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REPORT

GC-MS Analysis of the Rhizome Oil of *Lagenandra toxicaria* Dalz.

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ABSTRACT The methanol extract of *Lagenandra toxicaria* (Rhizome) was subjected to column chromatographic technique. The oil obtained, was subjected to GC-MS analysis and the chemical constituents present in the oil were identified as Methyl ester of 2-hydroxy benzoic acid, Diethyl phthalate, Oleic acid, Palmitic acid ethyl ester and Dioctyl phthalate. Diethyl phthalate was found to be the major constituent (89.46%).

(*Lagenandra toxicaria*, rhizome oil, GC-MS analysis, chemical constituents, bioactivities)

INTRODUCTION

Lagenandra toxicaria Dalz. (Figure 1) of Araceae is endemic to peninsular India [1]. It is a semi aquatic herb, found in marshes and along watercourses, often growing gregariously in semi evergreen forests at the altitude of 350 -1200 m. By tradition the plant is used in the preparations of ointments for skin itch and the rhizome is used in renal and cardiac ailments [2]. Rhizomes are considered carminative, tonic, diuretic and used in bilious complaints. The juice of the fresh plant is applied to wounds for quick healing [3]. Traditionally the plant is said to have insecticidal properties [4]. Effective utilization of any information requires its systematic evaluation. Recently, the plant was subjected to scientific studies and the rhizome oil of *Lagenandra toxicaria* has been shown to have antibacterial activity against the three human pathogens, namely *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* [5] by agar disc diffusion technique [6]. The rhizome oil was found to be more or less equally effective with that of the standard antibiotic chloramphenicol in the *in vitro* condition. The rhizome oil of *Lagenandra toxicaria* as well as possesses insecticidal and germicidal properties [7]. The oil was evaluated for its insecticidal activity against the storage pest *Tribolium castanaem* Herbst., by filter paper impregnation method and the LC_{50} value was found to be 0.069% in 24 hours. Low concentration (0.5µl/ml) of the oil in water totally inhibited the germination of seeds (*Cicer arietinum*, *Oryza sativa* and *Vigna radiata*), in the evaluation of germicidal activity carried out by the method of Rao and Singh [8]. There are about 12 species of *Lagenandra*, mainly in Sri Lanka [9], one species in North East India and four species in South India [10]. There has been no report of the phytochemical studies on the genus *Lagenandra* to date. Hence an attempt has been made to identify the chemical constituents of this useful medicinal plant, *Lagenandra toxicaria*. It is the first species of the genus subjected to phytochemical studies. This paper focuses on the GC-MS analysis of the *hitherto* unexplored, medicinally valued rhizome oil of *Lagenandra toxicaria* Dalz.

MATERIALS AND METHODS

The rhizomes of *Lagenandra toxicaria* were collected from Karayar, a Tirunelveli hill of Tamil Nadu, India, in the month of March 2003. Authentic specimens were deposited in the Department of Botany, St. John's College, Palayamkottai, Tamil Nadu, India. The rhizomes were collected in gunny bags and cleaned with water, chopped, dried and ground into powder.

Only glass containers were used for storing the powder and extract. Dry powder (300g) of *Lagenandra toxicaria* rhizome was extracted with two litres of methanol (8 hours / 3 times / 65°C), using a three litre round bottom flask fitted with a water condenser. After distilling the solvent, the extract was concentrated under reduced pressure.

The brown viscous methanol extract (20 g) was loaded onto a silica gel (60 - 120 mesh) column (4 x 25 cm) and eluted with nine different eluents of increasing polarity. The polarity of the eluents was increased, starting from n-hexane, n-hexane: ethyl acetate (4:1), n-hexane: ethyl acetate (3:2), n-hexane: ethyl acetate (2:3), n-hexane: ethyl acetate (1:4), ethyl acetate, ethyl acetate: methanol (3:1), ethyl acetate: methanol (1:1) to absolute methanol, (each 500 ml). The fractions were collected separately in 100 ml portions. A total of 45 fractions were obtained. The fractions from n-hexane: ethyl acetate (4:1) eluent, numbered 7 and 8 yielded greenish yellow oil (LT – oil), which were found to be the same by thin layer chromatographic analysis and hence pooled.

The oil was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant oil was subjected to GC-MS (Gas Chromatograph – Mass Spectrum) analysis. The oil (0.1 μl) was subjected to systematic GC and MS analysis using the SHIMADZU instrument, GC-MS P5000 (Japan). The length and diameter of the column (Carr. Gas Press (*k*Pa) : 24.50) was 20 m and 0.25 mm respectively. The initial temperature was 70°C and then risen by 10°C/min. to 300°C. The temperature 300°C was maintained for 20 minutes. The GC Parameters are presented in Figure 2. Five compounds (LT-1 to LT-5) were identified. The results are presented in Table 1.

