EXTRACTION AND IDENTIFICATION OF SOME CHEMICAL CONSTITUENTS FROM *EULOPHIA CAMPESTRIS* W. (GAMON-THANGA-ZIN) RHIZOME

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Abstract

The present research deals with the isolation and characterization of some chemical constituents of *Eulophia campestris* W. (Gamon-thanga–zin) rhizomes. *Eulophia campestris* W. is widely used in Myanmar as the traditional medicine for the diabetes, heart tonic, and aphrodisiac. Semi-quantitative elemental analysis of *E. campestris* rhizome was carried out by ED-XRF method. From the analysis, the elements of major composition such as K, Fe and Ca and minor composition such as Zn and Cu were observed in the sample. The isolation of some compounds from *E. campestris* rhizome was performed by column chromatographic method. Firstly, the dried powdered sample of *E. campestris* (250 g) was extracted with 70 % EtOH at room temperature. The concentrated extracts were partitioned with Pet-ether (60°-80°C) to remove the fat. The defatted residue was then extracted with ethyl acetate in a separating funnel. Ethyl acetate layer was evaporated to obtain a dry extract (4.0 g). After the extraction with ethyl acetate from *E. campestris* W. crude extract was separated by column chromatographic method on a silica gel column [(Toluene: EtOAc) (95: 5)], as eluent. The eluates were examined under UV lamps (254 nm & 365 nm) and on TLC plates by detecting with anisaldehyde reagent. The fractions with similar R_f values were combined. After combining together similar fractions, the isolated compounds, MM-1, MM-2 and MM-3 were obtained. The isolated compounds were also identified by UV, FT-IR, ^{1}H, ^{13}C NMR and GC-MS method. According to the chemical tests, UV, FT-IR, ^{1}H, ^{13}C NMR, and GC-MS methods, the isolated compounds could be deduced as 1,2-Benzene dicarboxylic acid, diisooctyl ester (MM-1, 0.01%), 3-cyclohexene-1-ol,5-(2-butenylidene)-4,6,6-trimethyl (MM-2, 0.011 %) and Bis -(2-Ethylhexyl) Phthalate ester (MM-3, 0.0075%) respectively.

Keywords: Column chromatographic method, *Eulophia campestris* W., Gamon-thanga-zin, GC-MS method, Spectroscopic method

Introduction

Description of the Plant

Genus *Eulophia* is highly diverse, occurs in a wide range of habitats, and belongs to family Orchidaceae. This plant produces two shoots, reproductive and vegetative, from their underground tubers. The genus *Eulophia* has a wide distribution and comprises over 230 species, which are widespread from tropical and Southern Africa, Madagascar and from neotropics to throughout tropical and subtropical parts of Asia and Australia (Cieslicka, 2006).

A terrestrial orchid *Eulophia campestris* is very common in the beach forests. The genus *Eulophia* species are widely found in the regions which possesses warm and humid, tropical climate with a temperature of 20° to 32°C and a mean relative humidity between 82 to 85% throughout the year. Traditional medicines with therapeutic utility have been used since antiquity and are still contributing a significant role in the primary health-care system. It is estimated that 70-80% of the world's population relies on traditional herbal medicines for their primary health care (WHO, 2008).

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Medicinal and Nutritional Uses

In Ayurvedic medicine, *Amarkand* is generally prescribed as expectorant, anabolic, tonic, diuretic, astringent, digestive, and soft purgative, and also recommended for the treatment of ear discharge, blood clotting, joint edema, and debility. In addition, it is also considered as a general tonic to promote strength and alleviates all the three "doshas". These are also used in stomatitis, purulent cough; and in the heart problems, dyscrasia, and scrofulous diseases of the neck; bronchitis, blood diseases, and as a vermifuge (Hossain, 2011). Some of the *Eulophia* species have been studied for their nutritional properties. *E. campestris* Wall. is available as a *salep* (flour of starch) in Indian markets, as food for children and convalescents (Artemas, 1923). Balance between nutrients and anti-nutrients were studied in *E. ochreata* Lindl. It was found that tubers had low values of all free carbohydrates and had a low content of anti-nutrients such as phytic acid and trypsin inhibitors (Aberoumand, 2009). The proximate composition and mineral constituents indicated that these tubers are a good source of plant fibers, proteins, and carbohydrates (Aberoumand and Deokule, 2009).

