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ISOLATION AND CHARACTERIZATION OF N-OCTA DECANOIC ACID FROM WHOLE AERIAL PARTS OF CENTELLA ASIATICA LINN.

Rashmi Saxena Pal[1]*, Yogendra Pal[2], Dr. Vijender Singh[3]
[1,2]Assistant Professor, Pharmacy Department, PSIT, Bhauti, Kanpur, UP, India.
[3]Director, BBS Institute of Pharmaceutical and Allied Sciences, Greater Noida, UP, India.
Email: rashmisaxenapal@gmail.com

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Abstract:

Various studies have already been performed involving the whole aerial parts of Centella asiatica (L.) (Umbelliferae), commonly known as gotukola or jalbrahmi and thus the present investigation has been carried out for the phytochemical study of ethanolic extract of the aerial parts of Centella asiatica. To perform this activity, the drug (1.5kg) was exhaustively extracted in 95% ethanol using Soxhlet apparatus. The column chromatography was performed then for isolating the various phytoconstituents using the solvents of increasing polarity from petroleum ether to methanol. The isolated compounds were structurally elucidated by using various spectral data analysis, i.e., IR, ^{1}H NMR, ^{13}C NMR and positive ion FAB MS. One of the isolated compounds was characterized as n-octadecanoic acid.

Keywords:

Whole aerial parts, Centella asiatica, Soxhlet, Column, n-octadecanoic acid

1. Introduction:

Centella asiatica (CA) is an important medicinal herb used in the orient,[1] which is also becoming popular day by day in the West[2].It is commonly known as *mandukparni* or Indian pennywort or *jalbrahmi* and has been used for various medicinal purposes in the Ayurvedic system of medicine in India for thousands of years and it is also listed in the historic *'Sushruta Samhita'*, an ancient Indian medical text [3,4.] Centella asiatica (L.) is a prostrate, faintly aromatic, stoloniferous, perennial, creeper herb, attains height up to 15cm (6 inches). Stem is glabrous, striated, rooting at the nodes. Centella asiatica flourishes extensively in shady, marshy, damp and wet places such as paddy fields, river banks forming a dense green carpet and rather than clayey soil, the sandy loam (60% sand) is found to be the most fertile soil for

its regeneration.[5] The chemical constituents of plant possess medicinal and nutraceutical applications and it is believed due to its biologically active components of triterpenes saponins.[6] The plant contains the glycosides viz Asiaticosides A & B, madecassosides and centellosides. The primary active constituents of CA are saponins (also called triterpenoids), which include asiaticosides, in which a trisaccharide moiety is linked to the aglycone asiatic acid, madecassoside and madasiatic acid.[8] Flavanoids such as kaempferol and quercetin are also present in the plant.[9] In Indian medicine the plant is important as a tonic for crude extract containing glycosides isothankuniside and thankuniside showed antifertility action in mice.[10-11] skin diseases and leprosy, and is reported to promote fibroblast proliferation and collagen synthesis.[12] The plant also is also used medicinally for its psychotropic uses.[13] Anti-protozoal activity is also exhibited by the alcoholic extract of the plant against Entamoeba histolytica.[14] Majority of studies have been performed on the various parts of Centella asiatica, so the present study involves the phytochemical investigation of ethanolic extract of the whole aerial parts of Centella asiatica.

2. Materials and Methods:

All melting points were determined in Centigrade scale in one-end open capillary on Perfit melting point apparatus and are uncorrected. IR spectra were recorded on Perkin Elmer spectrum RX 1 model. 1H NMR and 13C-NMR spectra were scanned on Bruker DRX-300 NMR (300MHz) instrument in $CDCl_3$ and D2O using Tetramethylsilane (TMS) and CDCl3 as the internal standard and coupling constants (J values) are expressed in hertz (Hz).Mass spectra were recorded by affecting electron impact ionization at 70eV on a Jeol SX-102 (FAB) mass spectrometer equipped with direct inlet probe system.

The m/z values of the more intense peaks are mentioned and the figures in bracket attached to each m/z values indicated relative intensities with respect to the base peak. The solvents used were of Qualigens LR grade. Silica gel (Qualigen 60-120 μm mesh) was used for column chromatography. TLC was performed on plates coated with silica gel G (Qualigen). Anhydrous sodium sulphate was used for drying all the solvents used during the research work.

2.1 Plant material:

The plant material was procured from AIMIL Pharmaceuticals, New Delhi. It was authenticated as Centella asiatica by Dr.M.P.Sharma, Reader, Department of Botany, Jamia Hamdard, New Delhi and a voucher specimen is preserved in the herbarium section of Department of Pharmacognosy, R.I.T., Greater Noida, Uttar Pradesh.

2.2 Extraction:

The plant material (1.5kg) was air dried, crushed to coarse powder, re-dried and was then exhaustively extracted with ethanol (95%) in a Soxhlet apparatus for 50 hours. The ethanolic extract was dried and dark brown mass 130gm (8.6%w/w) was obtained.

