Isolation and Identification of Bioactive Compounds from *Mesosphaerum suaveolens* L. (Kuntze) Aerial parts

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Abstract

Mesosphaerum suaveolens L. (Kuntze) is a perennial, erect, annual and aromatic herb that may grow up to height of 2m. *Mesosphaerum suvaveolen*s methanolic extract fractionization was carried out in chloroform. From chloroform fraction triterpenoid was isolated by column chromatography. Triterpenoid identification did via chemical test, physical properties, TLC pattern. Then, the confirmation of chemical constituents was carried out by instrumental techniques as; UV, IR, MS and NMR. The tetracyclic triterpenoid stigmasterol structure interpreted at lastly. The ethanobotanical survey claim the valuable plant used for treating inflammation and rheumatoid arthritis (RA), No doubt, the further investigation must be needed for determining different pharmacological activity and medicinal use of *Mesosphaerum suaveolens.* But, at this level the present study claim that the stated activity is because of stigmasterol, because in inflammation and RA the steroids and triterpenoid play a vital role.

Keywords *Mesosphaerum suaveolens,* Fractionisation, Isolation, Triterpenoid,

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Introduction

Mesosphaerum suaveolens, synonym *Hyptis suaveolens* the pig nut or chan (Family-Lamiaceae) or commonly called as 'vilayti tulsi' in Hydrabad, India (Annonymous, 2019). Root decoction of *Mesosphaerum suvaveolens* vastly known for its appetizing property (Chatterjee, 1997). A previous study shows that the plant root of *Mesosphaerum suvaveolens* contains 3β-hydroxyl-20 (29)-en -27 oic acid (Triguna *et al*., 1983). Previously it was reported to contain natural HIV ingrane inhibitor acid. Previously the triterpeniods structure were obtain from the roots *Mesosphaerum suaveolens w*hich were similar to betulin and betulinic acid. In the present article tetracyclic triterpenoid was isolated, characterized and identified via chemical reaction and spectroscopic method. *Mesosphaerum suaveolens* is traditionally used for the treatment of lung infection, cold, pain, fever, cramp and skin disease. *Mesosphaerum suaveolens* has also been reported to active against *Candida albicans* and gram positive and gram negative bacteria (Triguna *et al*., 1983).

Material and Methods

Collection and authentication of plant material

Plant material was collected from the bank side of Kadva dam, Tal. Igatpuri, Nashik, Maharashtra, India in November 2018. The identity of the plant material was did by local botanist and voucher specimen of the plant material has been deposited at Institute level.

Plant Material

*Mesosphaerum suaveolen*s aerial part (leaves, flowers, stems, seeds) was air dried at normal temperature (37±0.5ºC) for 5 to 7 days. Aerial part was finely powdered it was then pass through sieve #38 and kept in zip pack polyethylene bags for further use. The proper precautions were taken while storing the powder drug.

Chemicals and Instruments

Soxhlet apparatus & other common glassware and instruments used for the study. Solvents viz. chloroform, methanol, ethanol, toluene, ethyl. acetate and other reagents were procured from Loba Chemicals, Mumbai, India.

Extraction of plant material

Approximately 100 g powder was use for extraction using Soxhlet apparatus using methanol as solvent for 7 to 8 hr. Methanolic extract of *Mesosphaerum suaveolen*s collected in round bottom flask was then subjected to dryness by evaporation on water bath with respect to recovery of solvent by distillation. The halves of the obtained methanolic extract fractionize by chloroform to obtain chloroform extract of *Mesosphaerum suaveolen*s.

Chromatographic Study

TLC was carried out for the chloroform extract using Chloroform: Methanol (8:0.6) solvent system. The standard stigmasterol is dissolved in chloroform and spots are place on adsorbent layer. The plate was dried at room temperature and locations of spots was determined by spraying 1% concentrated H_2SO_4 in methanol as it produce colored spot which are visible in day light. After spraying, the plate is then placed in an oven and colored spots are observed. Column chromatographic study carried out using, silica gel (60–120 mesh size) slurry in benzene. Then, in fractionization part of chloroform extract (70-80 mg) is dissolved in 20 ml of chloroform which further triturated with 800 mg-1g silica gel (60-120) in mortar until the chloroform is get evaporated. The sample is loaded into the column up to 10%. Then after different fraction are eluted by the addition of solvent of different ratio of Benzene: Ethyl acetate. The TLC was performed of every fraction to determine the spot (Ushir *et al.*, 2011).