Figure 1 Leaves, stem and rhizome of *E. campestris* W. (Gamon-thanga-zin)

Materials and Methods

The rhizomes of *E. campestris* were collected from Kyaik-Htiyo hill, Mon State, Myanmar in cold season. Semi-quantitative elemental analysis of *E. campestris* rhizome was carried out by ED-XRF method (Griken, *et al.*, 1986). The isolation of some chemical compounds from *E. campestris* rhizome was carried out by solvent extraction and column chromatographic methods (Figure 2). The isolated compounds were confirmed by UV lamp and chemical reagent tests. The isolated compounds were characterized by UV, FT-IR, ^{1}H, ^{13}C NMR and GC-MS methods (Silverstein and Webster, 1998).
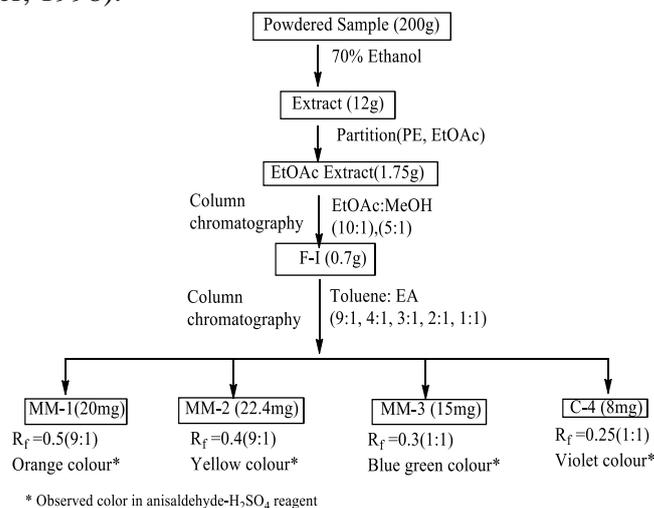

Figure 2 Flow diagram of isolation of compounds from *E. campestris* rhizome

Aim

To identify the isolated compounds of *E. campestris* rhizome by spectroscopic methods

Results and Discussion

## Semi-quantitative Elemental Analysis of *E. campestris* Rhizome

Semi-quantitative elemental analysis of *E. camprestris* rhizome was carried out by ED-XRF method (Griken, *et al*., 1986). From the analysis, the elements of major composition such as K (59.75%), Fe (16.89%) and Ca (14.87%) and minor composition such as Zn (4.97%) and Cu (3.52%) were observed in the sample (Figure 3). In human metabolism, potassium reduces the hypertension. Iron helps the function of blood in body. Calcium is very important element for the formation of teeth and bone.

Figure 3 ED – XRF spectrum of *E. campestris* W. rhizome

Isolation and Preliminary Confirmation of the compounds by UV and Reagent Test

Compounds (MM-1, 2 and 3) of EtOAc crude extract from *E. camprestris* rhizome were separated by column chromatographic method on silica gel G adsorbent. The polarity of eluent, toluene and ethyl acetate was successively increased from 9:1 to 1:1. According to the procedure in Figue 2, three compounds (MM-1, 2 and 3) were isolated from EtOAc crude extract. The isolated compounds were UV active (254nm & 365nm) and they were detected by spraying on the TLC chromatograms with anisaldehyde-H_2SO_4 reagent, iodine, 5% $FeCl_3$ and vanillin-sulphuric acid reagent. The results are shown in Figure 4 and Table 1.
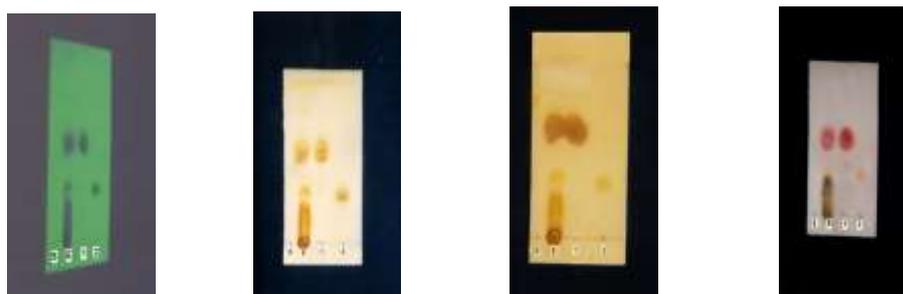
(a) UV_{254}nm   (b) I_2 reagent   (c) $FeCl_3$ (d) Vanillin & H_2SO_4
(S = Solvent,   E = Crude extract,   1 = MM-1, 2 = MM-3)

