RESEARCH ARTICLE

Preliminary Phytochemical Investigation, GC-MS Characterization, *In-vitro* Antioxidant Potential of Ethanolic Leaf Extract of Melastomataceae Species *Memecylon malabaricum* (C.B. Clarke) Cogn.

RR Manjula, Thirumal M*

Department of Pharmacognosy, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, India.

Received: 25th November, 2023; Revised: 12th December, 2023; Accepted: 20th January, 2024; Available Online: 25th March, 2024

ABSTRACT

The leaf extracts of *Memecylon malabaricum* were subjected to a detailed phytochemical investigation as part of this study. Based on the findings of this investigation, it was discovered that the leaf extracts included a wide range of bioactive components. These components included carbohydrates, glycosides, flavonoids, tannins, terpenes, phenols, and saponins. Throughout the whole of the experiment, the only extract that displayed phytosterols was the ethanolic extract. The findings of the DPPH, ABTS, total antioxidant assay-FRAP, superoxide anion radical scavenging assay, and total antioxidant assay-CUPAC assays demonstrated that all of the samples, but sample A in particular, had antioxidant qualities that were encouraging. This was the conclusion that was reached. A GC-MS analysis was used to thoroughly examine the chemical configuration of the leaves of *M. malabaricum*. According to the findings of this inquiry, 60 distinct components were examined. The legitimacy of the therapeutic potential of *M. malabaricum* is gained as a result of these results, which also pave the way for more study into the plant's possible applications in the fields of pharmacology and medicine.

Keywords: Melastomataceae, *Memecylon malabaricum* (C.B.Clarke) Cogn, GC-MS analysis of ethanolic leaf extract, Antioxidant activity, Total antioxidant DPPH, ABTS radical scavenging activity, FRAP, Superoxide anion radical scavenging activity.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.36

How to cite this article: Manjula RR, Thirumal M. Preliminary Phytochemical Investigation, GC-MS Characterization, *In-vitro* Antioxidant Potential of Ethanolic Leaf Extract of Melastomataceae Species *Memecylon malabaricum* (C.B. Clarke) Cogn. International Journal of Drug Delivery Technology. 2024;14(1):246-256.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

One of the most important components of Ayurvedic medicine is the phytoconstituents found in medicinal plants and herbs indigenous to India.[1] This kind of therapy offers a broad variety of treatments for a variety of conditions. The region of India known as the Western Ghats is home to between 250 to 350 distinct species of *Memecylon*, which may be discovered *via* exploration. Historically, these species have been used for the handling of a wide range of ailments, including but not limited to herpes, chickenpox, polyuria, menorrhagia, dysentery, bacterial infections, and inflammation.[2] In addition, these species are used in the treatment of dermatological and digestive conditions. The application of a paste made from cumin seeds and fresh shoot tips is one of the traditional treatments for *Malabaricum herpes*. This paste is applied to the particular location that is infected. In the Western Ghats mountains of India, there is a plant that is known as *Memecylon malabaricum* (C.B. Clarke) Cogn. This plant is a member of the family Melastomataceae and has a significant amount of phytoconstituents.[3] It is estimated that between 29 and 32 of the 39 distinct species of *Memecylon* may be found in the western ghats. Of these species, 21 are native to the region.[4] The *Memecylon lawsonii* Gamble, the *Memecylon lushingtonii* Gamble, the *Memecylon flavescens* Gamble, and the *Memecylon sisparense* Gamble are the four species that are considered to be rare or endangered according to the scientific community. Phytochemicals that are derived from species belonging to the genus *Memecylon* have the potential to revolutionize the pharmaceutical and herbal industries thanks to their capacity to alter the game.[5] Because of this, it would be feasible to develop novel herbal formulations and, in the long run, discover other phytoconstituents that are found in nature. The World Health Organisation (WHO) recommends performing preliminary phytochemical tests on herbs, sometimes called systematic screening of phytoconstituents. These tests aim to find and confirm the existence of bioactive

*Author for Correspondence: thirumam@srmist.edu.in

components in both the powder and extracts of the herbs.[6] In the family Melastomatacea, a variety of species have shown pharmacological or biological benefits. These effects include antioxidant, anti-inflammatory, anticancer, nephroprotective, antibacterial, anti-psoriatic, anti-diabetic, and antihelmintic properties. These species include *Memecylon* as one of them. It is likely that GC-MS analysis might be useful in evaluating the bioactive components that are present in plant extracts.[7] This is something that is definitely worth considering. Consequently, this would make fresh options available for pharmacological screening as well as further character evaluation research.

MATERIALS AND METHODS

Collection of Plant Material and Authentication

Dr. Madhavachetti, who is a member of the Department of Botany at Sri Venkateswara University in Thirupathi, studied the leaves of the *M. malabaricum* plant after it was removed from the Horsely hill area in Thirupathi. The university is located in Thirupathi.

Preparation of Extracts of the Plant Material

After shade-drying the plant material and grinding it into a coarse powder, the plant material is extracted using the soxhlation technique in an ethanol solvent for twenty-four hours. This process is repeated until the plant material is completely extracted. After the distillation process is complete, the residue is collected and placed in a desiccator to be dried.[8]

Preliminary Phytochemical Screening

Leaf powder and ethanolic leaf extract were both put through preliminary phytochemical screening independent from one another to make a comparison between the phytoconstituents. Compounds that are found in plants, such as glycosides, alkaloids, flavonoids, terpenoids, and phenols, amongst others.[9]

In-vitro Antioxidant Activity

DPPH method

A 0.1 millimolar solution of DPPH was combined with 5 microliters of the test chemical and 0.1 mL of the solution. This mixture was then placed into the wells of a 96-well plate. A spreadsheet in Excel was used to report the concentrations of the chemical that was being tested.[10] Additionally, extra blanks were produced by combining five microliters of the chemical concentration with 0.2 milliliters of either methanol or DMSO *via* a mixing procedure. This was done in addition to completing the reactions three times. After that, the plate was put into a dark chamber and used for incubation for 30 minutes so that the bacteria could grow. Measurements of the decolorization of the reaction mixtures were taken at a wavelength of 517 nm using a microplate reader that was manufactured by BioRad and is referred to as iMark. This measurement was performed after the incubation time had been completed. About 20 microliters of deionized water was added in the reaction mixture administered to the group in charge of monitoring the experiment. For the purpose of making a comparison with the control, we quantified the scavenging activity as a percentage of inhibition. We were able to determine the IC_{50} values, which are the concentrations at which the compounds are able to inhibit 50% of the DPPH radicals present in the environment. GraphPad Prism 6, which was used, gave rise to the possibility of doing this study.[11]