2.3 Preparation of Slurry:

The concentrated extract of the drug was taken and heated continuously on a water bath, gradually adding methanol in small portions with constant stirring till desired consistency was obtained. Weighed quantity of silica gel (60-120 mesh) was added slowly with mixing with a stainless steel spatula until a desired consistency was obtained. It was dried in air; the larger lumps were broken-up and finally passed through a sieve (No. 8) to get a uniform particle size.

2.4 Packing of Column:

The lower end of a clean dry column was plugged with adsorbent cotton. The column was then half filled with petroleum ether. Silica gel was added in small proportions and allowed to settle down gently until the necessary length of the column was attained. All the air bubbles were allowed to escape by running the column blank thrice with solvent. The dried silicagel slurry of the extract was packed in the column and plugged with the adsorbent cotton and then eluted successively in the order of increasing polarity with different solvents.

The development and elution of the column was carried out with successive series of solvents in various combinations, viz., petroleum ether, chloroform in petroleum ether (0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%) chloroform(100%), and methanol in chloroform. The fractions collected were subjected to thin layer chromatography. Chromatographically identical fractions were combined and concentrated.

2.5 Isolation of Phytoconstituents:

Elution of the column with petroleum ether- chloroform (3:2) afforded a colorless crystal of n-octadecanoic acid which was recrystallized from methanol, yield was 0.32(%w/w); Rf: 0.83 (chloroform: methanol, 8:2); m.p.:59-61^{O}C

IR max (KBr): 3380, 3021, 2926, 2857, 2359, 1655, 1593, 1432, 1216, 761cm^{-1}

^{1}HNMR ($CDCl_3$):δ 2.25(^{1}H,*d*,*J*=7.2Hz,H_2-2a), 2.20(^{1}H,*d*,*J*=7.2 Hz,H_2-2b), 1.57(2H,m,CH_2), 1.25(48H,*brs* 24CH_2) 0.87(3H,*t*.*J*=6.8 Hz,Me-28)

^{13}C NMR ($CDCl_3$): δ178.26(C-1), 44.28(CH_2), 36.62(CH_2), 38.819CH_2), 31.83(CH_2), 30.68(CH_2), 29.36(CH_2), 29.36(16 CH_2), 29.14(CH_2), 29.04(CH_2), 25.07(CH_2), 22.59(CH_2), 21.99(CH_2), 13.98 (CH_3-28).

ESI MS m/z: 424[M+] ($C_{28}H_{56}O_2$) (9.6)

3. Results:

The Compound, a fatty acid, was obtained as a colourless crystal from petroleum ether- chloroform (3:2) eluants. It gave effervescence with sodium bicarbonate solution and did not decolorize bromine water indicating saturated carboxylic acid nature of the molecule. Its IR spectra exhibited characteristic absorption band for carboxylic group at 3380, 1665 cm^{-1} and long aliphatic chain aliphatic chain at 761cm^{-1} .It had a molecular ion peak at m/z 424 in the mass spectrum consistent to the molecular formula of saturated fatty acid $C_{20}H_{56}O_2$. It indicated one double bond equivalent to the carboxylic group. The ^{1}H NMR spectrum of the compound showed two one proton doublet at δ 2.25(J=7.2 Hz) and 2.20(J=7.2Hz) assigned to methylene H_2-2 protons adjacent to the carboxylic group. A two proton multiplet at δ 1.57 and a broad signal at δ 1.25 (48H) were associated with the remaining methylene protons. A three proton triplet at δ 0.87(J=6-8Hz) was accounted to C-28 primary methyl protons .The ^{13}C NMR spectrum of displayed signals for carboxylic carbon at δ 178.26 (C-1), methylene carbons between 44.38-21.99 and methyl carbon at δ 13.98 (C-28).The absence of any signal beyond 2.25 in the ^{1} H NMR spectrum and between δ 178.26-44.38 in the ^{13} C NMR spectrum supported the saturated nature of the molecule. Based on these evidences the structure of the compound has been formulated as n-octadecanoic acid.

4. Discussions:

The result summarizes that n-octadecanoic acid, a fatty acid was isolated and characterized from ethanolic extract of the whole aerial parts of Centella asiatica, The chemical structure was elucidated by means of various physical (solvent extraction, TLC, Column chromatography) and spectral techniques.. In conclusion, n-octadecanoic acid obtained from ethanolic extract of the whole aerial parts of Centella asiatica in the manufacturing of various pharmaceutical dosage forms as a pharmaceutical aid.

It plays the role of a lubricant in the manufacturing of tablets and capsules.It also serves as a solubilizer in the preparation of many dosage forms.It is also used as an emulsifier.It is used in the preparation of various cosmetics as it is the natural component of cocoa butter and shea butter,therefore it is used as a base for the manufacturing of various creams and ointments. It is used in the manufacturing of detergents,soap,shampoos,lotions etc. It can harden soaps and give shampoos

a pearly color and consistency.It is also used to prepare candles. In our investigation, it appeared to be beneficial to serve for various human ailments and also fulfills other commercial purposes.