Figure 4 TLC spot test of isolated compound MM-1 and MM-3

Table 1 Physicochemical Properties of Isolated Compounds by Colour Reaction Tests

Isolated Compound	Solvent System	R_f	UV		Reagent				
			254nm	365nm	Anisal-dehyde	I_2	5%Fe Cl₃	Vanl. & S/A	Liberm.& Burchard
MM-1	Tol.:EA (9 : 1)	0.55	+	+	Orange	Yellow	No colour change	Yellow	-
MM-2	Tol.:EA (9:1)	0.46	+	+	Yellow	Yellow	-	Dark	-
MM-3	Tol: EA (1:1)	0. 3	+	+	Greenish blue	Yellow	No colour change	Yellow	-

Identification of Chemical Constituents from *E. campestris* Rhizome

Compounds: MM-1, 2 and 3 of EtOAc crude extract from *E. camprestris* rhizome were separated by column chromatographic method. The isolated compound MM-1, MM-2 and MM-3 were identified by UV, FT-IR, ¹H NMR, ¹³C NMR spectroscopic methods and GC-MS method. According to spectroscopic methods, the compound MM-1, MM-2 and MM-3 could be deduced as 1, 2-Benzene dicarboxylic acid, diisooctyl ester (0.01%), 3-cyclohexene-1-ol, 5- (2-butenylidene)-4,6,6-trimethyl (0.011 %) and Bis -(2-Ethylhexyl) Phthalate ester (0.0075%) respectively. The results of isolated compound MM-1 are shown in Figure 5, 6, 7, 8, 9, Table 2, 3, and 4.
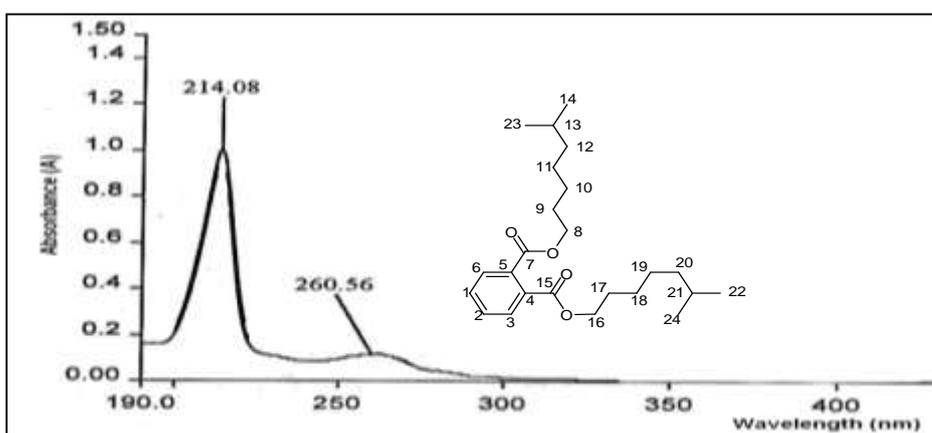

