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ISOLATION AND IDENTIFICATION OF SOME COMPOUNDS FROM THE AERIAL PARTS OF *Leucas aspera*

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ABSTRACT

Oleate of methoxy-methyl-n-pentyl-benzoquinone and 1-hexacosanol and sucrose were isolated from the chloroform and methanol extract respectively of the aerial parts of *Leucas aspera*. All of the isolated compounds were identified on the basis of the spectroscopic data analysis and chemical reactions.

Key words: benzoquinone, chromatography, crystallisation, *Leucas aspera*

INTRODUCTION

Leucas aspera is the plant locally known as Ghalkalosh / Dandakalosh which belongs to the family of *Labiatae*. It is a shrub and widely distributed all over Bangladesh. It is an important medicinal plant. The leaves of the plant are reported to have antibacterial activity against *Micrococcus pygogenes* and *Escherichia coli* (Rao and Narasimha, 1971; Rahman *et al* 2007). The flowers of the plant mixed with honey are very useful for cold and coughs. *Leucas aspera* has been reported to have insecticidal properties as well (Usher, 1974). Phytochemical studies on the plant have been shown it to contain oleic acid, linolenic acid, palmitic acid, ceryl alcohol, oleanolic acid, ursolic acid, β-sitosterol, α-sitosterol, diterpenes, quinines,13 and 16-trihydroxy-28-oleanolic acid, (Vanisha and Subadra,1998; Narayanan,1974; Pardhan *et al*,1990; Jam and Nath,1968; Sadhu *et al*, 2006; Mesbah *et al*, 2010; Badami and Patil,1975).

MATERIALS AND METHODS

Melting points of the isolated pure compounds were recorded by thin disc method on a Fischer John's electrothermal melting point apparatus. Column chromatographic and vacuum liquid chromatographic (VLC) separations were carried out using silica gel (60 Kiesel gel 60, 70-230 mesh, E. Merck) as the stationary phase. Precoated TLC plates (silica gel 60 kiesel guhr F_{254}, thickness 0.2 cm, E. Merck on aluminium foils) were used for the TLC examination as well as for the preparative thin layer chromatographic separation (PTLC). UV spectra were recorded in chloroform on a Pye Unicam, Model SP 8-100 UV spectrometer at the chemistry division of the Atomic Energy Research Establishment, Savar, Dhaka. IR spectra were recorded on a Shimadzu FTIR 8101 spectrometer as KBr pellet and as thin film. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz NMR spectrophotometer at the Dhaka Laboratory of Bangladesh Council of Scientific and Industrial Research (BSCIR).

The plants *Leucas aspera* were collected from the adjoining area of Tara under Manikgonj district. The aerial parts of the plants were cut into small pieces and then dried in the shade. The dried plant pieces were powdered in a grinding machine. The powdered plant material (3.5kg) was soaked in distilled pet. ether (40°-60°C) at room temperature for 72 hours. The pet.ether extract was collected and the extraction process was repeated two more times to ensure complete extraction. The solvent was evaporated to dryness under reduce pressure on a rotary evaporator at a temperature below 45°C when a greenish colored semisolid mass P (25g) was obtained. The residual plant material left after the extraction with pet. ether was then successively extracted at room temperature with chloroform and methanol. Chloroform extract gave a deep green semisolid mass C (20g), whereas the methanol extract gave a light green semisolid mass M (35g) on removal of the solvents. The present work was confined on the chloroform extract C and methanol extract M.

Separation of the compounds from chloroform extract C

Exactly 10.0 g of the chloroform extract **C** was subjected to vacuum liquid chromatographic

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separation over silica gel and eluted successively with pet. ether, gradient mixtures of pet.ether-chloroform, chloroform, 1:1 mixture of chloroform-ethyl acetate, ethyl acetate, mixtures of ethyl acetate-methanol and finally the column was washed down with methanol. The eluants were divided into 10 fractions according to their TLC behavior. Fraction no. 3(1.7 gm) gave a fairly well resolved spots on the TLC plates and was subjected to further study. The other fractions which did not give any distinct spots on the TLC plates were not studied further.

