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EVALUATION OF *IN VITRO* ANTIOXIDANT AND GC/MS SPECTROSCOPIC ANALYSIS OF MEMECYLON UMBELLATUM BURM F FOR ITS BIOACTIVE COMPOUNDS

Kannan Elangovan, Dhanraj Priyanka, Suryamurthy Anupriya, Zahiruddin Shahin Banu Kandasamy Murugesan*

CAS in Botany, University of Madras, Chennai 600025, Tamilnadu, India.

ABSTRACT

Medicinal plants plays a major role in the discovery of new therapeutic agents for drug development. In recent times there is more attention towards them, due to the presence of bioactive compounds and they have a dynamic role in prevention of various human diseases. *Memecylon umbellatum* Burm F (Family: Melastomataceae), is a traditional plant with potential ailing properties. In the present study, seed extracts of *M. umbellatum* were screened for phytochemicals using qualitative and quantitative analysis in order to discover and to improve the knowledge of traditional medicine. Based on phytochemical analysis, methanolic extract was evaluated for their *In Vitro* antioxidant potential and analyzed in GC-MS. Total antioxidant assay responded as well at 200 µg/mL, when compare to other solvent extracts methanol extract exhibited best activity. The highest total antioxidant capacity as BHT equivalents at 200 mg/g. Metal chelation effect of *M. umbellatum* displayed best activity in methanol extract 73.81% at 500 µg/mL GC-MS analysis revealed 8 major and minor phytochemical constituents in the methanolic seed extract of *M. umbellatum*.

Keywords: Anjani, Methanol, GC-MS, Memecylon, Phytocomponents.

INTRODUCTION

Nature has been a source of medicinal agents and importance of herbs in the management of human ailments cannot be overemphasized. Since early human history, natural products from plants have been investigated and utilized to treat major diseases. The natural products from the plants have been basis for the treatment of several human diseases [1]. Indian medicinal plants are widely used by all people either as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines.

Numerous physiological and biochemical processes in the human body may produce oxygen-centered free radicals and as well as overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), finally leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases in humans [2-4]. Antioxidant properties are the first links between chemical reactions and biological activity and it has been studied extensively for the past 10 years [5].

Number of plant products has been recommended as antioxidants, it has been recognized that naturally occurring substances in higher plants have antioxidant activity. Therefore, antioxidant activity is an important in-view of the free radical theory of aging and associated diseases. Flavonoids and other phenolic compounds of plant origin have been reported as scavenger ROS, thus they are viewed as promising therapeutic drugs for free radical pathologies [6-7]. Generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned that antioxidant activity of plants might be due to the presence of phenolic compounds [8].

Medicinal plants provide a large number of molecules that could be screened to find potential essential compounds for drug discovery [9-10]. Pharmaceutical and scientific communities have recently received the attention of medicinal plants, as the herbal remedies prepared from the whole plant are generally safe with fewer side effects if used in the proper therapeutic dosages [11].Iron is an essential element for life, due to its role in oxygen transport and catalytic activity of many enzymes, but it is toxcin large amounts. It has been demonstrated that mitochondria play a crucial role in iron homeostasis [12-13].

Corresponding Author :- **Kannan Elangovan** Email:- itsmeelangok84@gmail.com

Memecylon umbellatum Burm (Family: Melastomataceae) is small evergreen shrub or tree having young teeter branches and bears numerous umbellate cymes. The plant is known as "Anjani" in Sanskrit and "Ironwood tree" in English. Plants are distributed mostly in coastal regions of the Deccan peninsula, the eastern and southern part of India all along Western Ghats and in the Andaman islands[12].In Ayurveda, the leaves are used as a cooling astringent; used in conjunctivitis as a lotion; and given internally in leucorrhoea and gonorrhea. A lotion prepared from leaves is used to treat eye troubles. The leaves are reported to possess antiviral activity [13]. The literature survey revealed that, no proper reports or papers are available on antibacterial activity of seeds of *Memecylon umbellatum* Burm hence the present research work seems to be of enormous contribution, hence the present study was undertaken on *Memecylon umbellatum*, to screen antioxidant, phytochemical and GC-MS analysis for different phytoconstituents.

