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IN VITRO ANTIMICROBIAL ACTIVITY OF *β*-SITOSTEROL IDENTIFIED IN PETROLEUM ETHER EXTRACT OF FRUITS OF *HELICTERES ISORA* LINN

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ABSTRACT

Helicteres isora L is belongs to family Sterculiacae is a sub-deciduous shrub or small tree. . The species is native to Asia and Australia. It occurs, throughout India, from Jamuna eastwards to Nepal, Bihar and Bengal and southern India and Andaman Islands.The fruits are ground and used in colic pain, gripping bowels and flatulence. The fruits are astringent, acrid, refrigerant, demulcent, constipating, stomachic, vulnerary, haemostatic and urinary astringent. They are useful in colic, flatulence, diarrhoea, dysentery, wounds; ulcers, haemorrhages, and diabetes .The fruit extract of possess weak anti-HIV (I) activity. The root and stem barks are considered to be expectorant, demulcent, astringent and anti-galactagogue and are useful in colic, scabies, emphysema, gastropathy, diabetes, diarrhoea and dysentery. The fruits were also found to possess significant antispasmodic activity. In the present investigation, β- sitosterol was extracted by using soxhlet extraction method from the fruits of *Helicters isora* Linn and characterized by IR, spectral analysis. Initial results obtained from thin layer chromatography (TLC) showed the presence of β- sitosterol in the fruits sample with the R*f* value of 0.55. Further IR analysis of eluted bands showed the broad peak at 3426.3, 2936, 2832, 2366, and 1596.4 cm-1. Extracted β- sitosterol was further used to determine its antimicrobial activity by agar disk diffusion method. The antimicrobial activity ranged from 10 mm to 14 mm for *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus*. In comparison

with standard antibacterial compound Ampicillin (20µg/ml), it was found that purified β-sitosterol (20µg/ml) has antimicrobial activity almost equivalent to the standard.

KEYWORDS: *Helicteres isora* Linn, Fruit, Soxhelet extraction, IR, β-sitosterol, Antimicrobial activity,

INTRODUCTION

Helicteres isora L is belongs to family Sterculiacae, commonly known as East Indian screw tree, is a medicinally important, sub-deciduous shrub or small tree of reaching a height of 5-15 feet. Almost all parts of the plant are used in traditional medicinal system for curing various diseases.[1-3] Water extract of the fruits exhibits anti-HIV activity.[4] and reported to possess antispasmodic activity.[5] The roots showed significant hypolipidaemic and hypoglycaemic.[6-8] while bark has shown significant hypoglycaemic activity.[2] The species is native to Asia and Australia. It occurs throughout India.The fruits are ground and used in colic pain, gripping bowels and flatulence. The fruits are astringent, acrid, refrigerant, demulcent, constipating, stomachic, vermifuge, vulnerary, haemostatic and urinary astringent. They are useful in colic, flatulence, diarrhoea, dysentery, verminosis, wounds, ulcers, hemorrhages, epistaxis and diabetes .The fruit extract of possess weak anti-HIV (I) activity. The root and stem barks are considered to be expectorant, demulcent, astringent and anti-galactagogue and are useful in colic, scabies, empyema, gastropathy, diabetes, diarrhoea and dysentery.The fruits were also found to possess significant antispasmodic activity. chemical structure of β- sitosterol is similar to the cholesterol(fig1) . It is considered as a good biomarker due to its biological activity.[9] β- sitosterol is used as an antioxidant and an antidiabetic agent.[10] The Present investigation is aimed to isolate the β- sitosterol from petroleum ether extract of fruits of of *Helicteres isora* L, characterize by IR,UV,MS and NMR spectral analysis and to determine its antimicrobial activity against drug resistant microbes.

MATERIALS AND METHODS

Plant collection and identification

Shade dried fruits of *Helicteres isora* Linn. were obtained from the Himalaya Drug company, Dehradun, Uttarakhand. The specimen was identified and confirmed by the Botanical Survey of India, Dehradun, India.

Experimental Procedure

The solvents and chemicals used for this work are laboratory grade petroleum ether, chloroform, methanol, acetic anhydride, ferric chloride, glacial acetic acid, and sulphuric acid. Column chromatography (CC) was conducted on silica gel 60 (Kieselgel 100-200 µm particle size, E. Merck, Darmstadt, Germany) and TLC was performed on silica gel 60 F_{254} precoated aluminium plates (layer thickness 0.2 mm, E. Merck, Darmstadt, Germany). IR spectra were measured on a Shimadzu FTIR DRX-500 in KBr pellets. ^{1}H NMR (400 MHz), ^{13}C NMR (150 MHz) and 2D NMR spectra were recorded on Bruker Ultrashield 400 spectrophotometer with TMS as the internal standard.