ABTS free radical scavenging assay

In order to generate the ABTS free radical reagent, a solution of ammonium persulfate (APS) with a concentration of 2.45 mM was used in combination with a solution of ABTS that had a concentration of 7 mM. Both of these solutions were blended together. As a result, the generation of ABTS radicals was made possible. It was necessary to dilute the reagent one hundred times in order to attain the concentration that was wanted.[12]

After that, various amounts of the standard, which was Ascorbic Acid - SD Fine, using the Catalogue number F13A/0413/1106/62, and samples were added to 200 µL of the ABTS free radical reagent in separate wells of a 96-well plate. This was done in order to determine the concentration of the reagent. It was carried out in a manner consistent with the directions given in the Excel sheet. In order to allow the reaction time to gradually increase, the plate was then kept in a dark atmosphere at room temperature for a duration of ten minutes. This was done in order to allow the reaction time to gradually increase. This was done in order to ensure that the best possible results were obtained.[13]

After the reaction mixtures had been incubated, the absorbance of the mixtures was determined by using a microplate reader (iMark, BioRad) to measure the absorbance of the mixtures from 750 nm. It was claimed that the findings were presented in the form of a percentage of the group that was referred to as the control.[14]

The IC_{50} values were calculated with the assistance of Graph Pad Prism 6, which was used to draw the conclusions. The concentration of the chemical at which it can inhibit 50% of the ABTS radicals is represented by the numbers shown below.[15]

Total antioxidant assay-FRAP

While performing the total antioxidant Assay-FRAP, ten microliters of the test compound and the standard (Ascorbic Acid, SRL, Cat no. 23006) were added to a mixture that contained 0.04 milliliters of 0.2 M Na_3PO_4 buffer (pH 6.6) and 0.05 milliliters of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ solution. The mixture was then subjected to the total antioxidant assay-FRAP. According to the directions included in the Excel sheet, this was carried out in the appropriate manner. A strong vortex was performed on the reaction mixture, and then it was allowed to incubate at a temperature of 50°C for 20 minutes.[16]

The reaction was stopped by adding 0.5 mL of trichloroacetic acid to the liquid at a concentration of 10%. This was done after the incubation period had been completed. The second stage consisted of adding 50 µL of deionized water and 50 µL of a ferric chloride solution with a concentration of 1%. In order to conduct a comparison between the colored solution

and the blank, a microplate reader designed by BioRad and manufactured by iMark was used at a wavelength of 700 nm.[17]

In order to acquire the IC_{50} values, which are the concentrations at which the compounds block 50% of the ferric reducing antioxidant power (FRAP), Graph Pad Prism 6 was used. This tool was utilized for the purpose of generating the IC_{50} values.[18]

Superoxide Anion Radical Scavenging Assay

It can be seen in the Excel sheet that a variety of various concentrations of riboflavin solution were combined with extract and standard. Before initiating the reaction, the mixture was allowed to rest in an incubator at room temperature for a half an hour while being shown to light. This was done before the reaction began. When the reaction mixture was added to the mixture that had been incubated earlier, the mixture was thoroughly mixed following the addition. This occurred throughout the process of adding the reaction mixture.[19]

After that, an absorbance measurement was performed at a wavelength of 560 nm using a plate reader that was made in the United States by Bio-Rad. One of the names for the plate reader was an iMark. The measurement of the absorbance readings of the samples[20] accomplished authentication of the samples' ability to scavenge superoxide anion radicals.

In order to get the IC_{50} values, which indicate the concentration at which the samples can block fifty percent of the superoxide anion radicals, the software programme GraphPad Prism 6 was used. These values were obtained when the samples were tested.[21]

Total Antioxidant Assay - CUPRAC Assay

On a plate that included 96 wells, the test chemical, samples, and standard were applied to the wells designated for them at the different concentrations stated on the Excel sheet. The plate contained 96 wells. Following that, 200 microliters of the reagent combination were introduced into each well containing the tested sample. In every one of the three preparations of the reaction mixture, 200 µL of methanol and ten microliters of the necessary compound concentrations for the samples and the standard (Trolox - Ottokemi, Cat no. T7723) were added. This was done in order to ensure that the reaction mixture was accurate. Trolox - Ottokemi was the product that served as the benchmark. As was the case in the past, the procedure for generating boundary samples was the same.[22]

For the third step, which was to initiate the reaction, the plate was left in a dark environment for a period of 30 minutes. This was done in preparation for the upcoming stage. Following the incubation period, the absorbance of the decolorized reaction solutions was determined by using a microplate reader (iMark, BioRad) at a wavelength of 490 nm. The absorbance of the solutions was measured by doing this in order to get an accurate reading. The reaction mixture that was used as the control was composed of 20 microliters of deionized water. This water was used in place of the sample or standard. For the purpose of making a comparison with the control, we quantified the scavenging activity as a percentage of inhibition.[23]

The IC_{50} values, the concentrations at which the chemical blocks fifty percent of the CUPRAC radicals, were produced using the GraphPad Prism 6 software. These values show the inhibitory concentration at which the chemical is effective.[24]

Analysis of Phytochemical Substances using GC-MS Analysis

In order to analyze the phytochemical components that are present in the leaves of *M. malabaricum*, a method that is known as GC-MS was used. The study was supported using a Shimadzu 17A GC paired with a Shimadzu QP 2010 PLUS MS. The operating system used for the analysis was the Class VP Chromatography Data System version 4.3. Helium gas is used as the carrier in a gas chromatography (GC) examination that is carried out on a capillary column that is SPb-5 in size. During the process of injecting a 1-µl sample, the split mode injector is set to 2500 c, and the detector is pre-set to 2800 c. Both of these settings are being performed simultaneously.[25]

In this configuration, the electron multiplier is set to 900 volts, the ionization voltage to 70 electron volts, and the ion source temperature to 180°C. The flame ionization detector was used in the process of trying to identify the peaks that were included on the GC chromatogram. The NIST version 2.0 was used to analyze each and every peak. Immediately after that, we merged all of the data into a single peak table. The analytical technique known as GC-MS is considered to be one of the most reliable methods for determining whether or not plant extracts contain phytoconstituents that are bioactive.[26]

RESULT AND DISCUSSION

Preliminary Phytochemical Screening of Leaf *M. malabaricum* (C.B.Clarke) Cogn.