MATERIALS AND METHODS

Chemicals and reagents

Acetic acid, ammonium molybdate, chloroform, Dragendorff's reagent, ethanol, ether, $Fecl_2$, ferric chloride, ferrozine, hydrochloric acid, magnesium chips, mayer's reagent, methanol, molisch's reagent, ninhydrin, potassium hydroxide, pyridine, sodium phosphate, sodium nitroprusside, sulphuric acid were obtained from Sigma Aldrich, Hi-media, Merck

Collection and identification of plant

Seeds of *Memecylon umbellatum* Burm F.Melastomataceae family were collected in a sterile polythene bag from paranur, Chengalpat District, Tamilnadu, India and the plant was authenticated by A. Saraswathy, Director, Captain Srinivasan Murthi Institute of Ayurveda and Siddha drug development, Arumbakkam, Chennai-106 and voucher specimen was deposited in Madras University Botany Laboratory at CAS in Botany, University of Madras, Tamil Nadu, India.

Preparation of plant extract

Memecylon umbellatum was collected from the field and washed thoroughly with running tap water and rinsed in sterile distilled water by following method of [30]. The washed plant materials were shade dried at room temperature for 10 to 15 days. The shade dried plant parts were made into coarse powder using mechanical grinder. The powder was extracted with different solvents such as hexane, chloroform, ethyl acetate, methanol and aqueous in a Soxhlet apparatus for 8 to 16 h. The extract was concentrated using a rotary evaporator (Heidolph laborata, Germany) at various temperature under reduced pressure.

Screening of phytochemicals

Detection of Alkaloids

100 mg of powdered sample was dissolved in 5 mL of methanol and then filtered. Then 2 mL of filtrate was mixed with 5 mL of 1% aqueous HCl. 1 mL of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange-red precipitate was taken as positive. To the second tube Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids [31].

Shinoda's test for flavonoids

Five hundred milligram of sample was dissolved in 5 mL of ethanol, slightly warmed and then filtered. Few pieces of magnesium chips were added to the filtrate followed by addition of few drops of concentrated hydrochloric acid. A pink, orange, or red to purple coloration was taken as a confirmation for the presence of flavonoids [31].

Molisch's test – carbohydrates

Five hundred milligram of powdered sample was taken and dissolved in 5 mL of distilled water and then filtered.

Filtrate was added with few drops of Molisch's reagent, followed by addition of 1 mL of conc. H_2SO_4 by the side of the test tube. After two minutes, 5 mL of distilled water was added. Red or dull violet color formation at the interphase of the two layers was taken as positive test.

Legal's test - glycosides

The extract was hydrolyzed with HCL for few hours on a water bath and the hydrolysate was subjected to Legal's or Borntrager's test to detect the presence of glycosides. To the hydrolysate added 1 mL of pyridine and a few drops of sodium nitroprusside solution was added and then it was made alkaline with solution hydroxide solution. Appearance of pink to red color showed the presence of glycosides.

Ferric chloride test - tannins

1 to 2 mL of the extract and few drops of 5% aqueous ferric chloride solution were added. A violet color formation indicates the presence of tannins.

KOH test - phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

Ninhydrin test - protein and amino acids

1 mL of the extract was treated with few drops of ninhydrin reagent. Appearance of purple color shows the presence of amino acids.

Detection of Triterpenoids

10 mg extract was dissolved in 1 mL of chloroform

and 1 mL of acetic anhydride was added following of 2 mL of concentrated sulphuric acid. Formation of reddish violet color indicates the presence of triterpenoid.

Detection of Anthraquinones

5 mL of the extract solution was hydrolyzed with diluted concentrated sulphuric acid extracted with benzene. One mL of dilute ammonia was added to it. The result was indicating the rose pink coloration suggests the positive response for anthraquinones.

Liebermann–Burchard test for steroids

200 mg of powder sample was dissolved in 2 mL of acetic acid separately; solutions were cooled followed by the addition of few drops conc. H_2SO_4. Color development from violet to blue or bluish-green was taken as positive test steroidal ring.

Test for saponins

One gram of powdered sample was boiled in 10 mL of distilled water and then filtered. 3 mL of distilled water was added to filtrate and shaken vigorously for about 5 min. Formation of foam after shaking was taken as a confirmation for the presence of saponins.