Fraction no. 3 (1.7 g) was a pink colored gummy material, had an agreeable smell and found to be a mixture of several compounds. Total amount of the fraction was chromatographed on a silica gel column in n-hexane: chloroform (5:2) and eluted with the same solvent system. The eluants were divided into 11 sub-fractions according to their TLC behavior. On removal of the solvent fraction no. 3 yielded a yellow colored crystalline pure compound **C-1**(50 mg). Rest of the fractions was proved to be complicated mixtures as revealed by TLC. No further work was done on them.

Separation of the compounds from methanol extract M: 32 g of the extract **M** was subjected to vacuum liquid chromatographic separation on a silica gel column and eluted successively with pet. ether, mixtures of pet. ether-chloroform, chloroform, 1:1 mixture of chloroform-ethyl acetate, ethyl acetate, mixtures of ethyl acetate- methanol and finally with methanol. The eluants were divided into 11 fractions according to their TLC behavior. Fraction nos. 7 and 10 gave fairly well resolved spots on the TLC plates but rest of the fractions was complicated mixtures.

Fraction No.7 (2.0 g) was a greenish colored gummy material and showed four spots on the TLC plates at R_f 0.68, 0.61, 0.42, and 0.37 with a tailing from the baseline in 50% ethyl acetate in pet. ether. Crystallisation of the greenish colored gummy material from hot methanol yielded a brown colored precipitate (100 mg). TLC examination of the brown precipitate showed two spots on the TLC plates in different solvent systems and gave R_f 0.68 and 0.50 in 5% methanol in chloroform. Preparative thin layer chromatographic separation of this fraction on silica gel plates with 5% methanol in chloroform led to the isolation of a pure compound C-2 (12 mg) with R_f 0.68 in 5% methanol in chloroform.

Fraction No. 10 (7.0 g) was successively triturated with pet. ether, ethyl acetate and methanol. Methanol triturated fraction (3.0 g) showed two spots on the TLC plates at R_f 0.55 and 0.42 in 50% methanol in ethyl acetate with a long tailing from the baseline. Column chromatographic separation of this fraction on silica gel with ethyl acetate and gradient mixtures of ethyl acetate- methanol and finally with methanol led to the isolation of a pure compound **C-3** (50 mg) with R_f 0.42 in 20% methanol in ethyl acetate.

RESULTS AND DISCUSSION

The aerial parts of the plant *Leucas aspera* were successively extracted with pet. ether, chloroform and methanol. Vacuum liquid chromatographic separation of the chloroform extract yielded 10 fractions according to their TLC behavior. Most of the fractions were found to be complicated mixtures. However, column chromatographic separation of the fraction no. 3 led to the isolation of a pure compound which was designated as compound C-1. The methanol extract was divided into 11 fractions by vacuum liquid chromatographic separation. The present work was concentrated on the fraction nos.7 and 10 because they gave fairly well resolved spots on the TLC plates. Column chromatographic separation of the fraction nos. 7 and 10 gave two pure compounds which were designated as compound C-2 and C-3 respectively.

Compound C-1 was a yellow colored crystalline substance, m.p. 109-111°C and possessed an agreeable smell. It showed characteristic UV absorptions for quinonoid structures at λ_{max} 435, 286, and 207 nm. The absorptions were similar to those observed for benzoquinone with four substituents (Yamaguchi, 1970). The compound gave positive tests for benzoquinone with sodium dithionite and exhibited IR absorption at 1689 cm^{-1} for carbonyl function of the quinone structure. The compound also contained an ester function (ν_{max} 1745cm^{-1}).

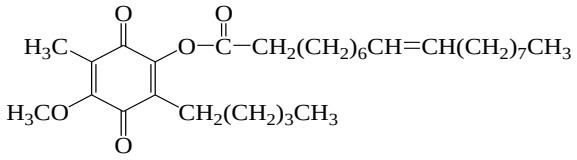
The presence of olefinic protons was supported by 2H triplet at δ 5.3 in the ^{1}H NMR spectrum. It showed a 3H singlet at δ 3.6 for methoxy group and a 3H singlet at δ 1.6 for methyl group attached to the benzoquinone nucleus. The 2H triplet at δ 2.8 and 2H unresolved peak at δ 2.05 indicated the presence of two methylene groups attached with the carbonyl carbon atom of the ester function and benzoquinone nucleus respectively. The huge unresolved peak at δ 1.4-1.2 was equivalent to 32H for 16 methylene groups of long alkyl chain. Two 3H triplets at δ 0.9 and 0.8 for the two terminal methyl groups of the two alkyl chains.

