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Chemical Investigation on the Chloroform Fraction of the Aerial Parts of *Leucas zeylanica*

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Abstract Chemical Investigation on the Chloroform Fraction of the Aerial Parts of Leucas zeylanica were studied. The plant materials were clipping into small pieces and dried in air absence of sunlight. The dried materials were grinded into powder in a Cyclotech Machine (200 mesh) and stored in an air tight container. The air dried powder 5 kg was successively extracted with n-hexane (3×72 h), chloroform (3×72 h) and MeOH (3×72 h) respectively. On removal of the solvent, the n-hexane extract gave a light green Mass (18.5 gm), chloroform extract gave a deep green Mass (43 gm). and MeOH extract gave a light yellow mass (55g). The extracts, were concentrated to dryness under controlled temperature 45℃. Fractionation and purification of chloroform extract by chromatographic method afforded the new compound (A). The compound was characterized by chemical and spectroscopic method.

Keywords TLC, PTLC, IR, NMR, MS

1. Introduction

The genus Leucas (Lamiaceae) comprises about 80 species [Hedge, 1990]. The highest species diversity has been found in East Africa [Ryding, 1998]. In India, 43 species are available [Murkerjee, 1940]. The previous taxonomic accounts of Leucas for the present Bangladesh area have been given by Hooker (1885), Prain (1903) and Kangilal et al. (1939) who reported only five species. But eight species of the genus Leucas R. Br. (Lamiaceae) have been recognized for Bangladesh are given below [Mahbuba Khanam et al., 2005]. 1) Leucas aspera, 2) Leucas biflora, 3) Leucas cephalotes, 4) Leucas ciliata, 5) Leucas indica, 6) Leucas mollissima, 7) Leucas vestita and 8) Leucas zeylanica. *Leucas zeylanica* is one of the important medicinal plant of Bangladesh. The Bengali name of the plant is Kusha or Shetadrone. The tribal name of the plant is Pai Thung Sa (Marma). It belongs to the Lamiaceae family. Its English name is slitwort. It is a small, terrestrial, herbaceous, annual erect plant or sometimes tufted, hispid and aromatic plant growing to a height of upto 45 cm, stipules absent. Stem is green in color; It is qudra angular plant. Leaves are elliptic in shape and green in color which occur opposite sides of stemps and large in number. These are subsessile leaves which are linear lanceolate or elliptic lanceolate which is 2.5 to 7.5 cm long. Not lobed or divided, blunt at the tip, obtuse entire or cerrulate, gradular hispid and coarsely dentate at the margin. Roots are mainly tap root and fibrous which is white or brown in color. It is cultivated in home gardens for use in local medicine and as a pot herb. It is widely distributed throughout Southeast Asia is consisting of the countries that are south of China and Japan, east of India, west of Papua New Guinea and north of Australia but is rather rare in East Asia. Juice of the herb is used in headache, colds, scabies and other skin diseases. Decoction of leaves is used as a lotion for ulcers of the nose. The leaves are anthelmintic, diaphoretic, stimulant and Kulnerary. They are applied topically to heal wounds. The leaves are used as a poultice to treat itch and vertigo. The entire plant is externally rubbed on abdomen after childbirth in human pregnant [Harborne J.B. et al., 1999]. The sap of the leaves is used for sores of eyes and nostrils. Also it is used as a vermifuge with children. The plant is used medicinally for coughs, toothaches and abdominal pains. In Malaysia, the leaves may be taken as a sedative and to teal wounds. The whole herb has a bitter taste, but is still used as a pot herb. This was the first report that *Leucas zeylanica* showed antifungal activity against dermatophytes [Babu et al., 2016]. The microbs such as E.Coli and Coliforms growth were inhibit when exposed to leaves extract of *Leucas zeylanica* [Zhang et al., 2016]. In China and Malaya poultice of leaves is used for wounds and sores. In Srilanka it is used for anorexia, flatulence, colic, in mixture, used to treat malaria. In Thailand leaves, roots and flowers are used for weaning. Poultice of leaves used for wound healing and to stop

