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Research Article

ISOLATION OF STIGMASTEROL AND β-SITOSTEROL FROM PETROLEUM ETHER EXTRACT OF AERIAL PARTS OF *AGERATUM CONYZOIDES* (ASTERACEAE)

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ABSTRACT

General phytochemical screening of the aerial parts of *Ageratum conyzoides* (Asteraceae) revealed the presence of steroids, terpenes, phenolic compounds, saponins, fatty acids, alkaloids. The aim of this study is to identify and characterize the bioactive principle from the aerial parts of the plant. It has wide folk medicinal use. For isolation of the compound, the dried aerial parts powder of *Ageratum conyzoides* was subjected to hot extraction with petroleum ether; this extract was saponified with alcoholic KOH and subjected to chromatography. Isolated compound were purified by chloroform. The isolation and purification afforded white crystalline powder which was subjected to physical, chemical and spectral identification by IR, ^{1}H-NMR, ^{13}C-NMR and GC-MS. The compound was concluded as stigmasterol and β-sitosterol.

Keywords: *Ageratum conyzoides*, Phytochemical, Stigmasterol, β-sitosterol, Fatty acids.

INTRODUCTION

The use of plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available. However, the systemic study of plants for detecting antimicrobial activity is of comparatively recent origin[15]. These investigations have been triggered by the emergence and spread of antibiotic resistant microorganisms causing the effective life-span of existing antibiotics limited. Hence the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of new anti-infective agents.

Ageratum conyzoides is a small herbaceous plant belongs to the family Asteraceae[1,2]. It is softly hairy, erect, branched, annual weed up to 80-90 cm in height. It is a tropical plant used in various parts of Africa, Asia and South America for curing various diseases. The plant has been reputedly being used as purgative, febrifuge, for opthalmia, colic, treatment of ulcers and wound dressing [3-5]. The antienteralgic and antipyretic properties of the plant were also indicated in a review on 'Medicinal plants from Senegal' [6]. In some African countries, the plant has been popularly use for skin diseases, wound healing, mental and infectious diseases, headaches and dyspnea, used in traditional medicine for its antiasthmatic, antispasmodic and haemostatic effects, uterine troubles, pneumonia by rubbing them on the chest of the patient [7-10]. Its roots, leaves, flowers and whole plants are used as medicine. A wide range of chemical compounds including alkaloids, coumarins, flavonoids, chromenes, benzofurans, sterols and terpenoids are present in this species.

The purpose of this study is to identify and characterize the bioactive principles from the aerial part of *Ageratum conyzoides.* In this paper, we report the isolation and characterization of known compounds from *Ageratum conyzoides* namely stigmasterol and beta-sitosterol.

EXPERIMENTAL

Collection, Identification and preparation of plant materials

The aerial parts of the plant were collected from Herbal Nature Park, Chuharpur, Yamuna Nagar, Haryana in the month September 2007. The plant was taxonomically identified, authenticated by Professor Dr. J. S. Sodhi, HOD, Botany Department, Guru Nanak Khalsa College, Yamuna Nagar, Kurukshetra University, Haryana and deposited with A. R. College of Pharmacy, Vallabh Vidhya Nagar, Gujarat. The aerial parts of the plant were manually separated was air dried, powdered, sieved, weighed and stored in air tight container and subsequently referred to as powdered drug.

Extraction and Isolation

Powdered (400g) aerial parts of *Ageratum conyzoides* was defatted exhaustively with petroleum ether (60-80°c) in a soxhlet extractor. The solvent was recovered under pressure to obtained dark greenish brown oily mass (5.6g), which was labeled as petroleum ether extracts (PEE) and kept in the refrigerator. The resulting marc was air dried at room temperature and then exhaustively extracted successively with solvents with increased polarity and concentrated under reduced pressure and labeled accordingly. The petroleum ether extract of aerial parts of the plant was saponified using 1M alcoholic KOH, to remove fatty material and then subsequently picked up in petroleum ether and the solvent was evaporated to yield 3g of unsaponified matter. This fraction contains lesser number of components than the unsaponified extract [16].

