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ANALYSIS OF BIOACTIVE CONSTITUENTS FROM THE LEAVES OF *MALLOTUS TETRACOCCUS* (ROXB.) KURZ, BY GAS CHROMATOGRAPHY - MASS SPECTROMETRY

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Keywords:

Mallotus tetracoccus, Phytochemicals, GC-MS analysis, Bis (2-ethyl hexyl) phthalate, Longiborneol, Phthalates

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ABSTRACT

Mallotus tetracoccus (Roxb.) Kurz. is found in Western Ghats of India. *Mallotus tetracoccus* is one of the medicinally important plants belonging to the family Euphorbiaceae, commonly known as “vatta kanni” in Tamil. In the present study the ethanolic extract of *Mallotus tetracoccus* has been subjected to GC-MS analysis. The major chemical constituents are Bis (2-ethyl hexyl) phthalate (46.78%), 3-methyl-2-(2-oxypropyl) furan (13.31%), E-8-methyl-9-tetradecen-1-ol acetate (6.63%), Octadecanoic acid, 2-oxo (4.46%) and Longiborneol (2.39%). Thus the extract of *Mallotus tetracoccus* was characterized by substantial levels of diesters (50%), alcohols (15%), alkanes (3%), sesquiterpenes (5%), terpenoids (13%), fattyacid (5%) and sugars (2.6%). The presence of some of these constituents in the plant extract provides the scientific evidences for the antipyretic, anti-inflammatory and hepatoprotective properties of the plant.

INTRODUCTION: Natural products have played an important role in the development of drugs and drug leads for various diseases including cancer [1]. The secondary metabolites from natural sources are good candidates for drug development because being elaborated within the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than totally synthetic drugs [2]. Thus a search for anticancer compounds from medicinal plants is on a rise.

Several species of the genus *Mallotus* are a rich source of biologically active compounds such as phloroglucinols, tannins, terpenoids, coumarins, benzopyrans and chalcones [3-9]. *Mallotus tetracoccus* (Roxb.) Kurz, are found in evergreen forests up to 1600m. The common names include *Mullu polavu,* Vatta (tamil)*, Thavatta, Vatta, Vatta kumbil, Vetta kumbil* (malayalam) and *Uppale mara* (kannada). The trees grow up to 5-15 m tall, leaf blades are triangular-ovate or ovate, sometimes 1- or 2-lobate, 10-25 × 9-20 cm, leathery, abaxially brownish tomentose, adaxially glabrous, base obtuse or truncate. The reported bioactivities of the extracts or the individual chemical constituents isolated from this genus include antipyretic [10], anti-inflammatory, hepatoprotective [11], antioxidant and radical scavenging activities [12]. Though there are many works reported on various species of Mallotus, a literature search revealed no references to previous work on *Mallotus tetracoccus* plant extract composition.

Thus the objective of the study was to identify the active compounds from *Mallotus tetracoccus* leaf extract by GC-MS analysis.

MATERIALS AND METHODS:

Collection of plant material: The leaves of *Mallotus tetracoccus* were collected from the Agasthiar Malai Reserved Forest, Western Ghats, South India, authenticated by the Director, Centre for Biodiversity and Forest Studies, Madurai Kamaraj University, and voucher specimens were deposited in the herbarium of Centre for Biodiversity and Forest Studies of our university (No. AM-03).

Preparation of powder and extract: Leaves (1 kg) were shade dried, powdered and extracted with ethanol for 6-8 hours using soxhlet apparatus. The extract was then filtered through muslin, evaporated under reduced pressure and vacuum dried to get the viscous residue. The ethanolic extracts of the plant was used for GC-MS analysis.

GC - MS analysis:

Preparation of extract: *2* µl of the ethanolic extract of *Mallotus tetracoccus* was employed for GC/MS analysis.

Instruments and chromatographic conditions: GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of components: Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS:

GC-MS analysis:

GC-MS chromatogram of the ethanolic extract of *Mallotus tetracoccus* is given in **Figure 1**.

