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ISOLATION AND CHARACTERIZATION OF β -SITOSTEROL FROM METHANOLIC EXTRACT OF *CORDIA DICHOTOMA* LINN. BARK

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ABSTRACT: *Cordia dichotoma* Linn. belonging the family Boraginaceae is locally called as Lasora in India. It is an important drug to cure diseases of the kidney, liver, spleen, heart and blood which attribute many medicinal properties in Ayurveda. The purpose of this study is to identify and characterize the bioactive principle from the bark of *Cordia dichotoma* Linn. Phytochemical screening methanolic extract of bark of *Cordia dichotoma* Linn. was done and showed the presence of phytosterol. Phytosterols were isolated from the methanol extract by admixing 100 g of extract with 200 mL of acetonitrile. The resulting white sterol crystals were analyzed by Thin Layer Chromatography and High Performance Liquid Chromatography (HPLC) for the purification. The sterol for structure elucidation was further studied by more sophisticated analytical techniques e.g. FTIR, ¹H-NMR, ¹³C-NMR and LC-MS. After various spectral analysis and interpretation of same, the isolated compound was identified as β -sitosterol.

INTRODUCTION: To treat infectious and other diseases is an age-old practice and possibly the only method available which is the use of plants and their preparations. The plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential crude drugs as well as a source of new anti-infective and treatable agents. *Cordia dichotoma* G. Forst. belonging the family Boraginaceae is locally called as Lasora in India. This plant is a medium sized tree having short crooked trunk mainly found in the North Western Tarai Region of Uttar Pradesh native of Tropical Asia and Australia.

It occurs in all the warmer parts of India i.e. the forest region ^{1,2,3}. Fruits of *Cordia dichotoma* (CD) are globose or ovoid glossy, yellowish brown, pinkish or nearly black when ripe, usually single seed surrounded by a transparent, sticky, sweet edible pulp ⁴. It is an important drug to cure diseases of the kidney, liver, spleen, heart and blood which attribute many medicinal properties in Ayurveda. Various parts of the plants are used as antipyretic, for anti-anemic effect, as a remedy for impotency and to treat gastric pain, asthma, mouth ulcers, bronchitis, diarrhea, rheumatism and dental caries ⁵. Traditionally bark of the plant is reported for the treatment of ulcerative colitis ⁶.

Various chemical constituents like alkaloid, carbohydrate, flavonoids, proteins, amino acids, phenols, tannins, glycosides and steroids are present in this species. The purpose of this study is to identify and characterize the bioactive principle from the bark of *Cordia dichotoma* Linn.

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In this paper, we reported the isolation and characterization of known compound from *Cordia dichotoma* Linn. namely β -sitosterol.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material:

Bark of *Cordia dichotoma* L. were collected from the sides of the railway track, Meerut cantonment, Uttar Pradesh, India. It was collected in August to December 2015. Herbariums were prepared for authentication of plant. One copy of herbarium file was deposited in Department of Pharmacognosy, IFTM University, Uttar Pradesh, India and one copy was deposited for authentication. Authentication of plant was done by National Botanical Research Institute, Lucknow, India. (Voucher no. NBRI/CIF/477/2015).

Extraction: The bark of CD were collected, cut, dried and powdered in a grinder. The powdered material was extracted in a Soxhlet apparatus carried out successively with petroleum ether (60-80), methanol and water by maceration. Each time before extracting with the next solvent, the powdered material was air dried at room temperature and extract the obtained was concentrated under reduced pressure to obtain a crude dried residue ⁷.

Phytochemical Screening:

Liebermann-Burchard Test: Methanolic extract 1mg was dissolved in chloroform and few drops of acetic anhydride were added to it, followed by concentrated sulphuric acid from the side of the tube. A transient colour development from red to blue and finally green indicated the presence of sterol.

Salkowaski Reaction: Methanol extract 1mg was dissolved in 2ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red colour in the chloroform layer indicated the presence of sterol ⁸.

Isolation of Phytosterols: Phytosterols were isolated from the methanol extract by admixing 100 g of extract with 200 mL of acetonitrile. The mixture was heated to a temperature of approximately of the boiling point of acetonitrile. This temperature was maintained for 10 to 15 min. At this time, an insoluble unsaponifiable portion, which comprises the undesirable gummy material,

formed a layer at the bottom of the vessel; the sterols and acetonitrile resolved in a clear solution. This sterol-acetonitrile solution was then decanted and formation of white sterol crystals ⁹.