Preparation and Extraction of Plant material

The fruits (2.0 kg) of *H. isora* were shade dried at room temperature, chopped into small pieces was exhaustively defatted with petroleum ether (60-80°C) and successively extracted with chloroform ($CHCl_3$) and then finally methanol (MeOH) using the soxhlet apparatus for 6 hours. Removal of the solvents under reduced pressure gave the respective extracts.

The yield of the extracts was determined to be 12 gm, 22 gm and 55 gm, respectively. TLC examination of petroleum ether extracts showed good patterns. Hence these extracts were used for chemical examination. These extracts were concentrated in vacuum and stored at 4°C until examined.

FIG -1 : Prepared plant material of *H. isora* fruit parts for analysis

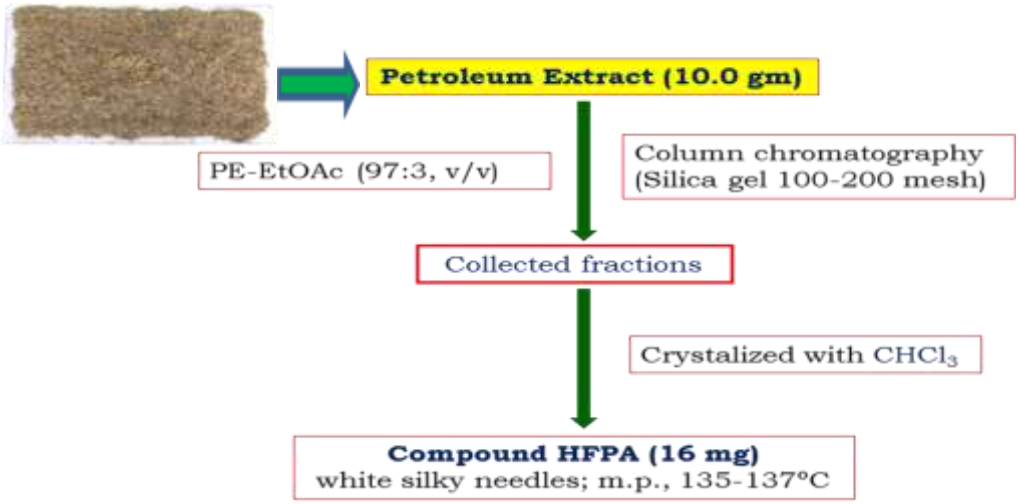
EXAMINATION OF PETROLEUM ETHER EXTRACT

The petroleum ether extract (6.0 gm) was column chromatographed over silica gel and eluted with varying amount of petroleum ether and ethyl acetate (EtOAc). Different fractions of 50 mL each were collected, examined on TLC and combined based on their TLC behavior. The

column on elution with petroleum ether-EtOAc (97:3, v/v) afforded one pure compound HFPA.

COMPOUND HFPA

Fraction eluted with petroleum ether-EtOAc (98:2, v/v) on concentration yielded a white solid which on crystallization from chloroform gave white silky needles (16 mg), m.p. 135-137°C). It gave pink to green color in Libermann-Burchard test indicative of a sterol. Melting point with an authentic sample of beta sitosterol was undepressed. Co-TLC and superimposable IR with the authentic sample confirmed its identity.



