In vitro antioxidant and anti-inflammatory properties of different solvent extracts of Memecylon talbotianum Brandis

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Abstract
Memecylon talbotianum Brandis is an important ethnomedicinal plant. The leaves of this plant were extracted with different solvents and evaluated for antioxidant and anti-inflammatory properties. Methanol extract exhibited with greater reactive oxygen species scavenging activity, DPPH (IC_{50} 4.25 mg / ml), ABTS (IC_{50} 1.28 mg / ml), SRSA (IC_{50} 3.33 mg / ml) and reducing power (IC_{50} 5 mg / ml) properties reported. Its capacity to inhibit xanthine oxidase (IC_{50} 12.65 mg / ml) and 15-lipoxygenase (IC_{50} 1 mg / ml), inflammatory mediators is reported.

Keywords: M. talbotianum, DPPH, antioxidant, XO, anti-inflammatory

Plants have been used as a source of traditional medicines from time immemorial to combat ailments and human sufferings. Due to its long period of practice traditional medicine has become an integral part of the culture of people. Abebe noted that more than 95% of traditional preparation of medicines is of plant origin. Evidently, traditional knowledge of medicinal plants is important in the development of new drugs. About 80% of the drugs discovered are based on information derived from ethnomedical investigation. Memecylon talbotianum Brandis., is a small tree belong to the family Melastomataceae commonly known as Alimmar or Kancan is mainly distributed in deciduous to evergreen Western Ghat forests. This plant is used as a folk medicine. Biological activity such as antioxidant, lipoxygenase, cyclooxygenase and antimicrobial activities have been reported only for methanolic extract of M. talbotianum. However various pharmacological activities like antidiabetic, antiviral, antispasmodic, anti-inflammatory, antimicrobial, antioxidant, antihelminthic, hepatoprotective activity, anti-insect activity, antispasmodic, nephroprotective, anti-pyretic activity have been reported for M. umbellatum. Analoges, antihyperlipidemic, anti-tumour, anti-ulcer, anti-mutagenic, anti-quotation, anti-choloretic, anti-carcinogenic, anti-arthritis and anti-fertility activities have also been reported in M. umbellatum and M. malabaricum. In the present study in vitro antioxidant and anti-inflammatory have been carried out to check the bioactive potential of different solvent extracts. The antioxidant properties were determined by superoxide scavenging activity and as reducing power assay, anti-inflammatory activity were determined based on lipoxygenase (LOX), xanthine oxidase (XO) the results were compared with standards like ascorbic acid, Gallic acid for antioxidant, quercetin for anti-inflammatory activity. The methanol, ethyl acetate and water extracts showed promising antioxidant, anti-inflammatory properties.

2. Materials and Methods
2.1 Plant sample collection
The Memecylon talbotianum plants were collected from Sringeri region in Western Ghats of Karnataka State, India during April 2012 and dried in shade. The plant was identified by plant taxonomist. The leaves were separated from the main plant and dried under shadow. Dried leaves were milled in a blender, and stored in the refrigerator at 4 °C.

2.2 Preparation of extracts
The dry leaf powder (250g) was sequentially extracted using different solvents of increasing polarity. Hexane, Ethyl acetate, Methanol and Water in Soxhlet apparatus (boiling point 55 to 64 °C). Resultant extracts were concentrated in a flash evaporator under reduced pressure.

2.3 Chemicals and Media
DPPH (1, 1 –Diphenyl-2-picryl hydrazyl), ferric chloride, Gallic acid, Ascorbic acid, Quercetin, potassium ferricyanide, trichloroacetic acid, nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), lipoxidase, lipoxygenase, linolic acid, xanthine oxidase, xanthine disodium salt and standard antibiotics were purchased from Sigma-Aldrich Chemicals (Bangalore, India). All other solvents and chemicals were of analytical grade and obtained from Merck, Bangalore India. The absorbance was measured by the Spectra Max 340PC Multimode plate reader (Molecular Devices).