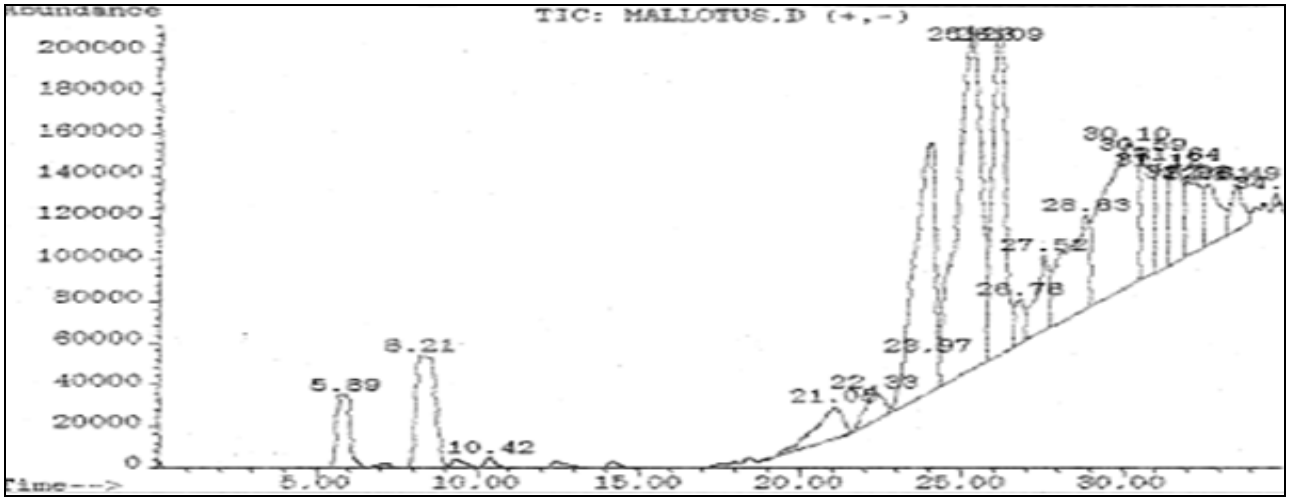


FIG. 1: GC-MS CHROMATOGRAM OF ETHANOLIC EXTRACT OF THE WHOLE PLANT OF *MALLOTUS TETRACOCCUS*

On comparison of the mass spectra of the constituents with the NIST library, fifteen peaks were obtained out of which thirteen phytoconstituents were characterized and identified (**Table 1**). The retention time (RT) is in minutes.

The various phytochemicals which contribute to the medicinal activity of the plant are listed in **Table 2**.

TABLE 1: PHYTOCOMPONENTS IDENTIFIED IN THE ETHANOLIC EXTRACTS OF THE WHOLE PLANT OF *MALLOTUS TETRACOCCUS* BY GC-MS

S. No.	RT	Name of the compound	Molecular Formula	MW	Peak Area (%)
1.	21.03	1, 2-benzenecarboxylic acid	$C_8H_6O_4$	166.14	2.34
2.	22.33	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	345.56	1.33
3.	25.23	Bis (2-ethyl hexyl) phthalate	$C_6H_4(C_8H_{17}COO_2)$	390.56	46.78
4.	27.32	d-mannitol, 1-o-heptyl	$C_6H_{14}O_6$	182.17	2.61
5.	28.83	E-8-methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268.43	6.63
6.	30.10	3-methyl-2-(2-oxopropyl) furan	C_5H_6O	82.102	13.31
7.	30.59	4-methyl-Z-4-hexadecen-1-ol	$C_{17}H_{34}O$	254.54	3.83
8.	31.12	Tetrapentacontane, 1.34-dibromo	$C_{50}H_1O_2$	703.35	2.90
9.	31.63	Octadecanoic acid, 2-oxo	$C_{18}H_{36}O_2$	284.48	4.46
10.	31.96	4-methyl-dodecec-3-en-1-ol	$C_{17}H_{34}O$	254.54	3.03
11.	32.61	Longiborneol	$C_{15}H_{26}O$	222.37	2.39
12.	33.49	p-menth-8(10)-en-9-ol, cis	$C_{10}H_8O$	154.25	1.49
13.	34.73	Dodecane, 1, 2-dibromo	$C_{12}H_{24}Br_2$	328.13	0.13

TABLE 2: ACTIVITY OF PHYTO-COMPONENTS IDENTIFIED IN THE ETHANOLIC EXTRACTS OF THE WHOLE PLANT OF *MALLOTUS TETRACOCCUS* BY GC-MS ANALYSIS