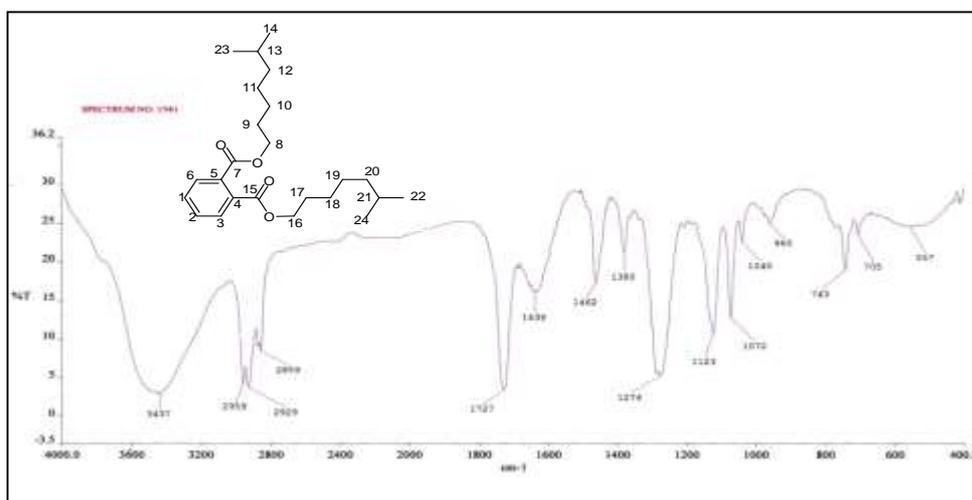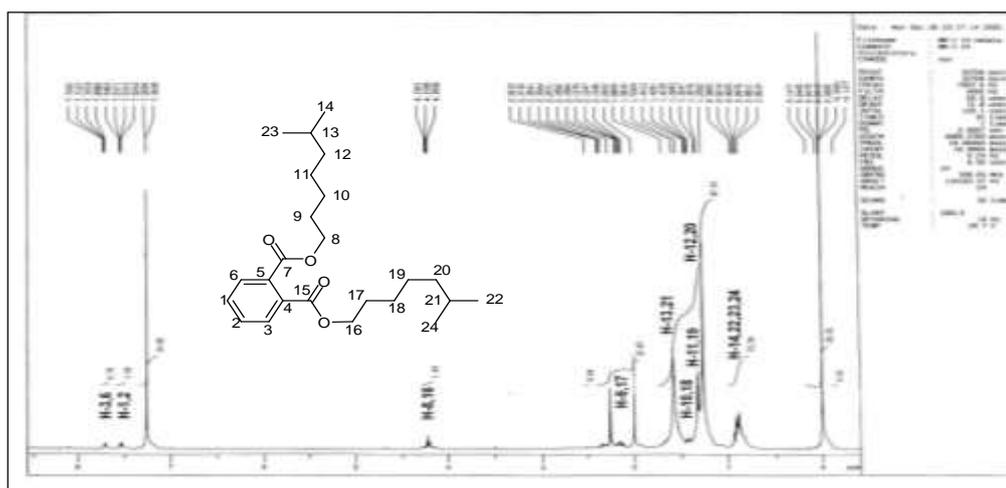**Figure 5**   UV spectrum of isolated compound MM-1

Figure 6 FT-IR spectrum of isolated compound MM-1

Table 2 FT-IR Spectral Data of Isolated Compound MM-1

Frequency Range (cm^{-1})	Mode of Vibration	Assignment
2929	ν_{asym} C-H	- CH$_3$ and -CH$_2$
2859	ν_{sym} C-H	- CH$_3$ and -CH$_2$
1727	ν C=O	Ester carbonyl
1638 (vw)	ν C=C	Ortho-disubstituted
1462	δ_{oop} -CH$_2$	-CH$_2$ group
1380	δ_{oop} -CH$_3$	-CH$_3$ group
1274	ν_{asym} C – O – C	Aromatic ester
1123	ν C – O	Alkyl substituent
1072, 1040	$\delta_{in\text{-}plane}$-CH$_2$ or -CH$_3$	- CH$_2$ or -CH$_3$ group
743 (vs)	ν C = C	Ortho-disubstituted

Figure 7 ^{1}H NMR spectrum of isolated compound MM-1 (400 MHz, CDCl$_3$)

Table 3 ^{1}H NMR Spectral Data of Isolated Compound MM-1 (400 MHz, CDCl$_3$)

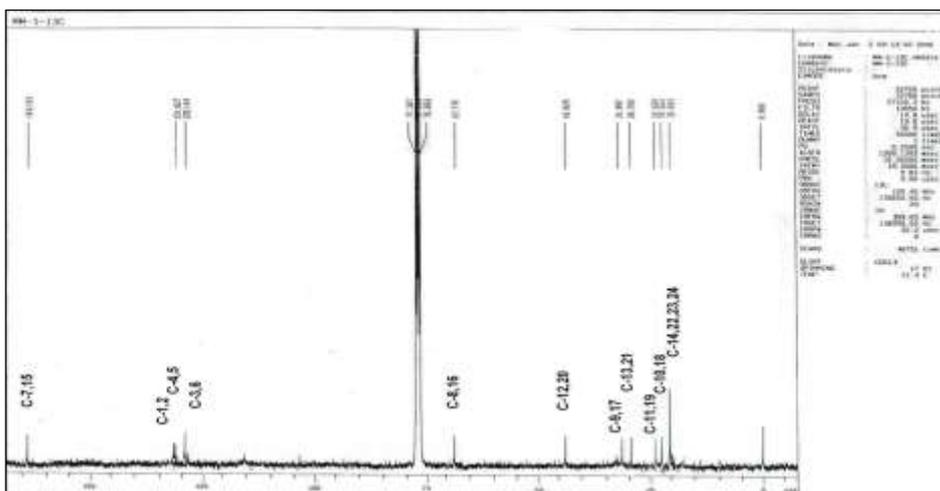| H – No.	δ_H (ppm)	Type of Proton	H – No.
H – 1, 2	7.53	dd	= CH
H – 3, 6	7.71	d	= CH
H – 8, 16	4.21	t	O – <u>CH$_2$</u>- CH$_2$
H – 9, 17	2.13	m	-CH$_2$-<u>CH$_2$</u>-CH$_2$
H – 10, 18	1.42	m	-CH$_2$-<u>CH$_2$</u>-CH$_2$
H – 11, 19	1.30	m	-CH$_2$-<u>CH$_2$</u>-CH$_2$
H – 12 , 20	1.25	m	-CH$_2$-<u>CH$_2$</u>-CH
H – 13 , 21	1.58	m	CH$_2$-<u>CH</u>-
H–14,22,23,24	0.90	m	<u>CH$_3$</u>-CH(CH$_3$)- <u>CH$_3$</u>

