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Review Article

ISOLATION AND CHARACTERIZATION OF *N*-HEPTACOSANYL OLEATE FROM THE WHOLE AERIAL PARTS OF *CENTELLA ASIATICA* LINN.

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ABSTRACT

Various studies have already been performed involving the whole aerial parts of *Centella asiatica* (L.) (Umbelliferae), commonly known as gotu kola or jalbrahmi, and thus, the present investigation has been carried out for the phytochemical study of an ethanolic extract of the aerial parts of *C. asiatica*. To perform this activity, the drug (1.5 kg) 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 using various spectral data analysis, i.e. infrared,^{1}H nuclear magnetic resonance (NMR),^{13}C NMR, and positive ion fast atom bombardment mass spectrometry. One of the isolated compounds was characterized as n-heptacosanyl oleate.

Keywords: Whole aerial parts, *Centella asiatica*, Soxhlet, Column, Heptacosanyl oleate.

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INTRODUCTION

Centella asiatica (Hydrocotyle asiatica), belonging to family Umbelliferae, is also known as gotu kola in Hindi [1]. It is known as karinga in Marathi and mandukparni in Sanskrit [2]. It is a prostrate, slender, tender, faintly aromatic herb, which has numerous creeping stoloniferous stems, rooted at nodes with long internodes [3]. The plant contains the glycosides, *viz*., asiaticosides A and B, madecassosides, and centellosides [4]. It contains the triterpene acids such as asiatic and madecassic acid [5]. Flavonoids such as kaempferol and quercetin are also present in the plant [6]. The plant contains volatile and fatty oil. The fatty oil consists of glycerides of palmitic, stearic, lignoceric, and oleic acids [7]. Centella is also rich in vitamin C, vitamin B1, vitamin B2, niacin, carotene, and vitamin A. The total ash contains chloride, sulfate, phosphate, iron, calcium, magnesium, sodium, potassium, etc. [8,9]. The plant shows various pharmacological activities such as gastric ulcer healing activity, which is shown by asiaticoside present in it [10]. Its crude methanolic extract shows antioxidant activity [11]. In Indian medicine, the plant is important as a tonic for skin diseases and leprosy and is reported to promote fibroblast proliferation and collagen synthesis [12]. The plant also serves for its psychotropic uses [13]. Alcoholic extract of the plant shows antiprotozoal activity against *Entamoeba* histolytica [14]. Crude water extracts of Centella plant along with other plant combinations show antiherpes simplex virus activities [15]. The majority of studies have been performed on the various parts of *C. asiatica*, so the present study involves the phytochemical investigation of ethanolic extract of the whole aerial parts of *C. asiatica*.

METHODS

All melting points were determined in centigrade scale in one-end-open capillary on Perfit melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin Elmer Spectrum RX 1 model. 1H nuclear magnetic resonance (NMR) and 13C-NMR spectra were scanned on Bruker DRX-300 NMR (300 MHz) instrument in $CDCl_3$ and D_2O using Tetramethylsilane and $CDCl_3$ 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 (fast atom bombardment) 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. Thin layer chromatography (TLC) was performed on plates coated with silica gel G (Qualigen). Anhydrous sodium sulfate was used for drying all the solvents used during the research work.

Plant material

The plant material was procured from AIMIL Pharmaceuticals, New Delhi. It was authenticated as *C. 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 the Department of Pharmacognosy, R.I.T., Greater Noida, Uttar Pradesh.

Extraction

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

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 the 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.

Packing of column

The lower end of a clean, dry column was plugged with absorbent 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 silica gel slurry of the extract was packed in the column and plugged with the adsorbent cotton and then eluted successively in 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 TLC. Chromatographically, identical fractions were combined and concentrated.

Isolation of phytoconstituents

Elution of the column with petroleum ether-chloroform (2:3) furnished:

Colorless crystals recrystallized from methanol: Chloroform (1:1).

Yield: 0.25 (%w/w).

Rf: 0.71 (mobile phase: 7: 3; benzene-chloroform).

mp: 53-55°C.

IRνmax (KBR): 3021, 2925, 2855, 2360, 1720, 1630, 1594, 1438, 1216, 1036, 929, 761/cm.

^{1}H NMR ($CDCl_3$): δ 5.18 (1 H, m, H-9), 5.30 (1 H, m, H-10), 3.47 (2 H, brs, H2-1'), 2.32 (1 H, d, J=7.2 Hz, H-2a), 2.27 (1 H, d, J=7.2 Hz, H-2b), 2.18 (2 m, H2-8), 1.61 (H2-2b), 1.26 (40 H, brs 20×CH_2), (32 H, brs 16×CH_2), 0.86 (3 H, t. J=5.4 Hz, Me-18'), 0.82 (3 H, t. J=6.5 Hz, Me-27').