RT	Name of the compound	Compound Nature	**Activity
21.03	1, 2-benzenecarboxylic acid	Diisononyl ester	Preservative
22.33	Di-n-octyl phthalate	Phthalic acid	Plasticizer, cosmetics
25.23	Bis (2-ethyl hexyl) phthalate	Diester of phthalic acid	Plasticizer
27.32	d-mannitol, 1-o-heptyl	Sugar alcohol	Osmotic diuretic agent, renal vasodilator
28.83	E-8-methyl-9-tetradecen-1-ol acetate	Alcohol	Insect pheromone
30.10	3-methyl-2-(2-oxopropyl) furan	Isoprene	Not reported
30.59	4-methyl-Z-4-hexadecen-1-ol	Alcohol	Insect pheromone
31.12	Tetrapentacontane, 1.34-dibromo	Alkane	Insect pheromone
31.63	Octadecanoic acid, 2-oxo	Saturated fatty acid	Preservative, cosmetics
31.96	4-methyl-dodecec-3-en-1-ol	Alkene alcohol	Not reported
32.61	Longiborneol	Sesquiterpenoid	Semiochemical, antibacterial, antifungal
33.49	p-menth-8(10)-en-9-ol, cis	Terpene alcohol	Flavour and fragrance agent
34.73	Dodecane, 1, 2-dibromo	Alkane	Not reported

The components may be grouped in to five main classes: Diesters (50%), alcohols (15%), alkanes (3%), sesquiterpenes (5%), terpenoids (13%), fatty acids (5%) and sugars (2.6%). The major constituent was found to be bis(2-ethylhexyl) phthalate at retention time of 23.97, 25.23 and 26.09. Phthalate is found at retention time of 23.03 minutes. Mannitol, a sugar alcohol is found at RT 27.32. 3-methyl-2-(2-oxopropyl) furan is said in RT 30.10, is said to be another major compound with a peak area of 13.31%. E-8-methyl-9-tetradecen-1-ol acetate is found next with RT value of 30.59. The sesquiterpenoid, longiborneol had a RT value of 32.62. p-menth- 8(10)-en-9-ol is a monocyclic terpene alcohol with a RT value of 33.49. It is used as flavour and fragnance enhancer. 1, 2-dibromo dodecane, an alkane had an RT value of 34.73. 4-methyl-dodecen-1-ol, an alkene had an RT value of 31.96.

The major phytochemical constituent's present in ethanolic extract of *Mallotus tetracoccus* are presented as mass spectra and compound structures are in (**Figure 2 to Figure 5**). They were identified as Bis (2-ethyl hexyl) phthalate (46.78%), E-8-methyl-9-tetradecen-1-ol acetate (6.63 %), Octadecanoic acid, 2-oxo (4.46%) and longiborneol (2.39%) respectively.

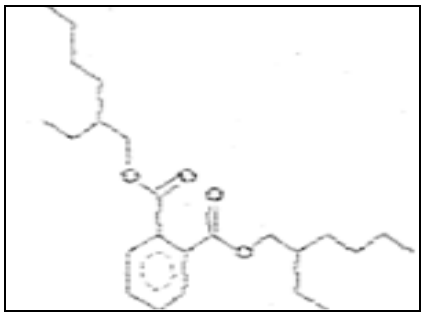
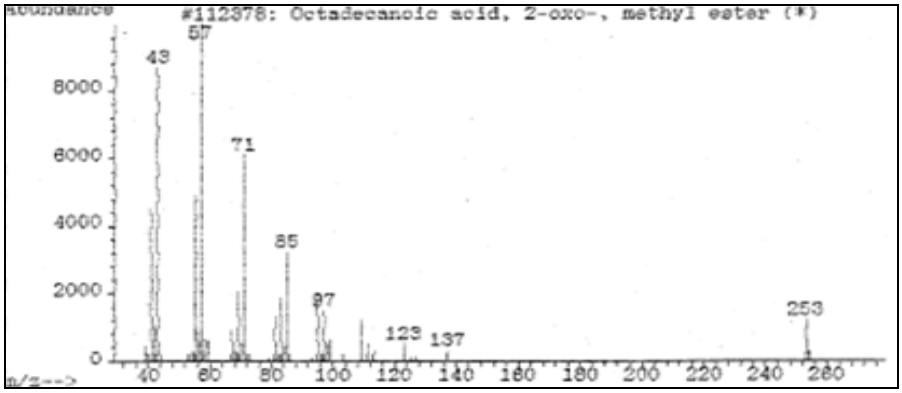


