PHYTOCHEMICAL SCREENING AND IN VITRO ANTIOXIDANT POTENTIAL OF MEMECYLON UMBELLATUM BURM LEAF EXTRACTS

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ABSTRACT

Objective: Different dry extracts of Memecylon umbellatum Burn leaf obtained by various solvents such as petroleum ether, chloroform, ethyl acetate, acetone, methanol and chloroform water (IP) was screened to reap the benefits of its antioxidant and free radical scavenging activity using ascorbic acid as a standard antioxidant. Methods: The in vitro free radical scavenging activity was evaluated using diphenyl picryl hydrazyl (DPPH) radical method using various concentrations of dry extract in distilled water (1, 2, 4, 8, 16, 20 μg/ml) against blank with ascorbic acid as a standard in same concentrations. Results: Among the all extracts, Methanol leaf extract has showed higher Antioxidant activity (84.65 ± 0.064 %) having IC50 Value 11.81 ± 0.033 μg/ml at 20 μg/ml. While, IC50 value for ascorbic acid was found to be 8.91 ± 0.084 μg/ml. Conclusion: The results clearly indicate that Methanol leaf extract of Memecylon umbellatum is effective in free radical scavenging. So in future, this may emerge as promising natural herbal source of powerful antioxidant.

Keywords: Memecylon umbellatum, DPPH reagent, Antioxidant activity, Ascorbic acid, IC50.

1 INTRODUCTION

The mammalian body has its own multifarious defense mechanism involving natural enzymatic (superoxide dismutatase, catalase and glutathione peroxidase) and non-enzymatic (thioreredoxin, thiols, and disulfide-bonding) antioxidants which counteract the harmful effects of free radicals and other oxidants. In normal metabolism, the levels of oxidants (i.e. free radicals) and antioxidants in humans are maintained in balance, which is necessary for sustaining optimal physiological conditions. Free radicals are generated as a result of impaired balance between reactive oxygen species (ROS) production and antioxidant enzymes. These are chemically unstable atoms or molecules that cause extensive damage to cells, causes damage to DNA molecule, lipids and proteins. If free radicals overcome the body’s ability to regulate them, a condition known as oxidative stress ensues. This could lead to number of life threatening diseases like cardiovascular disease, Parkinson’s disease, cancer, mild cognitive impairment, neural disorders, Alzheimer’s disease, ulcerative colitis, aging, diabetes, mellitus, anaemia, atherosclerosis, asthma. Protection against this type of disease can be enhanced by ample intake of various dietary food supplements (containing α-tocopherol, β-carotene, and ascorbic acid etc) and synthetic antioxidants, but these synthetic antioxidant capsules and dietary supplements are found to be less effective in various cases. This has attracted a great deal of research interest in natural antioxidants. Several herbs and spices including Ocimum sanctum, Cichorium intybus, Piper cubeba, Panica granatam, Allium sativum, Delonix regia, Terminalia chebula, Zingiber officinale etc have been reported to exhibit antioxidant activity. The majority of the antioxidant activity is due to flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins.

Memecylon umbellatum Burn. (Family: Melastomataceae) is a small evergreen shrub or tree grows up to 8-14 m tall having young tree branches and bears numerous umbellate cymes. The plant is known as “Anjani” in Sanskrit and “Ironwood tree” in English. Plants are distributed mostly in coastal regions of the Deccan peninsula, the eastern and southern part of India all along the Western Ghats and in the Andaman islands. Different dry extracts of Memecylon umbellatum have been investigated for its hypoglycemic activity using alloxan induced hyperglycemia Wistar albino rats. The results clearly indicate that Methanol leaf extract of Memecylon umbellatum is effective in free radical scavenging. So in future, this may emerge as promising natural herbal source of powerful antioxidant.

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2 MATERIAL AND METHODS

2.1 Plant material

The leaves of *Memecylon umbellatum* were collected in the month of March-April from Gaganbavda hills region, Maharashtra, India. The plant material was taxonomically identified by Dr. S. R. Yadav, Department of Botany, Shivaji University, Kolhapur, India (M.S.). The voucher herbarium specimen is deposited in the Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur.

2.2 Chemical

DPPH Reagent, Ascorbic acid (ACME Chemicals, Mumbai), All other chemicals are of analytical grade and procured from Loba Chem.

2.3 Methods

A standard curve was obtained using Ascorbic acid with the help of double beam UV/Visible spectrophotometer (Jasco-V-630).

