

COMPARATIVE EVALUATION OF ANTIDIABETIC AND ANTIOXIDANT POTENCY OF DIFFERENT EXTRACTS OBTAINED FROM *MEMECYLON* SPECIES

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ABSTRACT

Objective: *Memecylon* species is being extensively used in traditional medicine for the treatment of skin disorders and it is proved to possess antidiabetic and anti-inflammatory properties. The present investigation was to study the effect of different solvent extracts of five *Memecylon* species such as *M. umbellatum*, *M. talbotianum*, *M. edule*, *M. malabaricum* and *M. wightii* on antidiabetic and antioxidant effects.

Methods: Plant extracts were prepared using soxhlet apparatus using different solvents such as hexane, ethyl acetate, methanol and water and obtained extracts were subjected to antidiabetic (α -amylase and α -glucosidase inhibition assays) and antioxidant (2, 2-Diphenyl-2-Picryl Hydrazyl hydrate (DPPH), 2,2-Azino-bis (3-ethyl benzothiazoline-6-Sulfonic acid)diammonium salt (ABTS), Superoxide radical scavenging assay (SRSA) and reducing power assays) evaluated at different doses.

Results: Methanol extracts of all five *Memecylon* species exhibited effective antidiabetic and antioxidant properties among them methanol extracts of *M. malabaricum* and *M. talbotianum* have highest biological activity. For α -amylase IC₅₀ value for both *M. malabaricum* and *M. talbotianum* was found to be 100 and 130 μ g/reaction and IC₅₀ value for α -glucosidase was found to be 6.1 and 7.8 μ g/reaction respectively. For DPPH the IC₅₀ value was found to be 190 μ g/reaction, for ABTS 31-39 μ g/reaction, for SRSA 950-1200 μ g/reaction and for reducing power assay 420-490 μ g/reaction respectively.

Conclusion: The results indicate that methanol extracts of *M. malabaricum* and *M. talbotianum* possess potent *in vitro* antidiabetic and antioxidant activities compared to other *Memecylon* species.

Keywords: *Memecylon* species, Antioxidant, Antidiabetic, Methanol extracts

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INTRODUCTION

Oxidative stress is reduced by generating antioxidants from the plant and protects the body against free radical-mediated toxicities [1]. Several plant species are screened for antioxidant activities which are known to exhibit antidiabetic activity. However, it is estimated that more than 400 plant-derived products are reported to be used for the treatment of diabetes across the globe [2]. The antidiabetic effect of some plant extracts has been proved in both human and animal models of diabetes [3].

The genus *Memecylon* L. (Melastomataceae) is native to Asia, Africa, Madagascar and pacific islands and comprises about 300 species. In the India, the genus is mainly distributed in Western Ghats region represented by about 40 species of which 21 are endemic to peninsular India [4, 5]. The species of this family are small trees or shrubs mainly used in the treatment of skin diseases such as herpes, stomach disorders, snakebite, wounds etc., which is mainly used in traditional medicinal system [6, 7, 8] a few compounds have been investigated and also several pharmacological properties have been reported from the crude extracts of *Memecylon* species [9]. Since very few studies have thrown light on bioactive properties of *Memecylon* species such as *M. wightii* is very less, and their comparative analysis was also few. Therefore the present study was to determine the *in vitro* antidiabetic and antioxidant properties of different fractions obtained from *Memecylon* species such as *M. umbellatum*, *M. edule*, *M. talbotianum*, *M. malabaricum* and *M. wightii*.

MATERIALS AND METHODS

Chemicals

2,2-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid (ABTS), nicotinamide adenine dinucleotide reduced (NADH), phenazine methosulphate (PMS), nitro blue tetrazolium (NBT), potassium ferricyanide, ascorbic acid,

gallic acid, quercetin, ferrous sulfate 7 H₂O, α -amylase, α -glucosidase, 4-nitrophenyl- α -D-glucopyranoside, All other solvents, chemicals and reagents used were of analytical grade and obtained from reputed companies.

Plant materials and preparation of extracts

Five *Memecylon* species namely, *M. umbellatum* Burm, *M. edule* Roxb, *M. talbotianum* Brandis, *M. malabaricum* Clarke and *M. wightii* Thwaites were collected from different parts of Karnataka. Shade dried leaves of *Memecylon* species were powdered in an electric blender and extracted sequentially with hexane, ethyl acetate, methanol and water using soxhlet apparatus.