Chromatographic separation

A small quantity of unsaponifiable matter was dissolved in chloroform and this solution is spotted on TLC plates using pre-coated aluminium with silica gel 60 $F_{254.}$ Then the TLC plates were run by specific solvent system and viewed individually under UV light and also (5%) sulphuric acid in methanol reagent. Through several pilot experiments it was found that the compounds of unsaponifiable fraction were separated by the solvent system of chloroform and ethanol in the proportion of 9.8:0.2. The chromatograms when developed in iodine chamber yielded six to seven spots respectively and three spots at Rf (0.43, 0.64, 0.95) becomes reddish brown soon turns to purple or violet indicate zones for steroidal nucleus. Column chromatography of PEE was conducted using silica gel (Mesh 60-120) that was packed using wet packing method in hexane. The column was run using hexane, chloroform and methanol by gradient elution technique. TLC was used to monitor the eluates. A total of 158 eluates were collected. Similar fractions were pooled together. Further purification is carried out using preparative TLC. Spots were identified, scraped and eluates using petroleum ether and chloroform as solvents [15, 16].

Finally eluate ST yielded a single spot when subjected to TLC using several solvent systems including chloroform: ethanol (9.8:0.2), ethyl acetate: ethanol (9.8: 0.2), chloroform: ethyl acetate (4:1) and it showed to be homogenous compound. ST a white crystalline powder (100mg) with melting point (144-146°C) was further subjected to IR, Proton NMR (400MHz), Carbon-13 NMR (100 MHz) and GC-MS to ascertain the chemical structure.

Tests for alcohol

4g of cerric ammonium nitrate was dissolved in 10ml of 2N HNO_3, on mild heating. A few crystals of isolated compound were dissolved in

0.5ml of dioxane. The solution was added to 0.5ml of cerric ammoinium nitrate reagent and diluted to 1ml with dioxane and shaken well. The developed yellow to red color indicates the presence of an alcoholic hydroxyl group [18].

Tests for steroid

Salkowski reaction: A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. A reddish color was seen in the upper chloroform layer [18].

Liebermann-burchard reaction: A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by addition of 2-3 drops of acetic anhydride. Solution turned violet blue and finally green [18].

Spectroscopic characterization

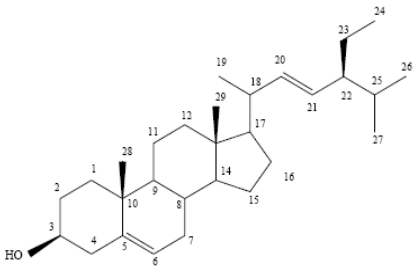
Different spectroscopic methods were used to elucidate the structure of isolated compound ST. Among the spectroscopic techniques IR, ^{1}H-NMR, ^{13}C-NMR and GC-MS were carried out. The infra red spectrum was recorded on FTIR Perkin Elmer, ^{1}H-NMR and ^{13}C-NMR spectra were recorded using $CDCl_3$ as solvent on Bruker Advance II 400 NMR spectrometer SAIF Panjab University, Chandigarh and GC-MS spectra were recorded at high resolution on a mass spectrometer (Perkin Elmer Auto system) at Sophisticated Instrumentation centre for Applied Research and Technology, Anand, Gujarat, India and the data are given in m/z values.

The IR absorption spectrum showed absorption peaks at 3373.6cm^{-1} (O-H stretching.); 2940.7 cm^{-1}and 2867.9cm^{-1} (aliphatic C-H stretching); 1641.6cm^{-1} (C=C absorption peak); other absorption peaks includes 1457.3cm^{-1} (CH_2); 1381.6cm^{-1} (OH def), 1038.7cm^{-1} (cycloalkane) and 881.6 cm^{-1}.

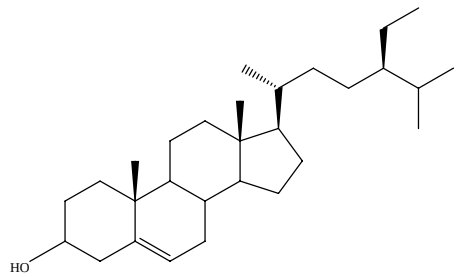
^{1}HNMR ($CDCl_3$, 400MHz) of ST: ^{1}HNMR has given signals at δ 3.2(1H, m, H-3), 5.26 (1H, m, H-6), 5.19(1H, m, H-23), 4.68(1H,m,H-22), 3.638(1H, m, H-3), 2.38(1H, m, H-20), 1.8-2.0 (5H, m) ppm. Other peaks are observed at δ 0.76-0.89 (m, 9H), 0.91-1.05 (m, 5H), 1.35-1.42 (m, 4H), 0.69-0.73 (m, 3H),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

^{13}CNMR ($CDCl_3$, 100MHz) of ST: ^{13}CNMR has given signal at 150.98, 145.2 (C-5), 139.8 (C-22), 121.7, 118.89(C-6), 79.03 (C-3), 55.3(C-14), 55.18(C-17), 50.45 (C-9), 48.3 (C-9), 40.8 (C-20), 40.1(C-12), 39.2 (C-13), 38.9 (C-4), 38.6 (C-12), 37.18 (C-1), 37.12 (C-10), 36.3 (C-8), 35.59(C-20), 34.29 (C-22), 34.24 (C-7), 32.66 (C-8), 29.86 (C-25), 29.71 (C-16), 28.41 (C-2), 28.1 (C-15), 27.4 (C-28), 26.1 (C-11, 26), 21.6 (C-27), 19.32 (C-19), 17.71 (C-21), 15.6 (C-18, 29).