RESULTS AND DISCUSSION

The oil obtained from *Lagenandra toxicaria* (**LT-oil**) through column chromatography, when subjected to GC-MS analysis showed five peaks indicating the presence of five compounds (labeled LT-1 to LT-5) (Figure 3). The compound LT-2 was found to be the major component of the oil at 89.46% followed by compound LT-1 at 9.87%. The other three compounds LT-3, LT-4 and LT-5 were present only in trace. The mass spectra of these compounds are presented in Figures 4 - 8. Comparison of the mass spectra of these compounds to those of the compiled data of Wiley 7n : 1 library attached to the mass spectrometer revealed compound LT-1 to be the methyl ester of 2-hydroxybenzoic acid. Compound LT-2 was found to be diethyl phthalate. Compound LT-3 was identified as oleic acid and compound LT-4 was identical to palmitic acid ethyl ester. Compound LT-5 was identified as dioctyl phthalate.

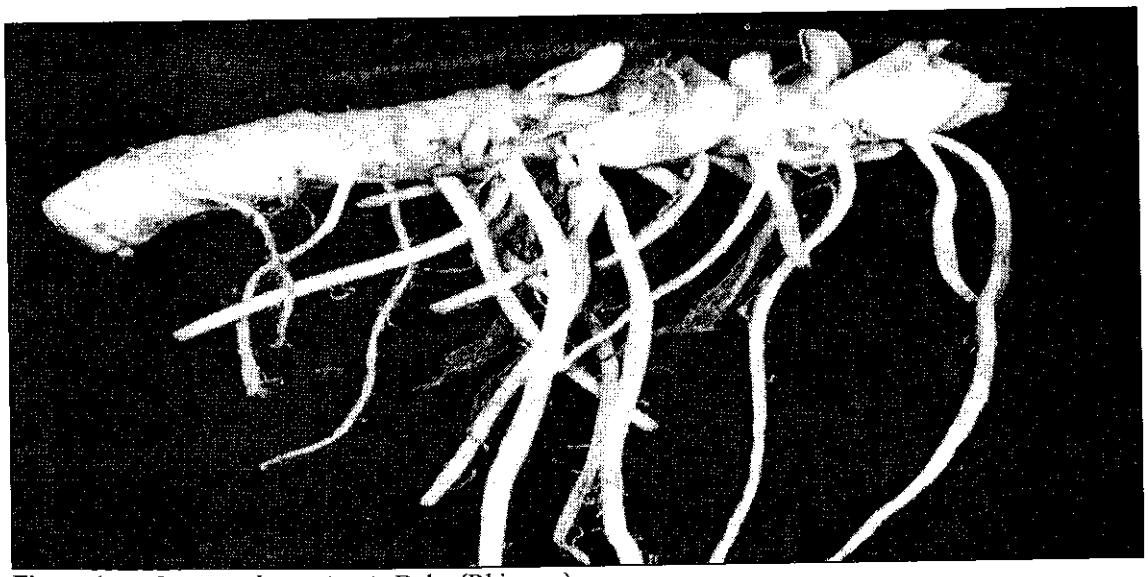


Figure 1. *Lagenandra toxicaria* Dalz. (Rhizome)