In vitro antioxidant activity

2, 2-Diphenyl-2-Picryl Hydrazyl hydrate (DPPH) radical scavenging activity

The DPPH free radical scavenging property of the different solvent extracts of *Memecylon* species was determined by the method described by Sultanova [10]. The DPPH (300 μ M) solution was prepared in methanol containing 100 μ l reaction mixture containing 5 μ l of plant extract (100 to 400 μ g/ml) and 95 μ l of DPPH solution. The radical scavenging reaction was carried out at 37 °C in the dark for 30 min and the absorbance was recorded at 517 nm. IC₅₀ values were evaluated which indicates the concentration of extracts required to scavenge 50% of free radicals. Positive control used was ascorbic acid.

2, 2-Azino-bis (3-ethyl benzothiazoline-6-Sulfonic acid) diammonium salt (ABTS) radical scavenging activity

The antioxidant activity measured using the ABTS method described by Re [11]. The ABTS solution was prepared by mixing 2.45 mmol potassium persulfate and 7 mmol ABTS and keeping in the dark for 12 h at room temperature. The prepared ABTS solution was diluted again with methanol to get an absorbance of 0.700 \pm 0.005 at 734 nm.

Different concentrations of test samples (20 to 80 µg/ml) were added to 2.95 ml of ABTS working a solution to give a final volume of 3 ml. The absorbance was recorded after incubation at room temperature for 30 min at 734 nm. Gallic acid was used as a positive control.

Superoxide radical scavenging assay (SRSA)

The superoxide scavenging capacity of the plant extract was determined by the method of Singh [12]. According to this method, different concentrations of the extract (320 to 2560 µg/ml) were prepared. Different concentrations of extracts were taken, and the total volume made up to 400 µl using phosphate buffer (0.1M pH 7.4) containing 1 ml of PMS (60 µM), 1 ml of NADH (677 µM) and 100 µl of NBT (144 µM) in phosphate buffer (0.1M pH 7.4). The reaction was carried out at room temperature for 5 min including blank. The color intensity was read using the spectrophotometer at 560 nm (Beckman Coulter, DU 730 Life Sciences).

Reducing power assay

The reducing power assay of different extracts of *Memecylon* species was evaluated according to the method described by Shen [13]. Different concentrations (250 to 1000 µg/ml) of the sample (1 ml) was mixed with PBS buffer (pH 7.4, 0.02M), 1.0 ml potassium ferricyanide (1.0%, w/v) and the reaction was incubated at 50 °C for 20 min. Then the reaction was terminated by adding 1.0 ml of tri chloroacetic acid (10.0%, w/v) again the solution was mixed with 0.4 ml ferric chloride (0.1%, w/v) and kept for 10 min. Different concentrations of ascorbic acid were used as a positive control. The increased absorbance of the reaction mixture indicated increased reducing power by reading the absorbance at 700 nm.

Determination of IC₅₀ value calculation for antioxidant assay

The concentration of the sample required for scavenging 50% of the radicals/to chelate metal ions was calculated as follows.

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where "A_{control}" is the absorbance of control, and "A_{sample}" is the absorbance of the sample. IC₅₀ values denote the concentration of a sample required to decrease the absorbance by 50%.

In vitro antidiabetic activity

Inhibition of α-amylase

The pancreatic α-amylase (PPA) inhibition assay was carried out in a microtitre plate based on the starch-iodine test [14]. The reaction mixture consisted of 40 µl of 0.02 M sodium phosphate buffer (pH 6.9, containing 6 mmol NaCl) and leaf extract at concentrations from 80 to 240 µg/ml (w/v). The reaction was initiated by addition of PPA (0.02 units) and soluble starch (1%, w/v) followed by incubation at 37 °C for 10 min. The reaction was stopped by addition of 1M HCl (20 µl). The color change by the addition of iodine reagent (5 mmol Iodine and 5 mmol Potassium iodide) was read at 620 nm on a microplate reader (VarioSkan Flash, Thermo Fisher Scientific, Finland). To eliminate the absorbance due to only extract/ enzyme/substrate, appropriate controls were included. The enzyme activity without extracts was used for all calculations. The known PPA inhibitor, acarbose (6.5 to 32.8 µg/ml) was used as positive control.

Inhibition of α-glucosidase

The enzyme source was α-glucosidase dissolved at a concentration of 0.1 U/ml in 0.1 M phosphate buffer (pH 7.0), containing BSA (2 g/ml) and sodium azide (0.2 g/ml). The substrate 1 mmol *p*-nitrophenyl-α-D-glucopyranoside (PNPG) (20 µl) was used. Twenty microliters of different concentration of leaf extract (5 to 15 µg/ml) was incubated for 5 min with the enzyme (50 µl) and the reaction was further continued by addition of substrate (50 µl). The reaction was terminated 5 min after by the addition of 50 mmol sodium hydroxide (3 ml) and the absorbance was read at 410 nm. To eliminate the absorbance produced by the extract/enzyme/substrate, appropriate controls were included. The enzyme activity without plant extract was used for all the calculations [15].