Following the completion of an initial phytochemical screening, it was found that extracts derived from the leaves of *M. malabaricum* (C.B.Clarke) Cogn. had a considerable quantity of phytoconstituents. Table 1 provides a summary of the findings that were made available to the researchers.

DPPH test for scavenging free radicals

Table 2, Figures 1 and 2 provides the findings obtained from the DPPH free radical scavenging test, which may be seen by

Table 1: Preliminary phytochemical screening of leaf *M. malabaricum* (C.B.Clarke) Cogn

Chemical test	*With powder*	*Aqueous extract*	*Ethanolic extract*
Carbohydrates	+	+	+
Proteins	-	-	-
Alkaloids	-	-	-
Glycosides	+	+	+
Flavonoids	+	+	+
Taninns	+	+	+
Terpenes	+	+	+
Phenols	+	+	
Phytosterols	-	-	+
Saponins	+	+	+

Table 2: The results of the DPPH tests for free radical scavenging

Sample code	*IC_{50} value (µg/mL)*
Ascorbic Acid	3.139 ± 0.047
ELE-MM	121.8 ± 0.02

Table 3: ABTS radical scavenging assay results

Sample Code	*IC_{50} value (µg/mL)*
Ascorbic Acid	1.306 ± 0.04
ELE-MM	49.17 ± 0.03

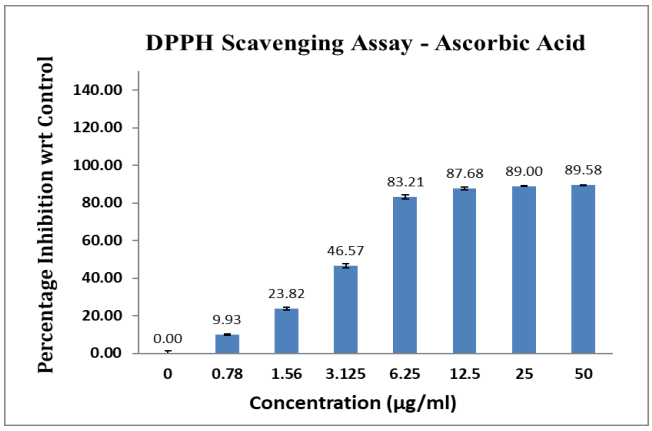


Figure 1: DPPH scavenging assay- ascorbic acid

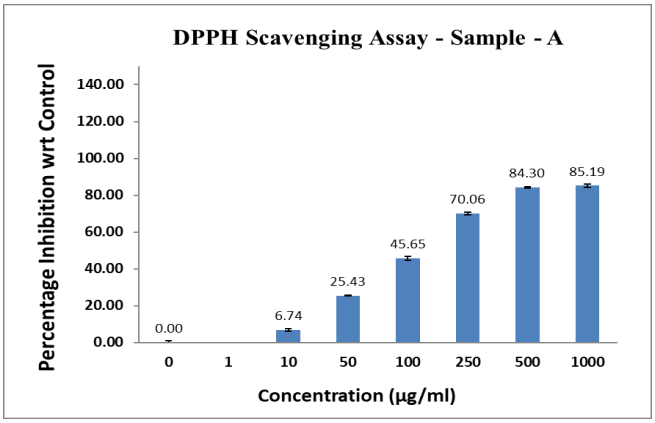


Figure 2: DPPH scavenging assay- ELE-MM

clicking on this link. In the case of ascorbic acid, which is a standard antioxidant, the IC_{50} value was determined to be 3.139 ± 0.047 µg/mL. Given these findings, it may be concluded that ascorbic acid has a powerful antioxidant impact. A comparison was conducted between the standard and the chemical that was being tested, designated as ELE-MM, which exhibited the ability to act as an antioxidant. Upon analysis, it was determined that the IC_{50} value for ELE-MM was 121.8 ± 0.02 µg/mL.

ABTS test for free radical scavenging

ELE-MM of the ABTS radical scavenging experiment exhibited a noteworthy level of antioxidant activity, as shown by an IC_{50} value of 49.17 ± 0.03 µg/mL. Table 3, Figures 3 and 4 indicates that ELE-MM exhibited a considerable degree of antioxidant activity. By virtue of its much lower IC_{50} value of 1.306 ± 0.04 µg/mL, ascorbic acid, which is often regarded as the most powerful antioxidant, exhibited its very powerful antioxidant characteristics.

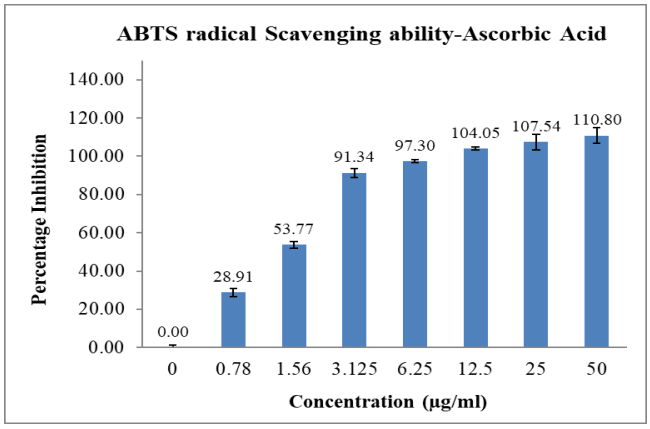


Figure 3: ABTS radical scavenging ability - ascorbic acid

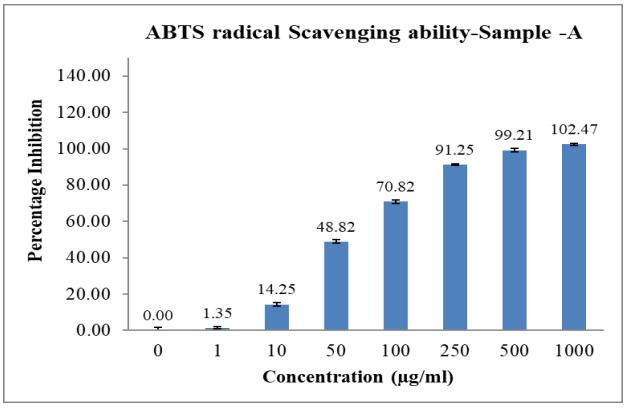


Figure 4: ABTS radical scavenging ability – ELE-MM

Total Antioxidant Assay-FRAP

A summary of the findings from the total antioxidant assay-FRAP is provided in Table 4, Figures 5 and 6, which is provided for your convenience. Furthermore, ascorbic acid had a noteworthy antioxidant activity, as shown by its IC_{50} value of 1.305 ± 0.024 µg/mL. ELE-MM, the chemical that is being investigated, had outstanding antioxidant properties, as seen by its IC_{50} value of 19.66 ± 0.079 µg/mL. This value is far higher than the standard, indicating that it is significantly more effective.

