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International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Phytochemical, antioxidant, and anti-diabetic analysis of leaf and stem extracts of *Memecylon malabaricum* (C.B.Clarke) Cogn.

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ARTICLE INFO

Article history:

Received: 19 June, 2020

Revised: 14 October, 2020

Accepted: 25 October, 2020

Published: 30 November, 2020

Keywords:

Antioxidant activity, GC-MS analysis, *Memecylon malabaricum*, Phytochemicals, α-amylase inhibition.

DOI:

10.25004/IJPSDR.2020.120604

ABSTRACT

Memecylon malabaricum (C.B. Clarke) Cogn. belongs to the family Melastomataceae is one of the important medicinal plants in the traditional system of medicine. It has been used to treat diabetes, bacterial infections, and skin disorders, including Herpes. Hence, the present study deals with the analysis of stem and leaf extracts through gas chromatography-mass spectroscopy (GC-MS) to identify the bioactive compounds. 29 in stem and 25 phytochemicals in leaf extract were identified. Based on the results of GC-MS analysis, further studies were carried out to estimate phenols, flavonoids, and total antioxidant activity. It was found that stem methanolic extract has a slightly more quantity of phenolics than the leaf extract. However, leaf extract has shown a considerable amount of flavonoids and better antioxidant activity than stem extract as revealed by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and reducing power assay. Further, inhibition of the activity of α-amylase by leaf extract was detected through preliminary studies, thereby supporting its traditional use to treat diabetes.

Introduction

Plants with known medicinal properties are the main sources of traditional medicine and form rich resources of ingredients in drug development. About 80% of the drugs discovered are based on the traditional knowledge of medicinal plants. Hence, commercially they are the essential components in the field of pharmaceuticals. The Royal Botanic Garden, Kew has documented 3, 69,000 flowering plants in 2016, of which 31,128 plants are identified as useful plants; out of these, 17,810 plants are mentioned as having known medicinal value.[1] The above-said data indicates the necessity to explore the vast number of remaining plants for their potential use.

The genus *Memecylon* belongs to the family Melastomataceae comprises about 526 species worldwide.[2] In India, it is represented by 42 species, of which 21 are endemic.[3,4] Several species are believed to have medicinal properties and are being used in treatment of diabetes, skin diseases apart from their antimicrobial and wound healing activities.[4-6] Many species of *Memecylon* are being shown to have antioxidant activity.[3,4,7,8]

M. malabaricum (C.B.Clarke) Cogn., endemic to Western ghats, is an important medicinal plant of the genus *Memecylon* in the traditional medicine system. It is used in the treatment of diabetes, various skin diseases, and stomach disorders. Identification of the active principles in the extracts is a prerequisite for the evaluation of their therapeutic value. Since the previous studies have indicated the pharmacological importance of this taxon, the present investigation deals with the comparative study of stem and leaf extracts by subjecting them to GC-MS analysis, antioxidant assays, and α-amylase inhibitory assay.[4,9-14]

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Material and Methods

Collection and Preparation of Extracts of the Source Material

The leaf and stem samples of *M. malabaricum* were collected from Agumbe, Karnataka (lat13°29'56" N and long 75°04'24.4" E) (Fig. 1A-C). Mature leaves and tender intermodal segments from main branches were thoroughly washed in running water for 60 minutes and shade dried for 30 days. Thus, shade dried samples were made into a coarse powder using the laboratory mixer-grinder and stored in an airtight container for further use.

A total of 10g of each sample was used to extract phytochemicals with Soxhlet apparatus for 12 hours. Methanol was used as a solvent. Thus obtained methanol extract was evaporated to dryness and the dried powder was stored at 4°C for further use in GC-MS, antioxidant, and α-amylase analysis.

Preliminary Phytochemical Screening

Screening for various bio-constituents was carried out as per the method described by Trease and Evans.[15] One gram of both samples were immersed in different solvents for 24 hours. The filtrates, after removing the debris, were used for screening the various phytochemicals.