Figure 8 ^{13}C NMR spectral data of isolated compound MM-1(100 MHz, CDCl$_3$)

Table 4 ^{13}C NMR Spectral Data of Isolated Compound MM-1 (100 MHz, CDCl$_3$)

| C – No.	δ$_C$ (ppm)	Interpretation
C-7, 15	163.7	Ester carbonyl C
C-4, 5	131.8	Aromatic C (q) with ortho substitution
C-1, 2	132.0	Aromatic methine C
C-3, 6	129.1	Aromatic methine C
C-8, 16	67.7	Oxygenated methylene C
C-9, 17	31.8	Aliphatic methylene C
C-10, 18	22.7	Aliphatic methylene C
C-11, 19	23.6	Aliphatic methylene C
C-12, 20	43.8	Aliphatic methylene C
C-13, 21	29.7	Aliphatic methine C
C-14,22,23,24	20.0	Aliphatic methyl C
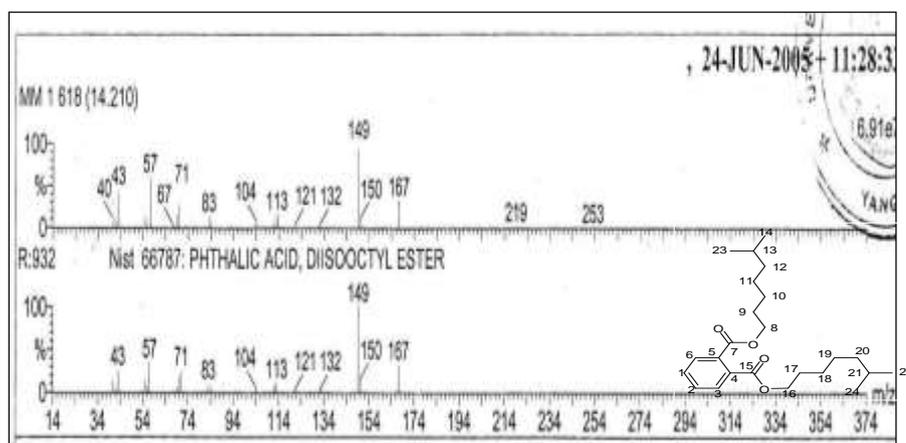
Figure 9 GC-MS spectrum of MM-1 compared with that of 1, 2-benzene dicarboxylic acid diisooctyl ester from GC-MS library data