ANTIOXIDANT ASSAY

Total antioxidant capacity using Phosphomolybdenum

The antioxidant activity of *M. umbellatum* was evaluated by Phosphomolybdenum method according to the procedure of [32]. An aliquot of 0.1 mL of each fraction (dissolved in respective solvent) was combined in a vial with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vial was capped and incubated in a water bath at 95°C for 90 min. After the incubation, samples were cooled to room temperature, and the absorbance of the mixture was measured at 765 nm against a blank. Percent inhibition was calculated by the following formula;

% inhibition = $1 - A_{\text{sample}} / A_{\text{control}}/100$

Determination of chelating effects of ferrous ions

The chelation of ferrous ions was estimated by method of [33]. Briefly, 50 µl of 2 mM $Fecl_2$ was added to 1 mL of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL). The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. Absorbance was measured at 562 nm. The percentage inhibition of ferrozine Fe_2+ complex formation was calculated and Na_2EDTA was used as positive control.

$$\text{Metal chelating \%} = \frac{Ao - A_s \times 100}{A_s}$$

A_0 = Absorbance of control, A_s = Absorbance of sample.

CHROMATOGRAPHY ANALYSIS

GC–MS analysis were carried out an SHIMADZU QP 2010T which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC–MS) instrument employing the following condition: capillary column −624ms (30m×0.32mm×1.8µm) operating in electron mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1.491mL/min and injection volume of 1.0mL, injector temperature was 140º C; ion source temperature of 200º C. The oven temperature was programmed from 45º C. Mass spectra were taken at 70eV. Interpretation of mass spectrum GC–MS was conducted using database of National institute standard and technology having more than 62,000 patterns. The spectrum of the unknown compounds stored in the NIST library. The compound prediction based on Dr. Duke phytochemical and Ethnobotanical Database by Dr. Jim Duke of the agricultural research service/USDA. The names of the components of the test material were ascertained.

RESULTS AND DISCUSSION

Preliminary Screening of Phytochemicals

The results of the phytochemical screening was carried out with methanol extract indicated the presence of different types of active constituents such as alkaloid, flavonoid, phenolic, saponin, tannin. Flavonoids have antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis. Steroids, abundant in many plants, have been shown to have anti-leukemic, antipyretic, diuretics, hypercholesterolemic effects and derivatives of steroids are active as anticancer and anti-viral agents and also have been reported to stimulate menstrual discharge and diminish secretion of milk [14]. Medicinal and healing properties of herbs are closely related to their chemical components such as acids, alkaloids, essential oils, steroids, saponins, tannins etc. and isolating these phytochemicals from the herbal extracts depends upon the solubility in various solvents. The results of previous studies which have reported that methanol is a better solvent for more consistent extraction of active compounds from medical plants when compared to other solvents, such as water [15-19].

Flavonoids have antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis.

Steroids, abundant in many plants, have been shown to have hypercholesterolemic effects, diuretics and also exhibit anti-leukemic, antipyretic, and derivatives of steroids are active as anticancer and anti-viral agents. Economical importance of saponins lies in their easy conversion to medicinally used steroidal hormones. Physiological benefits of phenolics from the plants have been recognized to their potential role towards modulating cell signal transduction pathways, inducing apoptosis and inhibiting lipid peroxidation [20-22].

Latex and resins of some plants contains terpenes and physiological function of these compounds protect against certain pathogens causing diseases both in human and animals [23]. Chemical analysis of this active constituents is hope to reveal some interesting applications

of the secondary metabolites isolated as well as their bioactivities.

Total antioxidant activity

The result of total antioxidant activity (TAC) is shown in Figure 1. The reduction of Mo (V) from Mo (VI) by the samples and successive formation of green phosphate/Mo (V) complex at acid pH. From the results, antioxidant capacity of different extracts was able to inhibit the Mo complex and this might be due to presence of the phenolics and flavonoid compounds in the plant extracts.

The *In Vitro* antioxidant assay involves direct inhibition of the reactive oxygen species, or the scavenging of free radicals [24].There are varieties of phenolic compounds present in the various natural sources and these phenolic compounds can be separated by the various solvents on the basis of difference in their polarity. The present research was carried out with the aim to discover the drugs as non-toxic that can scavenge the free radicals and there by halt the causation and progression of the diseases. The antioxidant activity of the phenolic compounds were attributed to its redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and have also metal chelating properties [25].