2.4 Preparation of extract

Leaves were sorted for foreign matter and dried under shade by spreading in thin layers using aluminum trays for 10 days. Electric grinder (Bajaj-make) was used for powdering soft tissues of leaves. Coarse powder of leaves (#40) was used for extraction.

2.5 Soxhlet extraction process

Extraction was carried out by standard procedure. One kg powder of roots of *Memecylon umbellatum* was used for extraction. Sample powder was packed gently in previously washed and dried cloth bag and solvent was placed from the top with the help of funnel to moisten the drug sample. 3.5 liter of solvent (ethyl acetate, methanol, chloroform water, chloroform, and petroleum ether) was placed in distillation flask and assembly was made air tight with sealing wax. Solvents were selected on the basis of extractive values and with their increasing order of polarity. Extraction was carried out at or slightly above the boiling point of each solvent. Extraction was carried out for 18 hours or on the basis of clarity of dropping solvent (saturation). The solvent was collected every time after completion of the process and powder was dried in hot air oven for 24h at 450C. The process was repeated for all the next solvents and finally the dried powder was macerated with 3.5 liter of chloroform water IP (0.25% v/v) at room temperature with frequent shaking. All the liquid extracts were subjected for physical analysis and are concentrated in a rotary film vacuum evaporator (Dolphin, Mumbai) and finally dried under reduced pressure. The residue was weighed, % yield was calculated. All the extracts were further dried over anhydrous calcium chloride and preserved in vacuum desiccators for further studies. Different extracts were abbreviated according to solvent and part of the plant and used throughout the work.

2.6 Physical evaluation of different liquid extracts of leaf

All the extracts were studied for physical evaluation with respect to color, pH, florescence, density, specific gravity, viscosity along with nature of solid residue obtained after concentration of the extracts with % yield has shown in Table 1.

<table>
<thead>
<tr>
<th>Name of extract</th>
<th>Extract color</th>
<th>pH</th>
<th>Fluorescence</th>
<th>Specific gravity</th>
<th>Density</th>
<th>Viscosity</th>
<th>yield of solids (g)</th>
<th>Nature of solid extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEEL</td>
<td>DG</td>
<td>6.8</td>
<td>R O DB</td>
<td>0.6908</td>
<td>0.8231</td>
<td>0.6124</td>
<td>8.810</td>
<td>Waxy</td>
</tr>
<tr>
<td>ChEL</td>
<td>G</td>
<td>6.2</td>
<td>R Y G</td>
<td>1.2990</td>
<td>1.5480</td>
<td>1.0290</td>
<td>2.062</td>
<td>Lumpy</td>
</tr>
<tr>
<td>SEEL</td>
<td>YG</td>
<td>6.5</td>
<td>Y P G</td>
<td>0.6582</td>
<td>0.7850</td>
<td>0.3474</td>
<td>0.375</td>
<td>Powder</td>
</tr>
<tr>
<td>EAEL</td>
<td>RB</td>
<td>5.4</td>
<td>- R BR</td>
<td>0.6437</td>
<td>0.7265</td>
<td>1.8547</td>
<td>3.065</td>
<td>Powder</td>
</tr>
<tr>
<td>ButEL</td>
<td>WR</td>
<td>5.8</td>
<td>- MG</td>
<td>0.6760</td>
<td>0.8058</td>
<td>2.5793</td>
<td>4.604</td>
<td>Powder</td>
</tr>
<tr>
<td>AEL</td>
<td>BR</td>
<td>7.2</td>
<td>Y MW</td>
<td>0.6994</td>
<td>0.7801</td>
<td>0.4835</td>
<td>2.243</td>
<td>Powder</td>
</tr>
<tr>
<td>EthEL</td>
<td>RB</td>
<td>7.1</td>
<td>- G</td>
<td>0.7902</td>
<td>0.8185</td>
<td>0.9625</td>
<td>8.544</td>
<td>Powder</td>
</tr>
<tr>
<td>MEL</td>
<td>RB</td>
<td>6.0</td>
<td>BR DG YW G</td>
<td>0.8240</td>
<td>0.8848</td>
<td>0.8556</td>
<td>28.36</td>
<td>Waxy</td>
</tr>
<tr>
<td>AqEL</td>
<td>RB</td>
<td>5.2</td>
<td>YG B G</td>
<td>1.0080</td>
<td>1.0124</td>
<td>1.0210</td>
<td>15.72</td>
<td>Powder</td>
</tr>
</tbody>
</table>

PEEL-Petroleum Ether extract leaf, ChEL-Chloroform Extract Leaf, SEEL-Solvent Ether Extract Leaf, EAEL-Ethyl Acetate Extract Leaf, ButEL- n-Butanol Extract Leaf, AEL- Acetone Extract Leaf, EthEL-Ethanol Extract Leaf, MEL-Methanol Extract Leaf, AqEL-Aqueous Extract Leaf, DG -Dark Green, G-Green, YG – Yellowish Green, RB-Reddish Brown, WR-Wine Red, BR-Brownish Red, RR-Reddish Brown, R-Red, G-Green, MG-Milky Green, MW-Milky White, B-Blue, DB-Black Dark, YW- Yellowish White.