Fig 1: A- Habit of *Memecylon malbaricum*; B- Twig with Inflorescence C- Twig with Ripen fruit

GC-MS Analysis

Methanolic extracts of leaf and stem were analyzed by GC-MS technique with an instrument Shimadzu QP 2010S with a Rxi-5sil MS column (30 m length ×0.25 mm ID × 0.25 µm thickness). Helium was used as carrier gas at approximately 1 mL/min at pulsed split less mode. The sampling time was 2 minutes and 1 µL of the samples were injected and the temperature was maintained at 260°C. The ion source and the interface temperatures was programmed by starting at 60°C then elevated to 280°C at a rate of 5°C/min, with a 2 minutes hold at 280°C. The ion source and the interface temperatures were set at 200°C and 280°C, respectively. The mass spectrophotometric detector was operated in electron impact ionization mode with energy of 70eV and scanning from m/z 50-500 at the scan speed of 1000. The event time for mass spectra was 0.50. Spectral data analyzed from Wiley 8 and NIST 11 libraries were used to identify the separated peaks obtained for the samples.

Estimation of Total Phenols

The samples' total phenolic contents were determined as per the Folin-Ciocalteau method[16] with slight modification. An aliquot of 0.1 mg/mL of the samples mixed with 0.2 mL of 2N FC reagent and allowed for 5 minutes incubation at room temperature. To this reaction mixture, 2 mL of 7% sodium carbonate was added. The final volume was made up to 10 mL by adding double distilled water and incubated for 90 minutes in the dark before the absorbance was measured at 765 nm using UV-Vis spectrophotometer (Elico, Sc164). Gallic acid was used as standard ($r^2 = 0.9$). The experiment was repeated thrice and expressed as milligram of Gallic acid equivalent per g (mg/GAE/g).

Estimation of Total Flavonoids

Total flavonoids was determined by following the Aluminium chloride method.[17] An aliquot of 3 mL of the samples was mixed with a mixture of 0.2 mL of aluminum chloride (10%) and 0.2 mL of potassium acetate (1M). The final volume was made up to 9 mL by the addition of 5.6 mL of double-distilled water. The reaction mixture was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm using UV spectrophotometer and was compared with the standard curve plotted against quercetin (Standard $r^2 = 0.9$). The total quantity of flavonoids was expressed in mg per quercetin equivalent per g of sample with the help of a calibration curve of quercetin (mg/QEE/g). The experiments were performed in triplicate.

Total Antioxidant Assay

The total antioxidant activity of the extracts was estimated by phosphomolydeum method.[18] A 3 mL of phosphomolybdeum reagent comprising sulphuric acid (0.6M), sodium phosphate (28 mM), and ammonium molybdate (4mM) was mixed with 0.3 ml of extracts before incubation at 95°C for 90 minutes. The absorbance was

measured at 695 nm against blank after cooling at room temperature. Methanol was used as blank and the activity is exposed as the number of the equivalent of ascorbic acid (Standard) in mg per g of the extract (mg/AA/g).

DPPH Radical-scavenging Activity

Free radical scavenging activity of the extracts was determined as per the method of Braca *et al*.[19] with slight modification. The methanolic solution of DPPH (0.002%) was mixed with serial dilutions of the extracts ranging from 60 to 600 µg/mL and incubated in dark for 15 minutes. Absorbance at 517 nm was recorded. Ascorbic acid was used as standard. The percent of scavenging activity was calculated by using the equation

$$\% \text{ of Scavenging activity} = (Ac\text{-}As)/Ac \times 100$$

Where Ac-absorbance of the control, As-absorbance of the sample. The activity was reported as IC_{50}, which represent the concentration of the extract needed to scavenge 50% of DPPH free radicals.

