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Research Article
Isolation and Characterization of β-Sitosterol from the Flowers of Bastard Oleaster (*Elaeagnus latifolia* Linn.)

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Abstract

Background and Objective: *Elaeagnus latifolia* Linn., commonly known as wild olive, has been used as a traditional medicine to treat a variety of ailments, including anticancer, antioxidant, free radical scavenging activities and as an astringent. The study aimed to isolate and characterize the bioactive components from the flowers of *Elaeagnus latifolia*. **Materials and Methods:** The dried coarsely powdered flowers were extracted with water for 48 hrs and subjected to chromatography, characterised by UV-Visible, FT-IR, MS, ^{1}H NMR and ^{13}C NMR. **Results:** Phytochemical screening revealed the presence of glycoside, phytosterols and saponins. ^{1}H NMR has revealed a signal at 7.44 ppm for O-H_3 proton, the signal at 8.7 ppm for H_{26}. Six methyl protons appeared at 0.81, 0.95, 0.97, 1.04, 1.29 and 1.32 ppm for H_{29}, H_{21}, H_{27}, H_{26}, H_{18} and H_{19}. ^{13}C NMR has given signal at 134.7 and 121.4 ppm for $C_5 = C_6$ double bond, 71.7 ppm for C_3 β-hydroxyl group, 19.4 ppm for angular methyl carbon atom for C_{19}, 29.6 ppm for C_{25}, 36.6 ppm for C_{20}, 56.5 ppm for C_{17}, 28.8 ppm for C_{16}, 42.3 ppm for C_{13}, 40.2 ppm for C_{12}, 50.1 ppm for C_9, 32.0 ppm for C_2, 37.2 ppm for C_1. The purification of the compound resulted in white crystals with melting points 140-146°C and molecular mass 414.71. **Conclusion:** Based on the spectral data analysis and chemical reaction, the isolated compound was concluded as β-sitosterol. This compound has not been previously isolated from the flowers of *Elaeagnus latifolia*.

Key words: β-sitosterol, *Elaeagnus latifolia*, Elaeagnaceae, flower, isolation, bastard oleaster, antioxidant

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Elaeagnus latifolia Linn. belongs to the family Elaeagnaceae. It is a straggling shrub with several branches and grows 3-5 m tall. It is commonly known as Bastard Oleaster[1]. It can be found in the hilly regions of India, Sri Lanka and China. In India, it is found at an altitude of 1500 m in the Northeastern states of Assam, Nagaland, Khasi and Jaintia Hills of Meghalaya, India[2,3].

Silvery white scales cover the stems, leaves, flowers and fruits. The leaves are many, ranging in length from 3.8-10 cm and width from 2.2-5 cm. Bees pollinate the flowers, which are straw-coloured, clustered and hermaphrodite. The perianth is 8 mm in length and is covered with silvery scales. Flowers bloom from August to November, while the fruits ripen from March to April.

Fruits range in colour from red to orange-red and can be eaten raw or preserved as a pickle. Different parts of this plant have been used for curing various ailments such as in cancer treatment[2], antioxidant and free radical scavenging activity[3].

This study aims to isolate and characterise the bioactive components from the flowers of *Elaeagnus latifolia*. In this paper, we report the isolation and characterization of the compound, β-sitosterol from the flower part. For the first time, this compound was isolated from the flowers of *Elaeagnus latifolia*.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Pharmacognosy, Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Mehsana, Gujarat, India, from May, 2013-April, 2017.

Collection and authentication of plant materials: The fresh flowers of *Elaeagnus latifolia* Linn. (Elaeagnaceae) were collected in bulk from the horticulture farm of ICAR Research Complex, NEH Region, Shillong, Meghalaya, India, in November, 2014. The collected specimens were identified and authenticated by the Botanist Dr. A.A. Mao, Scientist-F, Botanical Survey of India, Eastern Regional Centre, Shillong, India. The voucher specimen (Acc. No. 28949, 68401) was preserved for future reference. The herbarium was deposited in S.K. Patel College of Pharmaceutical Education and Research, Department of Pharmacognosy, Ganpat University, India.

Preparation of plant extract: The flowers were shade dried at room temperature for 10 days, coarsely powdered and stored in airtight containers for further studies.

Extraction and Isolation of the compound: The coarsely powdered flowers were subjected to extraction with water for 48 hrs using the Soxhlet apparatus. Extraction was repeated and the extract was filtered through Whatman filter paper. The filtrate was evaporated and dried under reduced pressure to yield an aqueous extract. The aqueous extract of the flower was dissolved in chloroform and was subjected to thin layer chromatography (TLC). TLC of the extract was carried out in various solvents using Precoated silica gel 60 F_{254} as an adsorbent which was procured from (E. Merck Ltd, Germany)[4]. The plates were developed and observed under UV at both 254 and 366 nm and showed prominent band separation with the solvent system toluene:chloroform:methanol (4:4:2) and later sprayed with 5% sulphuric acid. Thus, the R_f values were calculated.

