

## IN VITRO ANTIOXIDANT ACTIVITY, LIPOXYGENASE, CYCLOOXYGENASE-2 INHIBITION AND DNA PROTECTION PROPERTIES OF MEMECYLON SPECIES

SHAILASREE SEKHAR, SAMPATH-KUMARA KK, NIRANJANA S R AND PRAKASH H S\*

Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India.  
Email: hasriprakash@gmail.com

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### ABSTRACT

Traditionally several species of *Memecylon* are used to cure skin problems. However, scientific validation of the plant as a modern medicine is lacking. Therefore, in the present study methanolic extracts of three species of *Memecylon* i.e., *M. umbellatum*, *M. talbotianum* and *M. malabaricum* were assessed for their antioxidant, anti-microbial, DNA protection capacities, 5-lipoxygenase and human cyclooxygenase (COX-2) inhibition.

Methods: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) ABTS radical scavenging capacities for extracts in dose range of 50 µg/mL - 10 mg/mL were measured. 15-Lipoxygenase inhibition for these extracts at 10 - 200 µg/mL dose was studied. Human cyclooxygenase-2 was recorded for doses 10 and 50 µg/mL. DNA-nicking assay at 10 µg was recorded. Cell cytotoxicity was recorded by colorimetric MTT assay using doxorubicin as control for extracts (50 - 500 µg/mL).

Results: The IC<sub>50</sub> values for scavenging the DPPH radical ranged from 0.11 to 0.17 mg/mL and those for the ABTS radical cation from 2.1 to 3.7 mg/mL for the extracts. *M. malabaricum* leaf extract exhibited highest lipoxygenase inhibition capacity with an IC<sub>50</sub> value of 29.87 µg/mL. It also inhibited human COX-2 (80.6 % at 50 µg/mL). Methanolic leaf extracts could prevent DNA nicking by hydroxyl radicals, produced in the Fenton reaction. *M. malabaricum* leaf extracts inhibited growth of *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli*.

Conclusion: This study forms initial screening of these plants with further studies directed towards identification of bioactive molecules as potential lead candidates.

**Keywords:** *Memecylon*, Antioxidant, DNA protection, Lipoxygenase, Cyclooxygenase-2

### INTRODUCTION

In India, Western Ghats, is one of the 34 global biodiversity hotspots covering an area of 159,000 sq km with 4500 -15,000 plant species [1]. Almost 1,800 of these are endemic to the region and 500 plants have been identified to have potential medicinal value [2].

The genus *Memecylon* L. (family Melastomataceae) comprises of about 300 species in the world, of which 30 species has been reported from India [3]. *Memecylon umbellatum* Burm. f., is a small and semi evergreen shrub or tree bearing several umbellate cymes. The leaves are used to treat conjunctivitis, and internally to treat leucorrhoea and gonorrhoea [4]. Bark is used in the treatment of bruises [4, 5], snake bite [6] and skin diseases [7]. Pharmacological studies have shown biological activities such as anti-diabetic [8], anti-microbial activity [9] and anti-viral properties [10]. The plants contain a wide variety of phytoconstituents such as umbellactone, β amyrine, oleanolic acid, ursolic acid, sitosterol and tannins [11].

*Memecylon talbotianum* Brandis, endemic to the Western Ghats is a small tree mostly distributed in a small pocket of evergreen forest Central and south Sahyadri [12]. There have been no pharmaceutical reports on this specie of *Memecylon*. *M. malabaricum* Cogn. is traditionally used for the treatment of herpes. The young shoot tip paste along with cumin seeds is applied externally on the skin [13]. Methanol extracts of plants have been reported to have bactericidal activity against both Gram (+) and Gram (-) bacteria; and fungi [14]. A phytochemical study of the whole *Memecylon* genus reported the presence of 13 fatty acids, 12 methyltetradecanate, glucose, amino acids, carotenoids, a phenolic glycoside and possibly undefined saponins [15].

Antioxidant property is considered an important biological activity. Phenolic compounds are well known to possess this activity [16] along with anti-inflammatory, anti-cancer and DNA protective effects [17].

The present study was directed towards validation of ethno-biologically important *Memecylon* species for their bioactive properties viz., antioxidant, anti-microbial, DNA protection, 5-lipoxygenase and human cyclooxygenase-2 (COX-2) inhibition.

### MATERIALS AND METHODS

#### Plants

The whole plants of *Memecylon umbellatum*, *M. talbotianum* and *M. malabaricum* were collected from the Kigga region of the Western Ghats in, Chikmagalore District of Karnataka State. Herbarium specimens have been deposited in the herbarium at the Department of Studies in Biotechnology (*M. umbellatum* # IOE LP0001; *M. talbotianum* # IOE LP0002; *M. malabaricum* # IOE LP0003).