ABTS Radical Cation Scavenging Method

The assay was carried out with minor modifications of Re *et al*. method.[20] The ABTS radical cations ($ABTS^{+}$) were produced by mixing ABTS solution (2mM) with potassium per sulfate (17mM) in the dark for 12 to 16 hours at room temperature. Thus prepared $ABTS^{+}$ solution was diluted with methanol till an absorbance of 0.7 ± 0.02 was obtained at 734 nm. 0.1 mL of different concentrations of extracts ranging from 60 to 600 µg/mL were added to 2 mL of diluted $ABTS^{+}$ reagent before the absorbance was read at 734 nm. Ascorbic acid was used as standard, and the percent inhibition was calculated by the following formula.

$$\% \text{ of Scavenging activity} = (Ac\text{-}As)/Ac \times 100$$

Where Ac – absorbance of control, as-absorbance of sample. The percent of scavenging activity was plotted against the concentration of the extract to obtain the IC50 value. Lower the value, higher the radical scavenging effect.

Reducing Power Capacity

The assay was conducted according to the method of Oyaizu.[21] Different concentrations of methanolic extracts of samples ranging from 20–100µg/mL were added to 2.5 mL of phosphate buffer (0.2M) at pH 6.6. To this mixture, 2.5 mL of potassium ferricyanide (1%) was added before incubating at 50°C for 20 minutes. A 2.5 mL of 10% trichloroacetic acid (2.5 mL) was added to this mixture to terminate the reaction and centrifuged at 3000 rpm for 10 minutes. 2.5 mL of upper layer of the solution was taken and mixed with 2.5 mL of distilled water along with 0.5 ml of ferric chloride (0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture is an indication of increased reducing power.

α-Amylase Inhibition Assay

The inhibition of α amylase activity was determined using the modified method of Miller[22]. About 1g of each sample was homogenized with 10 mL of 0.1M phosphate buffer (pH 7). The homogenate was then centrifuged at 6000rpm for 10 minutes at 4°C. The supernatant obtained was further used. A volume of 200 µL of α-amylase solution was mixed with 200 µL of different concentration of extracts and was incubated for 10 minutes at 30°C. Thereafter 200 µL of the starch solution (1% in water) was added to each tube and incubated for 3 minutes at room temperature. The reaction was terminated by adding about 200 µL of dinitrosalicylic acid reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution) and boiled for 10 minutes in a water bath at 85–90°C. The mixture was cooled and was diluted by adding 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the extract with 200 µL of a buffer. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the formula.

$$\% \text{ α-amylase inhibition} = 100 \times (\text{absorbance of control} - \text{absorbance of sample})/(\text{absorbance of control})$$

The % of inhibition of α-amylase was plotted against the extract concentration and the IC_{50} values were calculated.

Data analysis

All the experiments were carried out in triplicate. Data were expressed as Mean ± Standard deviation and subjected to one-way analysis of variance (ANOVA). Significance F ratios between the group means were analysed by Duncan's multiple ranges at $p \leq 0.05$.

Results and Discussion

Preliminary phytochemical screening of both stem and leaf extracts are shown in Table 1. Methanol, ethyl acetate, n-Hexane, chloroform and water were used as solvents to screen the phytochemicals following standard methods. Methanol extract has shown the presence of a maximum number of phytochemicals compared to other extracts. Saponins are absent in all the extracts, except the leaf aqueous extracts. However, detection of saponins in the methanolic seed extract of *M.umbellatum* and leaf extract of *M.malabaricum* were reported by Vivek *et al*.[5] and Elangovan *et al*. [23], respectively. While Tejavathi *et al*. [24] have reported the absence of saponins in all the extracts of *M.flavescens*. Selection of a solvent plays a critical role in the extraction of biologically active compounds because of their presence in minimum quantities. Solvents with wide range of polarity were commonly used for the extraction. Since phenolic compounds are known to have a wide range of pharmacological activities, their maximum recovery from the extracts is required to employ various qualitative and quantitative analyses. Among the solvents used in the present study, the methanol was found to be best with

Table 1: Preliminary phytochemical screening of leaf and stem extracts of *M. malabaricum*