Determination of IC₅₀ for antidiabetic assay

The IC₅₀ value is the concentration of the extract, containing the enzyme inhibitor that inhibited the activity by 50%. The % of inhibition was calculated as follows:

$$\% \text{ Inhibition} = EC - (ET - TC) / EC$$

Where EC is the enzyme activity of control, ET is the enzyme activity of test, and TC is the test control.

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of product from substrate per min under the assay conditions.

Statistical analysis

Values in tables and graphs were means±standard deviations (n=3). The IC₅₀ values were calculated from linear regression analysis. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Student's t-test to determine the significant difference between samples with 95% confidence limit.

RESULTS

In vitro antioxidant studies

The DPPH, ABTS, SRSA and reducing power assay of 100-400 µg/reaction for DPPH, 20-80 µg/reaction for ABTS, 320-2560 µg/reaction for SRSA and 250-1000 µg/reaction for reducing power assay was calculated, and the IC₅₀ value was determined from the linear regression of the percent antioxidant activity against extract. The antioxidant activity for each extract was dose-dependent.

The methanol extracts of five *Memecylon* species showed the best DPPH, ABTS, SRSA and reducing power activity, ethyl acetate, water extracts showed moderate activity and hexane extracts showed lower antioxidant activity. The radical scavenging by methanol extract was in the range of 59.3 to 89.4%, 70.4 to 98%, 75.5 to 94.1% and 78 to 96% respectively. The methanol extracts of *M. malabaricum* and *M. talbotianum* showed highest radical scavenging activity of 89.4 and 89% in DPPH, 92 and 98% in ABTS, 94.1 and 92.9% in SRSA and 96 and 93% in reducing power assay respectively (tables 1-4).

Table 1: 2, 2-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging activity of *Memecylon* extracts

Plant	%DPPH radical scavenging activity of extracts			
	Hexane	Ethyl acetate	Methanol	Water
<i>M. umbellatum</i>	33.7±0.3	66.9±0.2 [350]*	59.3±0.1 [300]*	30.8±0.9
<i>M. edule</i>	29.8±0.4	29.3±0.8	50.7±0.4 [280]*	42.1±0.1
<i>M. talbotianum</i>	26±1.2	75±0.9 [200]*	89±0.3 [190]*	44±2.2
<i>M. malabaricum</i>	61.8±0.3 [400]*	83.3±0.7 [190]*	89.4±0.3 [190]*	78.8±0.9 [320]*
<i>M. wightii</i>	49.3±0.5	54.6±0.9 [350]*	80.3±0.2[370]*	40.5±0.3

Note: Ascorbic acid was used as a reference standard with 94.7±1.5% radical scavenging activity at 400 µg/reaction concentration and IC₅₀ of 436±1.2 µg.* Represents the IC₅₀ value of the extract in µg. Data are expressed as mean±SD (n=3)

Table 2: 2, 2-Azino-bis (3-ethyl benzothiazoline-6-Sulfonic acid) diammonium salt (ABTS) radical scavenging activity of *Memecylon* extracts

Plant	%ABTS radical scavenging activity of extracts			
	Hexane	Ethyl acetate	Methanol	Water
<i>M. umbellatum</i>	24±1.18	56±1.2 [60]*	89±1.2 [40]*	50±1.1 [69]*
<i>M. edule</i>	30.4±0.8	54.3±0.8 [58]*	70.4±1.08 [45]*	56.7±0.9 [66]*
<i>M. talbotianum</i>	49±1.2	80±2.1 [39]*	98±2.8 [31]*	61±3 [60]*
<i>M. malabaricum</i>	40.2±0.6	73.3±1.4 [42]*	92±1.4 [39]*	71±0.9 [53]*
<i>M. wightii</i>	34.3±0.6	69±1.2 [45]*	68.2±1.2 [43]*	59.2±1.8 [59]*

Note: Gallic acid was used as a reference standard with 98.7±1.5% radical scavenging activity at 80 µg/reaction concentration and IC₅₀ of 31±0.9 µg. **Represents the IC₅₀ value of the extract in µg. Data are expressed as mean±SD (n=3)