#### Preparation of leaf extract

Fresh leaves were thoroughly washed and dried under shade. They were ground to a coarse powder. Ten grams of ground material was suspended in 50 ml of methanol. The suspension was shaken for 60 min at 25 ±2°C, sonicated for 75 min at room temperature (RT) and filtered. The extraction process was repeated three times. The solvent was evaporated in a SpeedVac (Savant SPD 2010, Thermo Scientific) and the extracts were stored in dark at 4°C.

#### Chemicals and reagents

Linoleic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 15-lipoxygenase (soybean), trypsin, chloramphenicol, 3-(4,5-dimethylthylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Quercetin, ascorbic acid and butylated hydroxyl toluene (BHT) were purchased from HiMedia (Bangalore, India). Human COX-2 inhibition kit was obtained from Cayman, Ann Arbor, MI, USA. pBR322 was obtained from Merck Biosciences (Bangalore, India). All solvents and reagents from various suppliers were of analytical grade.

#### DPPH radical scavenging assay

The traditional DPPH method [18] was modified and used in this study. A 300 µM DPPH solution was prepared in methanol. Methanol extracts of medicinal plants, ascorbic acid or butylated hydroxyl toluene (BHT) and quercetin diluted in methanol were used in the assay at different concentrations. Five microlitres of sample was added to the DPPH solution (95 µL; absorbance of 0.68 ± 0.005 at

517 nm) in a microtiter plate and the mixture was incubated for 30 min in dark at RT. Scavenging of DPPH was recorded as reduction in absorbance at 517 nm using a Spectra Max 340PC Multimode plate reader (Molecular Devices). Antioxidant activity was expressed as IC<sub>50</sub> value.

#### ABTS radical scavenging assay

Antioxidant activity was measured using a UV-Vis spectrophotometer (Beckman Coulter, DU 730 Life Sciences) using the ABTS method [19]. The results were expressed in terms of total antioxidant capacity (TAC). The values are mean  $\pm$  SD of three independent experiments.

#### Estimation of total phenolic content

The total phenolic content of each sample was estimated using the Folin-Ciocalteu colorimetric method [20]. The absorbance of the resulting blue color was measured at 760 nm with UV-Vis spectrophotometer (Beckman Coulter, DU 730 Life Sciences). Quantification was done using standard curve with gallic acid. Results were expressed as gram of Gallic acid equivalents (GAE) per 100 gram of dry weight of sample (g GAE/100g DW). The values are mean  $\pm$  SD of three independent experiments.

#### Anti-microbial activity

##### Test microorganisms and bioassay for anti-microbial activity

All the microbial strains of human pathogens used in the anti-microbial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. These microbes include the gram negative bacteria such as *Escherichia coli* (MTCC 724), *Klebsiella pneumonia* (MTCC 661), *Salmonella typhi* (MTCC 733), *Shigella flexneri* (MTCC 1457); and gram positive bacteria such as *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* (MTCC 441). The agar well-diffusion method [21] was followed to determine the anti-microbial activity. Nutrient agar was used to culture the test microorganisms by spread plate method. Two 10 mm diameter discs were made and placed on these plates. Methanolic extract (10  $\mu$ l of 10 mg/ml) was loaded on 10 mm diameter sterile discs placed on agar medium under sterile conditions. Control experiments consisted of same volume of methanol. The plates were incubated at 37°C for 15-18 h and the diameter of inhibition zones was recorded. The results were compared and analyzed against the same concentrations of chloramphenicol under similar conditions of experiment. Experiment was repeated thrice with three replicates each time and the average values were recorded.

##### Lipoxygenase inhibition assay

A spectrophotometric assay for determination of LOX activity was used as reported [22] with slight modification. Soybean 15-lipoxygenase (15-LOX) was used for the assay. Inhibition experiments were run by measuring the loss of soybean 15-LOX activity (5  $\mu$ g) with 0.2  $\mu$ M linoleic acid (Sigma) as the substrate prepared in solubilized state [22] in 0.2M borate buffer (pH 9.0). Inhibition studies in presence of various concentrations of extracts (10, 25, 50, 100, 200  $\mu$ g/mL) and reference compound *viz.*, quercetin was recorded at 234 nm using UV-Vis spectrophotometer (Beckman Coulter, DU 730 Life Sciences). The inhibitory effect of the extracts was also expressed as percentage of enzyme activity inhibition. IC<sub>50</sub> indicating the concentration required to inhibit 50 % LOX activity was also calculated. Values of hydroperoxide content and lipoxygenase activity were calculated from equation,

$$\text{Specific activity (LOX)} = \Delta A \cdot V / \epsilon \cdot l \cdot c$$

where,  $\Delta A$  is the value of absorbance increase per min,  $V$  is the volume of incubation mixture,  $\epsilon$  is the extinction coefficient for linoleic acid ( $25 \times 10^{-3}$  mol/l/cm) [23],  $l$  is the length of the cuvette (1 cm) and  $c$  is the concentration of enzyme in mg (0.005). The values are mean of three independent experiments.