Metal chelating activity

Methanol extract of *M. umbellatum* exhibited better metal chelating capacity was 73.81% at 500 µg/mL concentration and it might contain significant quantity of antioxidant compounds as equivalents of EDTA to effectively reduce the oxidant in the reaction mixture. In this case, a concentration dependent chelating activity was shown in Fig 2. The chelating activity of EDTA was significantly higher than all extracts, but the methanol extract was closely related to the activity of standard followed by ethyl acetate, aqueous, chloroform and the lowest activity was obtained in hexane extract. Transition metal chelating activity depends on the ability of samples to chelate transition metals (Fe2+ or Cu+). Antioxidant capacity of EDTA has been used as a reference standard from which plant extracts with potential antioxidant activity are compared [26].

The chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion. The ability plays a vital role in antioxidant mechanism since it reduces the concentration of the catalyzing transition metal in lipid peroxidation mechanism [27, 28].

GC-MS analysis

The rapid and simple method outlined the active principles from the studied plant which is responsible for some therapeutic and aromatic effects. Results of the GC-MS analysis of ethanol extract provide 8 major and minor peaks (Table 3), the presence of phytoconstituents with different therapeutic potential. These compounds were identified through mass spectrometry attached with Gas Chromatography.

Nowadays efforts were focused on plants because of their usage of historical times and world's population rely on plants for the treatment of infections and noninfectious diseases. *M. umbellatum* contains various bioactive compounds the mass spectra of these compounds were matched with those found in the NIST/NBS spectral database and the data's are given.

However, isolation of individual phytoconstituents and screening for biological activities definitely will give successful results. From this present study, it could be concluded that importance of phytochemicals from medicinal plants and its uses in pharmaceutical industries.

Chlorozotocin is a cytostatic agent that is used in the investigational treatment of cancers of the stomach, large intestine, pancreas and lung. There is growing awareness in correlating the phytochemical components and their biological activities [29].

N -Butanol occurs naturally as a minor product of the fermentation of sugars and other carbohydrates, and is present in many foods and beverages. It is also a permitted artificial flavorant in the United States, used in butter, cream, fruit, rum, whiskey, ice cream and ices, candy, baked goods and cordials. In industrial use, 2-furoic acid is a preservative, acting as a bactericide and fungicide also considered an acceptable flavoring ingredient and achieved a generally recognized as safe (GRAS) status in 1995 by the Flavor and Extract Manufacturers Association (FEMA). Its derivatives also aid in the production of nylons are often used in biomedical research.

Commercial significant thiazoles include mainly dyes and fungicides. Thifluzamide, tricyclazole, and thiabendazole are marketed for control of various agricultural pests. Another widely used thiazole derivative is the non-steroidal anti-inflammatory drug Meloxicam. Hexadecanoic acid otherwise known as Palmitic acid is mainly used to produce soaps, cosmetics and release agents. These applications utilize sodium palmitate, which is commonly obtained by saponification of palm oil.

Table 1. Qualitative analysis of phytochemical constituent from *M. umbellatum*

S. No	Secondary metabolites	Seed methanol
1	Acids	-
2	Alkaloids	-
3	Carbohydrates	+++
4	Cardiac glycosides	+++
5	Coumarins	+++

6	Cyanin	++
7	Flavonoids	+++
8	Glycosides	+++
9	Phenols	+++
10	Quinones	+++
11	Saponin	++
12	Steroids	+++
13	Tannins	+++
14	Terpenoids	-
15	Triterpenoids	-

+++ - Strongly Positive ++ - Positive
+ - Trace - Not detected

Table 2. Quantitative analysis of secondary metabolites from *M. umbellatum*

S. No	Secondary metabolites	Methanol
1	Alkaloids	3.44
2	Acids	19.4
3	Anthraquinones	6.645
4	Coumarins	Nil
5	Fatty acid	35.7
6	Gallic acid	-
7	Glycosides	27.5
8	Phenols	9.86
9	Quinones	0.787
10	Saponin	1.22
11	Steroids	0.998
12	Tannins	2.77
13	Terpenoids	2.33
14	Tritrepenes	4.33

