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Research Article

ISOLATION AND CHARACTERIZATION OF PHYTOSTEROLS FROM *CORDIA MACLEODII* (HOOK F. AND THOMSON) BARK BY CHROMATOGRAPHIC AND SPECTROSCOPIC METHOD

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ABSTRACT

Aims and objectives: The study focuses on isolation and determination of the chemical constituents of the Cordia macleodii bark, used as medicinal plant in folklore system. The principal theme of the study is to develop applied chromatographic techniques for the separation, isolation and detection of the compounds.

Methods: A petroleum extract of bark were analyzed by GC/MS, IR, and UV. The structures were elucidated on the basis GC-MS library of reported data.

Result: Three known compounds Stigmasterol, Cholest-5-EN -3OL (3 Beta)-Carbonyl chlorinated, Camphesterol were determined from Cordia macleodii bark. These compounds were isolated from this plant for the first time.

Conclusion: From the present study, it is concluded that chromatographic and spectroscopy has potential as rapid and simple tools in the isolation and analysis of various compounds from Cordia macleodii bark.

Keywords: Gas chromatography-mass spectrometry, Infrared, Chromatography, Spectroscopy, Phytosterols, Cordia macleodii.

INTRODUCTION

The sterols and related compounds are normally found in the unsaponiable portions of plant materials. Sterols, especially, phytosterols have wide spread distribution in higher plants. They are grouped under triterpenes having cyclopentane perhydrophenanthrene ring system. They occur both in free form and as simple glucosides. Examples are sitosterol, stigmasterol, camphesterol etc. Phytosterols have distinct structural differences in comparison to animals sterols. Some of the animal sterols have been reported in certain plants [1,2]. Ecodyner, the insect mouthing hormones are also reported from some plants. It is speculated that they may serve as a protective measure against insect predation [3]. β-sitosterol and camphesterol and stigmasterol isolated from certain plants [4,5] are reported to possess anti-inflammatory activity.

Phytosterols and phytostanols, also referred to as plant sterols and stanols, are common plant and vegetable constituents and are, therefore, normal constituents of the human diet. They are structurally related to cholesterol but differ from the side chain.

The phytosterols are 28- and 29- carbon sterols, as distinct from cholesterol, which is a 27- carbon sterol. Cholesterol is the predominant sterol in animal fats and fish oils, but is very rare in vegetable oils. Both delta-5- and delta-7 sterols occur in vegetable oils. The delta-5-sterols have a double bond between carbons 5 and 6 in the steroid skeleton, whereas the delta-7-sterols have one between carbons 7 and 8. The major phytosterols are camphesterol, stigmasterol, and beta-sitosterol, all of which are delta-5-sterols. Camphesterol and beta-sitosterol has a saturated side chain; while the side chain of stigmasterol is unsaturated. The predominant 4 monomethylsterols in vegetable oils, obtusifoliol, gramisterol, cycloeucalenol and citrostadienol, are all delta-7 and delta-8 sterols, with the exception of cycloeucalenol, whose steroid skeleton has a 9, 19-propane ring. The most common 4, 4-dimethylsterols in vegetable oils, cycloartenol and 24-methylenecycloartanol, are also 9, 19-propanesterols [6,7].

These compounds contain a total of 27-30 carbon atoms the number of carbon atoms in the biosynthetic precursor squalene oxide). Their structures are closely related and varied depending on the extent of modifications of the ring system and side chain variations. Sterols are known to have a wide range of biological activities and physical properties. Plant sterols (that is., phytosterols), in particular, are important agricultural products for health and nutrition industries. They are useful emulsifiers for cosmetic manufacturers and supply the majority of steroidal intermediates and precursors for the production of hormone pharmaceuticals [8]. A number of plant sterol with specific structures is known to inhibit oxidative deterioration of oils serving as potential antipolymerization agents for frying oils. Hypocholesterolemic activities of some phytosterols (e.g., soy sterols, vegetable oil components and sitosterol) have been documented [9-12]. The saturated analogues of phytosterols, and their esters have been suggested as effective cholesterol-lowering agents offering cardiologic health benefits [12].