The resulting crystals were analyzed by Thin Layer Chromatography and High Performance Liquid Chromatography (HPLC) for the purification.

Purification for Single Sterol Compound: The isolated crystals was thin layer chromatographed in solvent system chloroform: methanol (9.8:0.2) and visualized by using spraying reagent phosphomolybdic acid solution. The obtained two spots were then separated by preparative CO-TLC and after multiple runs of sample thin layer chromatographically the spot with the R_f value 0.5 was pooled, evaporated the solvent and dried. The preparative glass plates were used for the separation.

The separated sterol compound was then analyzed by High Performance Liquid Chromatography (HPLC).

High Performance Liquid Chromatography:

Instrumentation: The HPLC system consisted of a Waters 600 controller pump, a U.V. Visible detector (wavelength tunable), a Phenomex C18 (250 X 4.60 mm), 5 μ m column and a Data ace software (Chromatography workstation), digital analytical weighing balance (Wesnar), vacuum filtration assembly.

HPLC Conditions: The mobile phase consisting of acetonitrile: methanol (7:3) was filtered through 0.45 μ membrane filter before use, degassed by and were pumped from the solvent reservoir in the ratio of 70:30 v/v was pumped into the column at a flow rate of 0.8 ml/min. The column temperature was 30°C. The detection was monitored at 210 nm and the run time was 12 - 15 min. The volume of injection loop was 20 μ l prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

Spectroscopic Studies: The sterol for structure elucidation was further studied by more sophisticated analytical techniques e.g. FTIR, ¹H-NMR, ¹³C-NMR and LC-MS. FT-IR spectroscopy (Bruker FT-IR Spectrometer, USA). ¹H and ¹³C-NMR spectra were recorded on a Bruker BioSpin

Advance III FT-NMR spectrometer, USA were recorded using CDCl_3 as solvent at Bio sapience Laboratory, Bhopal and LC-MS spectra were recorded at high resolution on Bruker's aurora M90 (USA) instrument.

RESULTS AND DISCUSSION: Methanolic extract of bark of *Cordia dichotoma* L. was showed the presence of phytosterol when tested. From the positive tests for steroids, it is assumed to be a compound containing steroidal nucleus. The isolated white crystalline needles like substance was observed melting point at 145 - 148 °C.

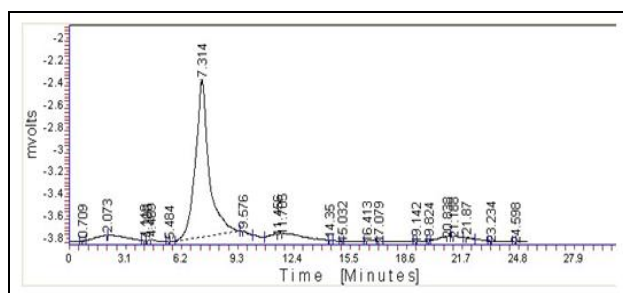


FIG. 1: CHROMATOGRAM OF THE ISOLATED COMPOUND PHYTOSTEROL

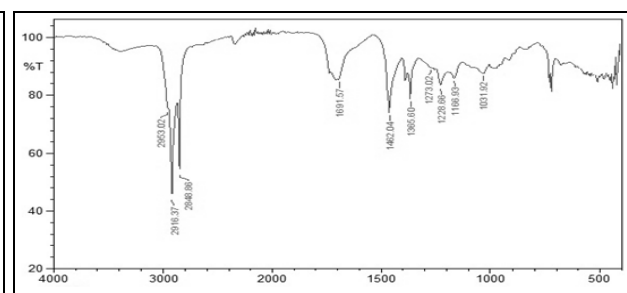


FIG. 2: FTIR SPECTRA OF PHYTOSTEROL COMPOUND

LCMS spectral analysis LC-MS spectroscopy showed the molecular ion peaks at 414 (413.5) that correspond to molecular formula, $\text{C}_{29}\text{H}_{50}\text{O}$. Ion peaks were also observed at m/z 397.5, 383.5,

371.1, 282.3. The molecular weight and fragmentation pattern indicate that the compounds presenting is β -sitosterol.