According to spectroscopic methods, the isolated compound MM-2 was deduced as 3-Cyclohexene-1-ol, 5-(2-Butenylidene)-4,6,6-trimethyl-, (Z, E). The results are shown in Figure 10, 11, 12 and Table 5.
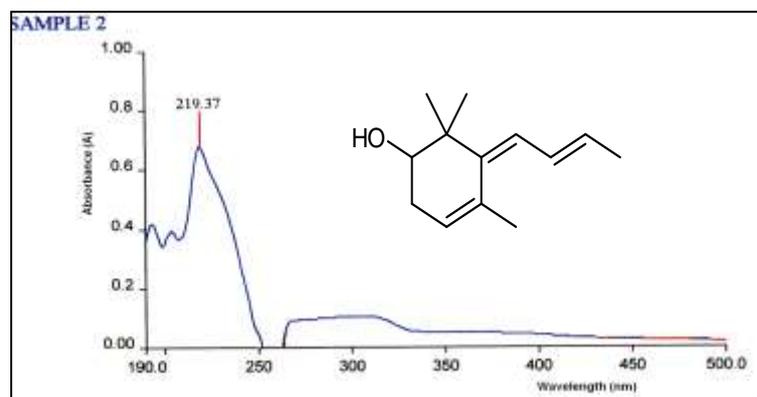
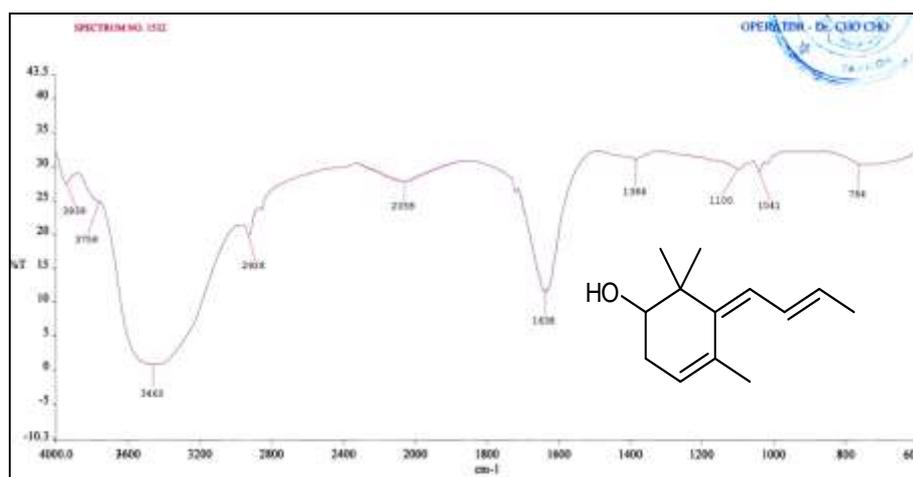**Figure 10** UV spectrum of isolated compound MM-2

Figure 11 FT-IR spectrum of isolated compound MM-2 (KBr)

Table 5 FT-IR Spectral Data of Isolated Compound MM-2

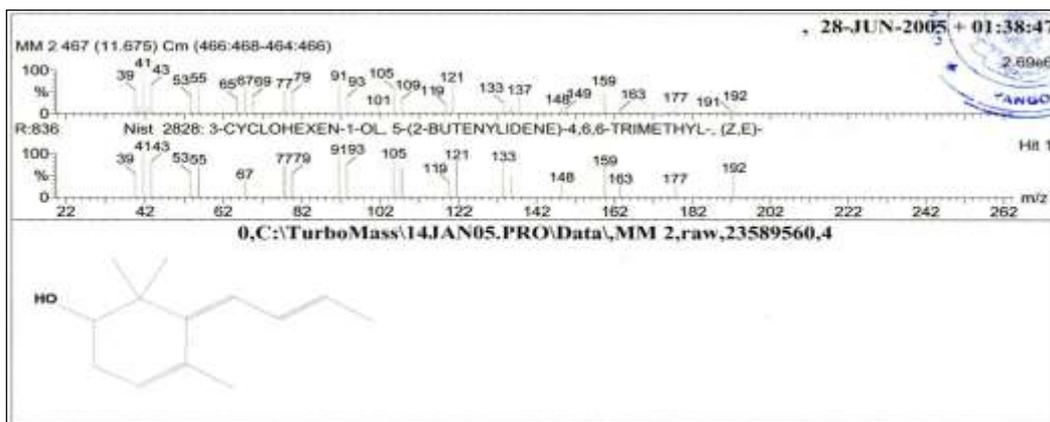| Frequency Range (cm^{-1})	Mode of Vibration	Assignment
3460	ν O-H	- OH group
2928	ν C-H (asym.)	- CH$_2$ &CH$_3$ groups
2870	ν C-H (sym.)	- CH$_2$ &CH$_3$ groups
1384	δ –CH$_3$ (out-of-plane)	Germinal dimethyl group
1638	νC=C	cyclohexene
1041	ν-OH	Cyclic sec. alcohol group
764	ν CH=CH (asym.)	Cyclic alkene

Figure 12   GC-MS spectrum of MM-2 compared with that of 3-cyclohexen-1-ol, 5-(2-butenylidene)-4, 6, 6-trimethyl-,(Z,E) from GC-MS library data