Cordia macleodii (Boraginaceae) commonly known as Dahipalas (Hindi). Bark is used for the treatment of jaundice. Wonderful wound-healer Bargarh district, Orissa [13]. Preliminary phytochemical analysis of C. macleodii bark indicated the presence of relatively high levels of flavonoids, alkaloids, steroids and terpenoids. It is necessary to establish specific method to isolate this molecule or fraction, and they should standardize. This research activity has contributed to new or renewed public interests worldwide in phyto-medicines.

METHODS

Plant materials

Fresh bark of C. macleodii collected from Orissa, India-in 2009 and the collected samples were identified, authentified by using various floras and texts and also matched with Pharmacognosy Departmental Herbarium no. Ref: No: 6046, I.P.G.T and R.A. of Gujarat Ayu University-Jamnagar, India.

Preliminary phytochemicals screening of the extract

The methanolic extract was testing to detect for the presence of different chemical groups of compounds as per the methods described in active pharmaceutical ingredient. Preliminary phytochemicals screening shows the presences of relatively high levels of flavonoid, alkaloids, steroids terpenoids, tannin, coumarines, etc. Isolation and identification of the marker compound from the bark and further investigation was under study.

Preparation of extract

The whole plant material was bark was shade-dried and crushed to make a coarse powder. The powder (660 g) was extracted (defeated) with 3 L of petrol-ether (60°-80°) by continuous extraction method (Soxhlet extraction) for 48 hrs. Solvent was distilled off, the extract was concentrated and dried under reduced pressure, which yielded a brownish mass. This crude extract was used for further investigation for potential of un-saponifiable properties.

Experimental

Separation of un-saponifiable fraction [14]

An accurately weighed pet-ether was taken in flask and added 40 ml of 20% methanolic potassium hydroxide and kept overnight at room temperature. Then next day the mixture was refluxed for 6 hrs. Moreover, then cooled at room temperature. Then add twice of its volume of distilled water and extracted with ether. Then combined ethereal extract was washed with distilled water till neutral to litmus paper and dried over anhydrous sodium sulfate. After this, the ether was evaporated to obtain unsaponifiable fraction (Fraction A). The aqueous portion left after ether extraction was named as saponifiable fraction. It was extracted acidified with 5 N H_2SO_4 and the aqueous layer was extracted 3-4 times with ether. Then ethereal layer was washed with distilled water and extractive dried over anhydrous Na_2SO_4, evaporated to obtain fatty acid portion (Fraction B).

Isolation of the compound from the unsaponifiable fraction A

The unaponifiable fraction (Fraction A) was tested for sterol with libermann-buchard reagent. It gives strong positive test. Then this compound was subjected to thin layer chromatography for designing solvent system. Then the unaponifiable fraction was concentrated and dried. For a given separation, column chromatography was carried out on Kieselgel 60 (60-70-mesh) (Merck), long narrow column. A fritted-glass disk may be seated at the end of the tube to act as a support for the packing material. The column is fitted at the end with a stopcock. A small fraction of dried sample was chromatographed on 30-50 times of its volume of silica gel 60-80° mesh size and prepared column. Isolation of the compound achieved using isocratic elution, with a solvent system n-hexane:di ethyl ether:GAA (7:3:0.5) to give 3 fractions. For these fractions were monitored on thin layer chromatography. Further fraction 1, 2, 3 were analyzed by infrared (IR), ultraviolet (UV)-visible and gas chromatography-mass spectrometry (GC-MS).

GC-MS protocol

GC-MS analysis was carried out on a Shimadzu GC-MS model no. QP 2010. sampler and gas chromatograph interfaced to a mass spectrometers (GC-MS) instrument employing the following conditions: Column elite-1 fused silica capillary column (30 mm×0.25 mm ID×1 µ M df, composed of 100% dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/minutes and an injection volume as per requirement (split ratio of 10:1) injector temperature 250°C; ion-source temperature 300°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minutes, to 200°C, then 5°C/minutes to 280°C, ending with an isothermal at 310°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da.

Fourier transform (FT) IR spectroscopy and UV/Visible protocol

FTIR analyses were carried out on model no. IR-200 thermo nicolet series instrument. Samples were ground with spectroscopy grade KBr, and a pellet of a homogeneous mixture was prepared using a hydraulic press. Care was taken for the maximum opacity of the pellet, and IR spectra was recorded between 4000/cm and 400/cm. A systronic λ-2 double beam UV/visible spectrophotometer-2201 was used for all spectral analysis. The instrument was preloaded with software, and the spectra were recorded in the range of 190-700 nm with background correction.

