



Isolation and Characterization of *n* – eicosanyl Lignocerate from the Whole Aerial Parts of *Centella asiatica* Linn.

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Accepted on: 23-06-2016; Finalized on: 31-08-2016.

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, ¹H NMR, ¹³C NMR and positive ion FAB MS. One of the isolated compounds was characterized as *n*-eicosanyl lignocerate.

Keywords: Whole aerial parts, *Centella asiatica*, Soxhlet, Column, *n*-eicosanyl lignocerate.

INTRODUCTION

Centella asiatica, commonly known as *mandukparni* or Indian pennywort or *jalbrahmi*, has been used as a medicine in the Ayurvedic tradition of India for thousands of years and listed in the historic 'Sushruta Samhita', an ancient Indian medical text.¹⁻² 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 & B, madecassosides and centellosides.⁴ 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.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, oleic acids.⁷ *Centella* is also rich in Vitamin C, Vitamin B₁, Vitamin B₂, niacin, carotene and Vitamin A. The total ash contains chloride, sulphate, 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 In Indian medicine the plant is important as a tonic for crude extract containing glycosides isothankunin and thankunin showed antifertility action in mice.¹¹⁻¹² Skin diseases and leprosy, and is reported to promote fibroblast proliferation and collagen synthesis.¹³ The plant also is also used medicinally for its psychotropic uses.¹⁴ Alcoholic extract of the plant shows anti-protozoal activity against *Entamoeba histolytica*.¹⁵ Centelloside and its derivatives are found to be effective in the treatment of venous hypertension. In addition, the total extract contains plant

sterols, flavonoids, and other components with no known pharmacological activity.¹⁶ 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*.

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 CDCl₃ 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.

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.

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.

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.

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.

Isolation of Phytoconstituents

Elution of the column with chloroform furnished colorless crystals recrystallised from methanol.

Yield: 0.17 (%w/w)

Rf: 0.82(mobile phase: 6:3:1; petroleum ether-chloroform-methanol)

mp: 65-67°C

Figure-1 shows the values of IRvmax (KBR) 3021, 2927, 2858, 2338, 1722, 1596, 1427, 1216, 1042, 928, 764cm⁻¹.

Figure-2 shows the value as follows ¹H NMR (CDCl₃): δ 4.48(1H, d, J= 6.6Hz, H2-1'a), 4.46(1H, d, J= 6.6Hz, H2-1'b), 2.30(1H, d, J= 6.9Hz, H2-2'a), 2.27(1H, d, J= 6.9Hz, H2-2'b), 1.61(4H, m, 2 x CH₂), 1.25(76H, brs 38 x CH₂), 0.87(3H, t, J =6.3 Hz, Me-24'), 0.85(3H, t, J =6.5 Hz, Me-21')

Figure-3 shows the values as ESIMS m/z 662[M+] (C₄₅H₉₀O₂) (19.8), 395(21.3), 265(8.9) (100%) 351(3.1).