	Methanol		*Ethyl Acetate*		*N-Hexane*		*Chloroform*		*Aqueous*	
Chemical Test	*Leaf*	*Stem*	*Leaf*	*Stem*	*Leaf*	*Stem*	*Leaf*	*Stem*	*Leaf*	*Stem*
Alkaloids	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	-	-	-	-	-	-	+	+
Tannins	+	-	-	-	-	-	-	-	+	-
Phenols	+	+	-	-	-	-	-	-	+	+
Terpenoids	+	+	-	-	-	-	-	-	+	+
Saponins	-	-	-	-	-	-	-	-	+	-
Coumarins	-	+	-	-	-	-	-	-	+	+
Carbohydrates	+	-	-	-	+	+	+	+	+	+
Phytosterols	+	+	-	-	-	-	-	-	-	-
Proteins and Amino acids	-	-	+	+	-	-	-	-	-	-

Table 2: List of chemical compounds characterized in the leaf sample of *M. Malabaricum* through GC-MS analysis

Sl. no	*Methanolic extract of leaf*	*Retention time*	*Peak area %*	*Molecular Weight g/mol*	*Molecular formula*	*Compound group*
1	Cyclopentane,1-bromo-2-fluro-, cis-	16.625	4.92	167.021	C_5H_8BrF	Halo alkanes
2	3-cyclopentylpropionic acid,but-3-yn-2-yl ester	16.759	3.84	194.270	$C_{12}H_{18}O_2$	Ester
3	Phenol,3,5-bis(1,1-dimethylethyl)-	19.240	1.83	206.323	$C_{14}H_{22}O$	Benzene
4	Beta.-D-Glucopyranose,16,-Anhydro-	20.367	8.50	162.141	$C_6H_{10}O_5$	Carbohydrate (monosaccharide)
5	Hexanoic acid, 1-cyclopentylethyl ester	20.567	2.73	212.333	$C_{13}H_{24}O_2$	Ester
6	Gamma.-Guanidinobutyric acid	20.692	7.69	145.162	$C_5H_{11}N_3O_2$	Acid
7	Morpholine,4-methyl-,4-oxide	20.847	2.34	117.146	$C_5H_{11}NO_2$	Heterocyclic
8	Octane,3-ethyl-2,7-dimethyl-	21.323	1.90	170.334	$C_{12}H_{26}$	Aldehyde
9	Pentadecanal-	22.733	3.87	226.398	$C_{15}H_{30}O$	Fatty aldehyde
10	5,5-diethylheptadecane	23.537	2.70	296.574	$C_{21}H_{44}$	Dialkyle alkanes
11	Neophytadiene	26.507	4.51	278.515	$C_{20}H_{38}$	Terpenoids
12	Octadecanoic acid, methyl ester	28.345	3.29	298.503	$C_{19}H_{38}O_2$	Ester
13	Methane, dichloro nitro-	29.092	1.41	129.93	$CHCl_2NO_2$	Hydrocarbon
14	Cis-sesquisabinene hydrate	38.307	2.41	222.3663	$C_{15}H_{26}O$	Terpene
15	3-cis-methoxy-5-cis-methyl-1R-cyclohexanol	38.999	4.34	144.214	$C_8H_{16}O_2$	--------
16	4-Methyl itaconate	39.042	1.75	144.1253	$C_6H_8O_4$	Ester
17	1-Heptatriacotanol	39.181	9.85	537.014	$C_{37}H_{76}O$	Hydro carbon
18	5,6-o-ethylbotanediyl-L-Ascorbic acid	39.317	4.07	--------	--------	Vitamin C
19	4-ethylbenzylamine,N,N-Diheptyl-	39.624	1.83	331.588	$C_{23}H_{41}N$	Amine
20	2(3H)-oxoninone,4,5,6,9-tetrahydro-,(Z)-	39.800	2.14	--------	--------	Ketone
21	Stigmast-5-En-3-ol, (3.Beta)-	39.975	7.04	414.718	$C_{29}H_{50}O$	Glycoside
22	Stigmasterol	40.082	6.28	412.702	$C_{29}H_{48}O$	Steroid
23	Piperidine,2,2,6,6-tetramethyl-	40.175	3.44	141.258	$C_9H_{19}N$	Heterocyclic compound
24	4-acetoxyquinoline-2-one	41.612	2.26	--------	--------	Ketone
25	Lupeol	43.585	5.05	426.729	$C_{30}H_{50}O$	Terpenoid

the maximum yield of 5.1 g in leaf and 2.0 g from stem extracts.