Table 3: Superoxide radical scavenging activity (SRSA) of *Memecylon* extracts

Plant	% Superoxide radical scavenging activity of extracts			
	Hexane	Ethyl acetate	Methanol	Water
<i>M. umbellatum</i>	40.5±1.8	89.2±0.2 [1200]*	84±1.3 [1100]*	49.6±1.4 [2400]*
<i>M. edule</i>	34.5±0.9	75.1±0.6 [1500]*	75.5±0.9 [1440]*	55.5±0.6 [2000]*
<i>M. talbotianum</i>	36.3±1.3	50±1.2 [2130]*	92.9±0.2 [950]*	82±1.2 [2000]*
<i>M. malabaricum</i>	42±1.2	94.1±1.2 [1200]*	94.1±0.4 [1200]*	84.6±1.3 [1290]*
<i>M. wightii</i>	34.6±2.2	80.5±1.4 [1200]*	84±0.6 [1200]*	52.5±0.9 [1900]*

Note: Gallic acid was used as a reference standard with 94.7±1.2% radical scavenging activity in 2560 µg/reaction concentration and IC₅₀ of 968±0.8 µg. **Represents the IC₅₀ value of the extract in µg. Data are expressed as mean±SD (n=3)

Table 4: Reducing power assay of *Memecylon* extracts

Plant	% Reducing power assay of extracts			
	Hexane	Ethyl acetate	Methanol	Water
<i>M. umbellatum</i>	53.4±1.2 [890]*	69.2±0.2 [710]*	90.2±1.2 [500]*	46.3±2.2
<i>M. edule</i>	57±2.6 [890]*	77±1.2 [800]*	78±0.9 [530]*	63.2±1.8 [680]*
<i>M. talbotianum</i>	12.9±2.9	85±2.1 [470]*	93±1.6 [420]*	83.8±0.9 [580]*
<i>M. malabaricum</i>	66.1±2.2 [699]*	96±0.9 [510]*	96±2.1 [400]*	86.1±2.3 [480]*
<i>M. wightii</i>	48±2.4	78±0.6 [690]*	81.2±1.3 [490]*	76.7±1.2 [590]*

Note: Ascorbic acid was used as a reference standard with 94.7±1.5% radical scavenging activity in 1000 µg/reaction concentration and IC₅₀ of 427.6±1.6 µg. **Represents the IC₅₀ value of the extract in µg. Data are expressed as mean±SD (n=3)

Table 5: Inhibition of α-amylase enzyme by plant extracts of *Memecylon* species

Plant	% inhibition of α-amylase enzyme			
	Hexane	Ethyl acetate	Methanol	Water
<i>M. umbellatum</i>	33.8±1.4	80.3±1.2 [120]*	94.4±1.4 [99]*	44.3±0.9
<i>M. edule</i>	34.3±0.9	73.3±0.6 [210]*	88±2.2 [112]*	66.8±2.4 [240]*
<i>M. talbotianum</i>	24±0.7	91±1.9 [120]*	94±3 [130]*	75±0.6 [210]*
<i>M. malabaricum</i>	48.1±1.2	82.3±1.8 [140]*	94.7±1.8 [100]*	69.8±0.6 [178]*
<i>M. wightii</i>	14.4±0.8	58.2±0.9 [125]*	88.4±1.6 [110]*	53.3±1.2 [159]*

Note: Acarbose was used as a reference standard with 98.2±2.4% inhibitory activity at 240 µg/reaction concentration and IC₅₀ of 135±0.9 µg. **Represents the IC₅₀ value of the extract in µg. Data are expressed as mean±SD (n=3)

In vitro antidiabetic studies**Inhibition of α -amylase and α -glucosidase activity**

Carbohydrate hydrolyzing enzymes α -amylase and α -glucosidase are responsible for glucose generation from the diet. α -amylase hydrolyzes alpha-1,4-glycosidic bonds and splits up starch components into smaller oligosaccharides and disaccharides, like maltose. The α -glucosidases hydrolyze disaccharides to monosaccharides. Inhibition of these two enzymes is important for management or control of diabetes. Methanol, ethyl acetate and water extracts of five *Memecylon* species have shown potent antidiabetic activity. Different concentrations of extracts ranging

from 80 to 240 μ g/reaction were tried for α -amylase, and 5 to 15 μ g/reaction were tried to check for α -glucosidase inhibitory activity.

The methanol extracts of five *Memecylon* species showed the best α -amylase and α -glucosidase inhibition activity. The inhibition of methanol extract ranged between 88 and 94.7% for α -amylase and 67 and 97.5% for α -glucosidase.

The methanol extract of *M. malabaricum* and *M. talbotianum* showed the highest antidiabetic activity of 94.7% and 94% for α -amylase and 89% and 97.5% for α -glucosidase activity. Hexane extracts showed lower activity, ethyl acetate and water extracts showed moderate inhibition for both α -amylase and α -glucosidase activity (table 5 and 6).

