding this research work. Our thanks extended to Botanical Survey of India, TNAU Campus for authenticating the plant specimen and SRM Pharmacy for spectrum analysis.

**Source of support:** Nil, Conflict of interest: None Declared