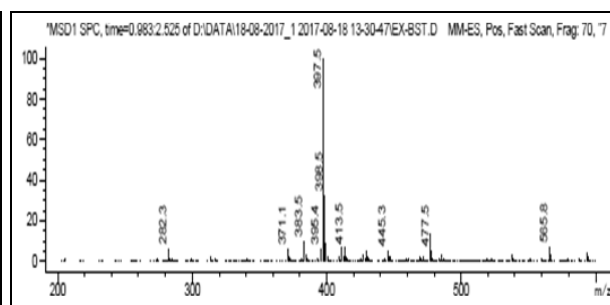
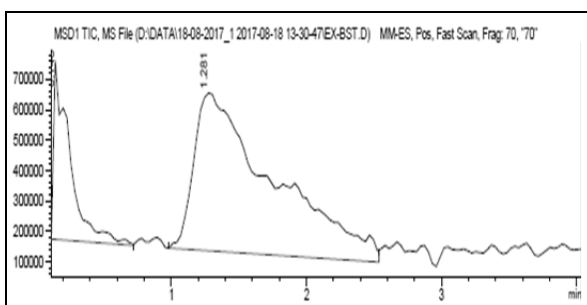


FIG. 3: LCMS SPECTRA OF ISOLATED COMPOUND

$^1\text{H-NMR}$ Spectral Analysis: $^1\text{H-NMR}$ (CDCl_3 , 400MHz) of ST: $^1\text{H-NMR}$ has given signals at δ 3.2(1H, m, H-3), 5.26 (1H, m, H-6), 5.19(1H, m, H-23), 4.68(1H, m, H- 22), 3.638(1H, m, H-3), 2.38(1H, m, H-20), 1.8-2.0 (5H, m) ppm Other peaks are observed at δ 0.76-0.89 (m, 9H), 0.91-1.05 (m, 5H), 1.35-1.42 (m, 4H), 0.69-0.73 (m, 3H), 1.8-2.00 (m, 5H), 1.07-1.13 (m, 3H), 1.35-1.6 (m, 9H) ppm.

$^{13}\text{C-NMR}$ Spectral Analysis: $^{13}\text{C-NMR}$ (CDCl_3 , 100MHz): $^{13}\text{C-NMR}$ has given signal at 140.77

(C-22), 121.7, 77.30 (C-3), 56.51(C- 14), 54.38 (C-17), 50.14 (C-9), 45.83 (C-9), 42.33 (C-20), 40.05.1(C-12), 39.78 (C-13), 38.84 (C-4), 37.27 (C-12), 37.02 (C-1), 3.51(C-10), 36.16 (C-8), 35.89(C-20), 35.46 (C-22), 33.95 (C-7), 32.42 (C-8), 29.16 (C- 25), 29.71 (C-16), 28.75 (C-2), 28.26 (C-15), 27.4 (C-28), 26.09 (C-11, 26), 24.31 (C-27), 19.83 (C-19), 18.71 (C-21), 15.6 (C-18, 29). The above IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and LC-MS spectral data and their comparison with those described in the literatures showed the structure of β -sitosterol.

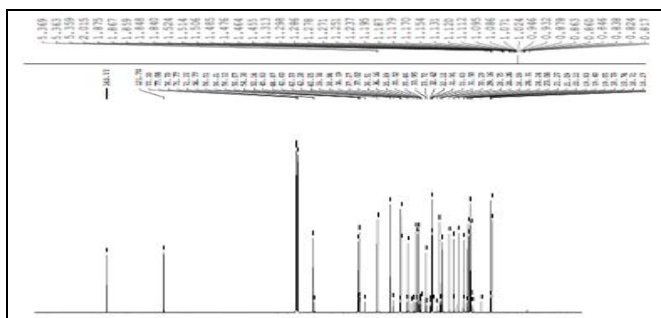


FIG. 4: NMR SPECTRA OF ISOLATED COMPOUND

Identification of Compound: After various spectral analysis, comparing the structure with spectral libraries and chemical tests the isolated compound was identified as $C_{29}H_{50}O$ β -sitosterol during interpretation.

CONCLUSION: The therapeutic effects of *Cordia dichotoma* have not been attributed to a specific compound however biological investigations have shown that β -sitosterol like phytosterol acts to attenuate heart disease and high cholesterol. It is also used for boosting the immune system and for preventing colon cancer, as well as for gallstones, hair loss, bronchitis, migraine and headache. Some men use β -sitosterol for enlarged prostate (benign prostatic hyperplasia or BPH). Some women use it for symptoms of menopause. β -sitosterol isolated from *Cordia dichotoma* L. could have the good future scope of this cheap medicinal plant.

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CONFLICT OF INTEREST: Nil

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