FTIR SPECRTUM

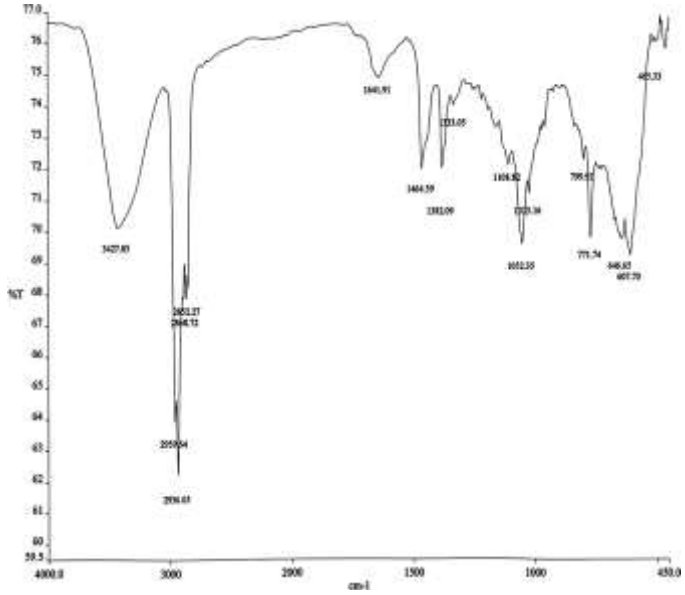


Fig 2 The FTIR spectrum

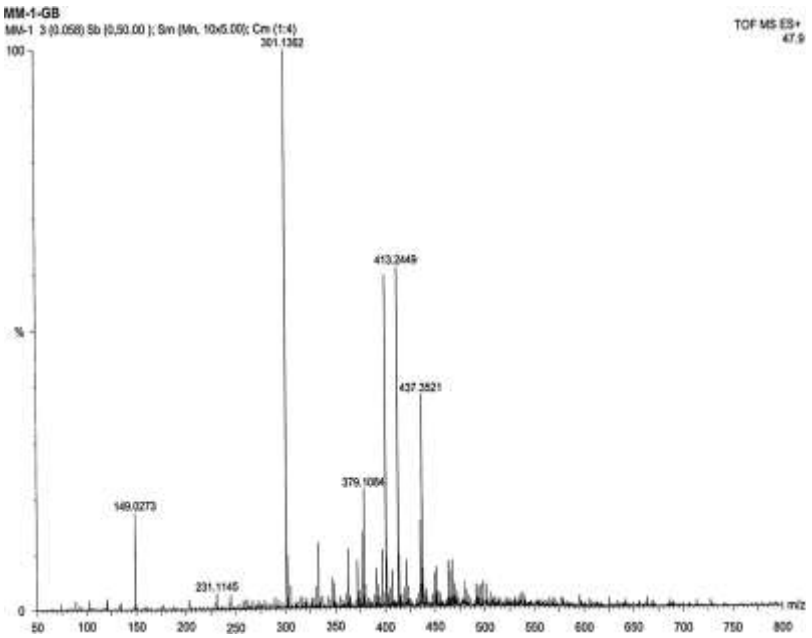


Fig 3 HR–ESI–MS spectrum

ANTIMICROBIAL ACTIVITY OF *β*-SITOSTEROL

The antimicrobial activity of the extracted compound was determined by the agar disk diffusion method of protocol given by Doughari, [11] with little modification. Ampicillin was used as a standard antimicrobial agent. *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* were used as the test organisms which were obtained from the department of Microbiology, SGRR Institute of medical and health sciences ,dehradun. The 6 mm sized disk were cut from Whatman filter paper and dipped in 20 µg/ml solution of puried β -sitosterol solution and placed on the agar plate. Similarly, filter paper discs were dipped in a Amicillin solution (20µg/ml) and used as control. All the plates were incubated at 300C for 24-48 hour. Antimicrobial activity was determined by measuring the "zone of inhibition" in millimeters as per the method of Jagessar *et al.*,[12]

RESULTS AND DISCUSSION

CHARACTERIZATION OF COMPOUND HFPA

This was obtained from the fractions eluted with petroleum ether-EtOAc (97:3, v/v) as a white solid which on crystallization from chloroform gave white silky needles (16 mg), m.p. 135-137°C. It responded positively Libermann-Burchard test which indicated the presence of a sterol moiety. Comparison of HFPA with the authentic (mixed melting point, Co-TLC and superimposable IR) confirmed its identity as *β*-sitosterol. Samples were sent to Depatment of

Instrumentation, IIT Roorkee, Uttarakhand, India for IR ,HR-MS and NMR spectral analysis.