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bleeding. Najat Nidhal et al. (2020) studied on Leucas zeylanica. In this study, thirty compounds were isolated from Leucas zeylanica, including two norditerpenoid, three flavonoid glycosides, six flavonoids, two phytosterols, two phenylpropanoids, five terpenoids, one aliphatic glycoside, one nucleobase, one amino acid, two alkaloids and one cytochalasin. Previous phytochemical screening on the genus Leucas zeylanica indicated that very little phytochemical study on *Leucas zeylanica* was carried out. Therefore, it demands more phytochemical investigations. Research project on the aerial parts of *Leucas zeylanica* has been undertaken to achieve the following objectives:

1. The present study has been undertaken for detailed investigation of the aerial parts of *Leucas zeylanica* with an aim for isolation, purification and structural elucidation of the various secondary metabolites.
2. Our principal objective was to isolate and purify steroid and terpenoid along with other constituents from the aerial parts of *Leucas zeylanica* as well as to determine the molecular structure of the isolated secondary metabolites by various physical and chemical methods.

2. Methods & Materials

The aerial parts of *Leucas zeylanica* were collected from the campus area of Chittagong University of Engineering & Technology. For the experimental purpose only the matured and fresh plants were collected during the month of December 2018.

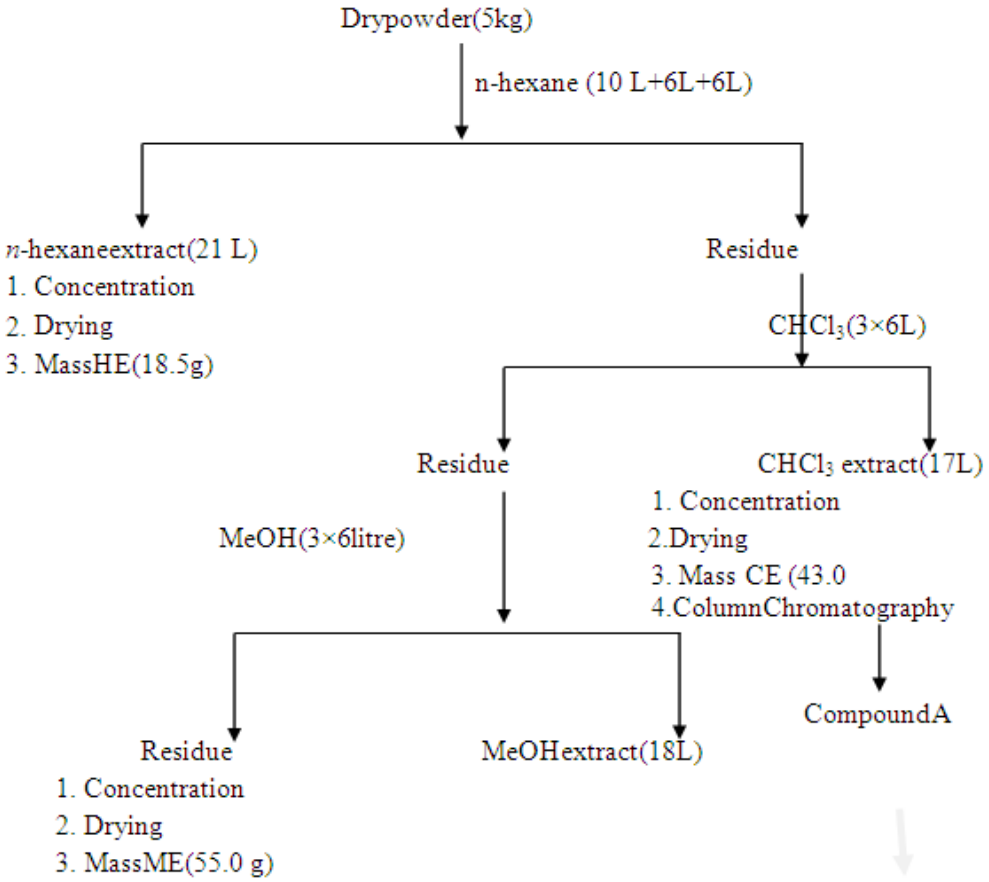
The plants were chopped into small pieces and dried in air in absence of sunlight. The dried materials were grinded into powder in a cyclotech-grinding machine (200 mesh). The powder was stored in polythene packets until used for extraction.