The preparative TLC method was used for the isolation of the compound. The thickness of the adsorbent layer, silica gel used was 2 mm as stationary phase (20×20 cm glass plates) and toluene:chloroform:methanol (4:4:2) were used as a solvent system. The band at R_f value 0.56 was identified as (*Elaeagnus latifolia*-5) EL-5 compound. EL-5 band was eluted with solvent (methanol) after scrapping the distinct band. The solvent was then filtered through Whatman filter paper. The filtrate was warmed and the temperature was decreased slowly until pure crystals were obtained.

EL-5, white crystals with melting point 140-146°C. It yielded a single spot when subjected to TLC using the solvent system toluene:chloroform:methanol (4:4:2). EL-5 was further subjected to UV, FT-IR, MS, ^{1}H NMR and ^{13}C NMR to ascertain the chemical structure.

Spectroscopic characterization: Analytical grade chemicals were purchased from M/s Merck Chemicals, India. Pure β-sitosterol was procured from Natural Remedies Pvt Ltd. Bengaluru, India. Among the spectroscopic techniques UV, FT-IR, MS, ^{1}H NMR and ^{13}C NMR were used to elucidate the structure of the isolated compound. Absorbance measurements were carried out on Thermo Scientific Evolution 201 UV-visible spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), the FT-IR spectrum was recorded on Agilent Cary 630 FT-IR spectrometer (Agilent Technologies, Santa Clara, California, USA). For FT-IR, the sample was mixed with KBr and pressed into discs of size 13 mm diameter×0.3 mm thickness. Agilent Technologies (LCMS Q-TOF) (Agilent Technologies, Santa Clara, California, USA), were used to record MS and the data were given in m/z values, ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance 500 NMR Spectrometer (Bruker, Billerica,

Massachusetts, USA) using the solvent $CDCl_3$ (Deuterated chloroform) and chemical shifts were observed relative to standard Tetramethylsilane.

RESULTS AND DISCUSSION

EL-5 showed a positive Liebermann Burchard test, assumed to be a compound containing a steroidal nucleus. EL-5, white crystals with melting point 140-146°C and showed R_f value 0.56 in solvent system toluene: chloroform:methanol (4:4:2). It yielded a single spot when subjected to TLC using the solvent system toluene:chloroform:methanol (4:4:2). The UV λ_{max} value in $CHCl_3$ was found to be 246 nm (Fig. 1).

Based upon the FT-IR Spectroscopy, the EL-5 compound was found to show distinctive peaks at 3370 cm^{-1} which implies the presence of -OH stretching. Absorption at 2941 and 2831 cm^{-1} is due to C-H stretching and 1626 cm^{-1} assigned to C = C stretch. This implies the presence of one double bond in the structure. Considering the nature of oxygen as hydroxyl and the presence of one double bond, the general formula for the compound can be considered as C_nH_{2n-6}. Therefore it can be a tetracyclic compound. Other absorption frequencies include 1403 cm^{-1} as a result of C-H bending, 1111 cm^{-1} is due to C-O bending and 1020 cm^{-1} signifies cycloalkane[5,6].

The exact molecular mass of the compound was found to be 414.71 which corresponds to the molecular formula $C_{29}H_{50}O$. Other fragment ions were m/z 304.30, 256.30, 149.02, 102.13 and 61.03[7].

^1H NMR ($CDCl_3$, 400 MHz) has revealed a signal at 7.44 ppm for O-H_3 proton, the signal at 8.7 ppm for H_{26}. Six methyl protons appeared at 0.81, 0.95, 0.97, 1.04, 1.29 and 1.32 ppm for H_{29}, H_{21}, H_{27}, H_{26}, H_{18} and H_{19}[6-8]. ^{13}C NMR ($CDCl_3$, 125 MHz) has given signal at 134.7 and 121.4 ppm for $C_5 = C_6$ double bond, 71.7 ppm for C_3 β-hydroxyl group, 19.4 ppm for angular methyl carbon atom for $C_{19,}$ 29.6 ppm for C_{25}, 36.6 ppm for C_{20}, 56.5 ppm for C_{17}, 28.8 ppm for C_{16}, 42.3 ppm for C_{13}, 40.2 ppm for C_{12}, 50.1 ppm for C_9, 32.0 ppm for C_2, 37.2 ppm for C_1 (Fig. 2a-b). The number of carbons extracted from ^{13}C NMR may reveal the structure of a steroid with 30 carbons[5-7]. Comparing the experimental data (R_f, melting point, UV, IR, MS and NMR) with those reported in the literature supports the proposed structure of the isolated compound (EL-5). Experimental data was also matched with the spectra of the pure β-sitosterol which was procured from Natural Remedies Pvt Ltd. Bengaluru, India. Thus, the proposed structure was assigned as β-sitosterol (Fig. 3). β-sitosterol undergoes an oxidative process like cholesterol, resulting in β-sitosterol oxides. This makes isolation of pure β-sitosterol a challenge due to the presence of sitosterol oxides[9].