##### Human cyclooxygenase (COX-2) inhibition

COX-2 inhibition was measured using a colorimetric human COX-2 inhibitor screening assay kit (Cayman Ann Arbor, MI, USA). The methanolic extract (10 and 50  $\mu$ g) was used for inhibition studies as per

manufacturer's protocol. The absorbance at 415 nm was read by using a microtitre plate reader Varioskan Flash with SkanIt Software 2.4.3 RE.

#### DNA protection studies

A DNA nicking assay was performed by using supercoiled pBR 322 plasmid [24]. Controls consisted of plasmid DNA subjected to reaction with Fenton reagent for 30 min under similar conditions. DNA protection effects against Fenton reagent was assessed by incubating methanolic extract (10  $\mu$ g) with plasmid DNA (0.5  $\mu$ g) for 30 min at 37°C and further to Fenton reagent for 30 min. The samples were electrophoresed in 1% agarose gel. The results were documented using XR+ Molecular Imager Gel documentation system (Bio Rad, USA).

#### Statistical analysis

All determinations including antioxidant capacity by DPPH, ABTS<sup>+</sup>, total phenolic content, anti-microbial activity, lipoxygenase inhibition assay, COX-2 and DNA nicking assay were conducted in triplicate. The reported value for each sample was calculated as the mean and standard deviation of three independent experiments.

### RESULTS

#### Antioxidant properties and total phenol content

The methanolic extracts of *Memecylon* spp. could scavenge free radicals in a concentration-dependent manner. The results were expressed as IC<sub>50</sub> value indicating the concentration of the methanolic extract required to scavenge 50 % of DPPH and ABTS<sup>+</sup>.

In DPPH assay *M. malabaricum* extract was most potent exhibiting IC<sub>50</sub> of 0.11  $\pm$  0.5 mg/mL followed by *M. umbellatum* with IC<sub>50</sub> of 0.14  $\pm$  0.61 mg/mL. The IC<sub>50</sub> for TAC was 0.17  $\pm$  0.4 mg/mL for *M. talbotianum*. The TAC of reference standards quercetin, butylated hydroxyl toluene (BHT) and ascorbic acid under similar conditions of the experiment were analyzed. The IC<sub>50</sub> values for scavenging DPPH radical for quercetin, BHT and ascorbic acid were 5.2  $\pm$  1.24  $\mu$ g/mL, 13.34  $\pm$  1.21  $\mu$ g/mL and 30.32  $\pm$  0.36  $\mu$ g/mL respectively (**Table 1**).

Antioxidant activity measured by ABTS<sup>+</sup> method indicated that methanolic extract of *M. malabaricum* had IC<sub>50</sub> of 2.1  $\pm$  0.21 mg/mL. *M. umbellatum* and *M. talbotianum* leaves exhibited TAC with IC<sub>50</sub> of 2.3  $\pm$  0.22 mg/mL and IC<sub>50</sub> of 3.7  $\pm$  1.13 mg/mL respectively. The IC<sub>50</sub> values for TAC with respect to the reference compounds quercetin was 6.13  $\pm$  0.66  $\mu$ g/mL, ascorbic acid was IC<sub>50</sub> of 76.4  $\pm$  0.13  $\mu$ g/mL and BHT exhibited IC<sub>50</sub> of 19.2  $\pm$  1.01  $\mu$ g/mL capacity to scavenge ABTS<sup>+</sup> (**Table 1**).

The amount of total phenolic content was estimated by Folin-Ciocalteu spectrophotometric method and reported as gallic acid equivalent/100 g dry weight (g GAE/100g DW). Among the leaf extracts, *M. talbotianum* leaves had a higher total phenolic content of 1.12  $\pm$  0.18 g GAE/100g DW. The leaves of *M. umbellatum* had 1.08  $\pm$  0.16 g GAE/100g DW of phenolic content. The *M. malabaricum* leaves had 1.96  $\pm$  0.49 g GAE/100g DW of phenolic content (**Table 1**).

#### Anti-microbial activity

In the present study all the extracts exhibited antimicrobial activity to various extents. The most effective extract tested was of *M. malabaricum* exhibiting clear effects on *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli* and had moderate effects on *Bacillus subtilis*. *M. umbellatum* leaves could slightly inhibit growth of *K. pneumonia*, *E. coli*, *S. typhi* and *B. subtilis*. *M. talbotianum* leaf extract showed clear and moderate anti-microbial activity against *E. coli* and *K. pneumonia* (**Table 2**).