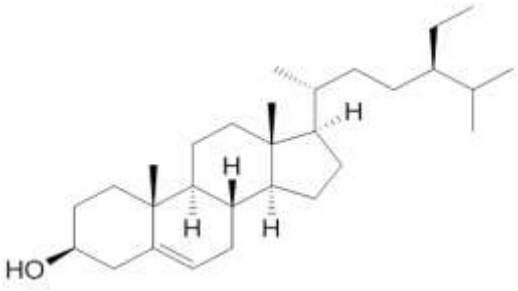


Figure 4: β-sitosterol

During the research , β-sitosterol was isolated from the fruits of *Helicteres isora* Linn by the soxhlet extraction method. β-sitosterol crystals were 'colourless needles' with the melting point 136-137°C. Dried crystals were reconstituted in chloroform and spotted on silica TLC plates. Presence of β-sitosterol was detected on plates as the pink spot at the R*f* value of 0.55 on staining with 50% H_2SO4 which is similar to the earlier reported by Karan *et al.*,.[13] Presence of β-sitosterol has been reported in various plants, such as leaves of *Ocimum sanctum*.[14] rhizomes of the *Stylochiton lancifolius.* [15], fruits of *Corylus colurna* Linn.[16] and *Solanum xanthocarpum*.[17] as well as in the tissue cultures of *Adhatoda vasica* & *Ageratum conyzoides*[18] and cell suspension culture of *Chrysanthemum coronarium* L.[19]The IR spectral analysis revealed a broad peak at 3426.3 cm-1 for OH group (Figure-2). The peaks at 2936, 2832, 2366 cm-1 indicate C=C group. The peaks at 1596.4 and 1032 cm-1 indicating the presence of C=O and C-O stretches, respectively. These data are comparable to the spectral data for β-sitosterol reported earlier.[20] Elemental analysis and molecular weight determination indicated C_{29}-$H_{50}O$ as its molecular formula. Similar results were reported by Karan *et al.*, (2012) in which IR peaks were obtained at 3426.89, 2924.52, 2855.1, 1738.51, 1057.31 cm-1.

ANTIMICROBIAL ACTIVITY OF *β*-SITOSTEROL

In the present study, the antimicrobial activity of the purified β-sitosterol was evaluated and the results are summarized in the Table-1;

Table: 1 - Antimicrobial activity of β-sitosterol

	Diameter of Zone of inhibition	
Test organism	**β-sitosterol**	**Ampicillin**
1 . *E.Coli*	14mm	18 mm

2. *Staphylococcus aureus*	13mm	18 mm
1. *Pseudomonas aeruginosa*	12mm	17 mm
4. *Bacillus cereus*	10mm	16 mm
5. *Staphylococcus mutans*	11*mm*	*16 mm*
5. *Aspergillus flavus*	10 mm	16 mm

The antimicrobial activity, determined by measuring the Zone of inhibition around the disc, ranged from 10 mm to 14 mm for the β- sitosterol. Ampicillin showed the inhibition zone for *E. coli* (18 mm), *S. aureus* (18 mm), *P. aeruginosa* (17 mm), *Bacillus cereus* (16 mm), *Staphylococcus mutans*(16 mm), *Aspergillus flavus*(16 mm), while β-sitosterol recorded 14 mm (*E. coli*), 13 mm (*S. aureus)*, 12 mm (*P. aeruginosa*), 10 mm (*Bacillus cereus*),11 mm(*Staphylococcus mutans*), 10 mm(*Aspergillus flavus*). Thus, the antimicrobial activity of the β-sitosterol (10mm to 14mm) was comparable to the standard Ampicillin (18 mm to 16 mm). Hence β- sitoterol is having higher antimicrobial activity against *E. coli* followed by *S. aureus*, *P. aeruginosa* and *Bacillus cereus* at lower concentration. Similar results were reported with the crude extracts of different plants containing β-sitoterol against various microorganisms (21,22). However, the concentrations used to be very high as compared to the present results. Thus these results prove the antimicrobial potential of β-sitosterol from the *Helicteres isora* and further, it can serve as an alternative chemical to treat the bacterial and fungal diseases.

CONCLUSION

β-sitosterol is a natural micro-nutrient which is found in the cells and membranes of all oil producing plants, fruit, vegetables, grains, seeds and trees. It has been proven to be a safe, natural and effective nutritional supplement and has shown amazing potential benefits in many diverse applications. Earlier experimental studies have shown its effectiveness as an anti-diabetic, antioxidant, anti-cancer, anti-ulcer, anti-inflammatory, antipyretic and anti-stress agent. This natural micro-nutrient is also an effective immune booster and used in the treatment of prostate enlargement and HIV. Our study contributes to establish β-sitosterol as potent antimicrobial agent at lower concentration against a wide range of bacteria. However, still more scientific evaluation and clinical trials are required to establish its therapeutic efficacy.

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