Since methanol extracts of both the stem and leaf have shown the maximum yields, it was subjected to GC-MS qualitative analysis. A total of 25 in leaf and 19 bioactive compounds in stem extracts were identified based on their retention time and percent peak area (Tables 2 and 3 and Figs. 2 and 3). GC-MS is considered as one of the authentic

Table 3: List of chemical compounds characterized in the stem sample of *M. Malabaricum* through GC-MS analysis

Sl. no	*Methanolic extract of stem*	*Retention time*	*Peak area %*	*Molecular weight g/mol*	*Molecular formula*	*Compound group*
1	Undecane,2-methyl-	16.353	2.48	170.34	$C_{12}H_{26}$	----------
2	Nonadecane	18.559	2.56	268.52	$C_{19}H_{40}$	Alkane Hydrocarbon
3	2,4-ditert-butylphenol	19.212	2.39	206.32	$C_{14}H_{22}O$	Phenol
4	Octadecanoic acid	20.450	1.94	284.48	$C_{18}H_{36}O_2$	Saturated Fatty acid
5	Heptadecane	21.314	2.23	240.47	$C_{17}H_{36}$	Straight Chain Alkane
6	Tridecanol, 2-ethyl-2-methyl-	23.537	1.00	242.44	$C_{16}H_{34}O$	Fatty Alcohols
7	Octane, 3-ethyl-2,7-dimethyl	25.761	0.69	170.34	$C_{12}H_{26}$	Alkane Hydrocarbon
8	Phytol, acetate	26.504	3.19	338.56	$C_{22}H_{42}O_2$	Acyclic diterpene
9	Decane,2,4,6-trimethyl-	29.797	0.63	184.36	$C_{13}H_{28}$	Methyle Hydrocarbon
10	Santalol,cis,.alpha.-	34.419	1.00	220.35	$C_{15}H_{24}O$	Terpene
11	Sebacic acid, 3-phenylpropyl propyl ester	34.579	0.42	362.50	$C_{22}H_{34}O_4$	Omega Di carboxylic acid ester
12	Carbonic acid,propargyl 2,2,2-trichloroethyl ester	38.025	0.48	231.45	$C_6H_5Cl_3O_3$	Ester
13	Methyl commate E	38.261	1.12	--------	--------	Ester
14	Glycerol .beta.-palmitate	38.883	11.53	330.50	$C_{19}H_{38}O_4$	Alcohol & ester
15	Pentadecanal-	38.967	5.82	226.40	$C_{15}H_{30}O$	Aldehyde
16	(s)-cembrene	39.133	35.65	272.47	$C_{20}H_{32}$	Terpenes
17	Stellasterol	39.723	7.32	398.67	$C_{28}H_{46}O$	Terpene (alcohol)
18	3-oxabicyclo(3.3.1)nonane	39.867	1.51	126.19	$C_8H_{14}O$	Hydrocarbon-alkane
19	Beta.-sitosterol	42.799	18.03	414.70	$C_{29}H_{50}O$	Alcohol/terpene