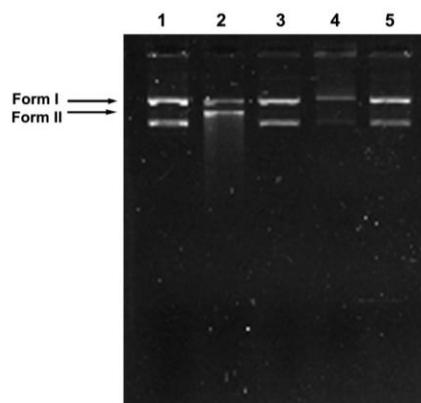
#### 15-lipoxygenase and human COX-2 inhibition activity

The LOX activity was monitored as an increase in the absorbance at 234 nm indicating the formation of hydroperoxylinoleic acid. All the *Memecylon* spp. leaf extract tested inhibited LOX in a concentration dependent manner. The highest inhibitory effect was observed for *M. malabaricum* with an IC<sub>50</sub> of 29.87  $\mu$ g/mL (**Table 3**). The *M. umbellatum* extract inhibited LOX with IC<sub>50</sub> of 39  $\mu$ g/mL. Amongst the three extracts *M. talbotianum* leaf extract

inhibited 96% of LOX activity at 200  $\mu\text{g}/\text{mL}$  and at a relatively higher  $\text{IC}_{50}$  value of 54.6  $\mu\text{g}/\text{mL}$  (Table 3). Complete LOX inhibition was observed at a concentration of 250  $\mu\text{g}$ . Human COX-2 inhibition revealed a dose dependent pattern with *M. malabaricum* inhibiting 80.58% at 50  $\mu\text{g}$  of extract. At the same concentration *M. umbellatum* and *M. talbotianum* inhibited 43.8% and 43% respectively (Table 4).

#### DNA protection studies

In this assay pBR322 plasmid DNA was exposed to Fenton reaction for 30 min at 37°C. It caused a change in native double stranded DNA band (Form I) to single-stranded, nicked plasmid DNA (Form II) initiating a differential pattern in agarose gel (Fig.1). Upon incubation of the plasmid DNA with 25  $\mu\text{g}$  of methanolic extract for 30 min at 37°C and further exposure to Fenton reagent under similar conditions as above, the scission of plasmid DNA was reduced or absent (Fig.1). Thus methanolic extracts had the capacity to scavenge the  $\cdot\text{OH}$  radicals produced by Fenton reagent protecting the pBR322 plasmid DNA. Of the different *Memecylon* spp. leaf extracts of *M. umbellatum* and *M. malabaricum* could effectively scavenge the potent free radicals. An absence or reduction in Form II and an increased Form I recorded indicated a notable protection offered by these test samples of medicinal plants.



#### DISCUSSION

Traditional knowledge of medicinal plants has clues to unknown potential novel drug molecules in the form of secondary metabolites [25]. Medicinal plant parts are rich in phenolic compounds, such as flavonoids, phenols (alkaloids, amines, betalains), terpenoids (eg, carotenoids) stilbenes, tannins, coumarins, lignans and lignins [20, 26]. Many secondary metabolites are used in pharmaceutical and food industry for their capacity to retard oxidative degradation of lipids, quench free radicals, chelate metals and improve nutritional quality of processed food [27].

Free radical(s) are oxygen-centered with at least one unpaired electron and are end products of several physiological and biochemical processes. They attack and damage cell membrane, cell organelles and DNA resulting in faulty translation of genetic information. This is implicated in contributing to cancer, atherosclerosis, aging, immunosuppression, inflammation, diabetes, neurodegenerative disorder etc.

Many plant species show antioxidant properties [28, 29]. The antioxidant capacities of the plant extracts largely depend on the composition of the extracts. DPPH, scavenging gave reliable information concerning the antioxidant ability of the tested compounds. ABTS<sup>+</sup> scavenging is also a recommended method for detecting the antioxidant potential for plant extracts as measurement at 734nm (long wavelength) eliminates colour interference by plant extracts<sup>20</sup>. The antioxidant capacity for the methanolic extract of *Memecylon* spp. reported as  $\text{IC}_{50}$  values for scavenging DPPH ranged from 0.11 to 0.17 mg/ml and for ABTS<sup>+</sup> the  $\text{IC}_{50}$  values ranged from 2.1 to 3.7 mg/ml. The methanolic extract of *M. malabaricum* leaves recorded the lowest  $\text{IC}_{50}$  of  $0.11 \pm 0.5$  and  $2.1 \pm 0.21$  mg/ml for scavenging DPPH and ABTS<sup>+</sup> respectively (Table 1). The potential of medicinal plants

antioxidant activity could be credited to the phenols in them. There is a large diversity of phenolic compounds in medicinal plants with antioxidant activity which may contribute to anti-cancer, anti-bacterial, anti-viral or anti-inflammatory activities to a greater extent [17, 29]. It is hypothesized that biological activity depends on the number and position of certain hydroxyl groups and other substituents in these molecules [30]. Total phenolic content of *Memecylon* spp. estimated in the present study could explain the antioxidant potential of the plant extracts. Studies have related plants richer in minerals and phenolic compounds with potent radical scavenging activity [31].