2.4. Antioxidant assays
2.4.1 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical scavenging activity
The DPPH free radical scavenging property of the different solvent extracts of Memecylon talbotianum was determined by the method described by Sultanova. The DPPH (300 µM) solution was prepared in methanol containing 100 µl reaction mixture containing 5 µl of plant extract and 95 µl of DPPH solution. The radical scavenging reaction was carried out at 37 °C in dark for 30 min and the absorbance was recorded at 517 nm. IC_{50} values were evaluated which indicates the concentration of extracts required to scavenge 50% free radicals. Positive control used was ascorbic acid. Scavenging effect was determined by the following formula:
DPPH inhibition (%) = ([A control – A sample]/A control) X100.
2.4.2 2, 2 - Azino - bis (3-ethylbenzothiazoline-6-Sulfonic acid) diammonium salt. (ABTS) radical scavenging activity

The antioxidant activity measured using the ABTS method described by Roberta et al. The ABTS radical solution was prepared by 2.45 mM potassium persulfate and 7 mM ABTS in dark for 12 h on incubating the mixture at room temperature. The prepared ABTS solution was diluted again with methanol to get an absorbance of 0.700 ± 0.005 at 734 nm. Different concentrations of test samples (50 μl) were added to 2 ml of working 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.

The percent inhibition was calculated from the following formula:

\[ \% \text{ inhibition} = \left( \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} \right) \times 100 \]

2.4.3 Superoxide radical scavenging assay (SRSA)

The superoxide scavenging capacity of the plant extract was determined by the method of Chen et al. According to this method different concentrations of extract (100-1000μg/ml) were prepared. Different concentrations of extracts were taken and the total volume made up to 400μl using phosphate buffer (0.1M pH 7.4) of different dilutions containing 1ml of PBS (60μM), 1ml of NADH (677μM) and 100μl of NBT (144μM) in phosphate buffer (0.1M PH 7.4). The whole reaction was incubated at room temperature for 5 min including blank. The color intensity was read using the spectrophotometer at 560nm. Percentage SRSA was determined using the following formula:

\[ \% \text{ SRSA} = \left( 1 - \frac{\text{Absorbance sample}}{\text{Absorbance control}} \right) \times 100 \]

2.4.4 Reducing power assay

The power reducing assay of different extracts of M. talbotianum was evaluated according to the method described by Chen et al. Different concentrations (20 to 100μg/ml) of the sample (1ml) was mixed with PBS buffer (pH 7.4, 0.02M), 1.0ml potassium ferricyanide (1.0%, w/v) and the reaction was incubated at 50°C for 20min. Then the reaction was terminated by adding 1.0 ml of tri chloro acetic acid (10.0%, w/v) again the solution was mixed with 0.4ml ferric chloride (0.1%, w/v) for 10min. Different concentrations of ascorbic acid (20 to 100μg/ml) was used as a positive control. The increased absorbance of the reaction mixture indicated increased reducing power by reading the absorbance at 700nm.

2.5 Anti-inflammatory assays

2.5.1 Lipoxygenase inhibition assay

Lipoxygenase (LOX) inhibition activity of different concentrations of Memecylon talbotianum was measured with linoleic acid as a substrate as described by Chen et al. The activity was measured in borate buffer (0.2 M, pH 9) at 234 nm after addition of lipoxygenase enzyme using linoleic acid (125 μM) as the substrate. The final enzyme concentration was 200 U/ml and different concentrations of plant extracts were added after dissolving it in DMSO solution. The enzyme solution was stored on ice and the enzyme activity was measured at regular intervals throughout the experimental period to ensure that enzyme activity was constant. The IC_{50} values were determined by linear interpolation by measuring points closest to 50% activity. The percent inhibition of LOX activity was calculated as follows:

\[ \% \text{ Inhibition} = \frac{[\text{Absorbance without plant extract} - \text{Absorbance with plant extract}]}{\text{Absorbance with plant extract}} \times 100 \]