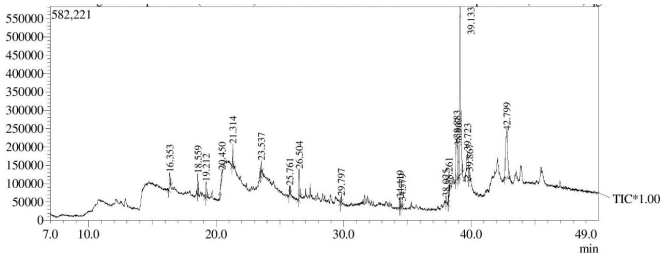

Fig. 2: The GC-MS chromatogram of Methanolic stems extract of *M.malabaricum*

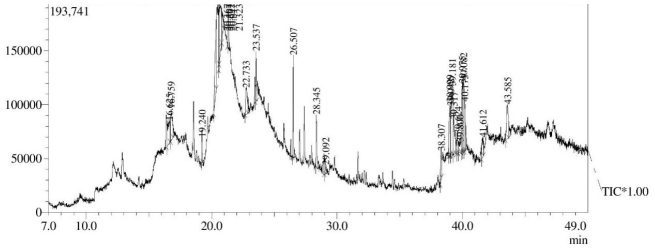

Fig. 3: The GC-MS Chromatogram of Methanolic leaf extract of *M. malabaricum*

techniques to identify the bioactive constituents.[25] Among the compounds identified in the stem extract, (S)-cembrene has present in the highest quantity of 35.65% followed by β-sitosterol (18.03%). Both these compounds are belonging to terpene group, which comes under phenolic compounds. Whereas, leaf extract has the maximum quantity of 1-Heptatriacotacol, a Hydrocarbon (9.85%) followed by ß-D-Glucopyranose,16,-Anhydro, monosaccharide (8.5%). However, Rajalakshmi[14] has identified 23 compounds in the leaf methanolic extract of *M.malabaricum* collected from Kerala; Palamitic acid vinly ester (25.01%) were found to be in large quantities. Variations in the synthesis of secondary metabolites may be due to the change in environmental conditions, which is, in turn, depends on the habitat.[26] Phenolic compounds are main class of secondary metabolites in plants that play a key role in plants' defense mechanisms. Many studies have shown a strong and positive correlation between the phenolic compounds and antioxidant potential.[27] Hence, the stem and leaf extracts' total phenols and flavonoid contents were determined employing standard procedures in the present study. The total content of phenolics in stem and leaf extracts were found to be 79.35 ± 0.45 mg/GAE/g and 76.61 ± 0.29 mg/GAE/g, respectively. However, Sivu *et al*.[4] have reported 25.76 ± 0.03 mg/GAE/g of total phenolics in the methanolic leaf extract of *M.malabaricum*. The total content of flavonoids in the present study were found to be 11.66 ± 0.13 mg/QE/g and 21.64 ± 0.15 mg/QE/g in stem and leaf samples, respectively. The phenolic content in the stem extract (79.35 ± 0.45) in the present study was slightly more than leaf extract (76.61 ± 0.29). However, the flavonoid content was significantly more in leaf extract (21.64 ± 0.15) than the stem (11.66 ± 0.13). The flavonoid content of methanolic leaf extract of

M.malabaricum was recorded by Sivu *et al.*[4], however, has shown 12.56 ± 0.05 mg/GA/g. Flavonoids as one of the widespread group of natural phenolic compounds possess a broad spectrum of pharmacological activities including antioxidants properties. Hence, the total antioxidant activity of stem and leaf extracts was determined by phosphomolybdeum method. Leaf extract has exhibited higher level of antioxidant potential of 50.06 mg/AAE/g than stem extract, which was recorded as 43.55 mg/AAE/g (Fig. 4). This observation does not correlate with GC-MS results where stem extracts have shown more phenolic compounds than the leaf extract. Lee *et al.*[28] However, it has been pointed out that antioxidant activity is a consequence of the synergism between different phenolic compounds and can not be attributed specifically to one constituent.