According to spectroscopic methods, the isolated compound MM-3 could be deduced as Bis (2-Ethylhexyl) Phthalate Ester. The results are shown in Figure 13, 14, 15, 16, Table 6 and 7.
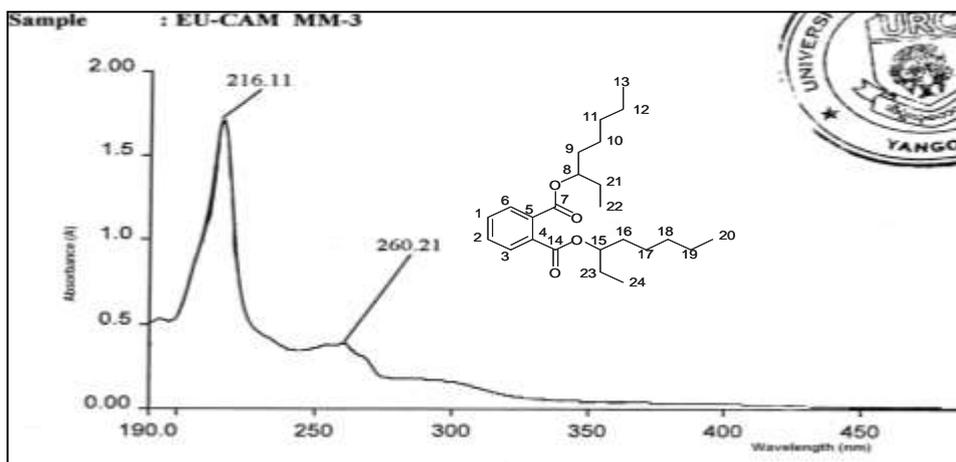

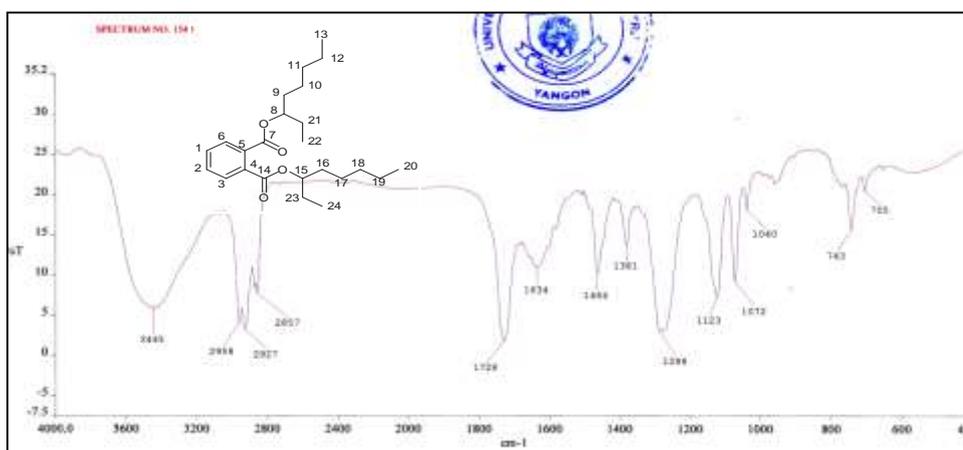**Figure 13**   UV spectrum of isolated compound MM-3

Figure 14   FT-IR spectrum of isolated compound MM-3 (KBr)

Table 6   FT-IR Spectral Data of Isolated Compound MM-3

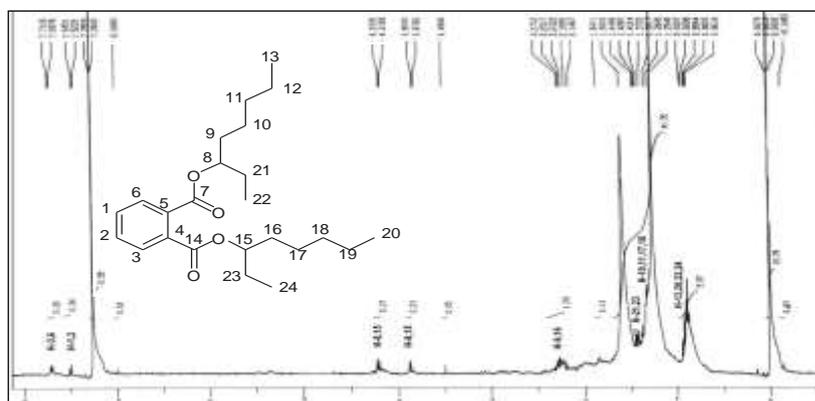| Frequency Range (cm^{-1})	Mode of Vibration	Assignment
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1728	ν C=O	Ester carbonyl group
1634 (vw), 743(vs)	ν C=C	-C = C-
1464	δ_{oop} -CH_2	-CH_2 group
1381	δ_{oop} -CH_3	-CH_3 group
1288	ν_{asym} C – O – C	Aromatic ester
1123	ν C – O	Alkyl substituent
1072, 1040	$\delta_{in-plane}$ -CH_2 or -CH_3	- CH_2 or -CH_3 group