2.5.2 Xanthine Oxidase Inhibition assay

Xanthine oxidase inhibitory activity was estimated by measuring the formation of uric acid from xanthine. The plant extracts of 1mg/ml was prepared in 50mM phosphate buffer (pH 7.0). The reaction was started by adding 2 μl of the sample containing xanthine oxidase (300 U/ml and different concentrations of plant extracts were added at 4°C in dark. The final enzyme concentration was 200 U/ml and different concentrations of plant extracts were added at 4°C in dark. The final enzyme concentration was 200 U/ml and different concentrations of plant extracts were added at 4°C in dark. The final enzyme concentration was 200 U/ml and different concentrations of plant extracts were added at 4°C in dark. The final enzyme concentration was 200 U/ml and different concentrations of plant extracts were added at 4°C in dark. The final enzyme concentration was 200 U/ml and different concentrations of plant extracts were added at 4°C in dark. The final enzyme concentration was 200 U/ml and different concentrations of plant extracts were added at 4°C in dark. The final enzyme concentration was 200 U/ml and different concentrations of plant extracts were added at 4°C in dark. The final enzyme concentration was 200

The percent inhibition of xanthine oxidase activity was calculated by the equation: (A_{control} - A_{sample}) / A_{control} x 100%.

2.6 Statistical analysis

All experiments were carried out in triplicate. The results of each sample were calculated as the mean and standard error of three independent experiments.

3. Results

3.1 Preparation of extracts

The current study was aimed at determining the bioactive potential of Memecylon talbotianum. The leaves were subjected to Soxhlet extraction by solvents with increasing polarity with quantitative yield, hexane (15.37 g); ethyl acetate (34.2 g); methanol (78.23 g) and water (12.32 g) from 250 g of leaves.

3.2 1, 1 − Diphenyl - 2-Picryl Hydrazyl (DPPH) radical scavenging activity

Different solvent activities of M. talbotianum leaves decreased the DPPH radical scavenging ability of the different solvent extracts of M. talbotianum leaves (Fig 1). The IC_{50} values in DPPH assays were found to be 4.25 mg/ml for methanol, 7.1 mg/ml for water extracts as ascorbic acid equivalent. Hexane and water extracts did not show any DPPH scavenging ability.

Fig 1: 1, 1 − diphenyl - 2-picryl hydrazyl (DPPH) radical scavenging activity of different solvent extracts of M. talbotianum leaf in comparison with standard ascorbic acid.

*significantly different from all the concentrations tested;  
*concentration with significant radical scavenging capacity when compared to different solvent extracts.
3.3 2, 2 - Azino - bis (3-Ethyl Benzothiazoline-6-Sulfonic acid) Diammonium salt (ABTS) free radical scavenging activity

The antioxidant activity determined by ABTS method indicated that the IC50 values of 1.28 mg/ml for methanol, 4.35 mg/ml for ethyl acetate and 7.14 mg/ml for water extracts and was reported as gallic acid equivalence (Fig 2). Hexane extract did not show any scavenging activity.

Fig 2: ABTS free radical scavenging activity of different solvent extracts of M. talbotianum leaf in comparison with standard Gallic acid

3.4 Superoxide radical scavenging assay (SRSA)

The percentage superoxide radical scavenging activity for various extracts in the present study identified IC50 values of 3.33 mg/ml for methanol. Both ethyl acetate and water extracts recorded 10 mg/ml concentration required for scavenging these harmful radicals (Fig 3). The scavenging activity is increased with increasing amount of methanol extract of the leaves of the M. talbotianum.

Fig 3: Superoxide radical scavenging activity (SRSA) of different solvent extracts of M. talbotianum leaf in comparison with standard Gallic acid

3.5 Reducing power assay

All the concentrations of methanol and ethyl acetate extracts of M. talbotianum showed significantly higher reducing power activities when compared to standard ascorbic acid (Fig 4). An IC50 of 5 mg/ml was identified as the required concentration to quench the ferric radicals in the reaction mixture. Other solvent extracts exhibited marginal ferric radical scavenging capacity.

Fig 4: Reducing power activity of different solvent extracts of M. talbotianum leaf with respect to standard ascorbic acid
3.6 Lipoxygenase inhibition assay

Different solvent extracts of *M. talbotianum* leaves inhibited LOX in a dose dependent manner (Fig 5). The maximum inhibitory potential was identified in methanol (IC\(_{50}\), 1 mg/ml) and ethyl acetate extracts (IC\(_{50}\), 1.36 mg/ml).