^{13}C NMR ($CDCl_3$): δ 174.15 (C-1), 118.01 (C-9), 117.96 (C-10), 60.11 (C-1'), 51.88 (C-2), 48.83 (CH_2) 38.84 (CH_2), 38.08 (CH_2), 37.64 (CH_2), 37.55 (CH_2), 36.61 (CH_2), 36.06 (CH_2), 34.79 (CH_2), 34.31 (CH_2), 31.82 (CH_2). 31.71 (CH_2), 29.59 (20×CH_2), 29.47 (CH_2), 29.34 (CH_2), 29.26 (CH_2), 29.13 (CH_2), 29.03 (CH_2), 24.87 (CH_2), 22.58 (CH_2), 13.94 (CH_3-18), 39.83 (CH_2-27').

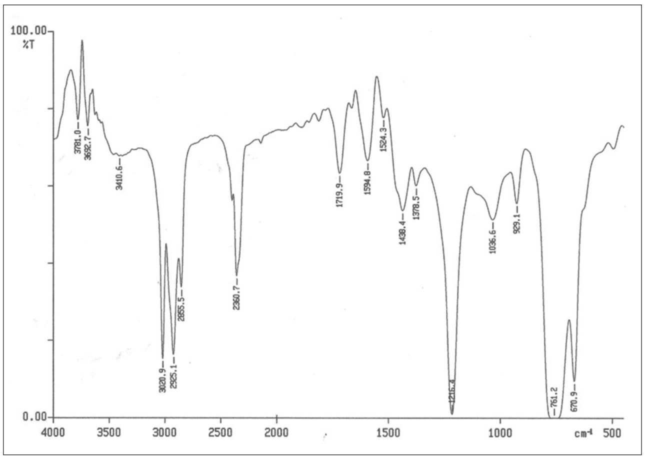

Fig. 1: Infrared spectra of compound n-heptacosanyl oleate

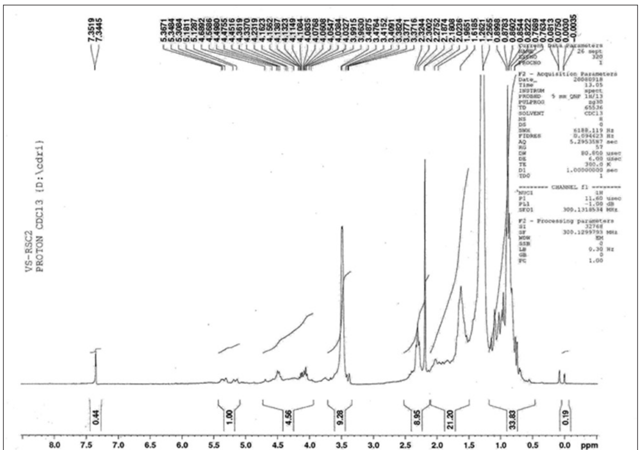

Fig. 2: ^{1}H nuclear magnetic resonance spectra of n-heptacosanyl oleate

ESIMS m/z 660 [M+] (C45H88O2) (19.8), 395 (21.3), 265 (8.9).

RESULTS

Compound CA-2, designated as n-heptacosanyl oleate, was obtained as a colorless crystalline mass from petroleum ether-chloroform (2:3) eluants. It decolorized bromine water indicating unsaturated nature of the molecule. Its IR spectrum showed characteristic absorption band for ester group (1720/cm), unsaturation (1660/cm), and long aliphatic chain (761/cm). It had a molecular ion peak at m/z 660 consistent with a molecular formula of an unsaturated fatty ester, C45H88O2. It indicated two degrees of unsaturation which were adjusted one each in the vinylic linkage and ester function. A large number of ion fragments recorded in the mass spectrum with a uniform difference of 14 mass units supported the presence of a long aliphatic chain in the compound. The absence of [M-Me]+ ion peak indicated the straight chain nature of the molecule (Stoianova, et al., 1969).

The mass spectrum displayed ions relating to CnH_{2n+1}, CnH_{2n}, and CnH_{2n-1} fragments and in higher abundance for the lower fragments suggesting long aliphatic chain of the molecule. The important ion peaks generating at m/z 265 [$CH_3(CH_2)$ 7CHCH=CH(CH_2) 7CO]+ and 395 [O(CH_2)26CH_3]+ supported that n-hepta cosanyl alcohol was esterified with oleic acid (Scheme 2).