The test for the radical scavenging of superoxide anion

The results of the superoxide anion radical scavenging assay are shown in Table 5, Figures 7 and 8, which was completed after the experiment was finished. Gallic acid, a typical antioxidant, can scavenge superoxide anion radicals, as seen by its IC_{50} value of 1.139 ± 0.29 µg/mL. This figure demonstrates the potency of gallic acid in this regard. A considerable antioxidant potential was exhibited by ELE-MM, as evidenced by its IC_{50} value of 7.232 ± 0.245 µg/mL, when compared to the standard.

Table 4: Results of total antioxidant assay-FRAP

Sample code	*IC_{50} value (µg/mL)*
Ascorbic acid	1.305 ± 0.024
ELE-MM	19.66 ± 0.079

Table 5: Results of superoxide anion radical scavenging assay

Sample code	*IC_{50} value (µg/mL)*
Gallic Acid	1.139 ± 0.29
ELE-MM	7.232 ± 0.245

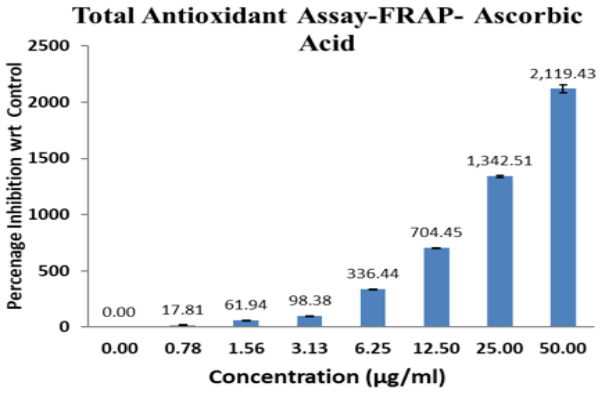


Figure 5: Total antioxidant assay-FRAP - ascorbic acid

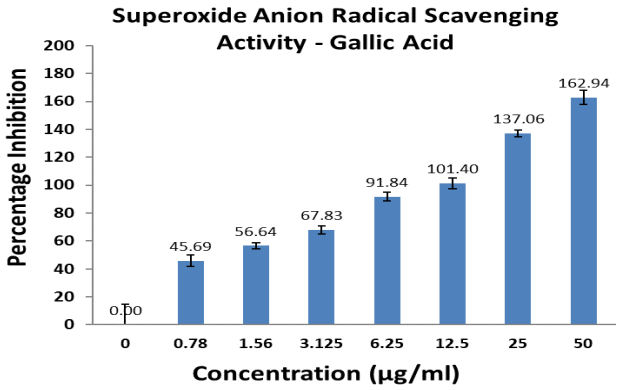


Figure 7: Superoxide anion radical scavenging Activity- Gallic acid

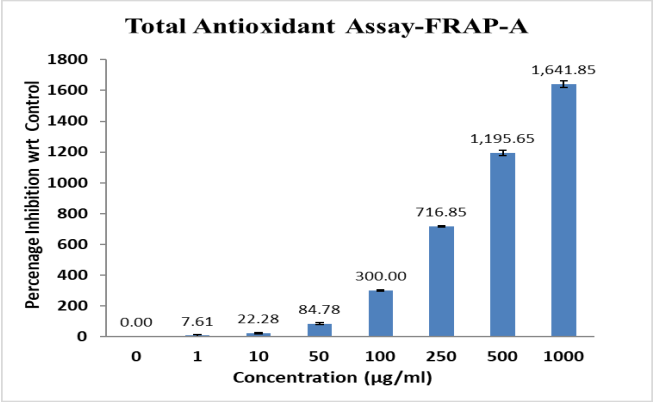


Figure 6: Total antioxidant assay-FRAP ELE-MM

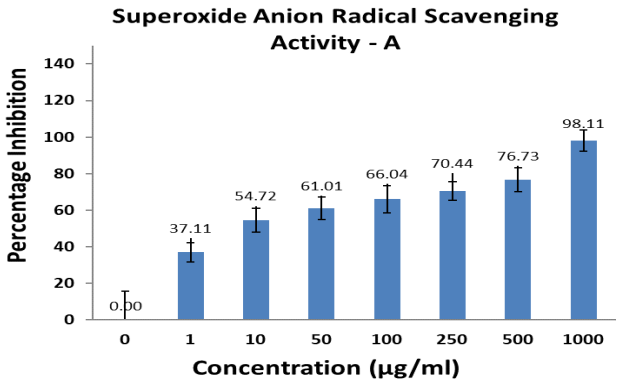


Figure 8: Superoxide anion radical scavenging activity of ELE-MM

Total Antioxidant Assay - CUPRAC Assay

Based on the total antioxidant assay - CUPRAC Assay findings, it was determined that both the standard (Trolox) and ELE-MM exhibited a considerable degree of antioxidant activity. Trolox, which had outstanding antioxidant activities, demonstrated its efficiency by exhibiting an IC_{50} value of 2.07 ± 0.295 µg/mL (Table 6, Figures 9 and 10). ELE-MM had a remarkable antioxidant capacity, as shown by its IC_{50} value of 15.88 ± 0.035 µg/mL, which is a significant value. Based on the present findings, it can be concluded that ELE-MM has the capability to efficiently target and neutralize CUPRAC radicals.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

For the purpose of conducting an investigation of the phytochemical components that were extracted from the leaves of *M. malabaricum* trees, the GC-MS method was used. The results of the investigation revealed the existence of a sizable