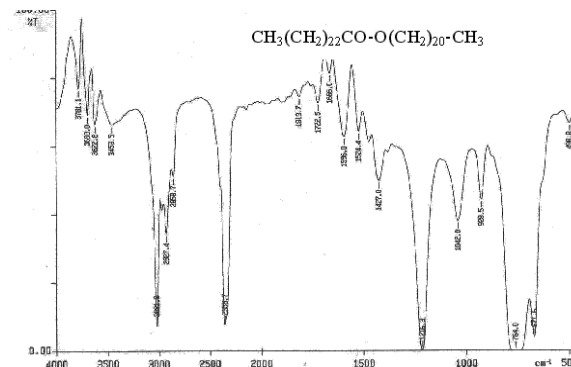


Figure 1: I.R Spectra of Eicosanyl Lignocerate

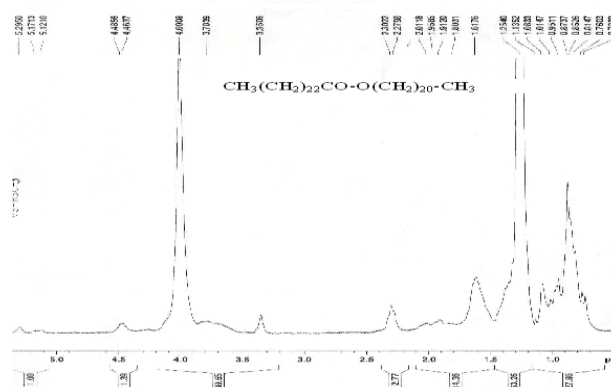


Figure 2: ¹H NMR Spectra of Eicosanyl Lignocerate

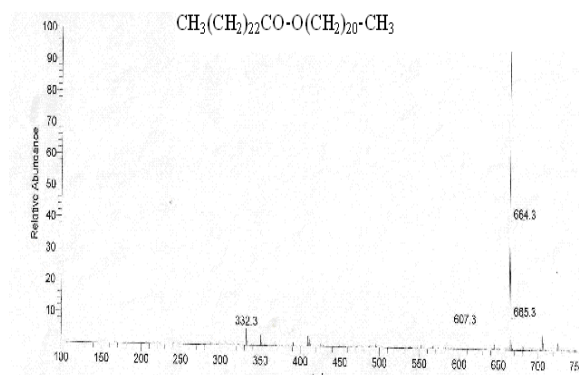


Figure 3: Mass Spectra of Eicosanyl Lignocerate

RESULTS

Compound named n- eicosanyl lignocerate, was obtained as a colorless crystallized mass from chloroform eluants. It did not decolorize bromine water indicating saturated nature of the molecule. As shown by Figure-1, Its IR spectrum exhibited characteristic absorption band for ester group (1722 cm⁻¹) and long aliphatic chain (764 cm⁻¹). As per Figure-3, it has a molecular ion peak at m/z 662

consistent with molecular formula of a saturated fatty acid ester $C_{45}H_{90}O_2$. The formation of an ion peak at m/z 351 $[CH_3(CH_2)_{22}CO]^+$ indicated that lignoceric acid was esterified with eicosanyl alcohol. According to Figure-2, the 1H NMR spectrum of the compound exhibited four one-proton doublets at δ 4.48 ($J=6.6$ Hz) and 4.46($J=6.6$ Hz) assigned to oxygenated methylene H2- 1 protons and at δ 2.30 ($J=6.9$ Hz) and 2.27($J=6.9$ Hz) ascribed to methylene H2-2 protons adjacent to ester group. A four proton multiplet at δ 1.61 and abroad signal at 1.25 (76H) were associated with the remaining methylene protons. Two three-protons triplet at δ 0.87($J=6.3$ Hz) and 0.85($J=6.3$ Hz) were attributed to terminal C-24 and C-21' primary methyl protons, respectively. The absence of any signal beyond δ 4.48 supported saturated nature of the molecule. Alkaline hydrolysis of compound yielded lignoceric acid. On the basis of the foregoing discussion the structure of the compound has been elucidated as n-eicosanyl lignocerate.

DISCUSSION

The result summarizes that n-eicosanyl lignocerate, 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-heptacosanyl oleate obtained from ethanolic extract of the whole aerial parts of *Centella asiatica* is used as in the synthesis of various pharmaceutical dosage forms. It is used for its emulsifying and solubilizing properties. It also serves as a tablet and capsule lubricant. It is also commonly found in shampoos, soaps, lotions and oils. It 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 used in the hardening and strengthening of the waxes. 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. The majority of the fatty acids esters are generated from tallow, palm kernel, tall oil, soya oil and sunflower oil. Consumers of fatty acids and esters are food stuff, cosmetics, soap and other personal care products, synthetic lubricants, paper, water treatment, as metal working fluids and in oil field applications. 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.

Acknowledgement: The authors are thankful to the Head, Department of Pharmacognosy, for providing laboratory facilities and to the Head, SAIF, CDRI, Lucknow, for spectral analysis. The authors are also thankful to Dr. M. P. Sharma for authenticating the drug.

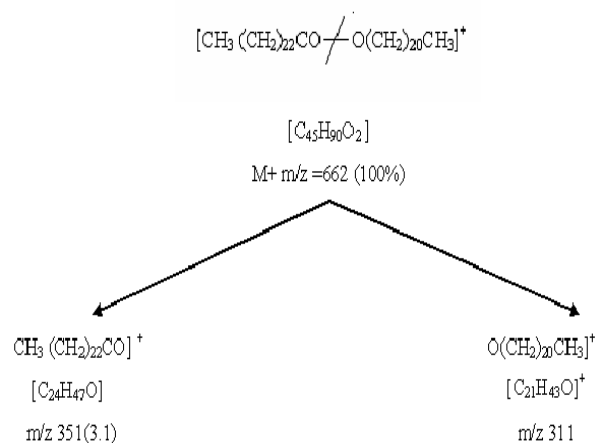


Figure 4: Mass Fragmentation Pattern of Eicosanyl Lignocerate

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Source of Support: Nil, Conflict of Interest: None.