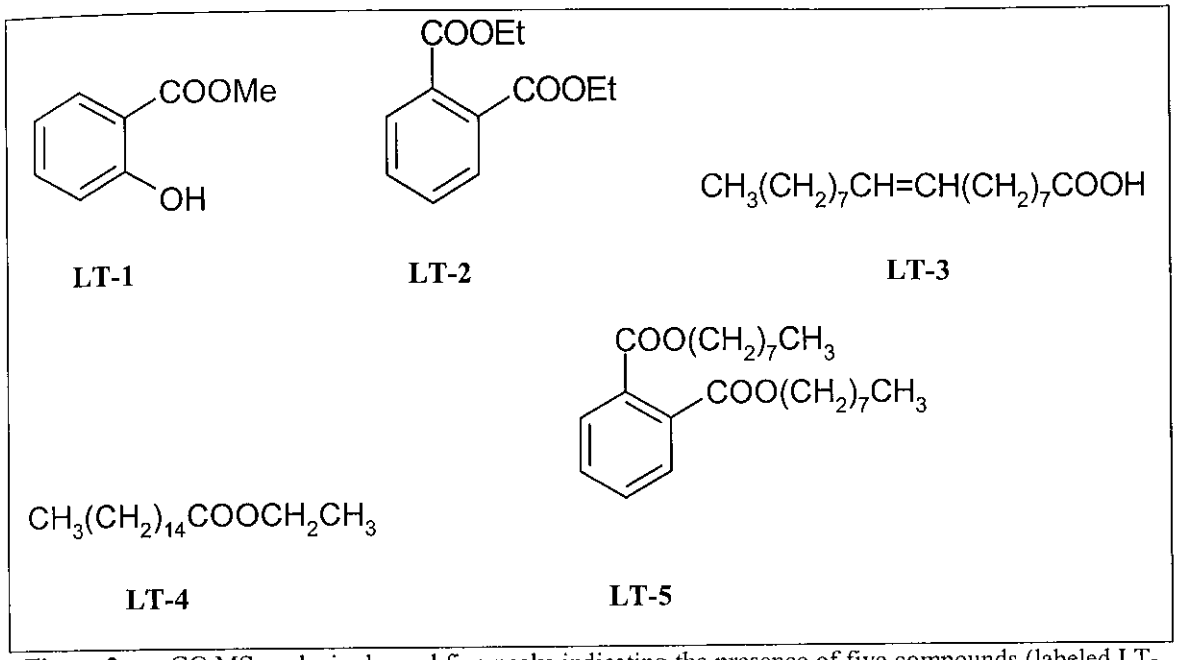
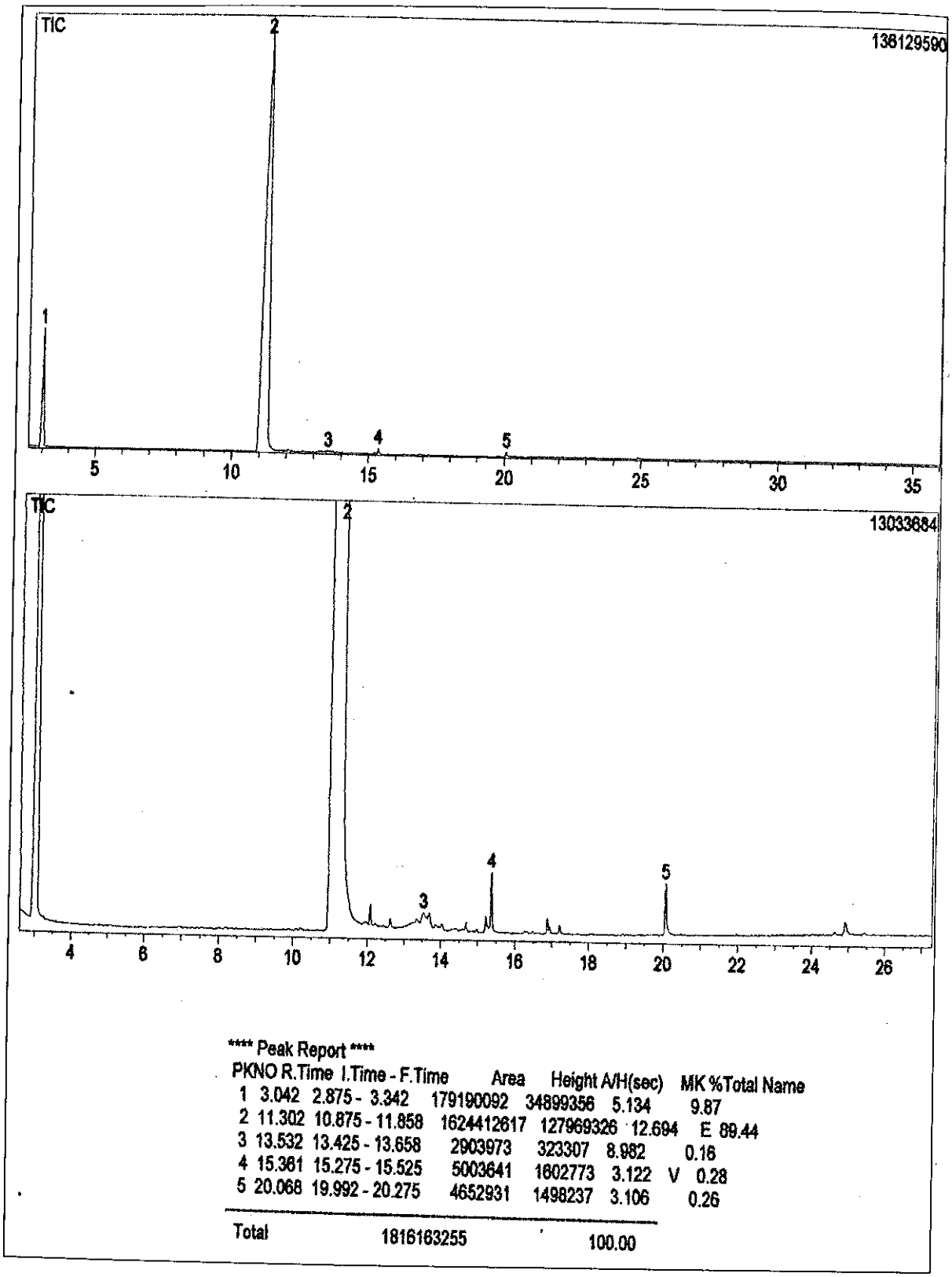


Figure 2. GC-MS analysis showed five peaks indicating the presence of five compounds (labeled LT-1 to LT-5)

Table 1. GC- MS analysis of the oil fraction of methanol extract of the rhizome of *Lagenandra toxicaria* Dalz.