Table 6: Results of Total Antioxidant Assay - CUPRAC assay

Sample code	*IC_{50} value (µg/mL)*
Trolox	2.07 ± 0.295
ELE-MM	15.88 ± 0.035

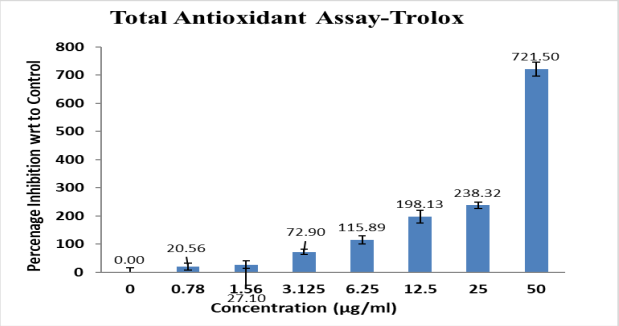


Figure 9: Total antioxidant wssay – Trolox

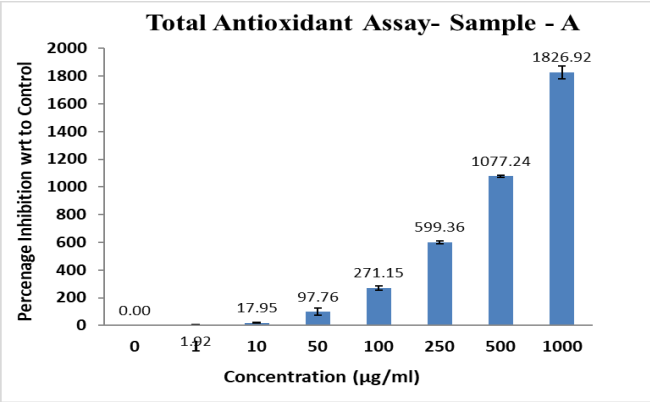
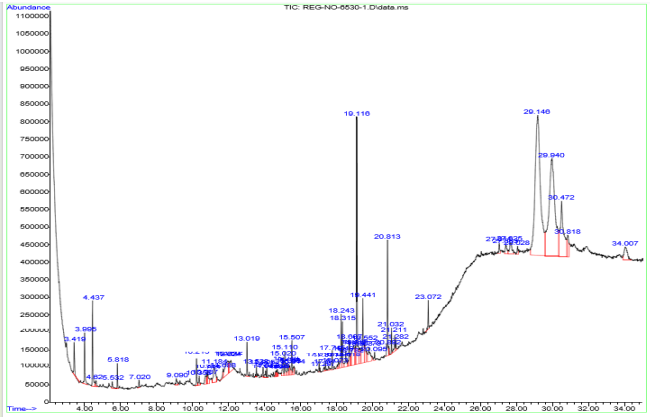
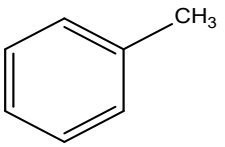
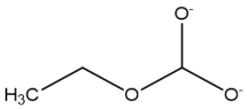
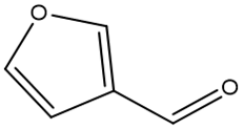
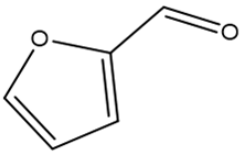
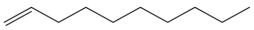
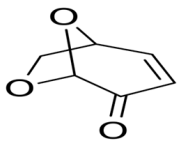
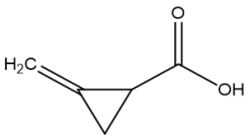
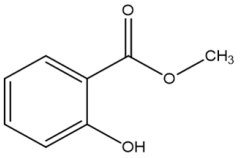


Figure 10: Total antioxidant assay – ELE-MM

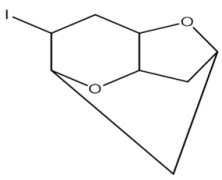
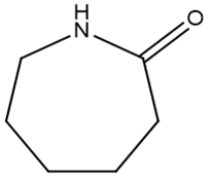
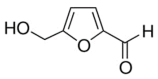
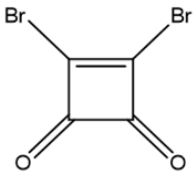
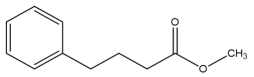
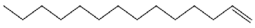
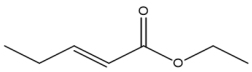
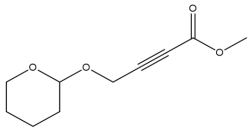
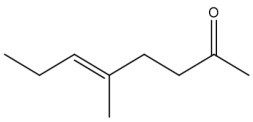
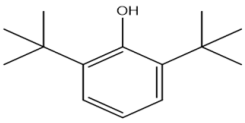
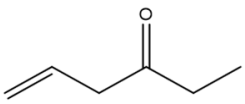
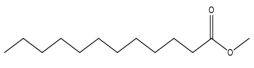
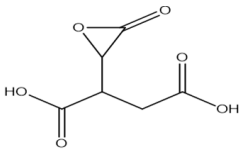
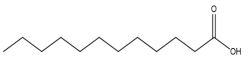


Graph 1: GC-MS graph of ethanolic leaf extract of *M. malabaricum*

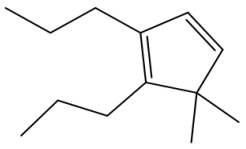
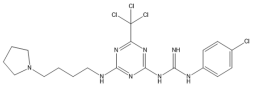
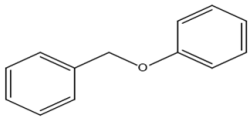
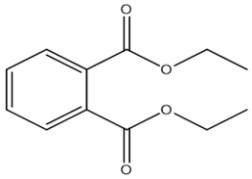
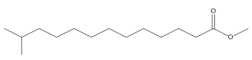
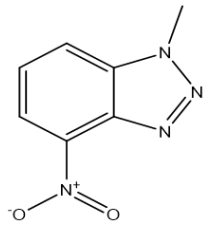
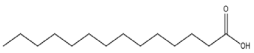
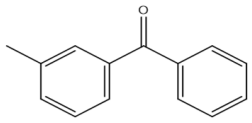
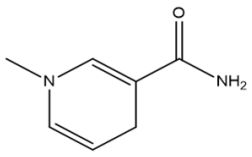
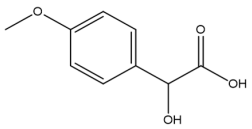
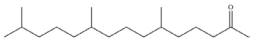
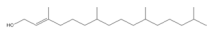
Table 7: GC-MS analysis of the phytochemical constituents extracted from the leaves of *M. malabaricum*