Table 3. Shows compounds identified from methanolic seed extract of *M. umbellatum*

S. No	R. Time	Name of the Phytoconstituents	Molecular Formula	Mol. Wt. (g/mol)	Peak Area (%)
1	3.566	Chlorozotocin	$C_9H_{16}ClN_3O_7$	313.69	0.81
2	3.958	1-Butanol	$C_4H_{10}O$	74.12	9.08
3	4.801	Glycerin	$C_3H_8O_3$	92.094002	0.90
4	5.803	1H-Pyrazole	$C_3H_4N_2$	68.08	10.45
5	6.209	4-Methyl-5H-furan-2-one	$C_5H_6O_2$	98.0999	0.67
6	7.270	1, 6-Octadien-3-ol3, 7-dimethyl-	$C_{10}H_{18}O$	154.2493	0.67
7	9.201	2-Furancarboxylic acid	$C_5H_4O_3$	112.0835	19.16
8	9.913	2-Furancarboxylic acid	$C_5H_4O_3$	112.0835	112.08
9	10.160	Pyrrolidine carboxamide	$C_5H_{10}N_2O$	114.1457	33.98
10	10.683	3-Furanmethanol	$C_5H_6O_2$	98.1	10.18
11	11.351	Thiazole	C_3H_3NS	85.13	2.99
12	12.919	D-Allose beta	$C_6H_{12}O_6$	180.16	0.99
13	17.726	Phthalic acid	$C_6H_4(COOH)_2$	166.14	0.54
14	17.886	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	0.66
15	18.205	Phthalic acid	$C_6H_4(COOH)_2$	166.14	1.88
16	23.274	Diisooctyl Phthalate	$C_{24}H_{38}O_4$	390.56	0.86