PEAK NO.	RETENTION TIME	NAME OF THE COMPOUND	AREA (%)
1	3.04	Methyl ester of 2 hydroxy benzoic acid	9.87
2	11.30	Diethyl phthalate	89.46
3	13.53	Oleic acid	0.15
4	15.36	Palmitic acid ethyl ester	0.26
5	20.07	Dioctyl phthalate	0.26



**** Peak Report ****

PKNO	R.Time	I.Time - F.Time	Area	Height	A/H(sec)	MK	%Total	Name
1	3.042	2.875 - 3.342	179190092	34899356	5.134		9.87	
2	11.302	10.875 - 11.858	1624412617	127969326	12.694	E	89.44	
3	13.532	13.425 - 13.658	2903973	323307	8.982		0.16	
4	15.361	15.275 - 15.525	5003641	1602773	3.122	V	0.28	
5	20.068	19.992 - 20.275	4652931	1498237	3.106		0.26	
Total			1816163255				100.00	

Figure 3. GC-MS chromatogram of the oil obtained from *Lagenandra toxicaria* (LT - oil) showing five peaks

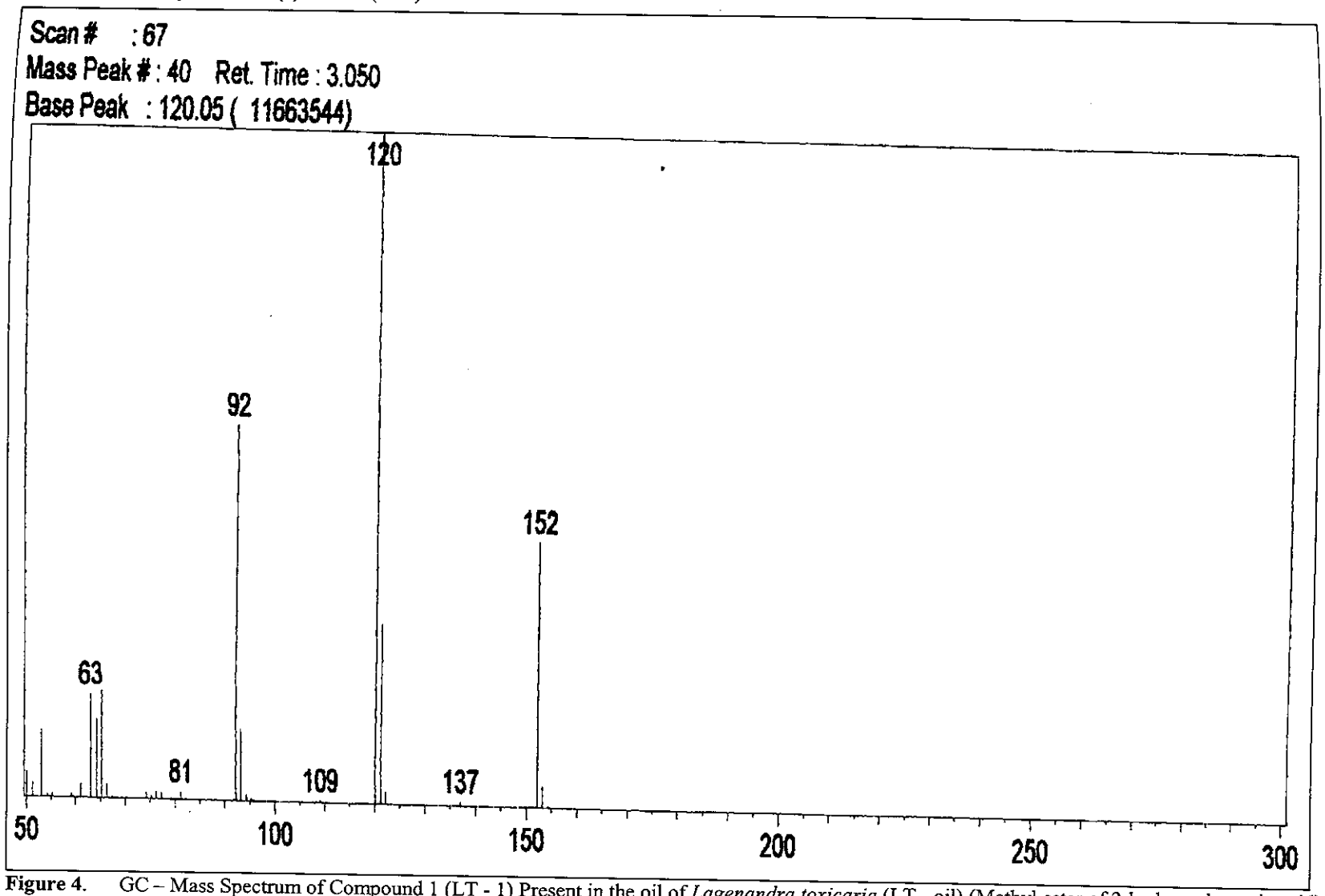


Figure 4. GC – Mass Spectrum of Compound 1 (LT - 1) Present in the oil of *Lagenandra toxicaria* (LT - oil) (Methyl ester of 2-hydroxy-benzoic acid)

