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Isolation & Structural Characterization Of Β Sitosterol From Methanolic Extract of *Cordia Obliqua* Fruit

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Abstract
Bioactive components that support or advance health and are found in the nexus of the food and pharmaceutical sectors are known as phytochemicals. These phytochemicals offer excellent therapeutic potential for treating a wide range of diseases. The characterization of phytomedicines is critical for compound identification. Spectroscopy and Chromatography are two essential ways for identifying substances. FTIR, Infrared (IR), NMR and mass spectrometry are extensively used techniques for identifying bioactive compound in herbal medicines. This study deals with Isolation & structural characterization of β -sitosterol from methanolic extract *Cordia obliqua* fruit**.** The plant material was collected & subjected to extraction by methanolic & aqueous extract. The % yield obtained was 1.09% for methanolic extract. The methanolic extract was enriched with carbohydrate, flavonoid, glycoside, sterol & protein.

Keywords: Cordia obliqua, Phytochemicals, Isolation & identification, Bioactive, β -sitosterol,

Introduction

The use of herbal plants in ancient times exemplifies the history of bioactive compounds. People had no understanding about bioactive chemicals previously, although their applications were sufficiently diversified in several fields. Given the wide range of bioactive chemicals and the vast number of plant species, it is critical to develop a standardized and integrated strategy to identifying these compounds with human health benefits. Numerous phytochemicals have been discovered to exhibit high antioxidant, antibacterial, and herbicidal activities in addition to their physiological functions in plants. A variety of bioactive chemicals have been identified, purified, and used in a variety of industries, including food, pharmaceutical, cosmetic, and agricultural. Exploration of active medicinal plants and their natural bioactive compounds has thus become critical in order to capitalize on the potential extra qualities of natural sources (Kellogg *et al*., 2016; Altemimi *et al*., 2017).

Although contemporary chromatographic and spectrometric techniques have made bioactive compound analysis easier than ever before, success is still dependent on extraction processes, input parameters, and the exact composition of plant parts. The matrix qualities of the plant component, solvent, temperature, pressure, and time are the most prevalent factors influencing extraction procedures The growing understanding of the dynamic chemical composition of varied bioactive molecules has been a pioneer fuel for the advancement of bioactive analysis during the last decade (Cieśla *et al*., 2016; Fu *et al*., 2019).

As a result of these significant technological and technological advancements, the pharmaceutical, food additive, and natural pesticide industries have been interested in bioactive compounds derived from natural sources. Bioactive chemicals, by definition, coexist with other compounds found in plants. Plant parts such as leaves, stems, flowers, and fruits can be used to identify and characterise bioactive chemicals (Ligor *et al*., 2018).

Cordia obliqua Willd. plant (Common name-Clammy Cherry) belongs to family Boraginaceae. It is a medium-sized deciduous tree that grows quite quickly. It has anthelmintic, purgative, diuretic, expectorant, antipyretic, hepatoprotective, and analgesic effects, according to traditional medicine. The fruits can be used as pickles and are edible. Paper sheets are pasted with mucilage-derived gum, which is also employed as a matrix component in tablet formulations. Pharmacological tests proved the antibacterial, hypotensive, respiratory stimulant, diuretic, and anti-inflammatory properties of the *C. obliqua* plant (Aimey *et al.*, 2020; Gupta and Gupta, 2015).

The β sitosterol has been found in a variety of dietary and nondietary plants. Its membrane stabilising impact on cell membrane has been described. It is a natural micronutrient found in higher plants and is found in healthy people's serum and tissues at concentrations 800-1000 times lower than endogenous cholesterol. Sitosterolin, its glycoside, is also detectable in serum, albeit in lower concentrations. The β sitosterol is widely regarded as a safe, natural, and effective nutritional supplement with numerous potential benefits. The administration of BS to rats was shown to be free of genotoxicity and cytotoxicity. Without causing significant toxicity, BS exhibits antioxidant, antimicrobial, angiogenic, antioxidant, immunomodulatory, antidiabetic, anti-inflammatory, anticancer, and antinociceptive properties. There are some nutraceutical products on the market that include β sitosterol. Their producers assert numerous beneficial advantages in the absence of strong experimental data (Rashed, 2020; Khan *et al*., 2022; Saeidnia *et al*., 2014). This study deals with isolation & identification of beta sitosterol from methanolic extract of *C. obliqua*

Materials & methods

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Collection of plant

Fruits of *Cordia obliqua* were collected from local area of Bhopal in the month of January 2023.

Extraction using hot continuous extraction method

Plant materials of *Cordia obliqua* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with methanol and distilled water using Soxhlet extraction method. Shade dried powdered of fruit of *Cordia obliqua.* Plant materials extracted in Soxhlet apparatus for 48 h. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods (Tiwari *et al.*, 2011).