RESULTS AND DISCUSSION

Identification of components

Identification and interpretation of compound by mass spectrum GC-MS was conducted using a database of National Institute Standard and Technology having more than 62,000 patterns and also Nibs. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. This compound also characterization by IR and UV. Isolated fraction of unsaponifiable of C. macleodii bark was subjected to spectral detection.

Compound 1 separated at retention time-35.53 and its molecular weight is 412, GC-MS fragment, had a mass $[M+H]^+$ 412. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments m/z 55 (100), 83, 81, 41, 105, 145, 159, 255, 394, and 412. In IR spectral analysis presence of -OH group (peak at 3600/cm), -CH3, -CH2 group (peak at 2949 and 2847/cm respectively), and unsaturation (peak at 1739 and 1663/cm) were observed C=O group, aromatic C=C (peak at 1539, 1508/cm), and 1096/cm indicate secondary alcohol, 773, 742, 669/cm are in plane and out of plane banding (Fig. 1a-c).

Compound 2 separated at RT-37.13 and its MW is 448, GC-MS fragment of compound had a mass $[M+H]^+$ 448. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments m/z 43 (100), 55, 81, 41, 105, 159, 213, 255, 394, 412, 414, and 448. IR spectral analysis presence of -OH group (peak at 3651/cm), -CH3, -CH2 group (peak at 2968, 2931, 2912, 2850/cm respectively), and unsaturation (peak at 1710) were observed C=O group, aromatic C=C (peak at 1587, 1458/cm), and 1099/cm indicate secondary alcohol, 956/cm for C=C aro. Sub, 883-748/cm -O-sub and P-sub of benzene and 717,656,601/cm are in a plane and out of plane banding (Fig. 2a-c).

Compound 3 separated at RT-25.56 with GC-MS fragment, had a mass $[M+H]^+$ 400. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments m/z 43 (100), 55, 73, 98, 129, 145, 157, 213, 255, 347, 400. In IR spectral analysis presence of -OH group (peak at 3479/cm), -CH3, -CH2 group (peak at 2937, 2912, 2848/cm respectively), unsaturation (peak at 1739 and 1707/cm) were indicates C=O group (cyclic ketone), aromatic C=C (peak at 1591, 1583/cm), C-H, 1157/cm indicate tertiary alcohol,

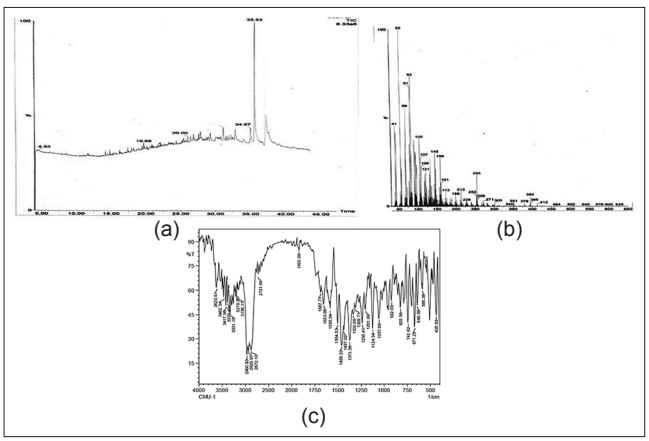

Fig. 1: Gas chromatography graph (a), mass spectrometry graph (b) and infrared graph (c) of isolated compound-1 from unsaponifiable fraction

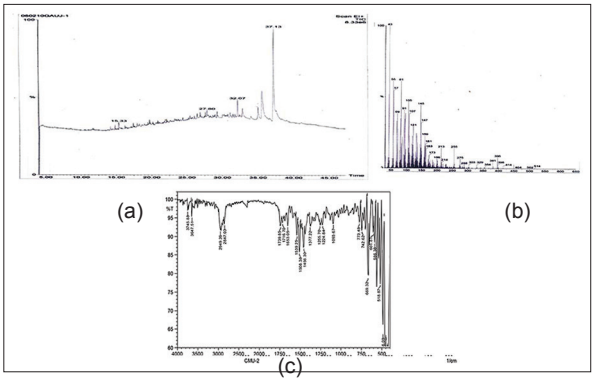

Fig. 2: Gas chromatography graph (a), mass spectrometry graph (b) and infrared graph (c) of isolated compound-2 from unsaponifiable fraction