Figure 15 ^{1}H NMR spectrum of isolated compound MM-3 (400 MHz, CDCl$_3$)

Table 7 ^{1}H NMR Spectral Data of Isolated Compound MM-3 (400 MHz, CDCl$_3$)

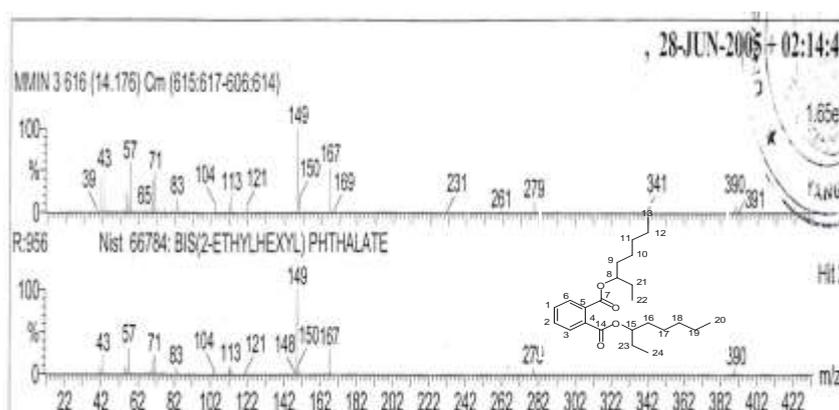| H – No.	δ_H (ppm)	Multiplicity	Type of Proton
H – 3, 6	7.71	dd	= CH
H – 1, 2	7.53	d	= CH
H – 8, 15	4.21, 3.87	q	O – C̲H̲- (CH_2)$_2$
H – 9, 16	2.3	m	-CH-C̲H̲$_2$-CH_2
H – 10, 11, 17, 18	1.25	m	-CH_2-C̲H̲$_2$-CH_2
H – 21, 23	1.4	m	-CH_2-C̲H̲$_2$-CH_2
H–13, 20, 22, 24	0.90	Triplet	-CH_2 – C̲H̲$_3$

Figure 16   GC-MS spectrum of isolated compound MM-3 compared with that of bis (2-ethylhexyl) phthalate easter from GC-MS library data

Conclusion

This research concerns with the isolation and characterization of some chemical compounds from *E. campestris* (gamon-thanga-zin) rhizome. The rhizome samples were collected from Kyaik-htiyo hill, Mon State, Myanmar. Semi-quantitative elemental analysis of *E. camprestris* rhizome was carried out by ED-XRF method. From the analysis, the elements of major composition such as K (59.75%), Fe (16.89%) and Ca (14.87%) and minor composition such as Zn (4.97%) and Cu (3.52%) were observed in the sample. In human metabolism, potassium reduces the hypertension. Iron helps the function of blood in body. Calcium is very important element for the formation of teeth and bone. The extraction and isolation of *E. camprestris* rhizome were performed by column chromatographic separation method. Form the separation, the pure isolated compounds; MM-1, MM-2 and MM-3 were obtained. The identification of isolated compounds from *E. camprestris* rhizome was carried out by the modern spectroscopic method such as UV, FT-IR, ^{1}H NMR, ^{13}C NMR and GC-MS methods. According to the spectroscopic method, the isolated compounds; MM-1, MM-2 and MM-3 could be deduced as 1,2-benzene dicarboxylic acid diisooctyl ester, 3-cyclohexen-1- ol, 5-(2-butenylidene)-4,6,6-trimethyl-,(Z,E) and bis (2-ethylhexyl) phthalate easter, respectively. According to the experimental results, *E. camprestris* rhizome may be used in the medicinal formulation for human health.

Acknowledgements

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