S. No.	*Name of the compound*	*Mol. formula*	*Mol. weight*	*Compound group*	*Structure*	*Chemical name*
1	Toluene	C_6H_8	92.14	aromatic		Methyl benzene
2	Ethyl orthoformate	$C_3H_6O_3$	90.08	orthoformate		
3	3-Furaldehyde	$C_5H_4O_2$	96.08	aldehyde		Furan-3-aldehyde
4	Furfural	$C_5H_4O_2$	96.08	aldehyde		Furan-2-aldehyde
5	1-Decene	$C_{10}H_20$	140.27	alkene		Deca-1-ene
6	Levoglucosenone	$C_6H_6O_3$	126.11	eneone		
7	Methylenecyclopropanecarboxylic acid	$C_5H_6O_2$	98.1	acid, alkene		2-methylenecyclopropanecarboxylic acid
8	1-Dodecene	$C_{12}H_{24}$	168.32	alkene		Dodeca-1-ene
9	Methyl Salicylate	$C_8H_8O_3$	152.15	phenol, ester		Methyl ester of Salicylic acid

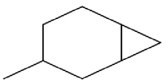
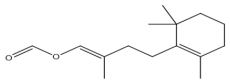
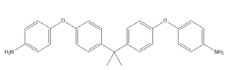
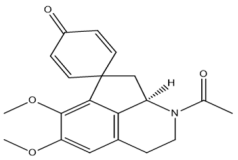
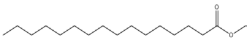
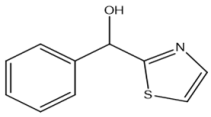
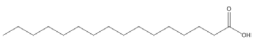
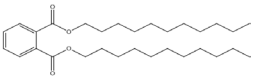
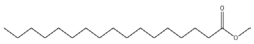
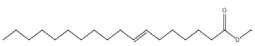
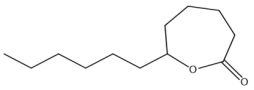
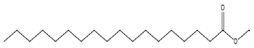
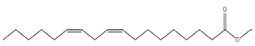
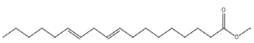
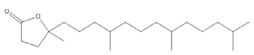
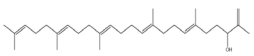
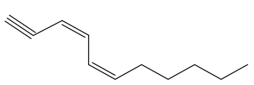
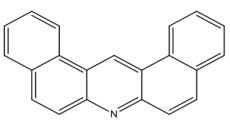
Cont....

10	5-Iodo-2,7-dioxa-tricyclo[4.3.1.0(3,8)]decane	$C_8H_{11}IO_2$	266.08	ether, halogen		5-Iodo-2,7-dioxa-tricyclo[4.3.1.0(3,8)]decane
11	Caprolactam	$C_6H_{11}NO$	113.16	amide		azepan-2-one
12	5-(hydroxymethyl)-2-furfural	$C_6H_6O_3$	126.11	alcohol, aldehyde		5-(hydroxymethyl)furan-2-carbaldehyde
13	3,4-Dibromo-3-cyclobutene-1,2-dione	$C_4Br_2O_2$	239.85	ketone, halogen		3,4-Dibromo-3-cyclobutene-1,2-dione
14	Benzenebutanoic acid, methyl ester	$C_{11}H_{14}O_2$	178.23	ester		methyl ester of benzenebutanoic acid
15	1-Tetradecene	$C_{14}H_{28}$	196.37	alkene		Tetradeca-1-ene
16	Ethyl trans-2-pentenoate	$C_7H_{12}O_2$	128.17	ester		trans-Ethyl-2-pentenoate
17	2-Butynoic acid, 4-[(tetrahydro-2H-pyran-2-yl)oxy]-, methyl ester	$C_{10}H_{14}O_4$	198.22	ester		2-Butynoic acid, 4-[(tetrahydro-2H-pyran-2-yl)oxy]-, methyl ester
18	5-methyl-5-Octen-2-one	$C_9H_{16}O$	140.22	ketone, halogen		5-methyl-5-Octen-2-one
19	2,6-bis(1,1-dimethylethyl)- Phenol	$C_{14}H_{22}O$	206.32	alcohol		2,6-di-tert-butylphenol
20	5-Hexen-3-one	$C_6H_{10}O$	98.14	alkene, ketone		hex-5-en-3-one
21	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214.34	ester		methyl dodecanoate
22	dl-Isocitric acid lactone	$C_6H_6O_6$	174.11	acid, ketone		2-(3-oxooxiran-2-yl)succinic acid
23	Dodecanoic acid	$C_{12}H_{24}O_2$	200.32	acid		Dodecanoic acid

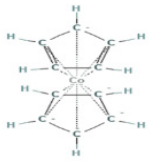
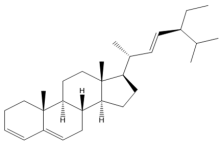
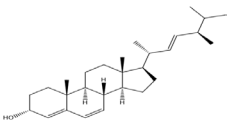
Cont....

24	1,3-Cyclopentadiene, 5,5-dimethyl-1,2-Dipropyl	$C_{13}H_{22}$	178.31	cyclic alkene		5,5-dimethyl-1,2-dipropylcyclopenta-1,3-diene
25	1-[p-Chlorophenyl]-3-[4-[[4-[1-pyrrolidinyl]butyl]amino]-6-trichloromethyl-S-triazin-2-yl]guanidine	$C_{19}H_{24}Cl_4N_8$	506.26	guanidine, amine, halogens		1-(4-chlorophenyl)-3-(4-((4-(pyrrolidin-1-yl)butyl)amino)-6-(trichloromethyl)-1,3,5-triazin-2-yl)guanidine
26	Benzene, (phenoxymethyl)-	$C_{13}H_{12}O$	184.09	ether		(benzyloxy)benzene
27	1-Tetradecene	$C_{14}H_{28}$	196.37	alkene		tetradec-1-ene
28	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	ester		diethyl phthalate
29	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	242.40	ester		methyl 12-methyltridecanoate
30	1H-Benzotriazole, 1-methyl-4-nitro	$C_7H_6N_4O_2$	178.15	nitro		1-methyl-4-nitro-1H-benzo[d][1,2,3]triazole
31	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	acid		Tetradecanoic acid
32	Methanone, (3-methylphenyl) phenyl	$C_{14}H_{12}O$	196.24	ketone		phenyl(m-tolyl)methanone
33	1-Octadecene	$C_{18}H_{36}$	252.48	alkene		Octadea-1-ene
34	1-Methyl-3-carbamoyl-1,4-dihydropyridine	$C_7H_{10}N_2O$	138.17	amide		1-methyl-1,4-dihydropyridine-3-carboxamide
35	Benzeneacetic acid, .alpha.-hydroxy-4-methoxy	$C_9H_{10}O_4$	182.17	acid, alcohol, ether		2-hydroxy-2-(4-methoxyphenyl) acetic acid
36	1,13-Tetradecadiene	$C_{14}H_{26}$	194.36	alkene		tetradeca-1,13-diene
37	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268.48	ketone		6,10,14-trimethylpentadecan-2-one
38	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53			(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol

Cont....