FIG. 2: THE MASS SPECTRUM ANALYSIS AND STRUCTURE OF BIS (2-ETHYL HEXYL) PHTHALATE (46.78 %)



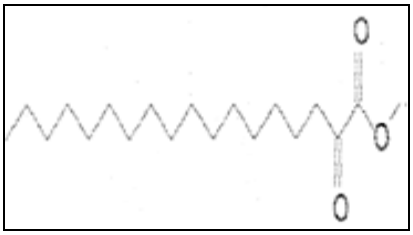
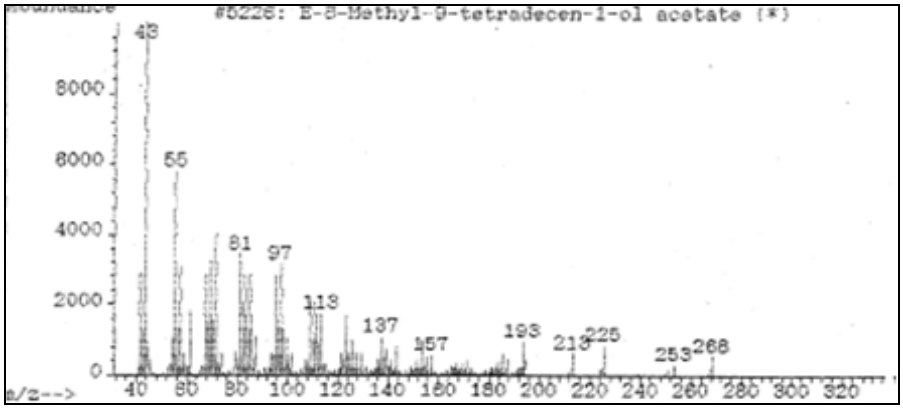


FIG. 3: THE MASS SPECTRUM ANALYSIS AND STRUCTURE OF OCTADECANOIC ACID, 2-OXO (4.46%)



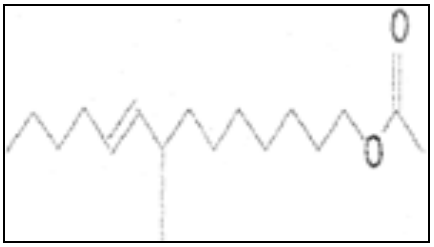
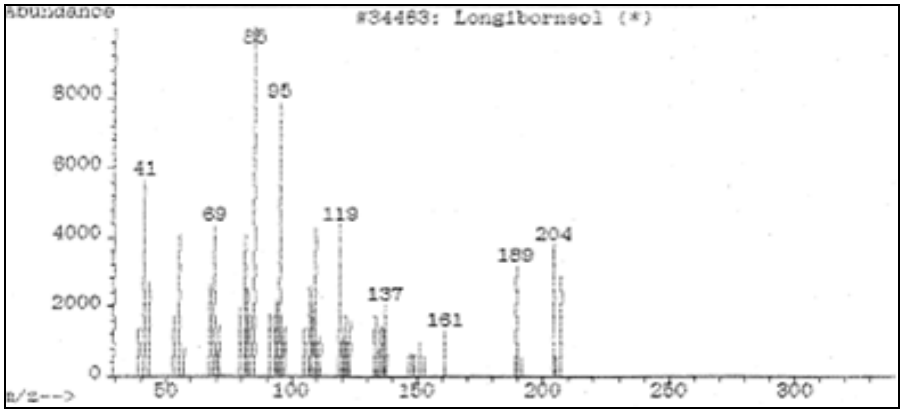


FIG. 4: THE MASS SPECTRUM ANALYSIS AND STRUCTURE OF E-8-METHYL-9-TETRADECEN-1-OL ACETATE (6.63 %)



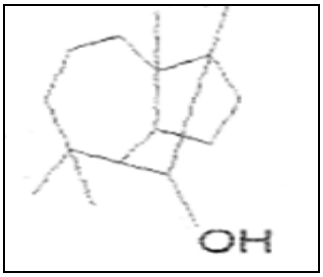


FIG. 5: THE MASS SPECTRUM ANALYSIS AND STRUCTURE OF LONGIBORNEOL (2.39%)

DISCUSSION: Fatty acid esters, derived from vegetable oils, are important plasticizers. Plasticizers are important plastics additives and when added to thermoplastics they increase their flexibility, transparency, and durability. The most common types of synthetic plasticizers are the dialkyl or alkyl aryl esters of phthalic acid (phthalates) and the most widely-used phthalates are the dioctyl phthalate, the diisodecyl phthalate, and the diisononyl phthalate. The level of phthalates in the environment is low as they are subjected to relatively rapid photochemical and biological degradation.