Solvents and Chemicals

All the solvents and chemicals used in the extractions and experiments were procured from E. Merk(Germany) or BDH (England) or Aldrich (America) and were either meant for laboratory use or were of analytical reagent grade. All solvents, like DCM chloroform, ethyl acetate, methanol and rectified spirit were distilled prior to use for extraction, chromatographic separation or any analytical purpose.

Extraction and fractionation of *Leucas zeylanica*

The air-dried powder (5kg) of *Leucas zeylanica* was successively extracted with n- hexane (3×72 h), $CHCl_3$ (3×72 h) and MeOH (3×72 h) respectively as described in the fractionation Scheme 1. On removal of the solvent, the n-hexane extract gave a light green Mass HE (18.5g), $CHCl_3$ extract gave a deep green Mass CE (43.0 g) and the MeOH extract gave a light yellow Mass ME (55.0 g).



Scheme 1. Extraction and fractionation of plant materials

Table 1. Column chromatographic seperation of Mass CE(5.0g)

No. of fractions	No.of Test tube	Eluting solvent	Observation	R_f values	Yield
CF1	1-10	*n*-hexane-EtOAc (10:1)	Nospot	-	-
CF2	11-21	*n*-hexane-EtOAc (10:1)	Onespot	~ 0.66	4.4% (220.0 mg)
CF3	22-60	Ethylacetate	Mixture		90% (4.5 g)

Table 2. Column chromatographic seperation of Mass CF3 (4.5g)

No. of ractions	No. of Test tube	Eluting solvent	Observation	R_f values	Yield
F1	1-7	$CHCl_3$:EtOAc:MeOH (1:1:1)	Nospot	-	-
F2	8-20	$CHCl_3$:EtOAc:MeOH (1:1:1)	Onespot	~ 0.58	6.1% (275.0 mg)
F3	21-30	$CHCl_3$:EtOAc:MeOH (1:1:1)	Onespot	~ 0.54	4.33% (195.0 mg)
F4	31-50	Methanol	Mixture		24.4% (1.10g)

Examination of the crude Mass CE

The crude Mass CE was soluble in $CHCl_3$ and EtOAc, partially soluble in petroleum ether and *n*-C_6H_{14} but insoluble in water. TLC of Mass CE was performed in different solvent systems on silica gel plates. The suitable solvent system for the best resolution was *n*-CH:EtOAc (10:1) in which one spot was observed with Rf value ~ 0.66. But a considerable amount of the crude remained in the base showing no sign of movement.

Separation of the crude Mass CE by CC

Mass CE (5.0 g) was dissolved in minimum quantity of $CHCl_3$ and adsorbed onto silicagel and finally recovered in the powder form for application into the column. The drypowder was then placed over a column (90 cm×8 cm) of silica gel made in *n*-C_6H_{14}.The elution was carried out with the solvent system *n*-C_6H_{14}:EtOAc(10:1). Portion of about 5 ml volume were collected at regular intervals. The eluents were monitored by TLC and pooled into three different fractions CF1, CF2 and CF3 depending on their TLC behaviour. Fraction CF1 being the void volume did not show any spot on TLC. Results of the chromatographic separation are in the **Table 1.**

Isolation of compound A from fraction CF2

Fraction CF2(220.0mg) was a white amorphous solid. It was soluble in $CHCl_3$ and EtOAc. On purification by repeated crystallization from the solvent system *n*-C_6H_{14}: EtOAc(8:1), it gave a white solid Mass(180.0mg). It gave a single spot with Rf value 0.66 in *n*- C_6H_{14}:EtOAc (10: 1) solvent system and was named as compound A. Fraction CF3(4.5g) keeps in the vacuum desiccators for further analysis.

Examination of crude Mass CF3

Mass CF3 was soluble in $CHCl_3$, EtOAc and MeOH. The Mass CF3 when examined by TLC using silica gel plates in different solvent systems, a minimum of two different spots could be visualized which indicated that CF3 might be a mixture of more than two different compounds. In the solvent system $CHCl_3$:EtOAc:MeOH (1:1:1), the Mass CF3 showed two distinct spots with Rf values ~0.58 and ~0.54 but a considerable amount of the crude remained in the base showing no sign of movement.