39	Bicyclo[4.1.0]heptane, 3-methyl	C_8H_{14}	110.20	alkyl		3-methylbicyclo[4.1.0]heptane
40	1-Buten-1-ol, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, formate, (E)-	$C_{15}H_{24}O_2$	236.35	aldehyde		(E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-1-en-1-yl formate
41	Benzenamine, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis	$C_{27}H_{26}N_2O_2$	410.51	amine		4,4'-((propane-2,2-diylbis(4,1-phenylene))bis(oxy))dianiline
42	Spiro[2,5-cyclohexadiene-1,7'(1'H)-cyclopent[ij]isoquinolin]-4-one, 1'-acetyl-2',3',8',8'a-tetrahydro-5',6'-dimethoxy-, (R)-	$C_{20}H_{21}NO_4$	339.39	ketone		(R)-1'-acetyl-5',6'-dimethoxy-2',3',8',8a'-tetrahydro-1'H-spiro[cyclohexa[2,5]diene-1,7'-cyclopenta[ij]isoquinolin]-4-one
43	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	ester		methyl-palmitate
44	2-Thiazolemethanol, .alpha.-phenyl	$C_{10}H_9NOS$	191.25	alcohol		phenyl(thiazol-2-yl)methanol
45	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	acid		palmitic acid
46	1,2-Benzenedicarboxylic acid, diundecyl ester	$C_{30}H_{50}O_4$	474.72	ester		diundecyl phthalate
47	1-Hexadecanol	$C_{16}H_{34}O$	242.44	alcohol		hexadecan-1-ol
48	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284.48	ester		methyl heptadecanoate
49	7-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	alkene, ester		(E)-methyl octadec-7-enoate
50	2-Oxepanone, 7-hexyl	$C_{12}H_{22}O_2$	198.30	lactone		7-hexyloxepan-2-one
51	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.50	ester		methyl stearate
52	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	alkene, ester		(9Z,12Z)-methyl octadeca-9,12-dienoate
53	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.47	alkene, ester		(9E,12E)-methyl octadeca-9,12-dienoate
54	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324.54	lactone		5-methyl-5-(4,8,12-trimethyltridecyl)dihydrofuran-2(3H)-one
55	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	$C_{30}H_{50}O_4$	426.72	alkene, alcohol		(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-1,6,10,14,18,22-hexaen-3-ol
56	(3E,5Z)-3,5-Undecadien-1-yne	$C_{11}H_{16}$	148.24	alkene, alkyne		(3E,5Z)-undeca-3,5-dien-1-yne
57	Dibenz[a,j]acridine	$C_{21}H_{13}N$	279.33	aromatic		dibenzo[a,j]acridine

Cont....

58	Cobalt,(.eta.-2,4-cyclopentadien-1-yl)[(1,2,3,4,5,6-.eta.)-1-phenylboratabenzene]-					
59	Stigmastan-3,5,22-trien	$C_{29}H_{46}$	394.68	alkene		(8S,9S,10R,13R,14S,17R)-17-((2R,5S,E)-5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene
60	Ergosta-4,6,22-trien-3.alpha.-ol	$C_{28}H_{44}O$	396.65	alkene		(3R,8S,9S,10R,13R,14S,17R)-17-((2R,5R,E)-5,6-dimethylhept-3-en-2-yl)-10,13-dimethyl-2,3,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol

number of distinct chemical substances. The Table 7, Graph 1 that follows offers a statistical overview of the compounds that were discovered, including the chemical names of the compounds, their molecular weights, the compound groups that they belong to, and their molecular molecules:

DISCUSSION

The outcomes of research that explore the antioxidant activities and phytochemical composition of leaf extracts obtained from *M. malabaricum* provide useful insights into the usage of the plant for therapeutic reasons. Both of these qualities are investigated in the leaf extracts. The initial phytochemical screening revealed several bioactive chemicals, including carbohydrates, glycosides, flavonoids, tannins, terpenes, phenols, and saponins. These compounds were shown to be present in the plant under investigation. In terms of pharmacological actions, these compounds exhibit a wide range of possibilities. *M. malabaricum* has the potential to be effective in both traditional and alternative forms of healing, taking into mind the data that have been presented here.

Excellent potential for antioxidant activity was indicated by the samples examined in several tests, including the DPPH, ABTS, total antioxidant, superoxide anion, and CUPRAC assays, among others. Extracts of *M. malabaricum* can battle diseases brought on by oxidative stress, despite the fact that their IC_{50} values are not identical to those of common antioxidants such as ascorbic acid and trolox. In order to determine which phytochemical components are responsible for this action and whether or not these components have any synergistic effects, it is important to perform more study. More research is required.

Through the use of GC-MS, researchers were able to get comprehensive knowledge on the phytochemical composition of the leaves of *M. malabaricum*. It has been shown that this particular species of plant contains a broad range of chemical compounds, such as aromatic hydrocarbons, aldehydes, alkenes, acids, esters, ketones, ethers, alcohols, amines, nitro compounds, and lactones. These are just a few examples of the many types of chemicals that have been discovered. Certain compounds, such as toluene, furfural, methyl salicylate, and caprolactam, are known to contain antioxidant and antibacterial characteristics. This is a well-known fact. In addition, alkene compounds have the ability to function as building blocks in the process of constructing molecules that have bioactive properties. As a result of the existence of compounds that include nitrogen and esters, respectively, there is a chance that the drug will have pharmacological effects as well as insecticidal effects.