2.7 Phytochemical screening

About 500 mg of each dried extract was dissolved in 100 ml of respective solvent and solution obtained was subjected for Phytochemical screening using different specific and general reagents. Samples were prepared as per the requirement of procedure and tests were repeated for final confirmation of phytoconstituents. The positive phytoconstituents present in different parts with various solvents have shown in Table 2.
2.8 Screening of extracts for in-vitro antioxidant activity using DPPH Assay

Extracts showing presence of triterpenes and polyphenolic compounds were screened for antioxidant activity using DPPH reagent. DPPH assay is most widely used method for determination antioxidant potential. Its use has been previously reported for species Acacia caesia[47], Aerva Lanata[48] etc.

2.8.1 Reagents for antioxidant activity

1. DPPH Reagent: Methanolic solution of DPPH (0.1 mM): 39.4 mg of DPPH was dissolved in one liter of analytical grade methanol.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable free radical, characterised by the delocalisation of the spare electron over the molecule as a whole. So this does not dimerize unlike the other free radicals. The delocalization of electron also gives rise to the deep violet color. When antioxidants react with DPPH, which is a stable free radical is reduced to the DPPHH i.e. 1 - 1 diphenyl - 2 – picryl hydrazine and as consequence there is loss of this violet color. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

2.8.3 Procedure

The DPPH scavenging activity was performed using a solution of 0.1 mM DPPH in methanol solution and 1.0 ml solution was added in 3.0 ml of test samples of each dry extract having concentrations as 1, 2, 4, 8, 16 and 20 μg/ml in methanol and kept in darkness. Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding the extract. Ascorbic acid at concentration 1, 2, 4, 8, 16, 20 μg/ml was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

\[ \text{DPPH Scavenged (\%) = } \frac{A_{\text{Control}} - A_{\text{Test}}}{A_{\text{Control}}} \times 100 \]

Where ‘A control’ is the absorbance of the control reaction and ‘A test’ is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the different extract was expressed in % DPPH radical scavenged and the results are given in Table 3.

Table 2: Phytochemical screening of leaf extracts of Memecylon umbellatum

<table>
<thead>
<tr>
<th>Extract</th>
<th>Sugars</th>
<th>Alk.</th>
<th>Tannins</th>
<th>Glycosides</th>
<th>Steroids</th>
<th>Proteins</th>
<th>Org. acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>NR</td>
<td>HT</td>
<td>CT</td>
<td>a</td>
<td>c</td>
<td>s</td>
</tr>
<tr>
<td>AqEL</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MEL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EthEL</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ButEL</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AEL</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>EAELE</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ChEL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SEEL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PEEL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

AqEL-Aqueous Extract Leaf, MEL-Methanol Extract Leaf, EthEL-Ethanol Extract Leaf, ButEL-Butanol Extract Leaf, AEL-Acetone Extract Leaf, EAEEL-Ethyl Acetate Extract Leaf, ChEL-Chloroform Extract Leaf, SEEL-Solvent Ether Extract Leaf, PEEL-Petroleum Ether Extract Leaf, Alk-Alkaloids, Gly-Glycosides, Org.acids-Organic acids, R-Reducing sugars, NR-Non Reducing sugars, HT-Hydrolysable Tannins, CT-Condensed Tannins, a-anthracene glycosides, c-Cardiac glycosides, s-Saponin glycosides, f-Flavanoidal glycosides, co-Coumarin glycosides, ST-Sesquiterpene, TT-Triterpene, + Positive, - Negative.
IC\textsubscript{50} value was determined to express antioxidant activity. It is the concentration of fractions that inhibits the formation of DPPH radicals by 50%. The lower IC\textsubscript{50} value represents the higher antioxidant activity of the tested sample.