Separation and Identification of phytoconstituents by column Chromatography

Glass column pack with silica for column 80 to 120 size. The stationary phase should be dry and activated by heating. Prepare methanolic plant extracts 2gm., mix with silica and load the sample onto the top of the column. Use a small amount of solvent to ensure even distribution. Slowly add the solvent (Toluene: Ethyl acetate 9:1 v/v) to the column. Allow the solvent to percolate through the stationary phase and carry the compounds with different polarities down the column. Collect the eluted fractions in separate vials or tubes. Monitor the elution process using techniques like thin-layer chromatography (TLC) to track the movement of compounds. Analyze each collected fraction (A) using techniques like IR, NMR and MASS spectroscopy and chemical tests to identify the presence of specific phytoconstituents.

Fractions with similar compounds combined to simplify further analysis. Compare the obtained results with known standards, literature, and databases to identify the phytoconstituents present in the fractions.

Results & discussion

In case of *Cordia obliqua* the aqueous extract have higher percentage yield of 4.57 % and methanolic extract observed to have percentage yield of 1.09 %. The methanolic extract of *Cordia obliqua* found to contain carbohydrate, flavonoid, glycosides, sterol & proteins while only phenol, lignin & glycosides found to be absent in aqueous extract.

Table 1: Percentage yield of *Cordia obliqua*

Sr. No.	Extract	*Cordia obliqua*
1.	Methanol	1.09%

Table 2: Phytochemical Test of *Cordia obliqua* (Fruit)

Sr. No.	Test	Methanol Extract
1.	Carbohydrate Test Fehlings Test Benedicts Test	 + +
2.	Phenol Ferric Chloride Test	 -
3.	Flavonoid Lead Acetate Test Alkaline Test	 + +
4.	Alkaloid Wagner's Test	 -
5.	Tannin Gelatin Test	 -
6.	Lignin Labat Test	 -
7.	Saponin Foam Test	 -
8.	Glycoside Conc. H_2SO_4 Test	 +
9.	Sterols Salkowski Test	 +
10.	Proteins Xanthoproteic Test	 +
11.	Diterpenes Copper Acetate Test	 -

Results of separation and Identification of phytoconstituents by column Chromatography

From the fractions, eluted out from toluene: ethyl acetate (9:1v/v) gives isolated gives compound A. In TLC profile, compound-A showed reddish colour at R_f 0.65 when detected under day light. On recrystallization with methanol, compound-A (35 mg) gave white to off white crystalline compound, Compounds-A was then subjected to determine then melting point, IR, NMR and mass spectral analysis, to elucidate the structure of this isolated compounds.

Table: FT-IR Interpretation of Isolated compound A

S. No.	Peaks cm^{-1}	Peak Assigned
1	3325	O-H str.
2	2842-2963	C-H str. (Alkane)
3	1716	CH_3 Group
3	860	Cyclohexane Ring