The antioxidant potential of the stem and leaf extracts are studied in the present study by subjecting them to DPPH radical scavenging assay, ABTS assay, and reducing power capacity method since any single method cannot fully evaluate the antioxidant capacity of a particular compound. The samples have exhibited remarkable DPPH free radical scavenging ability at different concentrations (Fig. 5). The percent inhibition, concentrations, and IC_{50} values were calculated. DPPH is a stable free radical with absorption at 517 nm. The color change from purple to yellow with varying degrees determine the scavenging potential of the extract. In the present study the highest capacity to neutralize DPPH radicals was found for the leaf extract with IC_{50} value of 173.16 µg/mL at the concentration of 150 µg/mL compared to stem extract, which has shown the IC_{50} value of 226.97 µg/mL. The scavenging activity of DPPH is directly proportionate to the concentration of the extracts. However, Shivu *et al.*[4] have recorded the IC_{50} value of 207.24 ± 0.03 in the methanolic leaf extract of the same taxon. Simultaneously, Vivek *et al.*[5] have found high scavenging of radicals with IC_{50} value of 6.26µg/mL in the leaf extract. Variations in the activity recorded in the same taxon is attributed to different geographical and climatic conditions in which they grow.[29]

The DPPH assay has good repeatability and most used assay to assess the antioxidant potential of the extracts. However, Bondet *et al.*[30] and Brand-William[31] have found that most phenolic antioxidants react slowly with DPPH to reach the steady-state (6 hours). Shalaby and Shanab[32] have therefore suggested that the ABTS method, which has extra flexibility to reach steady-state readily within 30 minutes at various pH, is better assay than DPPH. ABTS assay measures the relative ability of the extracts to scavenge the ABTS free radicals against ascorbic acid that was used as a standard. The change in color from blue to colorless indicates the scavenging capacity of the extracts. Percent of scavenging activity ranges from32.30 ± 1.2-72.68 ± 1.5µg/mL with IC_{50} value of 298.28µg/mL in leaf extract and 30.5 ± 1.5-69.0 ± 0.6 µg/mL with IC_{50} value of 374.60µg/mL in stem extract (Fig. 6).

The reducing power assay serves as a significant reflection of the antioxidant activity.[33] It measures the reducing capacity of the samples from ferric (Fe^{+3}) to ferrous (Fe^{+2}). The reducing power of the samples is measured on the changes in the color of the reaction mixture from yellow to various shades of green and blue depending on the concentration. The leaf extract has shown significant reducing capacity than the stem extract

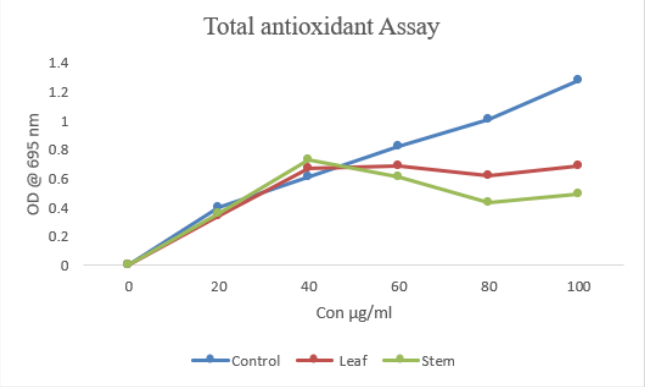

Fig 4: Total antioxidant activity of Methanolic extracts of leaf and stem of *M.malabarium.*

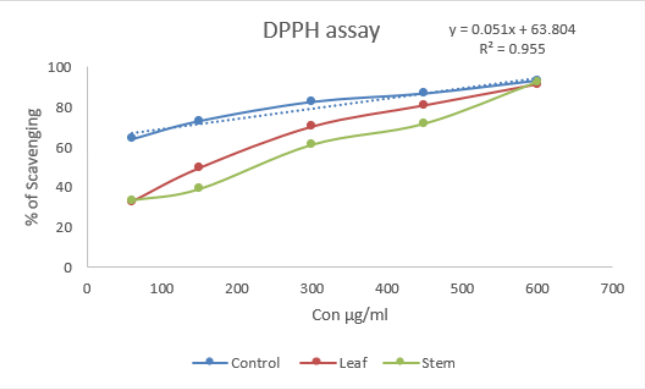

Fig. 5: DPPH radical scavenging activity of Methanolic extracts of leaf and stem of *M.malabaricum*