Separation of the crude Mass CF3 by CC

Mass CF3 (4.5 g) was dissolved in minimum quantity of $CHCl_3$ and adsorbed onto silica gel and finally recovered in the powder form for application into the column. The dry powder was then placed over a column (90 cm×8 cm) of silica gel made in $CHCl_3$. The elution was carried out with the solvent system CHCl3:EtOAc: MeOH (1:1:1). Portion of about 5 ml volume were collected at regular intervals. The eluents were monitored by TLC and pooled into four different fractions F1, F2, F3 and F4 depending on their TLC behaviour. Fraction F1 being the void volume did not show any spot on TLC. Results of the chromatographic separation are in the **Table 2.**

3. Results & Discussion

Compound A (180 mg) was an amorphous solid, m.p 170-172℃. Compound A did not give Salkowski and Liebermann-Burchard reaction for steroid and terpenoid. The IR spectrum of A showed broad absorption for O-H group at ν_{max} 3380 cm^{-1} and a weak absorption at V_{max}1603 cm^{-1} for C=C function in the molecule. The ^{13}C NMR spectrum of A showed the presence of 32 carbons in the molecule (Table 3). The mass spectrum of A exhibiteda molecular ion peak at m/z:481.5632[M+1] consistent with the molecular formula $C_{32}H_{48}O_3$. The ^{13}C DEPT spectrum of A revealed the presence of 13 methylene carbons, 11methine carbons and 3 methylcarbons in the molecule. The absorption

positions of all 32 carbons in ^{13}C NMR spectrum are given in Table 3. The ^{1}H NMR spectrum of A showed the presence of 8 aromatic protons attached to C2(7.22t), C3(6.42t), C4(7.69m), C5(7.42t), C6(6.40t), C2'(6.35d), C5'(6.37d), and C6'(7.09t). The absorption positions of two olefinic proton sattached to C20'(5.84d), 3 methine protons attached to C7(5.36s), C9'(4.96t) and C23'(1.43) are shown in ^{1}H NMR spectrum of A. Absorption positions of 12 sets of methylene protons and 3 sets of methyl protons are given in the Table 3. Absorption patterns, number of carbons and protons of compound A suggest the presence of two side chains in the molecule. The molecular ion peak of compound A m/z:481.5632 [M+1] along with other mass peaks m/z: [M+1] 463, 410, 340, 284, 218, 182,162, 123 etc can be explain on the given structure of A. The important H-H correlations in 2D COSY spectrum and H-C correlations in HMBC spectrum are presented in Fig-1 and Table- 3. Thus on the basis of IR,^{1}H NMR, ^{13}C NMR and mass spectra, structure **1** was confirmed to the compound A. Compound A is thus characterized as 4'-[7-[hydroxyl (phenyl) methoxy]-3'-isopentyl benzyl] tridec-8'-en-9'-ol and is reported first time from *Leucas zeylanica*.

4. Conclusions

The principal objective of this research work was to isolate biologically active compounds along with other secondary metabolites from the aerial parts of Leucas zeylanica as well as to determine the molecular structure of the isolated compounds. We are successful to finding out some of them. We are able to isolate and establish the structure of the compound A. The structure determination of the mentioned compound from Leucas zeylanica was first time reported from the plant body.