The ^{13}CNMR spectrum of the compound showed 31 carbon signals. Two signals at δ 174 and 173.5 are readily attributable to the two carbonyl carbon atoms of the benzquinonoid moiety. Four other low field signals at δ 133, 132, 130.5 and 130 are appropriate for the other four carbon atoms of benzoquinone nucleus. The signal at δ128.6 and two signals at δ 105 and 104 are also appropriate for the carbonyl carbon atom of the ester function and two olefinic

carbons respectively. Two signals at δ 60.2 and 52 readily provide support for the methoxy and methyl carbon atoms respectively attached to benzoquinone nucleus. The other carbon atoms of the compound are observed at high field as expected.

The presence of ester has been ascertained by IR spectrum and the fact that oleic acid has been shown to be present in *Leucas aspera*, it may be that the acid is present in the compound as an ester. This would also satisfy our observation that two olefinic protons are present in the molecule. The total number of protons 50 and the total number of carbons 31 as suggested by the ^{1}H NMR and ^{13}C NMR spectra respectively, together with the fact that the compound is a tetra-substituted benzoquinone with an ester function, the molecular formula for the compound can be computed as $C_{31}H_{50}O_5$.

On the basis of these assumptions, compound **C-1** is suggested to possess the following structure.



Compound C-1

Compound C-2 was a white colored solid material and melted at 78-80°C. The infrared spectrum of the compound showed a strong absorption band at 3417 cm^{-1} indicating the presence of OH group in the molecule. The absorption bands at 2926, 2855 and 1456 cm^{-1} are due to the aliphatic C-H stretching and bending vibrations respectively. The absorption band at 1070 cm^{-1} is due to the C-O stretching vibration. The ^{1}H NMR spectrum is also in agreement with that expected from a long chain alcohol. It showed a broad 1H singlet at δ 2.15 for the hydroxyl (OH) proton, a 3H triplet at δ 0.93 for the terminal methyl group and a 2H triplet at δ3.65 for the methylene protons of the type $–CH_2$-C***H***$_2$OH respectively. An intense 46H unresolved singlet at δ1.23 for the 23 methylene protons of the long alkyl chain and a 2H multiplet at δ1.59 is attributed to the methylene protons of the type CH_2-C***H***$_2$-CH_2OH. The above spectral data and comparision with reported literature values (Chatterjee and Majumdar 1969) can be nicely accommodated if the compound C-2 is 1-hexacosanol.

$CH_3(CH_2)_{23}CH_2CH_2OH$

Compound C-2

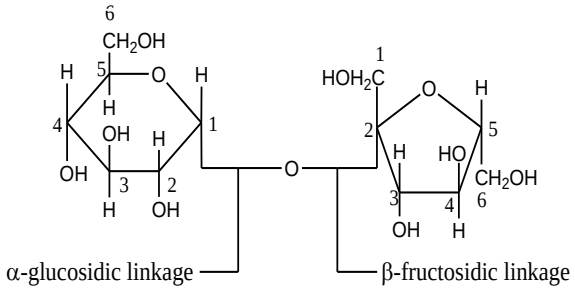
Compound C-3 (50 mg) was a colorless crystalline substance and melted at 185-186°C. It showed a single spot on the TLC plates at R_f 0.42 in 20% methanol in ethyl acetate when sprayed with aniline-diphenylamine-phosphoric acid reagent. The behavior of the spot towards the spraying reagents suggested it to be a sugar molecule. The compound also gave positive tests for sugar with Molisch and phenol-sulphuric acid reagents. TLC examination of the compound showed that it had same R_f as sucrose and hydrolysis of the compound with 2M trifluoroacetic acid showed two spots on the TLC plates which corresponded to that for glucose and fructose, spots for glucose and fructose appeared as blue and brown when sprayed with aniline-diphenyl amin-phosphoric acid reagent.