The 1H NMR spectrum showed two one-proton multiplets at δ 5.30 and 5.10 assigned to vinylic H-10 and H-9 proton, respectively. A two-proton broad signal at δ 3.41 was attributed oxygenated methylene H2-1' proton. Two one-proton doublets at δ 2.32 (J=7.2 Hz) and 2.27 (J=7.2 Hz) were ascribed to methylene H2-2 protons adjacent to ester function. Two multiplets at δ 2.18 and 1.61, integrated for two protons each, were accounted correspondingly to methylene H2-8 and protons H2-11 nearby by the vinylic carbons. Two broad signals at δ 1.26 (40 H) and 1.25 (32 H) were associated with the remaining methylene protons. Two three-proton triplets at δ 0.86 (J=5.4 Hz) and 0.82 (J=6.5 Hz) were accommodated to terminal C-18 and C-27' primary methyl protons, respectively. The 13C NMR spectrum of exhibited signal for ester carbons 174.15 (C-1), vinylic

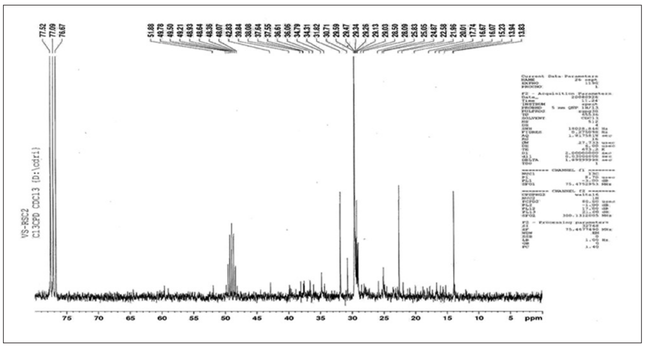

Fig. 3: ^{13}C nuclear magnetic resonance spectra of compound n-heptacosanyl oleate

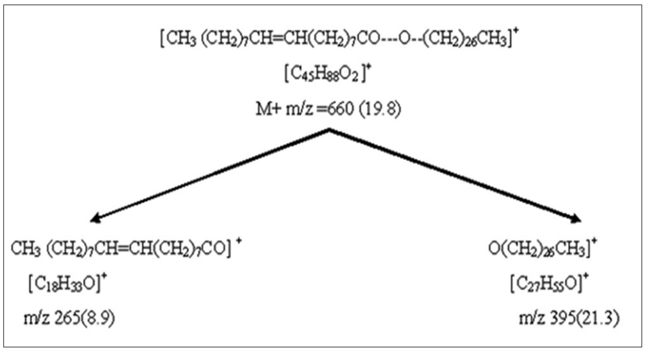

Fig. 4: Mass fragmentation pattern of n-heptacosanyl oleate

carbons at δ 118.01 (C-9) and 117.96 (C-10) oxygenated methylene carbon at 60.11 (C-1') remaining methylene carbons between δ 51.88-22.58 and 39.83 (C-27'). Alkaline hydrolysis of the compound yielded oleic acid (TLC comparable). On the basis of the aforementioned evidence, the structure of compound has been established n-heptacosanyl octadec-9-enoate.

DISCUSSION

The result summarizes that n-heptacosanyl oleate, a fatty acid was isolated and characterized from the ethanolic extract of the whole aerial parts of *C. asiatica*, the chemical structure was elucidated using various physical (solvent extraction, TLC, and column chromatography) and spectral techniques.

CONCLUSION

N-heptacosanyl oleate obtained from the ethanolic extract of the whole aerial parts of *C. asiatica* in the various pharmaceutical dosage forms; it is used as an emulsifying agent, solubilizing agent, tablet and capsule lubricant. It is also commonly found in lotions, detergents, soaps, and shampoos and is a natural component of cocoa butter and shea butter. One of the most popular uses of it is in the production of candles. It is often used to harden the wax and strengthen the candle. It also has an impact on the melting point of the wax, improving the durability and consistency of the candle. For these reasons, it can be found in most craft stores in the candle making section. It is also commonly used in the production of soap. In fact, soap may have been accidentally discovered in the ancient world by people trying to extract oil from animal fat; this process was likely similar to how n-heptacosanyl oleate is extracted from animal fat. Soap made from animal fat, however, suffers the drawback of having low water solubility, which can result in a residual film on bathtubs and skin. Therefore, rather than as a primary ingredient, this acid is usually used as an additive. It can harden soaps and give shampoos a pearly color and consistency. In our investigation, it appeared to be beneficial for various human ailments and other purposes.

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