FAB-MS spectroscopy showed the molecular ion peaks at 414 that correspond to molecular formula, $C_{29}H_{50}O$. Ion peaks were also observed at m/z 367, 271, 255, 229,189, 175, 161, 133, 121, 105, 107, 95, 81, 69, 55, 41.



Stigmasterol ($C_{29}H_{48}O$; Mol.Wt. 412.69)



β-sitosterol ($C_{29}H_{50}O$; Mol.Wt: 414.71)

RESULTS AND DISCUSSION

From the positive tests for steroids and alcohols given by ST, it is assumed to be a compound containing steroidal nucleus. The ST is white crystalline needles like substance with melting point 144-146^o c. On subjection to IR spectroscopic analysis, the observed absorption bands are 3373.6cm^{-1} that is characteristic of O-H stretching. Absorption at 2940.7 cm^{-1}and 2867.9 cm^{-1} is due aliphatic C-H stretching. Other absorption frequencies include 1641.6 cm^{-1} as a result C=C stretching however this band is weak, at 1457.3 cm^{-1} is a bending frequency for cyclic $(CH_2)n$ and 1381.6 cm^{-1} for $-CH_2(CH3)_2$ γ. The absorption frequency at 1038 cm^{-1} signifies cycloalkane. The out of plane C-H vibration of unsaturated part was observed at 881cm^{-1}. These absorption frequencies resemble the absorption frequencies observed for Stigmasterol. The proton NMR showed the proton of H-3 appeared as a multiplet at δ 3.2 and revealed the existence of signals for Olefinic proton at δ 5.19 (m), 4.68(m), 4.6(m) and 2.38(m). Angular methyl proton at 0.69(s), 0.80(s) and 1.02(s) corresponds to C_{18} and C_{19} proton respectively [13, 14].

The ^{13}C-NMR has shown recognizable signals 145.2 and 121.7 ppm, which are assigned C_5 and C_6 double bonds respectively as in Δ^5 spirostene [11]. The value at 19.32 ppm corresponds to angular carbon atom (C_{19}). Spectra show twenty nine carbon signal including six methyls, nine methylenes, eleven methane and three quaternary carbons. The alkene carbons appeared at δ145.2, 139.8, 121.7 and 118.89 [11, 13, 14].

The weak molecular ions were given at m/z 414 and the characteristic peaks were given at m/z 367 that corresponds, to (M-45) or loss of HO^+=CH-CH_3. These suggest that the sample ST contains two compounds with molecular weight of 414 and 412. Other ion peaks are m/z 271, 273 due to the formation of carbocation by β bond cleavage of side chain leading to the loss of $C_{10}H_{21}$ and $C_{10}H_{23}$ that corresponds to the M-141and M-143. The molecular weight and fragmentation pattern indicate that the compounds presenting ST are β-sitosterol and stigmasterol respectively.

The dehydration of fragment at m/z 273 would yield m/z 255, which on successive dealkylation would yield ions at m/z 188, 189, 175, 161, 148, 135, 121, 108, 95, 82, 69, 55, 41. The above I.R., ^{1}H-NMR, ^{13}C-NMR and MS spectral data and their comparison with those described in the literatures showed the structure of ST to be the mixture of β-sitosterol and stigmasterol in which may have maximum portion of stigmasterol. The only difference between the two compounds is the presence of C_{22}=C_{23} double bond in Stigmasterol and C_{22}-C_{23} single bond in β-sitosterol hence, the lack of practical difference in their R_f despite the use of several solvent systems. Furthermore, literatures have shown that sitosterol is difficult to be obtained in pure state [12-14, 17].

CONCLUSION

From the above findings, β-sitosterol and stigmasterol were isolated from petroleum ether extract of the aerial parts of *Ageratum conyzoides* and chemical structures elucidated respectively. It was carried out by means of various physical (solvent extraction, TLC, Column chromatography) and spectral techniques.

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