The infrared spectrum of the compound showed a broad band at 3400-3200 cm^{-1} indicating the presence of hydroxyl group. The ^{1}H NMR spectrum of the compound showed a 1H doublet at δ5.27 for the proton attached to the anomeric C-1 of the glucose unit and two 1H triplets at δ 3.63 and 3.21 are assignable to those protons attached to C-2 and C-4 of glucose part, whereas the 1H doublet of doublet at δ 3.31 for the proton attached to C-3 of glucose unit and two 1H and 2H multiplets at δ 3.76 and 3.74-3.75 for the protons attached to the C-5 and C-6 of glucose unit respectively. The ^{1}H NMR spectrum of the compound also showed a 2H singlet at δ 3.53, a 1H triplet at δ3.91, a 1H doublet at δ 4.08 which are appropriate for the protons attached to C-1, C-4 and C-3 of the fructose moiety respectively. The protons attached to C-5 and C-6 of fructose part was noticed by 1H and 2H multiplets at δ 3.81 and 3.75 respectively. The ^{13}CNMR data of the compound C-3 are shown in the following table.

Table 1. ^{13}C NMR chemical shifts of the compound C-3

	Carbon No.	δ Values
Glucose	1	92.90
	2	71.90
	3	70.02
	4	60.91
	5	73.36
	6	73.20
Fructose	1	62.14
	2	104.48
	3	82.17
	4	77.20
	5	77.80
	6	63.20

The above IR, ^{1}HNMR and ^{13}CNMR data and comparision with reported literature values (Lu *et al* 2007 and LEY and REID, 1979) can be nicely accommodated if the compound C-3 is sucrose.



Compound-3

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REFERENCES

Ahmad UM, Rahman AM, Jewena N and Hassan MM. 2010. Quinones of the aerial parts of *Leucas aspera*. Jahangirnagar University Journal of Science. 33 (1): 127-132.

Badami RC and Patil KB. 1975. Minor seed oils. X: Physico-chemical characteristics and fatty acid composition of seven minor oils. Journal of Oil Technologist's Association of India.7: 82–84.

Chatterje SK and Majumdar DN. 1969. Chemical investigation of *Leucas aspera*. Journal of the Institution of Chemists (India). 41: 98–101.

Jam MP and Nath HB. 1968. Examination of the component fatty acids of the oil from the seeds of *Leucas aspera*. Journal of Indian Chemical Society. 6: 34–36.

Pradhan B, Chakraborty D and Subba G. 1990. A triterpenoid lactone from *Leucas aspera*. Phytochemistry. 29: 1693–1695.

Rahman MS, Sadhu SK and Hasan CM. 2007. Preliminary antinociceptive, antioxidant and cytotoxic activities of *Leucas aspera* root. Fitoterapia. 78: 552–5.

Rao B and Narasimha GV. 1971. Antimicrobial action of some essential oils. IV. Effect of organic compounds. Riechstoffe, Aromen, Koerperpfl egemittel. 21(14, 16): 10–12.

Sadhu SK, Okuyama E, Fujimoto H and Ishibashi M. 2006. Diterpenes from *Leucas aspera* inhibiting prostaglandin-induced contractions. Journal of Natural Product. 69:

Lu YQ. Zhang FC and Zhang M, 2007. Chemical constituents of *Ixeris sonchifolia*. Journal of Chinese Pharmaceutical Sciences.16: 214–217.

Narayanan CR, 1974. Synthesis of some bridge triterpene ethers. Journal of Organic Chemistry. 39: 2639. 988–94.

Usher G, 1974. A Dictionary of Plants Used by Man, Constable and Co. LTD, Great Britain. pp. 352.

Vanisha NS and Subadra S,1998. Beta carotene content of green leafy vegetables of western India by HPLC. Journal of Food Science and Technology. 35 (4): 365.

Yamaguchi K. 1970. Spectral Data of Natural Products, Volume 1. Elsevier Publishing Company. pp. 417-421.