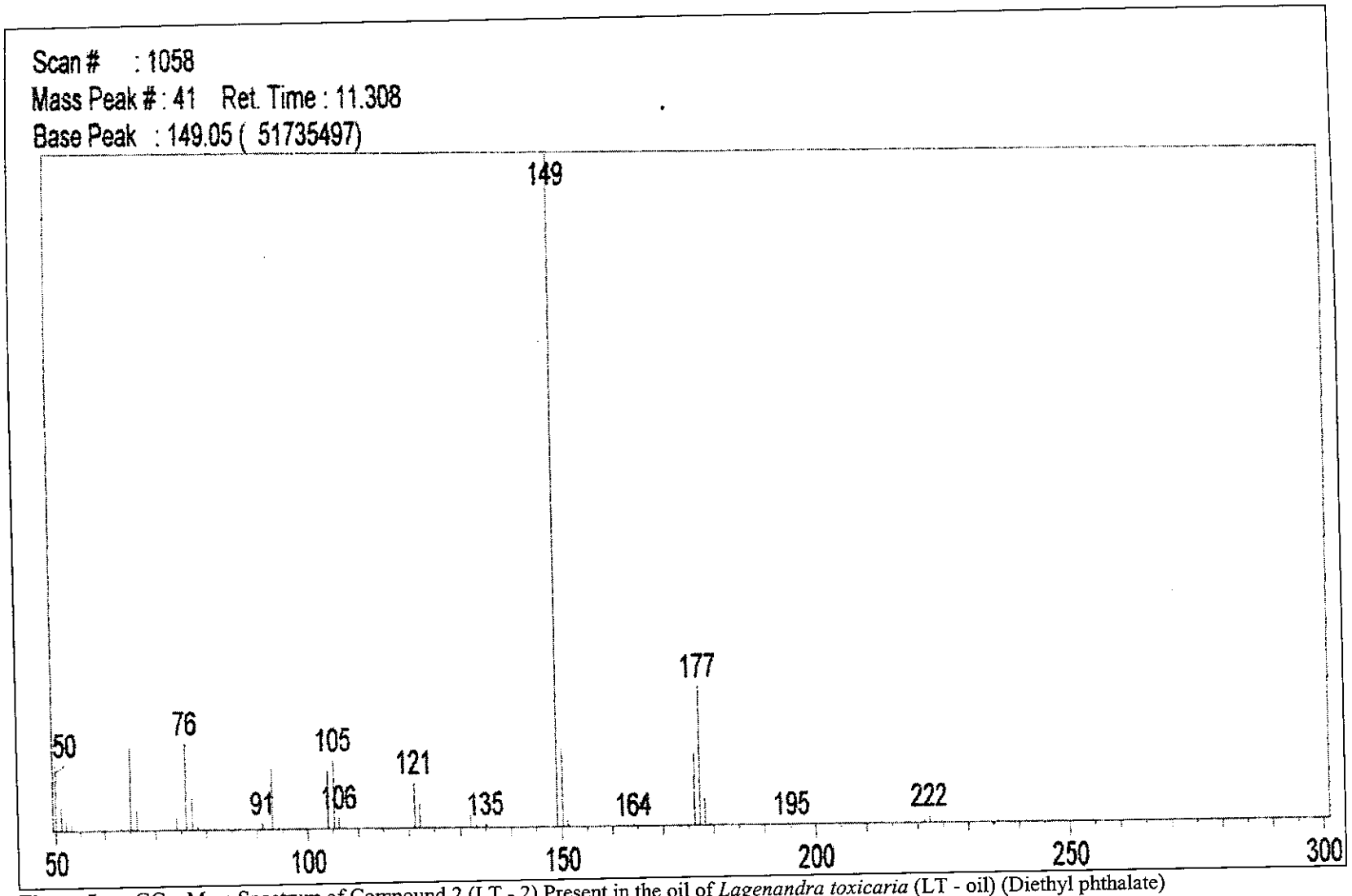


Figure 5. GC – Mass Spectrum of Compound 2 (LT - 2) Present in the oil of *Lagenandra toxicaria* (LT - oil) (Diethyl phthalate)