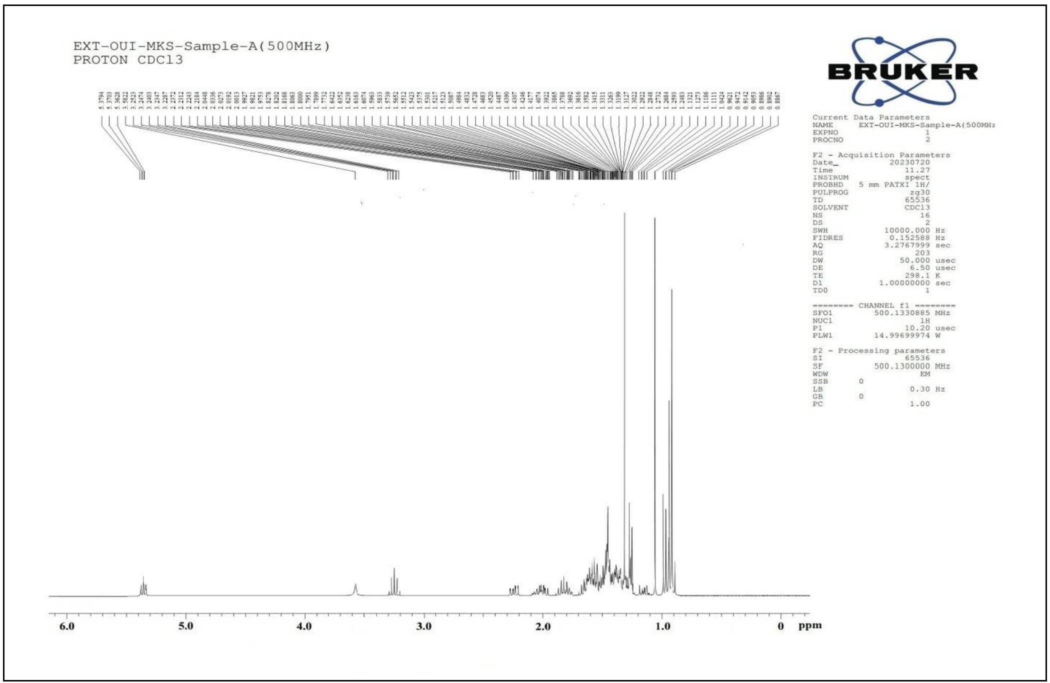


Figure: Graph of NMR for isolated fraction A

Table: Results of NMR Interpretation

S. No.	σ- Value	Pattern	J- Value	No. of Protons	Assignment
1	5.37	t	4.55, 3.55	1	CH
2	3.58	s	-	1	OH
3	3.25	m	-	1	CH
4	2.23	q	-	1	CH_2
5	2.04	m	-	1	CH_2
6	1.98	m	-	1	CH_2
7	1.82	m	-	1	CH
8	1.79	m	-	1	CH_2
9	1.64-1.13	m	-	27	CH, CH_2, CH_3
10.	1.04	s	-	3	CH_3
11	0.96	d	7.45	3	CH_3
12	0.91	d	4.45	6	CH_3
13	0.90	t	4.2, 1.55	3	CH_3

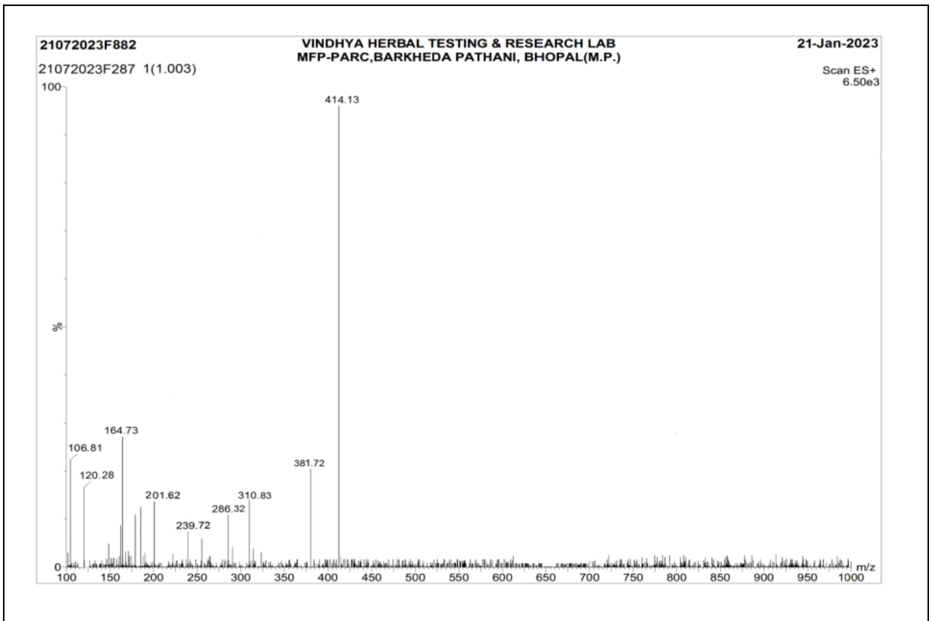
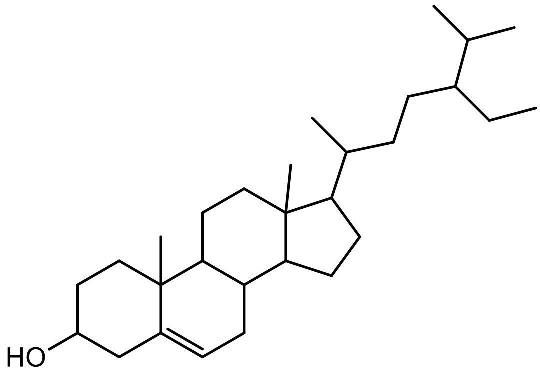


Figure: Graph of MASS for isolated fraction

MS: 414, (Molecular ion peak); 381, 164, 106
On the basis of interpretation of of above data following structure has been proposed



IUPAC Name: 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl 2,3,4,7,8,9,10,11, 12,13,14, 15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol
MF: $C_{29}H_{50}O$
MW: 414.39

Conclusion

The isolation and structural characterization of β-sitosterol from the methanolic extract of *Cordia obliqua* fruit have been successfully achieved. β-sitosterol is a well-known phytosterol widely found in various plant sources and is recognized for its potential health benefits. The compound's structural formula has been elucidated, revealing a complex arrangement of carbon atoms in the cyclopenta [a] phenanthrene nucleus. The successful isolation and structural characterization of β-sitosterol contribute to the understanding of the chemical composition of *Cordia obliqua* fruit and its potential bioactive constituents. β-sitosterol, as a phytosterol, has been associated with various biological activities, including anti-inflammatory, antioxidant, and cholesterol-lowering properties. This finding adds to the knowledge of the potential health-related benefits that *Cordia obliqua* fruit may offer.
This study underscores the significance of natural product chemistry in uncovering novel compounds from plant sources and highlights the importance of structural elucidation for accurate compound identification. Further studies can explore the bioactivity and potential applications of β-sitosterol isolated from Cordia obliqua fruit, contributing to the field of natural product-based drug discovery and functional foods.

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