However, the natural occurrence of phthalates in a wide variety of plants is already in the literature [13-15] and in fact fatty foods such as milk, butter, and meats are found to be the main sources of natural bis(2-ethylhexyl) phthalate and other phthalates [16]. 1, 2-Benzenedicarboxylic acid bis(2-ethylhexyl) phthalate has been isolated from a marine alga, *Sargassum weightii*, and apart from its plasticizing ability it was also found to have antibacterial effect on a number of bacteria [17]. There is a first report on isolation of 1, 2-benzenedicarboxylic acid bis(2-ethylhexyl) ester (a dioctyl phthalate ester) from the seeds of *Ricinus communis* [18]. Di-isooctyl phthalate has been reported from Limonium bicolour Kuntze [19] and *Dracaena cochinensis* (Lour.)SC [20]. Butyl and isobutyl phthalates were also reported from *D. cochinensis.* Di-(2-ethyl hexyl phtahalate has been isolated from the leaves of *Cassia auriculata* Linn [21].

Bis (2-ethyl hexyl) phthalate was reported from the roots of *Euphorbia hylonoma* Mazz [22]. Phthalates are reported to have antimicrobial and other pharamacological activities. Bis (ethyl hexyl) phthalate reported from *Streptomyces bangladeshiensis* show antimicrobial activity against gram positive bacteria and some pathogenic fungi [23]. Di (2-ethyl hexyl) phthalate isolated from *Alchornea cordifolia* reported to lower anti-inflammatory activity [24]. Di-isooctyl phthalate isolated from *Nigella glandulifera* Freyn. was identified as inhibiting melanogenesis [25]. The extract of *Gongronema latifolium* decne contains Phthalic acid, monoterpenes, and several compounds to be responsible for the activity against bacterial isolates from HIV infected patients [26]. The essential oil of *Leea indica* (Burm. F) Merr flowers showed phthalic acid esters (95.6%) as major constituents, had good antibacterial and antifungal activity [27]. Ethanol extracts of Rosa laevigata contains 1, 2-benzenedicarboxylic acid dinonyl ester, which was able to reduce Abeta-induced neurotoxicity, which is useful for the prevention of oxidative stress- induced neuro-degenerative disorders [28]. Phthalates also are reported to affect the human beings. Di (-2-ethylhexyl) phtalate (DEHP), a commonly used plasticizer is harmful to human health [29], affects functioning of liver in rats [30].

Triterpenes are reported recently for their antitumor, anticancer, antiviral, antimicrobial and anti-inflammatory activity. The GC-MS analysis of leaf extract of *Finlaysonia obovata*, a mangrove plant has showed the presence of triterpenoids, octadecanoic acid and several other constituent to be responsible for antibacterial activity [31].

Longiborneol was the main compound of the sesquiterpene group, with only 1.3% of the total of all the compounds. The GC-MS analysis of essential oil of Liverwort *Scapania undulata,*showed the presence of sesqiterpene hydrocarbons such as isolongibornene also helminthogermacrene [32]. Longiborneol present in many plants has shown various properties such as antimicrobial [33], antitumor and antifungal [34]. Dodecanoic acid is also found in human milk (5.8% of total fat), cow's milk (2.2%), and goat's milk (3.5%).

CONCLUSION: The present study has been found useful in the identification of several constituents present in the ethanolic extract of the leaves of *Mallotus tetracoccus.* The presence of various bioactive compounds (identified as phthalate esters, phthalate, alkanes, esters, alcohols, sugar, sesquiterpenoids) justifies the use of the whole plant for various ailments by traditional practitioners.

Vegetable oil-derived plasticizers such as phthalates are benign and not only make plastic material flexible but they also offer benefits such as its resistance to migration, evaporation and leaching, and the stability to light and heat, thus offer environmental friendly plastic for future use. It could be concluded that *Mallotus tetracoccus* plant is of phytopharmaceutical importance. However isolation of individual

phytochemical constituents and subjecting it to biological testing will definitely give fruitful results.

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