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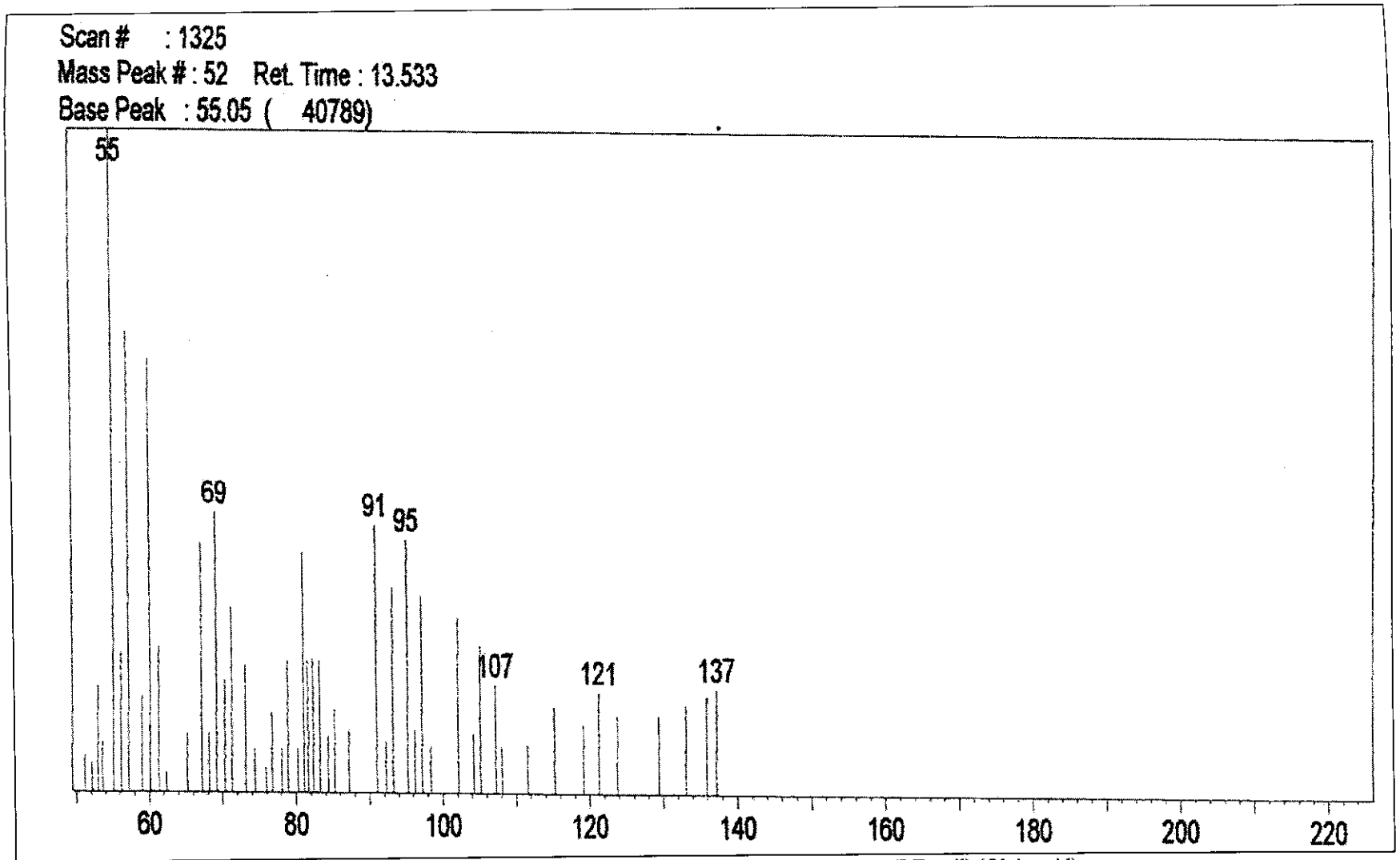

Figure 6. GC – Mass Spectrum of Compound 3 (LT - 3) Present in the oil of *Lagenandra toxicaria* (LT - oil) (Oleic acid)