In the present study all the extracts exhibited antimicrobial activity to various extents (Table 2). It could be attributed to the presence of secondary metabolites as suggested in previous reports [32]. In a recent study the *M. umbellatum* leaves inhibited Gram (-) bacteria [9]. Similarly, medicinal plants used in treatments have been tested for antimicrobial activity [33, 34, 35]. Essential oil from *Tanacetum polycephalum* traditionally used in treatment of arthritis and psoriasis, also exhibited bactericidal activity against pathogenic bacteria [33]. Stem bark extracts from *Alafia multiflora* traditionally applied on wounds and leg ulcers exhibited good anti-bacterial activity [34]. Lipoxygenases (LOXs) (LOX; EC 1.13.11.12) are a family of non-heme iron-containing dioxygenases catalyzing the biosynthesis of leukotrienes. Leukotrienes function as initiators of inflammation and their inhibition is considered to be partly responsible for the anti-inflammatory activity [36]. In the present study methanolic extracts *M. malabaricum* showed good anti-LOX activity with an  $\text{IC}_{50}$  value of 29.87  $\mu\text{g}/\text{ml}$  in comparison to *M. umbellatum* and *M. talbotianum* (Table 3). LOX inhibition was used to evaluate anti-inflammatory activity of a few medicinal plants used in Limousin country. *Filipendula ulmaria* (Meadowsweet) recorded LOX inhibition with  $\text{IC}_{50}$  of 60  $\mu\text{g}/\text{ml}$  and *Urtica dioica* (Nettle) methanolic extract inhibited LOX with  $\text{IC}_{50}$  of 348  $\mu\text{g}/\text{ml}$  [37]. In another study, eight methanolic extract out of 18 undomesticated plants of South Africa showed significant inhibition of 5-lipoxygenase (5-LOX) activity. *Bidens pilosa* extract exhibited  $\text{IC}_{50}$  of 21.8  $\mu\text{g}/\text{ml}$  and *Emex australis* extract recorded  $\text{IC}_{50}$  of 81.4  $\mu\text{g}/\text{ml}$  for LOX inhibition [38].

LOXs are sensitive to antioxidants as antioxidants are involved in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxo- or lipidperoxy-radicals. This could lead to less availability of lipid hydroperoxide substrate required for LOX catalysis [23]. Another hypothesis proposed indicated that inhibition by antioxidant could be attained via chelation of its non-heme bound iron [39] or by reduction of its ferric form [40], suggesting a competitive kind of inhibition as reported for *Mahonia aquifolium* [23]. We would like to speculate that LOX inhibition could be due to antioxidant properties of the methanolic extract with the mechanism of action to be elucidated. Previous studies suggested that compounds with antioxidant or anti-inflammatory activities inhibit tumor promotion and cell proliferation [41]. DNA involved in replication and transcription represents the most targeted macromolecule. Hydroxyl radicals ( $\cdot\text{OH}$ ) are produced in mammalian cells, catalyzed by transition metals (e.g., Fe[II] ions generated via reduction of Fe[III] by  $\text{O}_2^-$  in the presence of  $\text{H}_2\text{O}_2$  via the Fenton reaction). DNA strand breaks can occur when the deoxyribose chain interacts with  $\cdot\text{OH}$  radicals [42]. Most anti-cancer agents are believed to act mainly by quenching the free radicals or by direct interaction with DNA [17]. In the present study the preincubation of the circular plasmid DNA with 25  $\mu\text{g}$  of methanolic extract of *Memecylon* spp. protected the DNA from nicking (Fig. 1). Protection offered could be mediated through ability of extracts to quench the  $\cdot\text{OH}$  radicals produced by Fenton reaction.