Physical and Chemical Study

Isolated compound subjected for physical studies like organoleptic character, solubility, melting point and chemical test.

Structural Interpretation

The compound separated by column chromatography was then subjected for instrumentation analysis**.** The spot observed in a given fraction was subjected to recrystallization with chloroform and then it was subjected to following spectroscopic analysis (Fontanel *et al.*,

1998). The UV absorbance of the isolated compound was determined by the UV-Spectrophotometer- 1800 (Jasco made). For determining the various functional groups present in the isolated compound, IR spectroscopy (OMNIC Software) was done. NMR has been an extremely important tool for elucidation of molecular structure, especially the stereochemistry and configuration. The techniques reveal position of proton in a complex molecule. It is excellent choice for identification test in pharmaceutical analysis. NMR (Brucker advance II 400 Mhz) has found much application in the determination of impurities and minor components in mixtures because of ease, speed and specificity of the analysis.

Result and Discussion

Chromatographic Study

The TLC finger printing shows seven spots after spraying 1% concentrated sulphuric acid reagent (Table 1). The Rf value of stigmasterol in chloroform extract found to be 0.677, which matches with the standard stigmasterol. In TLC fraction 18 to 27 shows compounds with similar Rf value for solvent system Chloroform: Methanol (8:0.6) followed by 1 % H_2SO_4 spraying then kept at 50^{o}C in oven for 10 min. The fraction 18 to 27 prepared by mixing after evaporation of solvents it gives yellowish white solid mixtures of compounds. These mixtures of compounds subjected for preparative TLC to obtained single pure compounds by using the solvent system Chloroform: Methanol (9**:**0.6). From this one pure compound was obtained and labeled as; MSC-1. Further MSC-1 were subjected to spectroscopic analysis.

Physical and Chemical Study

Is a off white color powder, Soluble in Chloroform, methanol, ethyl acetate and ethanol, It's melting point is 175-177^{o}C. It also gives Salkovaski Chemical test positive which is confirmatory test for steroids.

Spectroscopic Analysis

The UV absorbance was determined at the wavelength (λmax) 271.50 nm with the comparison of the standard sample of stigmasterol at wavelength (λmax) 271.50 nm. The wave numbers found in the FTIR spectra are identified by correlating with standard wave number given in (Silverstain and Websters, 2007). The wave number for O-H stretch (3353.62 cm^{-1}) was found which identical assignment for stigmasterol is as it shows 1-OH group carbon number 3 position (Fig. 1). C-H stretching observed in IR spectrum of MSC-1 at absorbance 2934.82 cm^{-1}, 2866.10 cm^{-1}, 1459.39 cm^{-1}, 1382.97cm^{-1}.The C=O stretching (1054.74 cm^{-1}) is also observed. MSC-1 was also analyzed by ^{1}HNMR spectrometer. In ^{1}HNMR spectrum, the 7H proton appeared as a double of doublet at δ 0.96 which were identical with chemical shift of H-5, H-6, H-8, H-9 and 6-H olefinic proton showed a multiplet at δ 1.11 which were identical with chemical shift of 13-H and 29-H. Two -CH_3 group also appeared at δ

1.11 of H-13 and H-29. These assignments are in good agreement for the structure of stigmasterol.

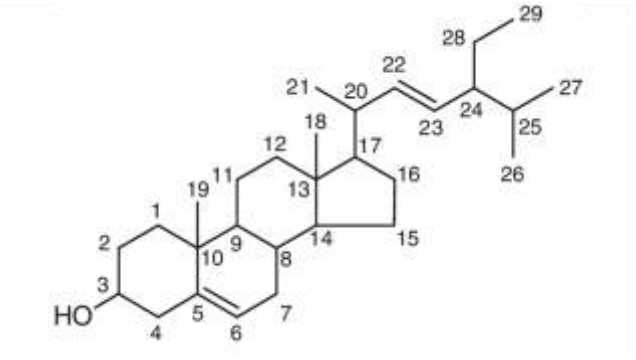

Figure 1: Predicted structure of MSC-1 as, Stigmasterol

Conclusion

After studying the physical and chemical properties, UV, FTIR and ^{1}HNMR spectrum; we can predict the structure of MSC-1 as, it is stigmasterol (Fig.1).

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