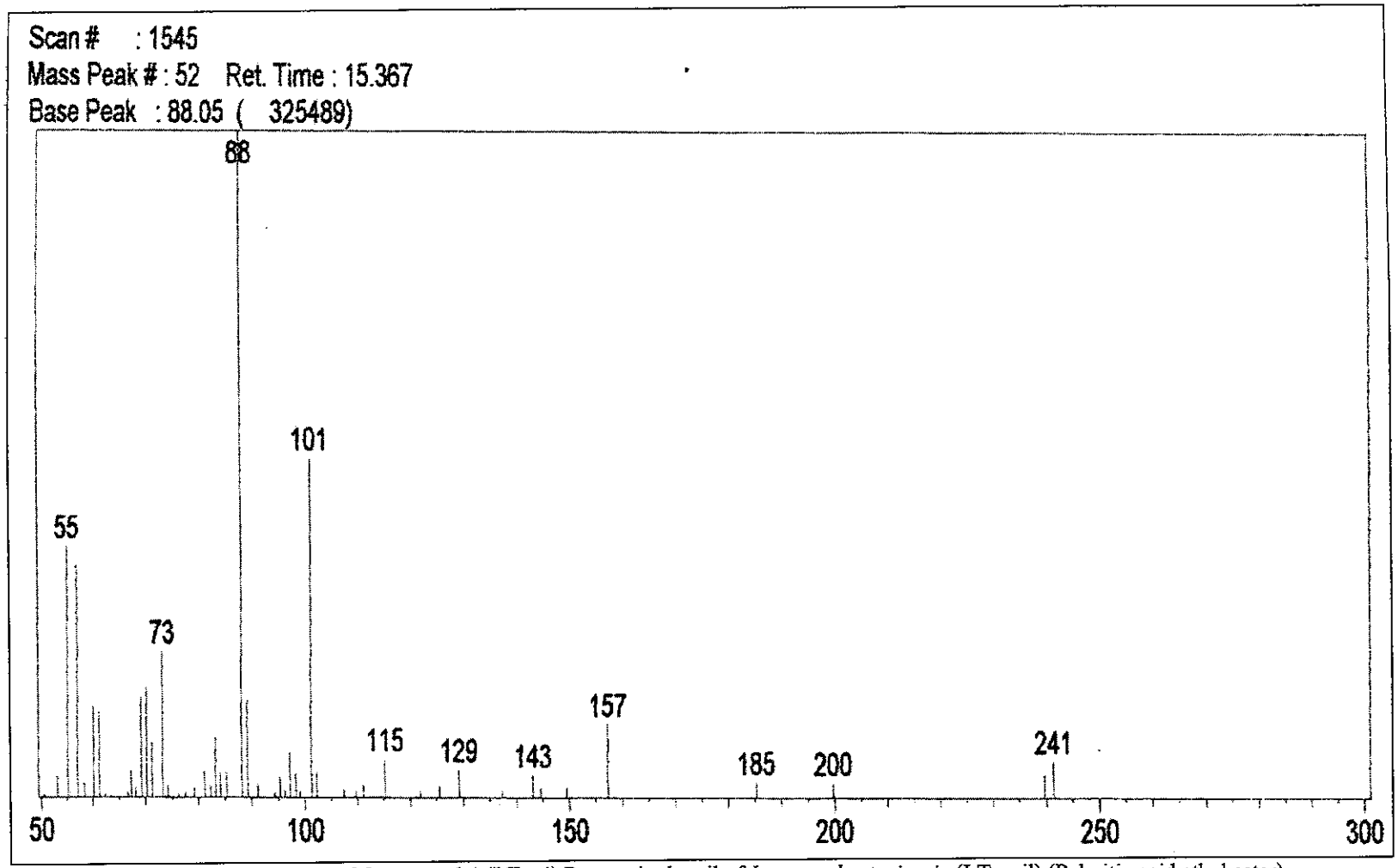

Figure 7. GC – Mass Spectrum of Compound 4 (LT - 4) Present in the oil of *Lagenandra toxicaria* (LT - oil) (Palmitic acid ethyl ester)