Figure 1. Total antioxidant activities of *Memecylon umbellatum* seed extracts

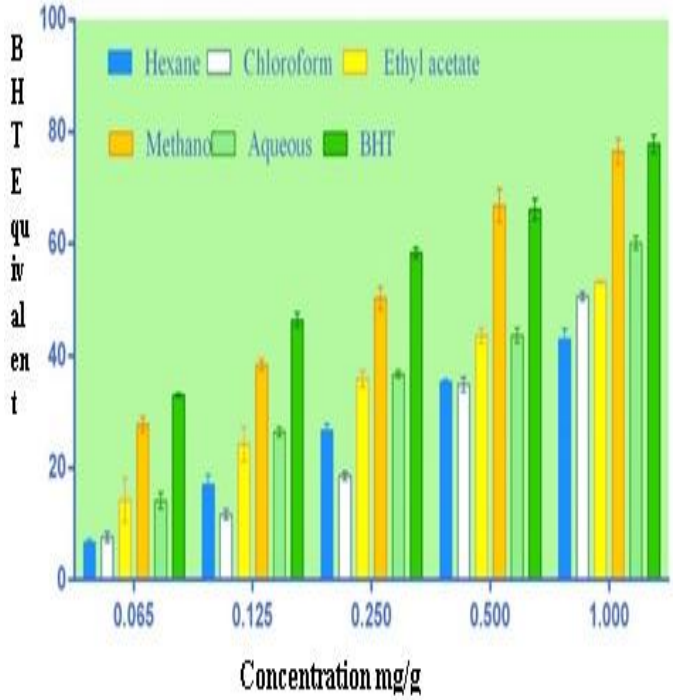


Figure 2. Metal chelating activities of *Memecylon umbellatum*

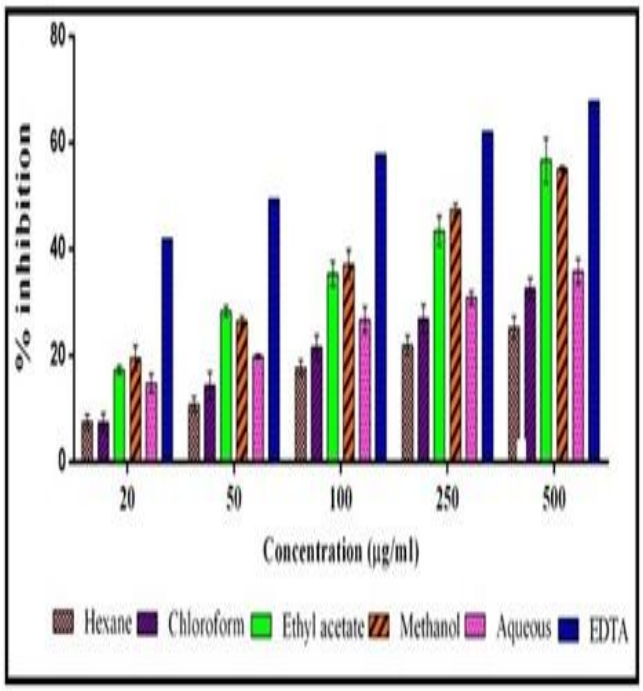