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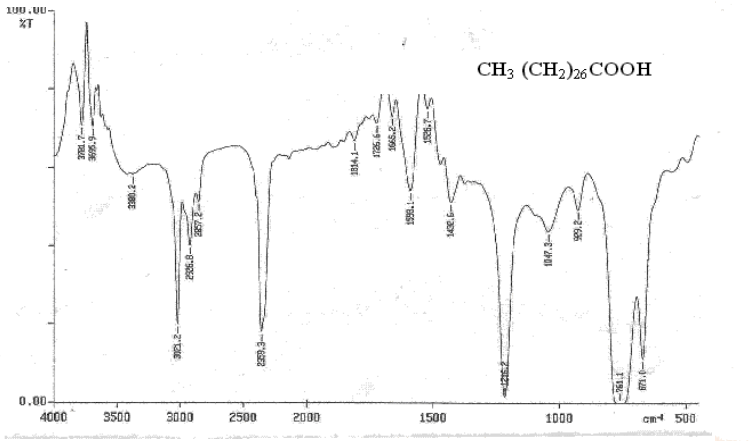
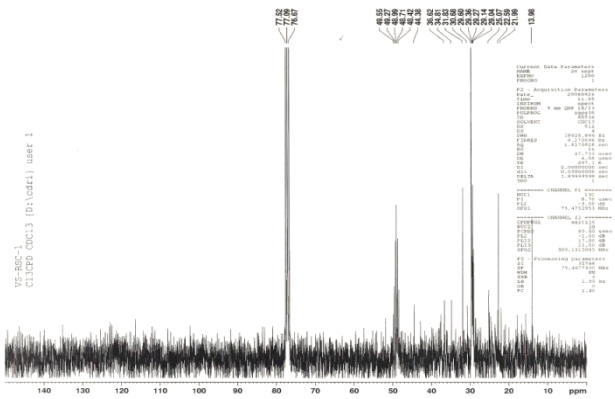
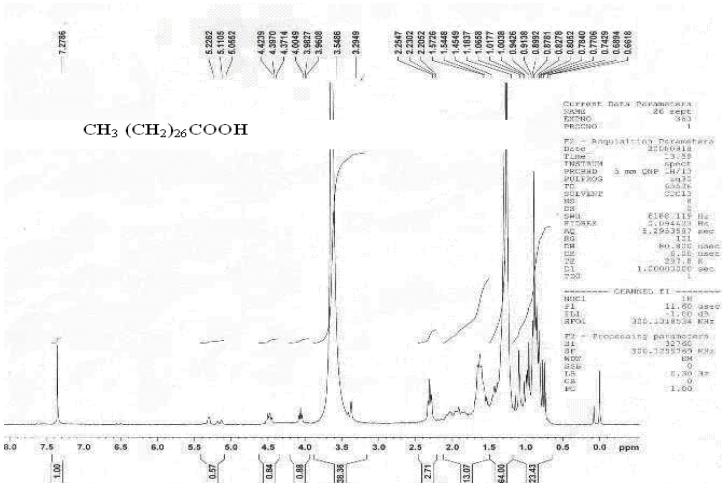
6.1 Figures at a glance:



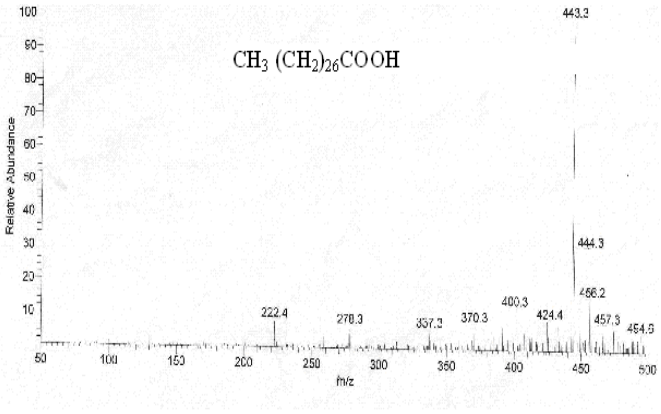
I. R Spectra of n-octadecanoic acid



2.3 ^{13}C NMR Spectra of Compound CA-1(n-octadecanoic acid)



^{1}H NMR Spectra of Compound n-octadecanoic acid



Mass spectra of Compound n-octadecanoic acid

$CH_3\ (CH_2)_{26}CO\ OH\ (m/z)=424$

$(M - OH)$ $\qquad$ $(M^+ - COOH)$

$CH_3\text{-}(CH_2)_{26}\text{-}C{\equiv}O^+$ $\qquad$ $CH_3\text{-}(CH_2)_{26}^+$

Mass fragmentation pattern of compound n-octadecanoic acid.

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Corresponding Author:

Rashmi Saxena Pal*,

Email: rashmisaxenapal@gmail.com