*Memecylon* spp. has been used to treat skin problems with strong anti-inflammatory and analgesic activity [43]. In the present study, all the three species of *Memecylon* leaves exhibited antioxidant activities credited to the presence of high phenolic content in the samples. Earlier studies on *Memecylon* have reported a broad spectrum anti-microbial activity for the methanolic extracts [9, 14]. The present work confirms the same against human bacterial pathogens. Among the different species of *Memecylon* screened, *M. malabaricum* leaves exhibited potent LOX and human COX-2

inhibition capacity *in vitro* thereby inhibiting PGE<sub>2</sub> production. As inhibition of these inflammatory mediators is considered in part treatment for inflammation [35] this study partially confirms traditionally reported anti-inflammatory capacity of *Memecylon* spp. Reports on "dual inhibitors" inhibiting LOX and COX-2 for effective

management of metabolic processes underlying osteoarthritis with a balanced arachidonic acid metabolism in the body has been highlighted [44-48]. The leaf extracts scavenged OH radicals produced during Fenton reaction as assessed for their capacity to protect DNA.

**Table 1: Antioxidant activity and total phenolic content of *Memecylon* extracts**

Medicinal plant	Total phenolic content <sup>a</sup>	Total antioxidant capacity (%TAC) <sup>b</sup>	
		DPPH (IC <sub>50</sub> )	ABTS (IC <sub>50</sub> )
Ascorbic acid		30.32 ± 0.36 µg/ml	76.4 ± 0.13 µg/ml
Quercetin		5.2 ± 1.24 µg/ml	6.13 ± 0.66 µg/ml
BHT		13.34 ± 1.21 µg/ml	19.2 ± 1.01 µg/ml
<i>Memecylon umbellatum</i>	1.08 ± 0.16	0.14 ± 0.61 mg/ml	2.3 ± 0.22 mg/ml
<i>M. talbotianum</i>	1.12 ± 0.18	0.17 ± 0.4 mg/ml	3.7 ± 1.13 mg/ml
<i>M. malabaricum</i>	1.96 ± 0.49	0.11 ± 0.5 mg/ml	2.1 ± 0.21 mg/ml

<sup>a</sup> Total phenolic content is expressed in grams (g) gallic acid equivalent (GAE)/100g dry weight (DW)

<sup>b</sup> Each result is expressed as mean ± S.D. (n = 3)

**Table 2: Anti-microbial activity of *Memecylon* extracts on test microorganisms\***

Plant extract	<i>Klebsiella pneumonia</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Shigella flexneri</i>	<i>Bacillus subtilis</i>
Standard antibiotic	+++ <sup>e</sup>	+++ <sup>e</sup>	+++ <sup>e</sup>	+++ <sup>e</sup>	+++ <sup>e</sup>	+++ <sup>e</sup>
Chloamphenicol						
Medicinal plant						
<i>Memecylon umbellatum</i>	~ <sup>b</sup>	~ <sup>a</sup>	~ <sup>b</sup>	~ <sup>b</sup>	~ <sup>a</sup>	~ <sup>b</sup>
<i>M. talbotianum</i>	+ <sup>c</sup>	~ <sup>b</sup>	++ <sup>d</sup>	~ <sup>b</sup>	~ <sup>a</sup>	+ <sup>c</sup>
<i>M. malabaricum</i>	++ <sup>d</sup>	++ <sup>d</sup>	++ <sup>d</sup>	~ <sup>b</sup>	~ <sup>a</sup>	+ <sup>c</sup>

a = -, no anti-microbial capacity; b = ~ slight anti-microbial capacity, 1-3mm inhibition zone; c = +, moderate anti-microbial capacity, 3-4mm inhibition zone; d = ++, clear anti-microbial capacity, 4-10mm inhibition zone; e = +++; clear anti-microbial activity >10mm inhibition zone.

\* The results are representative values of three independent experiments (n=3).

**Table 3: Inhibition lipoxygenase activity by *Memecylon* extracts**

Medicinal plant	Final concentration of methanolic extract (µg)	Lipoxygenase specific activity x 10 <sup>-1</sup> (U/mg LOX)	LOX inhibition (%)	IC <sub>50</sub> (µg/ml)
Lox activity (control)	-	2.3	-	-
<i>Memecylon umbellatum</i>	10	1.72	25.06	39.19 <sup>b</sup>
	25	1.47	35.81	
	50	0.89	61.46	
	100	0.12	94.86	
	200	ND	100	
<i>M. talbotianum</i>	10	2.13	8.56	54.6 <sup>c</sup>
	25	1.62	18.97	
	50	1.33	42.12	
	100	0.56	75.58	
	200	0.32	96.82	
	250	ND	100	
<i>M. malabaricum</i>	10	1.78	22.6	29.87 <sup>a</sup>
	25	1.35	41.26	
	50	0.15	84.91	
	100	ND	100	
	200	ND	100	

Each result is expressed as mean (n = 3); ND- not detected. \*Means in the same column followed by a different letter are significantly different (p < 0.05) according to analysis of variance

**Table 4: Inhibition of human cyclooxygenase (COX)-2 activity by *Memecylon* extracts**

Medicinal plant	Final concentration of methanolic extract (µg/ml)	COX-2 inhibition (%) <sup>*</sup>
<i>Memecylon umbellatum</i>	10	21.16 <sup>a</sup>
	50	43.8 <sup>b</sup>
<i>M. talbotianum</i>	10	23.79 <sup>a</sup>
	50	43 <sup>b</sup>
<i>M. malabaricum</i>	10	35.96 <sup>c</sup>
	50	80.58 <sup>d</sup>

Each result is expressed as mean (n = 3). \*Means in the same column followed by the same letter are not significantly different (p < 0.05) according to analysis of variance.