Figure 3.GC-MS spectrogram of the *M. umbellatum* methanol extract

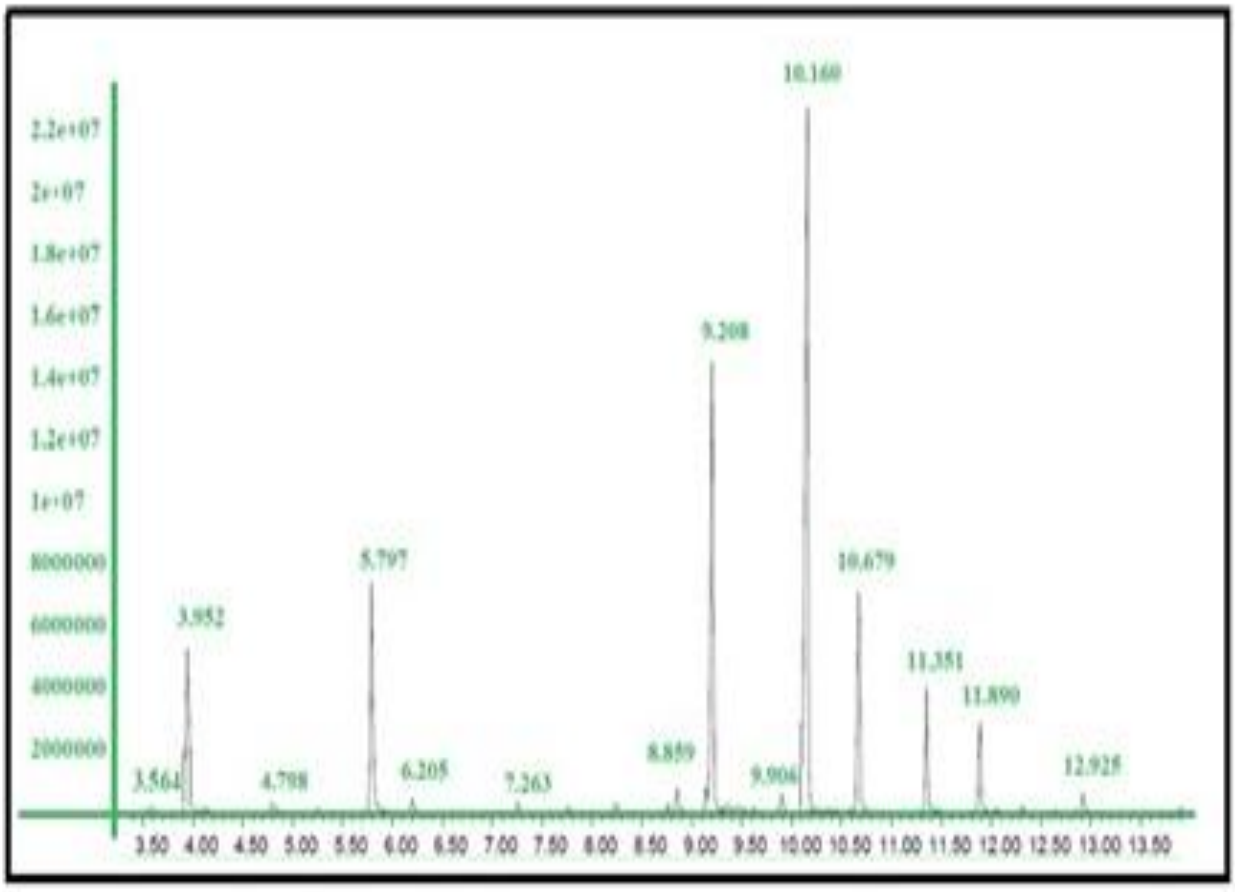
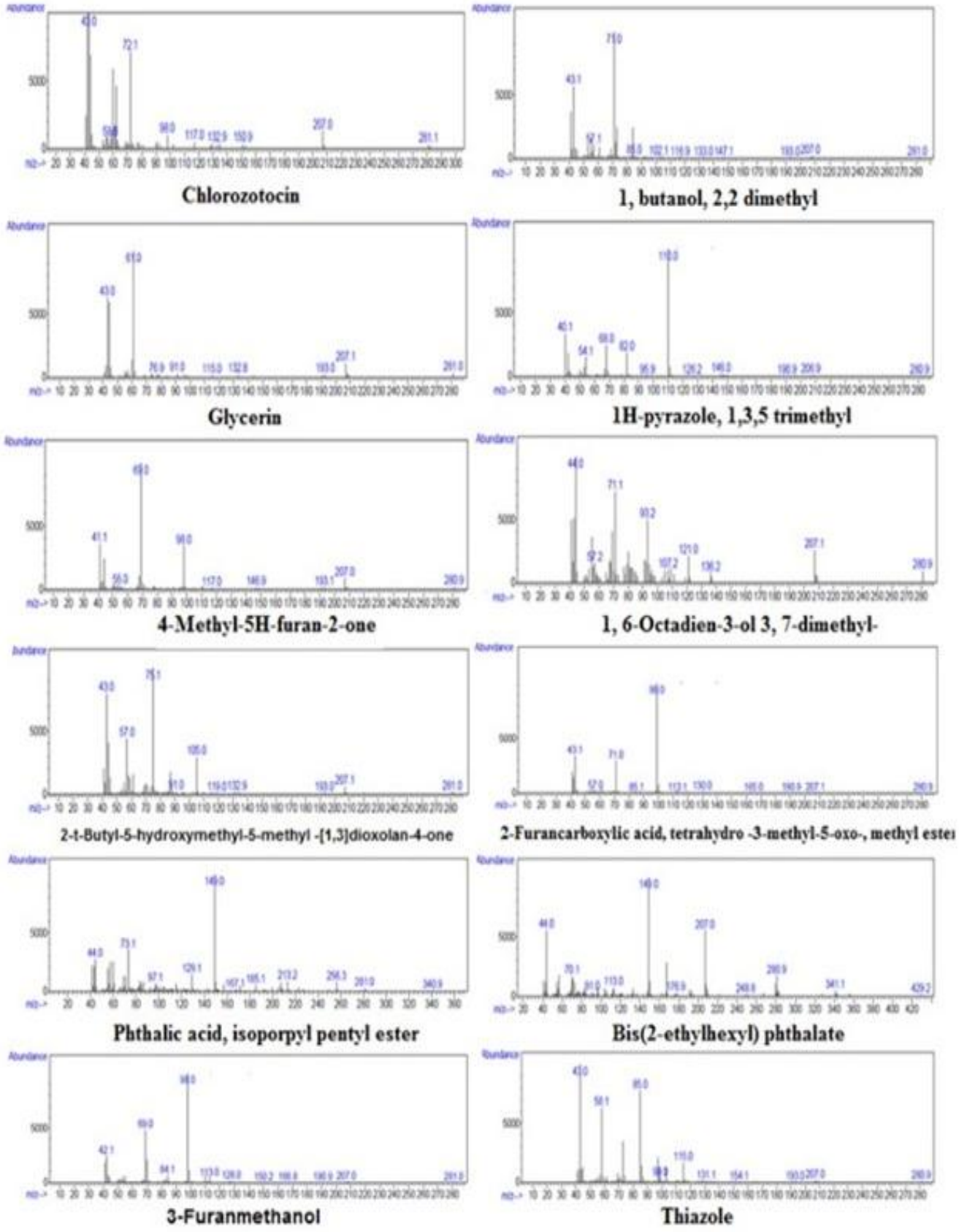
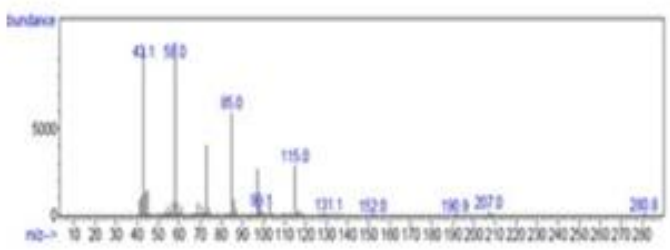
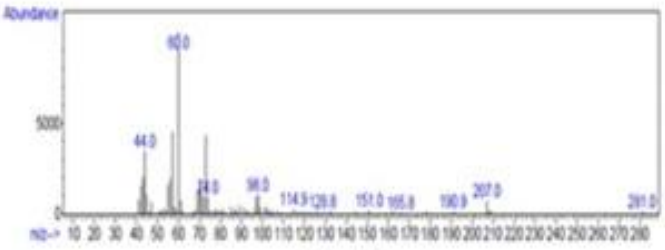