EL-5 compound was isolated as white crystals and showed a positive Liebermann Burchard test, assumed to be a compound containing a steroidal nucleus. The UV λ_{max} value in $CHCl_3$ was found to be 246 nm. Based upon the functional group analysis it was found that the nature of oxygen was hydroxyl and presence of one double bond, the general formula for the compound can be considered as C_nH_{2n-6}. Therefore, it can be a tetracyclic compound[5,6].

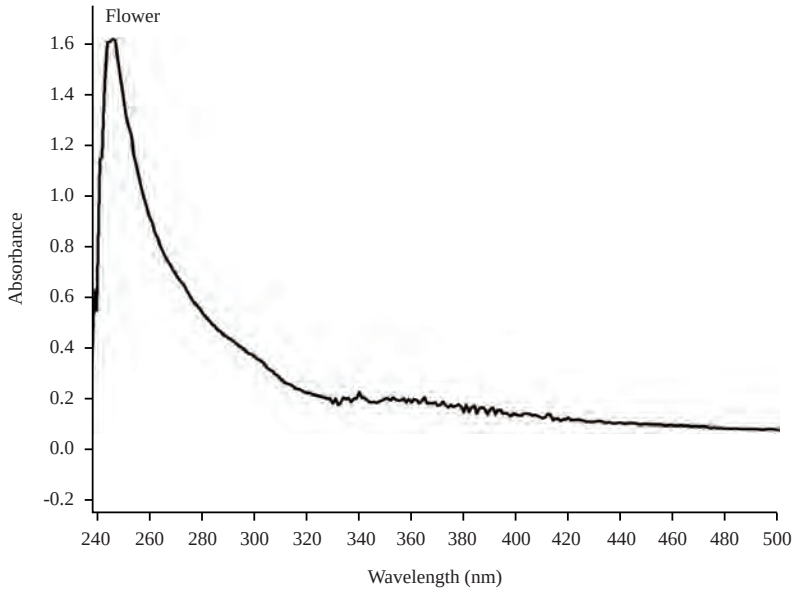

Fig. 1: UV spectra of EL-5 compound

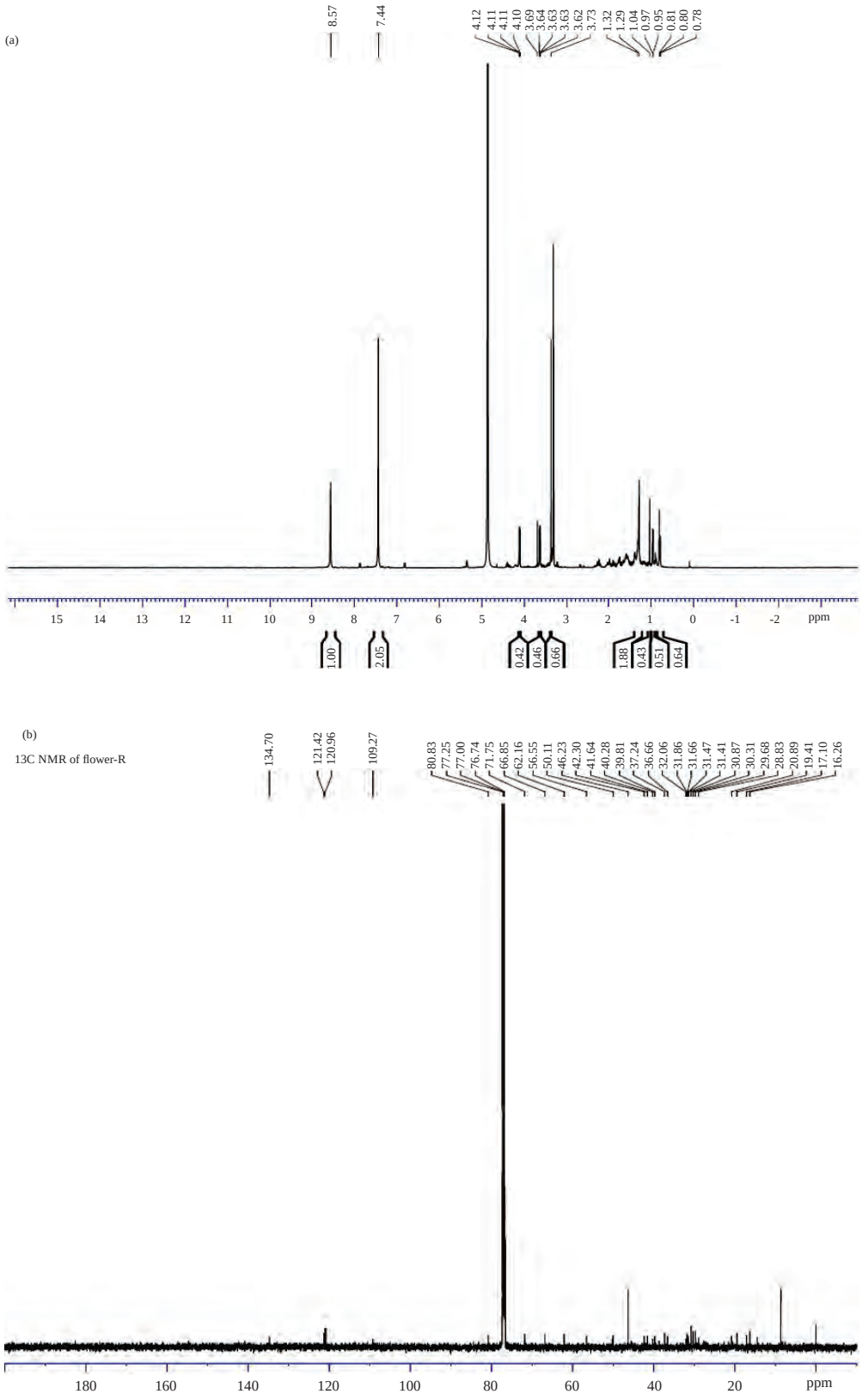

Fig. 2(a-b): NMR spectra of EL-5 (*Elaeagnus latifolia*) compound, (a) ^{1}H NMR spectra of EL-5 compound and (b) ^{13}C NMR spectra of EL-5 compound

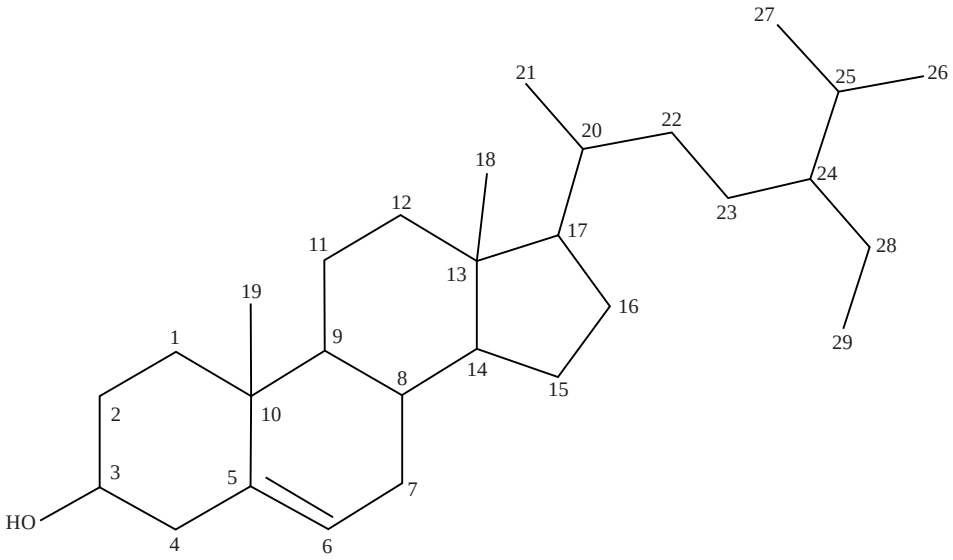

Fig. 3: Structure of β-sitosterol

Elaeagnus latifolia, the underutilized wild edible plants of North East India. As per the literature survey the indigenous plant, *Elaeagnus latifolia* is used in various pharmacological activities like cancer treatment[2], antioxidant and free radical scavenging activity[3]. In this study, we isolated and characterized the bioactive components from the flower of *Elaeagnus latifolia*. As this plant has so many health benefits, it can be used as a traditional herbal plant for the treatment of different kinds of ailments.

CONCLUSION

Based on UV, FT-IR, MS, NMR spectral data, physical properties and comparing with those described in the literature, the isolated compound was identified as β-sitosterol. Further studies on EL-5 may be taken up to explore the possible use of the compound in therapeutic activity.

SIGNIFICANCE STATEMENT

This study discovers the plant, *Elaeagnus latifolia* contains β-sitosterol as one of the active constituents. β-sitosterol is a plant sterol beneficial for antioxidant activity. This study will help the researcher to uncover the isolation of β-sitosterol from the plant *Elaeagnus latifolia* that many researchers were not able to explore. Thus, a new theory on these active constituents and possibly other constituents may be arrived at.

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