## CONCLUSION

Effective antioxidant activities for the *Memecylon* spp. reported in the study were observed by assessing their capacity to quench DPPH and ABTS<sup>+</sup> radicals. These activities could be possible due to the presence of phenolic compounds. Also observed was inhibition of 15-LOX and human COX-2 activities. *M. umbellatum* and *M. malabaricum* could scavenge hydroxyl radicals and thus protect DNA. However, further investigations are required to determine the bioactivities in these test samples of medicinal plants.

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## REFERENCES

- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. *Nature* 2000;403:853-858.
- Gadgil M. Western ghats: A lifescape. *J Indian Inst Sci* 1996;76:495-504.
- Henry AN, Chithra V, Balakrishnan NP. Flora of Tamil Nadu. 1989. Series 1. Vol. 3. Botanical Survey of India, Coimbatore, India.
- Anonymous. The Wealth of India Publication and Information Directorate, CSIR, Hillside, New Delhi, India. 1998.
- Puratchikody A, Nagalakshmi G. Wound healing activity of *Memecylon umbellatum* Burm. *J Plant Sci* 2007;292:179-186.
- Kshirsagar RD, Singh NP. Some less known ethno-medicinal uses from Mysore and Coorg districts, Karnataka state, India. *J Ethnopharmacol* 2001;75:231-238.
- Karuppawamy S. Medicinal plants used by Paliyan tribes of Sirumalai hills of Southern India. *Indian Nat Prod Rad* 2007;6:436-442.
- Amalraj T, Ignacimuthu S. Evaluation of the hypoglycemic effect of *Memecylon umbellatum* in normal and alloxan diabetic mice. *J Ethnopharmacol* 1998;62:247-250.
- Murugesan S, Pannerselvam A, Tangavelou AC. Phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* Burm. *J Appl Pharm Sci* 2011;1:42-45.
- Rajakumar N, Shivanna MB. Ethno-medicinal application of plants in the Eastern region of Shimoga District, Karnataka, India. *J Ethnopharmacol* 2009;126:64-73.
- Killedar SG, More HN. Estimation of tannins in different parts of *Memecylon umbellatum* Burm. *J Pharm Res* 2010;3:554-556.
- Viswanaathan MB, Lakshmanan KK. *Memecylon talbotianum* Brandis (Melastomataceae): A new record for the Eastern Ghats of Peninsular India. *Indian J For* 1990;13:174-175.
- Prakasha HM, Krishnappa M, Krishnamurthy YL, Poornima SV. Folk medicine of NR Pura taluk in Chikmagalur district of Karnataka. *Indian J Traditional Knowl* 2010;9:55-60.
- Hullatti KK, Rai VR. Antimicrobial activity of *Memecylon malabaricum* leaves. *Fitoterapia* 2004;75:409-411.
- Buchmann SL, Buchmann MD. Anthecology of *Mouriri myrtilloides* (Melastomataceae: Memecyleae), an Oil Flower in Panama. *Biotropica* 1981;13:7-24.
- Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med* 1996;20:933-956.
- Lopaczynski W, Zeisel SH. Antioxidant, programmed cell death and cancer. *Nutr Res* 2001;21:295-307.
- Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wiss Technol* 1995;28:25-30.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol Med* 1999;26:1231-1237.
- Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 Chinese medicinal plants associated with anticancer. *Life Sci* 2004;74:2157-2184.
- Perez C, Pauli A, Bazerque P. Antibiotic assay by agar-well diffusion method. *Acta Biol Med Exp* 1990;15:113-115.
- Kemal C, Louis-Flamberg P, Krupinsky-Olsen R, Shorter AL. Reductive inactivation of soybean lipoxygenase I by catechols: A possible mechanism for regulation of lipoxygenase activity. *Biochemistry* 1987;26:7064-7072.
- Rackova L, Oblozinsky M, Kostalova D, Kettmann V, Bezakova L. Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *J Inflammation* 2007;4:15-21.
- Lee JC, Kim HR, Kim J, Jang YS. Antioxidant activity of ethanol extract of the stem of *Opuntia ficus-indica* var. saboten. *J Agric Food Chem* 2002;50:1619-1624.
- Buenz EJ, Schnepfle DJ, Bauer BA, Elkin PL, Riddle JM, Motley TJ. Techniques: Bioprospecting historical herbal texts by hunting for new leads in old tomes. *Trends Pharmacol Sci* 2004;25:494-498.
- Surveswaran S, Cai YZ, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chem* 2007;102:938-953.
- Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem* 2007;105:940-949.
- Ansari JA, Inamdar NN. The Promise of Traditional Medicines. *Int J Pharmacol* 2010;6:808-812.
- Tapiero H, Tew KD, Ba GN, Mathe G. Polyphenols: do they play a role in the prevention of human pathologies? *Biomed Pharmacother* 2002;56:200-207.
- Cai YZ, Sun M, Xing J, Luo Q, Corke H. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sci* 2006;78:2872-2888.
- Seal T. Determination of nutritive value, mineral contents and antioxidant activity of some wild edible plants from Meghalaya State, India. *Asian J Appl Sci* 2011;4:238-246.
- Kosalec I, Pepeljnjak S, Bakmaz M, Vladimir-Knezevic S. Flavonoid analysis and antimicrobial activity of commercially available propolis products. *Acta Phar* 2005;55:423-430.
- Amiri H. Chemical composition, antibacterial and antioxidant activity of the essential oil of *Tanacetum polycephalum* Schütz. *Bip Int J Bot* 2007;3:321-324.
- Ikegbunam NM, Okpata OO, Ugwu MC and Esimone CO. Authentication of the antimicrobial activity of some indigenous herbal remedies used in the treatment of typhoid and urinary tract infections in Anambra State, Nigeria. *Int J Pharm Pharm Sci* 2013;5:73-75.
- Anago E, Lagnika L, Gbenou J, Loko F, Moudachirou M, Sanni A. Antibacterial activity and phytochemical study of six medicinal plants used in Benin. *Pak J Biol Sci* 2011;14:449-455.
- Ammon HPT, Annazodo MI, Safayhi H, Dhawan BN, Scrima R. Curcumin: a potent inhibitor of leukotriene B4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL). *Planta Med* 1992;58:226.
- Trouillas P, Calliste CA, Allais DP, Simon A, Marfak A, Delage C, Duroux JL. Antioxidant, anti-inflammatory and anti-proliferative properties of sixteen water plant extracts used in Limousin countryside as herbal teas. *Food Chem* 2003;80:399-407.
- Akula US, Odhav B. *In vitro* 5-Lipoxygenase inhibition of polyphenolic antioxidants from undomesticated plants of South Africa. *J Med Plant Res* 2008;2:207-212.
- Lin JK, Tsai SH, Lin-Shiau SY. Anti-inflammatory and antitumor effects of flavonoids and flavanoids. *Drugs Future* 2001;26:145-157.
- Gutierrez-Lugo MT, Deschamps JD, Holman TR, Suarez E, Timmermann BN. Lipoxygenase inhibition by anadanthoflavone, a new flavonoid from the aerial parts of *Anadenanthera columbrina*. *Planta Med* 2004;70:263-265.