Table 6: Inhibition of α -glucosidase enzyme by plant extracts of *Memecylon* species

Plant	% inhibition of α -glucosidase enzyme			
	Hexane	Ethyl acetate	Methanol	Water
<i>M. umbellatum</i>	44.5 \pm 0.9	64 \pm 2.1 [10.9]*	84 \pm 2.3 [8]*	56.5 \pm 0.6 [13.3]*
<i>M. edule</i>	50.5 \pm 0.5 [12]*	56 \pm 1.8 [8.7]*	67 \pm 2.2 [8]*	56 \pm 2.2 [11]*
<i>M. talbotianum</i>	41.2 \pm 1.2	95 \pm 1.9 [9]*	97.5 \pm 1.8 [6.1]*	74 \pm 2.4 [6.2]*
<i>M. malabaricum</i>	59.5 \pm 0.2 [11]*	90 \pm 0.6 [8.7]*	89 \pm 1.8 [7.8]*	74 \pm 0.9 [9]*
<i>M. wightii</i>	50.5 \pm 0.6 [13]*	72 \pm 0.9 [13]*	74 \pm 2.3 [11]*	62 \pm 1.2 [14]*

Note: Acarbose was used as a reference standard with 97.2 \pm 1.4% inhibitory activity at 15 μ g/reaction concentration and IC₅₀ of 9.2 \pm 0.8 μ g. *Represents the IC₅₀ value of the extract in μ g. Data are expressed as mean \pm SD (n=3)

DISCUSSION

Traditional knowledge about the therapeutic potential of plants is necessary to isolate and identify biologically active products from plants [16]. Therefore isolation and identification of bioactive compounds present in a crude extract serve as the building block for the development of new type of therapeutics with new mechanisms of action [17]. Bioactive properties such as antioxidant, anti-inflammatory, antimicrobial and antidiabetic are very important to identify potentials of plant sources for drug development. Hence an attempt has been made to screen *Memecylon* species for their medicinal value. The present study clearly demonstrated that the methanol extracts of *Memecylon* species have significant antioxidant property when assessed by DPPH, ABTS, Superoxide radical scavenging and reducing power assays. Antioxidant activity of *Memecylon* species are in the order *M. malabaricum*>*M. talbotianum*>*M. umbellatum*>*M. edule*>*M. wightii*. The phytochemicals are known to play an important role in bioactivity of medicinal plants. In the quantitative phytochemical analysis, phenolic content was much more than flavonoid content [7]. The alkaloids, phenolic compounds, tannins, flavonoids have been associated with various degrees of antioxidant [18] and antidiabetic activities [19]. Therefore the antioxidant and antidiabetic effects observed in the present study might be due to the activity of some of the classes of compounds present in *Memecylon* species. The result of antioxidant evaluation revealed that *Memecylon* species could be used as a potential source of natural antioxidants. The high antioxidant properties make them useful in different areas of pharmaceutical, nutraceutical and cosmeceutical applications.

In Indian traditional medicine, many of the medicinal plants have been tested to treat diabetes [20] and their active principles have been isolated [21]. Although different classes of drugs are available to control diabetes, still it is a challenging task to bring a better molecule which is devoid of undesirable adverse effects with reference to existing drugs. Therefore the search for more effective and safer hypoglycemic agents is continuing to be an important area of active research and the efficacy of antidiabetic medicinal plants have been increasingly documented [20]. In the present study, *M. malabaricum* and *M. talbotianum* methanol extracts showed highest α -amylase enzyme inhibition activity compared to acarbose and in α -glucosidase enzyme inhibition. *M. talbotianum* showed highest

enzyme inhibition activity, followed by *M. umbellatum*, *M. edule* and *M. wightii*. Rajesh [22] reported antidiabetic and anti-hyperglycemic potential of *M. umbellatum* for different solvent extracts like hexane, ethyl acetate and methanol. Inhibition of α -amylase, non-enzymatic glycosylation of hemoglobin, the glucose diffusion assay and the glucose uptake by the yeast cells were used to evaluate antidiabetic potential. Methanol extracts showed higher antidiabetic potential which supports present findings.

CONCLUSION

In conclusion, the results of the present study suggest that methanol extracts of *M. malabaricum* and *M. talbotianum* have potent activity, *M. umbellatum*, *M. edule* and *M. wightii* have moderate antidiabetic and antioxidant activities. The results provide useful information on free radical scavenging and antidiabetic activity of plant materials.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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