Figure 4. Components detected in the seed methanol extract of *Memecylon umbellatum*

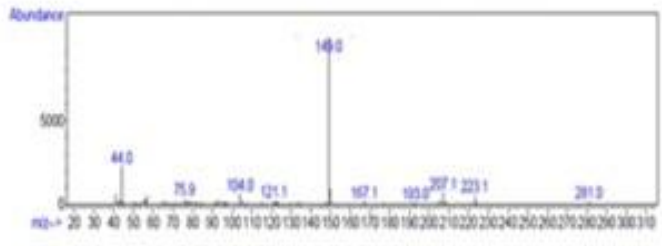




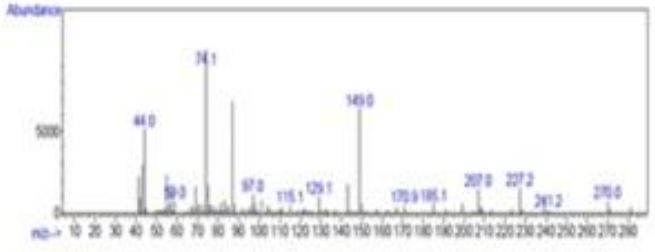
2-Methoxyethyl 3-Methylbutanoate



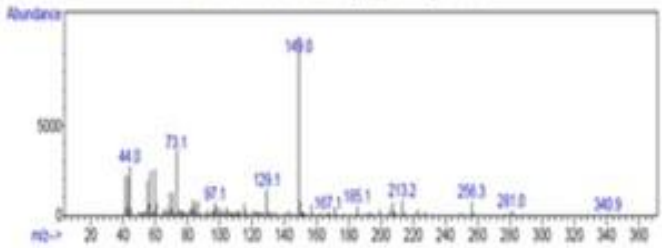
D- Aldose



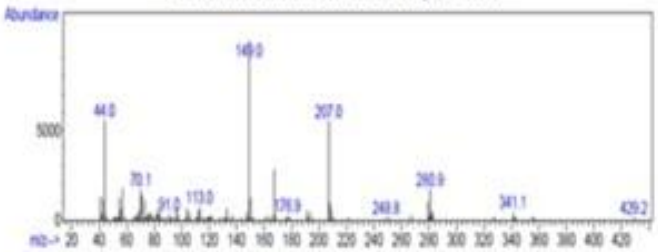
Phthalic acid butyl hexyl ester



Hexadecanoic acid methyl ester



Pthalic acid, isopropyl pentyl ester



Bis (2ethylhexyl) pthalate

CONCLUSION

Medicinal plants and their products are used to control diverse diseases and disorders of human beings. The results obtained in this present study are noteworthy, not only with respect to the antioxidant activities of the polar and non-polar solvent extracts, but also with respect to its content of various phytochemical components as well as this is the first report on the chemical composition and *IN VITRO* antioxidant activities of the seed extract of *Memecylon umbellatum Burm* F.GC-MS is very simple method, only few minutes to complete and it can be applied for screening the phytoconstituents from the medicinal plants. Results of this study will contribute to the recent research on natural products in many areas such as food, medicine, natural therapy and pharmacy. So that it might be utilized for the development of traditional medicines and further investigation is to elute the novel active bioactive compounds from the medicinal plants, also for a better understanding of mechanism of action, in vivo studies are needed which may create a new way to treat many incurable diseases and disorders.

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