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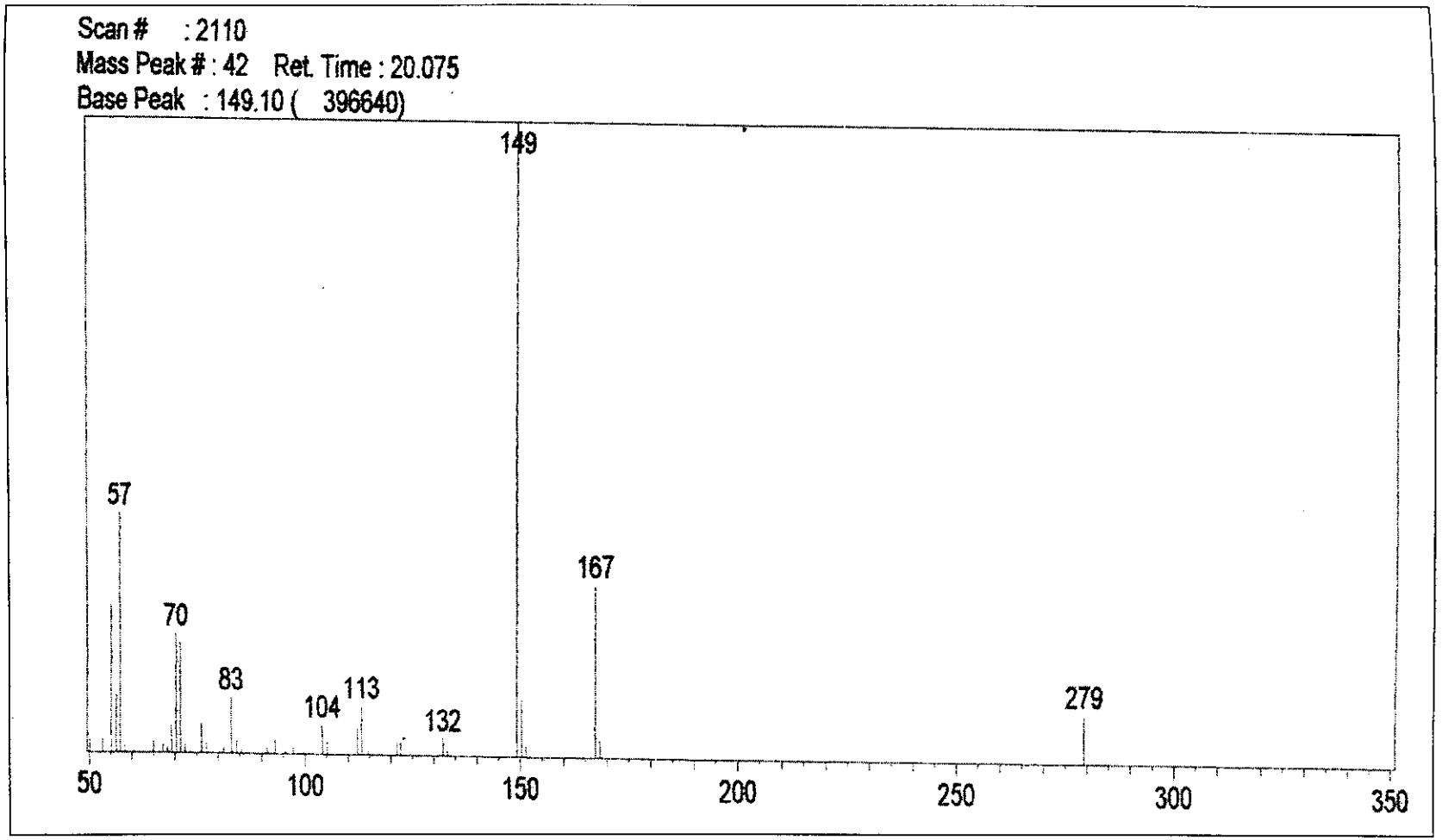

Figure 8. GC – Mass Spectrum of Compound 5 (LT - 5) Present in the oil of *Lagenandra toxicaria* (LT - oil) (Dioctyl phthalate)

Literature survey reveals that benzoic acids and its esters have been used externally as antiseptic lotions, ointments and mouthwashes [11]. It is more effective as a preservative in foods and pharmaceutical products at low pH [12]. Methyl ester of 2-hydroxy-benzoic acid is the major constituent of "winter green oil" (Wiley 7n:1 library), an essential oil, generally obtained from the leaves of a shrub *Gaultheria procumbens* (Ericaceae) from the Eastern United States and Canada, in which it occurs as a monotriphosphate (13). It is also extracted from the bark of *Betula lenta* (Betulaceae) and *Gaultheria fragrantissima*, which are used in North America in the formulation of oral hygiene, cosmetic and external pharmaceutical products [14].

The chemical compounds as reported in the present study; from *Lagenandra toxicaria* showed their pharmacological interest. The antibacterial, insecticidal and germicidal activities of *Lagenandra toxicaria* rhizome oil as referred above might be due to the toxic effect of these chemical constituents. Diethyl phthalate was found to be the major constituent (89.46%) of *Lagenandra toxicaria* rhizome oil. Diethyl phthalate has been reported as an antibacterial agent against *Campylobactor jejuni, Escherichia coli, Listeria monocytogenes* and *Salmonella enterica* [15].

Dioctyl phthalate, an antimicrobial compound was also isolated from the methanol extract of the marine brown alga *Sargassum wightti* by purifying through silica gel column and thin layer chromatography [16].

Diethyl phthalate has lethal toxicity. It affects certain organs at low concentration, specifically the reproductive organs, lungs, kidney and liver [17]. In the present study, diethyl phthalate is the major constituent of the rhizome oil of *Lagenandra toxicaria* hence possesses insecticidal activity in addition. Gogaoi *et al.* [18] observed reduction in germination of seedling growth of rice by addition of phenolics like benzoic and coumaric acids. Rice seedlings lost photo and geotropisms on application of 100 ppm of dioctyl phthalate [19]. Phthalates are the natural germination inhibitors [20]. Therefore, the oil inhibited germination of seeds.

CONCLUSION

The identification of chemical compounds from *Lagenandra toxicaria* carried out in this effort is a pioneer work, which helps to shed some light to the importance of this plant to mankind. The plant is poisonous in the unprocessed form, and self-medication with this wild plant is not advisable. The present study may serve as a guide in the selection of the plants with antibiotic activity for further work on the isolation and elucidation of the active compounds. An extensive research and development work should be undertaken, for their better economic and therapeutic utilization.

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SERIES B

PHYSICAL SCIENCES