Fig 5: Lipoxygenase inhibition activity by different solvent extracts of *M. talbotianum* leaf in comparison with standard quercetin

![Lipoxygenase inhibition assay](image)

* significantly different from all the concentrations tested;
* concentration with significant radical scavenging capacity.

3.7 Xanthine Oxidase Inhibition Assay

In the present study, *M. talbotianum* solvent extracts showed concentration-dependent inhibition of xanthine oxidase (Fig 6) with the highest inhibitory effect by methanol extract (IC\(_{50}\), 12.65 µg/ml) followed by ethyl acetate (IC\(_{50}\), 15.87 µg/ml) and water extracts (IC\(_{50}\), 24.19 µg/ml).

Fig 6: Xanthine Oxidase Inhibition Assay for different solvent extracts of *M. talbotianum* leaf in comparison with standard quercetin

![Xanthine Oxidase Inhibition Assay](image)

* significantly different from all the concentrations tested;
* concentration with significant radical scavenging capacity.

4. Discussion

Ethnobotanical information provides vital clues as sources of new drugs or active compounds for various critical ailments. *M. talbotianum* had not attracted much attention of researchers. In this study, we have used the extracts of this plant to confirm its usefulness in the traditional medicines. Several artificial drugs defend against oxidative damage, but they have adverse side effects to combat these effects it is safe to consume natural antioxidants from plant origin and traditional medicines. Free radicals play a major role in biological damages, and DPPH is a stable radical showing a maximum absorbance at 515nm. Lowest concentration of DPPH radical is mainly due to the scavenging ability of the soluble constituents. The extent of yellowing indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. Another recommended method for measuring antioxidant potential is through ABTS assay which eliminates color interference by plant extracts by measuring the absorbance at 734nm (long wavelength). Because of the ease and convenience, ABTS assay is widely used to determine the free radical scavenging properties.

The results show that the *M. talbotianum* bioactives possess hydrogen donating capabilities and acts as an antioxidant. The superoxide anion scavenging activity was determined by the auto oxidation of hydroxylamine hydrochloride. The scavenging activity is measured by the inhibition of generation of O\(_2^-\) free radicals. These radicals can liberate highly reactive radicals responsible for enormous biological toxicity and cell damage through lipid peroxidation. The Fe\(^{3+} / \ Fe^{2+}\) transformation in the presence of methanol and water extracts of the leaves of *M. talbotianum* was evaluated with the reducing capacity of a compound. The reducing power is increased with the increasing amount of sample methanol extract of the leaves of the *M. talbotianum*. In general, the solvent extracts of *M. talbotianum* leaves showed promising radical scavenging activity. Methanol and water extracts showed the highest antioxidant activity. The radical scavenging activity is of significant in searching for a suitable lead in developing drugs.

Lipoxygenases (LOXs) are the enzymes which play a major role in the biosynthesis of leukotrienes which acts as a proinflammatory molecules mainly responsible for the anti-inflammatory activity. *Memecylon* species traditionally involved in the treatment of various skin ailments with proven anti-inflammatory and analgesic activity. In the present study, methanol, ethyl acetate and water extracts of *M. talbotianum* leaves exhibited anti-inflammatory properties which prove the presence of phenols and flavonoid compounds. Xanthine oxidase is an enzyme which generates superoxide and uric acid which catalyzes the oxidation of hypoxanthine to xanthine. It has been proven that Xanthine oxidase inhibitors mainly used in the treatment of liver disorders and gout which is caused mainly by the generation of uric acid and superoxide anion radical. Methanol, ethyl acetate and water extracts of *M. talbotianum* leaves showed good xanthine oxidase inhibitory effect. Anti-
inflammatory properties through Xanthine oxidase inhibitory studies have been reported in many medicinal plants examples include *Swietenia mahagoni*<sup>25</sup>, *Adenanthera poyoina* and *Anteganon leptopus*<sup>26</sup>.

5. Conclusion

The study had shown that *M. talbotianum* has promising antioxidant, anti-inflammatory; characterization of bioactive compounds from this plant will have greater pharmaceutical applications in the treatment of several human diseases.

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References