CONCLUSION

The phytochemical examination of leaf extracts from *M. malabaricum* led to the discovery of a wide variety of bioactive chemicals. These compounds include alkaloids, glycosides, flavonoids, tannins, terpenes, phenols, and saponins. To summarize, the leaves of the plant were the subject of the inquiry that was carried out. Because these compounds possess properties such as antioxidants, antibacterial agents, and anti-inflammatory agents, *M. malabaricum* has the potential to be used in both traditional and contemporary healthcare systems. This potential therapeutic usefulness is underscored by the fact that these chemicals display these attributes. According to the findings of the bioassay, the samples exhibited a significant degree of antioxidant activity. This is a piece of news that is positive for the prospective medicinal uses of these compounds. The investigation examined the plant's chemical composition *via* the use of GC-MS. Based on the findings of this inquiry, it was determined that a number of compounds are known to contain a variety of pharmacological and biological activities. It is necessary to conduct additional research in order to achieve the following objectives: isolating and characterizing individual molecules; gaining an understanding of the distinct bioactivities of these molecules; and evaluating the potential of these molecules for the development of medications and therapies that are based on natural products. The findings of this study have, in conclusion, led to a rise in our understanding of the therapeutic capabilities of *M. malabaricum*. Furthermore, they have set a platform for further research work in the fields of pharmacology and medicine.

ACKNOWLEDGEMENT

The authors thank SRM College of Pharmacy, SRM Institute of Science and Technology for the kind support throughout the end of the research work.

REFERENCES

1. Kumar S, Dobos GJ, Rampp T. The significance of ayurvedic medicinal plants. Journal of evidence-based complementary & alternative medicine. 2017 Jul;22(3):494-501.
2. Begum NS, Ravikumar K, Ved DK. Ethnobotanicals of the Western Ghats. Ethnobotany of India Volume 2: Western Ghats and West Coast of Peninsular India. 2016 Dec 1.
3. Sachin C, Ajay P. Indian medicinal plants used in liver disease: a short review. Pharmacognosy Journal. 2011 Jan 1;3(19):91-4
4. Manikandan G, Ramasubbu R. Antimicrobial activity of leaf extracts of *Memecylon* heyneanum Benth. ex Wight & Arn.: An endemic tree species of Southern Western Ghats. Advances in Zoology and Botany. 2020;8(3):258-68..
5. Rajasekharan PE, Wani SH, editors. Conservation and utilization of threatened medicinal plants. Springer International Publishing; 2020 Jul 20.
6. Velavan S. Phytochemical techniques-a review. World Journal of Science and Research. 2015;1(2):80-91.
7. Dhila P, Shukla M, Khurana S, Banerjee AG. Multiple pharmacological properties and uses of an edible herb-Cassia tora. Food Ther Health Care. 2021; 3 (4): 106–114.
8. Odey MO, Iwara IA, Udiba UU, Johnson JT, Inekwe UV, Asenye ME, Victor O. Preparation of plant extracts from indigenous medicinal plants. International Journal of Science and Technology. 2012 Dec;1(12):688-92.
9. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020 Mar;8(2):603-8.
10. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. Journal of food science and technology. 2011 Aug;48:412-22.
11. Dawidowicz AL, Wianowska D, Olszowy M. On practical problems in estimation of antioxidant activity of compounds by DPPH method (Problems in estimation of antioxidant activity). Food chemistry. 2012 Apr 1;131(3):1037-43.
12. Nenadis N, Wang LF, Tsimidou M, Zhang HY. Estimation of scavenging activity of phenolic compounds using the ABTS•+ assay. Journal of agricultural and food chemistry. 2004 Jul 28;52(15):4669-74.
13. Shah P, Modi HA. Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. Int. J. Res. Appl. Sci. Eng. Technol. 2015 Jun;3(6):636-41.
14. Herraiz T, Galisteo J. Endogenous and dietary indoles: a class of antioxidants and radical scavengers in the ABTS assay. Free radical research. 2004 Mar 1;38(3):323-31.
15. Miller NJ, Rice-Evans CA. Factors influencing the antioxidant activity determined by the ABTS radical cation assay. Free radical research. 1997 Jan 1;26(3):195-9.
16. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry. 1996 Jul 15;239(1):70-6.
17. Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition research. 2003 Dec 1;23(12):1719-26.
18. Dudonne S, Vitrac X, Coutiere P, Woillez M, Mérillon JM. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of agricultural and food chemistry. 2009 Mar 11;57(5):1768-74.
19. Noda Y, Anzai K, Mori A, Kohno M, Shinmei M, Packer L. Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectrometer system. Iubmb Life. 1997 Jun;42(1):35-44.
20. Tao H, Zhou J, Wu T, Cheng Z. High-throughput superoxide anion radical scavenging capacity assay. Journal of agricultural and food chemistry. 2014 Sep 24;62(38):9266-72.
21. Kaur P, Arora S. Superoxide anion radical scavenging activity of Cassia siamea and Cassia javanica. Medicinal chemistry research. 2011 Jan;20:9-15.
22. Apak R, Güçlü K, Özyürek M, Karademi˙ r SE, Altun M. Total antioxidant capacity assay of human serum using copper (II)-neocuproine as chromogenic oxidant: the CUPRAC method. Free radical research. 2005 Sep 1;39(9):949-61.
23. Campos C, Guzmán R, López-Fernández E, Casado Á. Evaluation of the copper (II) reduction assay using bathocuproinedisulfonic acid disodium salt for the total antioxidant capacity assessment: The CUPRAC–BCS assay. Analytical biochemistry. 2009 Sep 1;392(1):37-44.
24. Çekiç SD, Kara N, Tütem E, Başkan KS, Apak R. Protein–incorporated serum total antioxidant capacity measurement by a modified CUPRAC (CUPRIC reducing antioxidant capacity) method. Analytical letters. 2012 May 1;45(7):754-63.
25. Pandian RS, Noora AT. GC-MS Analysis of Phytochemical Compounds Present in the Leaves of Citrus medica. L. Research Journal of Pharmacy and Technology. 2019;12(4):1823-6.
26. Sasikala P, Ganesan S, Jayaseelan T, Azhagumadhavan S, Padma M, Senthilkumar S, Mani P. Phytochemicals and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of Taraxacum officinale (L). Journal of Drug Delivery and Therapeutics. 2019 Jan 15;9(1):90-4.