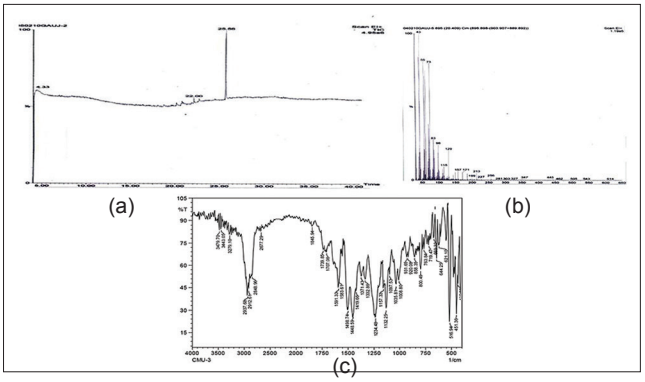

Fig. 3: Gas chromatography graph (a), mass spectrometry graph (b) and infrared graph (c) of isolated compound-3 from unsaponifiable fraction

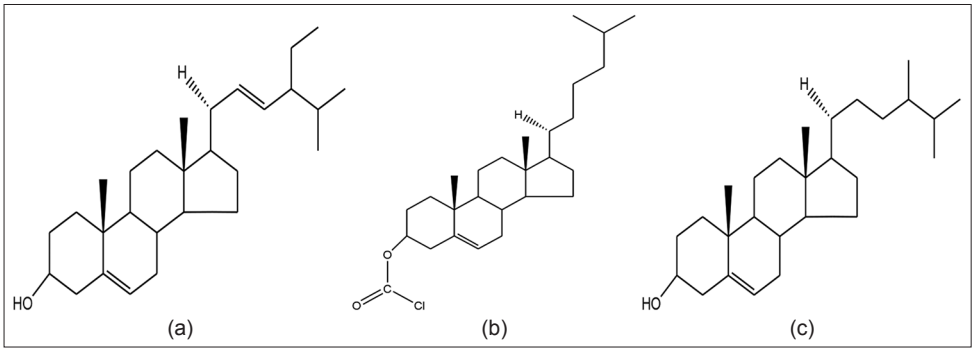

Fig. 4: Structure of the compound (a) Stigmasterol (b) Cholest-5-EN -3OL (3-beta) -carbonyl chlorinated (c) Camphesterol

800-773/cm- meta di sub of benzene and 742,669/cm are in plane and out of plane banding (Fig. 3a-b).

The UV-spectra of samples is taken to identify the presence of basic chromophores to support the structural elucidate of the samples. UV-spectra of compound for $1_{\lambda max}$ at around 220-270 nm, for the compound $2_{\lambda max}$ at around 220-280 nm and for compound $3_{\lambda max}$ at around 250-340 nm was observed which suggest the presence of extended S-bond system, aromatic rings and phenolic-OH group.

The sterol fraction was analyzed by GC/MS. From above spectra tentatively identified three compounds. The spectral analysis enabled us to identify 3 known compounds, stigmasterol, cholest-5-EN -3OL (3 beta) -carbonyl chlorinated, camphesterol, were identified within this study. The structure of these compounds was confirmed by comparison with published data in GCMS library. β-sitosterol and camphesterol and stigmasterol isolated from certain plants [4,5] are reported to possess anti-inflammatory activity all these compounds are isolated from C. macleodii bark for the first time and compounds 1, 2, 3, though all are already known from the Boraginaceae family (Figure 4).

CONCLUSION

The study permitted the isolation of three known compounds from the petroleum extract of the bark of C. macleodii it is being new for the species. The majority of them have reported biological activity, which support the traditional use of the plant. Some of the compounds have other interesting biological effects, for which the bark of C. macleodii could be investigated. The results obtained with the phytochemical analysis, extraction techniques, chromatographic and spectroscopy methods indicated their potential as rapid and simple tools in the isolation and analysis of various natural products. It is, however, evident that further experiments are still needed in the studied areas especially with activity guided isolation in order to reach their full potential in natural product chemistry.

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