41. Simon A, Allais DP, Duroux JL, Basly JP, Durand-Fontanier S, Delage C. Inhibitory effect of curcuminoids on MCF-7 cell proliferation and structure-activity relationships. *Cancer Lett* 1998;129:111-116.
42. Henle ES, Roots R, Holley WR, Chatterjee A. DNA strand breakage is correlated with unaltered base release after gamma irradiation. *Radiat Res* 1995;143:144-150.
43. Nualkaew S, Rattanamanee K, Thongpraditchote S, Wongkrajang Y, Nahrstedt A. Anti-inflammatory, analgesic and wound healing activities of the leaves of *Memecylon edule* Roxb. *J Ethnopharmacol* 2009;121:278-281.
44. Celloti F, Durand T. The metabolic effects of inhibitors of 5-lipoxygenase and of cyclooxygenase 1 and 2 are an advancement in the efficacy and safety of anti-inflammatory therapy. *Prostaglandins Other Lipid Mediat* 2003;71:147-62.
45. Martel-Pelletier J, Lajeunesse D, Reboul P, Pelletier JP. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. *Ann Rheum Dis* 2003;62:501-9.
46. Shelly MM, Hawkey CJ. Cox-Lox inhibition: Current evidence for an emerging new therapy. *Int J Clin Pract* 2003;57:301-4.
47. Brune K. Safety of anti-inflammatory treatments-new ways of thinking. *Rheumatology (Oxf)* 2004;43:116-20.
48. D'Mello S, Gadhwal MK, Joshi U and Shetgiri P. Modelling of COX-2 inhibitory activity of flavonoids. *Int J Pharm Pharm. Sci* 2011; 3:33-40.