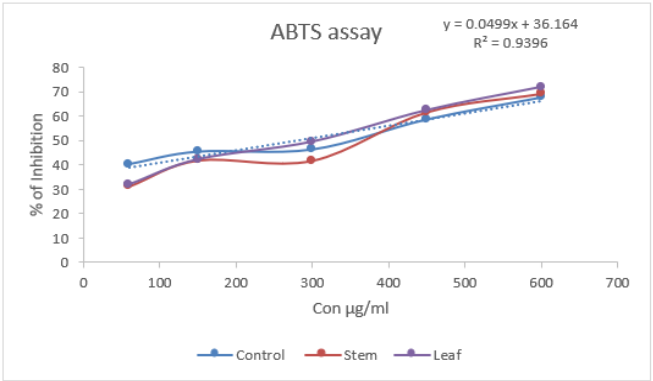

Fig. 6: ABTS radical scavenging activity of Methanolic extracts of leaf and stem of *M.malabaricum*

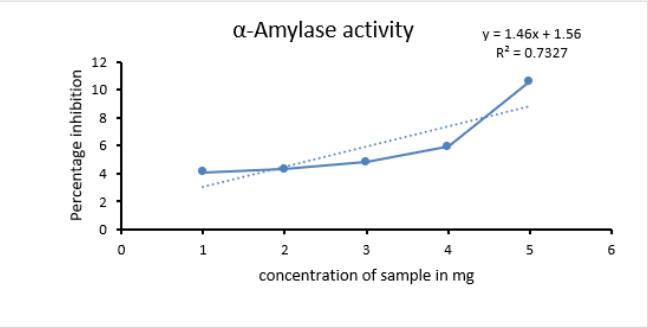

Fig. 7: Inhibition of α - amylase activity by *M.malabaricum* leaf extract

in the present study. The reducing power has increased with increasing concentration.

The three assays conducted in the present study have shown that leaf extract has better antioxidant activity than the stem extract. However, the GC-MS studies have revealed that stem extract has contain more phenolic compounds that has reflected in the total phenolic estimation. While total flavonoid estimation has shown more content in leaf extract than the stem extract. These results observed in the present study is in accordance with the other authors who are of the opinion that antioxidant activity cannot be attributed to a single specific compound but it is due to the synergism between different phenolic compounds.[28,34] Phenolic content and antioxidant activity of the two samples studied have shown linear correlation in all the three assays conducted in the present investigation with the correlation coefficients are above $R^2 = 0.9$.[35]

Plant phenols and flavonoids are the naturally occurring anti-diabetic agents, which are known to show an inhibitory effect on carbohydrate hydrolyzing enzyme activity.[36,37] α-amylase and α - glucosidase are the two enzymes required to hydrolyse the starch into maltose and then to glucose. Though various prescribed anti-diabetic drugs can manage diabetes mellitus, they have shown several side effects. Hence, a naturally occurring specific inhibitor for the activity of α-amylase is the need of the hour. Piparo *et al.*[38] have concluded from their studies on flavonoids and flavonols that are naturally occurring flavonoids act as inhibitors of human α-amylase that can be further exploited to manage postprandial glycemia. Since *M. malabaricum* is used in traditional medicine to manage diabetes, the preliminary studies on the inhibition of α - amylase by the crude extract was determined in the present study. The extract has shown dose-dependent activity and a maximum of 10.60% of inhibition at 5 mg/L. From 1 to 5mg/L of the sample, the percent inhibition has shown a steady increase (Fig. 7). The earlier report by Ramaiah[11] on the anti-diabetic activity of methanolic leaf extract of *M.malabaricum* has also indicated that the activity is dose-dependent. Further critical studies are needed to ensure anti-diabetic activity.

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HOW TO CITE THIS ARTICLE: Tejavathi DH, Sumalatha BS. Phytochemical, antioxidant, and anti-diabetic analysis of leaf and stem extracts of *Memecylon malabaricum* (C.B.Clarke) Cogn.. Int. J. Pharm. Sci. Drug Res. 2020;12(6):606-613. **DOI:** 10.25004/IJPSDR.2020.120604