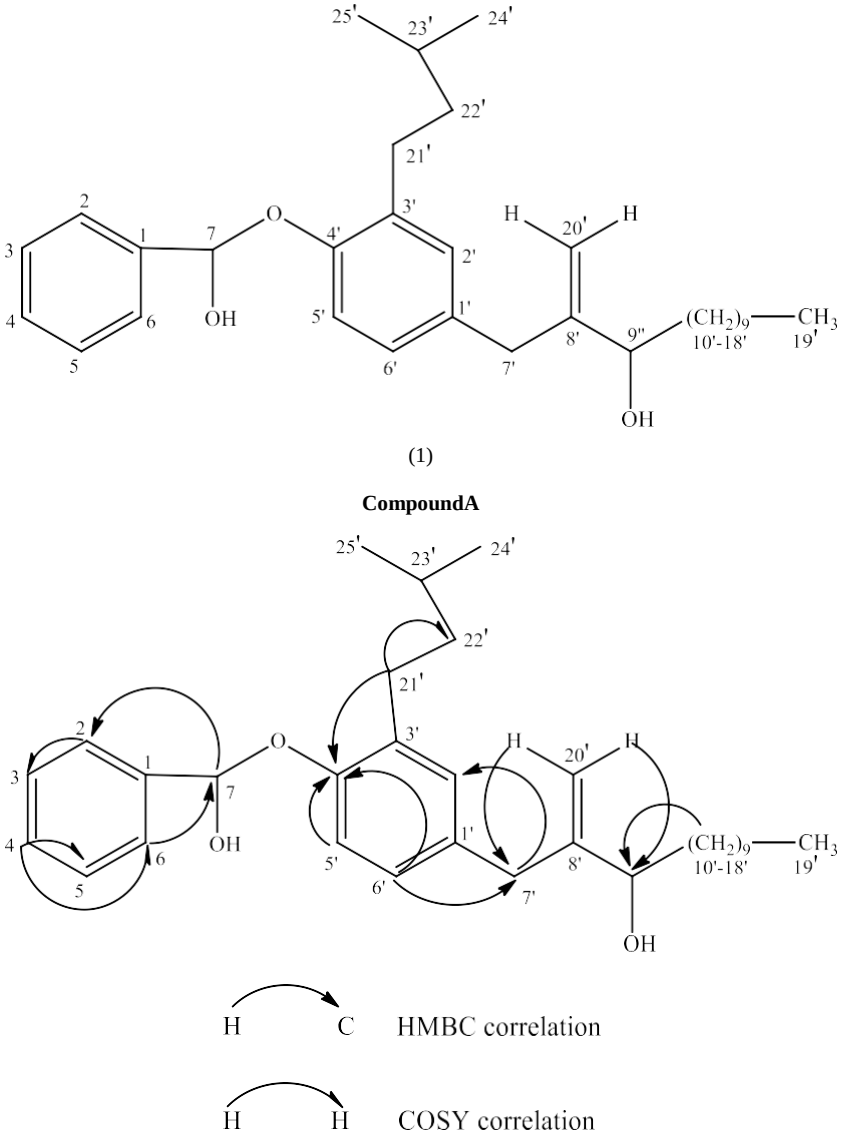


Figure 1. Important COSYand HMBC correlations of compound A

Table 3. ^{13}C NMR and ^{1}H NMR spectral data of Compound A recorded in $CDCl_3$

CarbonNos.	$^{13}C,\delta$	$^{1}H, \delta$	COSY	HMBC	CarbonNos.	$^{13}C, \delta$	$^{1}H, \delta$	COSY	HMBC
1	129.2				10'	33.5	1.64(m)	H-9',11'	C9'
2	107.8	7.22(t)	H-3	C7	11'	29.5	1.27		
3	128.8	6.42(t)	H-2,4	C2	12'	29.3	1.27		
4	127.1	7.69(m)			13'	29.2	1.27		
5	128.7	7.42(t)	H-4,6		14'	29.2	1.27		
6	128.6	6.40(t)	H-5,6		15'	29.1	1.27		
7	102.8	5.36(s)		C2	16'	29.0	1.27		
1'	129.1				17'	29.2	1.27		
2'	128.4	6.35(d)		C4'	18'	28.9	1.27		
3'	139.2				19'	14.0	.88(s)		
4'	156.9				20'	114.0	5.84(d)		C7',C9'
5'	130.3	6.37(d)		C4'	21'	37.2	2.37(t)	H-22'	C4'
6'	127.0	7.09(t)		C4'	22'	24.7	1.27		
7'	53.8	4.93(s)		C2',C4'	23'	31.9	1.43		
8'	127.3				24'	11.9	.90(s)		
9'	77.2	4.96(t)	H-10'		25'	11.5	.90(s)		

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