3 RESULTS AND DISCUSSION

3.1 Evaluation of different liquid extracts of leaf

Most of the extracts have shown different color in different solvents. Some of the extracts have showed typical florescence either in day or short (254nm) and long (366nm) wavelengths. Leaf extract showed maximum pH 7.2 for acetone and minimum pH 5.2 for aqueous extract. Specific gravity was found highest (1.2990) for chloroform extract and lowest (0.6437) for ethyl acetate. Also maximum viscosity (2.5793cp) for n-butanol and minimum (0.3474cp) for solvent ether was observed. The maximum % yield 28.36 for leaf was found for methanol and minimum 0.375 % for solvent ether.

3.2 Phytochemical screening of different extracts

Polar solvents used in the process of extraction have shown the presence of polar constituents such as mono and disaccharides, proteins amino acids different glycosides like anthracene, cardiac, flavanoidal, saponin and coumarin type glycosides. Polyphenols like tannins, organic acids, minerals and triterpenes were also found in most of the polar extracts while non polar solvents showed positive tests for sterols, aglycones of different glycosides, fatty acids and polysaccharides. Phytochemical screening of different liquid extracts showed the presence of reducing, nonreducing sugars, tannins and proteins (both hydrolysable and condensed) in almost every extract except for Chloroform (ChEL), Solvent Ether (SEEL) and Petroleum Ether Extract (PEEL). No traces of alkoids were detected in any of the extract. Chloroform (ChEL), Solvent Ether (SEEL) and Petroleum Ether Extract of leaf (PEEL) shows dearth of different glycosides, while other extract showed existence of cardiac, saponin, flavanoidal and coumarin glycosides. Being nonpolar in nature ChEL, SEEL and PEEL showed presence of Steroids and terpenes. Some extracts shows presence of organic acids also.

3.3 Screening of different extracts for In-vitro antioxidant activity

In the present study, in vitro antioxidant activity of the different leaf extract of Memecylon umbellatum was investigated by DPPH radical scavenging assays. It is probably due to the presence of phytochemicals like polyphenolics, steroids, glycosides and saponins, highly responsible secondary metabolite for antioxidant activities in these species. Methanol leaf extract showed 84.65 ± 0.064 % antioxidant activity which is higher than other extract [Pet. Ether (3.17 ± 0.084), Chloroform (4.76 ± 0.036), Ethyl acetate (77.24 ± 0.059), Acetone (78.83 ± 0.167), n-Butanol (82.01 ± 0.188) Ethanol (49.20 ± 0.068) and Aqueous extract (11.11 ± 0.058)] at 20 μg/ml. Pet. Ether, Chloroform and aqueous leaf extracts showed very feeble antioxidant activity. Antioxidant activity of different extracts was found to be in order as follow, Methanol > n-butanol > Acetone > Ethyl acetate > Ethanol > Aqueous > Chloroform > Pet. Ether.

IC\textsubscript{50} value, a guide for antioxidant value was determined from % antioxidant activity has been shown in figure 1.

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**Table 3: Antioxidant activity and IC\textsubscript{50} value of different extracts of Memecylon umbellatum**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvent</th>
<th>% Antioxidant activity of different extracts at various concentrations (µg/ml)</th>
<th>IC\textsubscript{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard</td>
<td>12.36 ± 0.305</td>
<td>8.91 ± 0.054</td>
</tr>
<tr>
<td>2</td>
<td>Petroleum ether</td>
<td>0.37 ± 0.105</td>
<td>315.45 ± 0.059</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>0.18 ± 0.205</td>
<td>4.67 ± 0.036</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl acetate</td>
<td>0.27 ± 0.105</td>
<td>12.94 ± 0.016</td>
</tr>
<tr>
<td>5</td>
<td>Acetone</td>
<td>0.28 ± 0.105</td>
<td>5.07 ± 0.018</td>
</tr>
<tr>
<td>6</td>
<td>n-Butanol</td>
<td>0.29 ± 0.105</td>
<td>12.19 ± 0.089</td>
</tr>
<tr>
<td>7</td>
<td>Ethanol</td>
<td>0.30 ± 0.105</td>
<td>4.92 ± 0.068</td>
</tr>
<tr>
<td>8</td>
<td>Methanol**</td>
<td>0.31 ± 0.105</td>
<td>11.01 ± 0.033</td>
</tr>
<tr>
<td>9</td>
<td>Aqueous</td>
<td>0.32 ± 0.105</td>
<td>90.10 ± 0.055</td>
</tr>
</tbody>
</table>

*Indicates ±SD (n=5) ** indicates more potent extract & significance (p<0.05)
Methanol leaf extract showed significant ($p<0.05$ Graphpad instat 3) IC$_{50}$ value (11.81 μg/ml) compared to standard i.e. ascorbic acid (8.91 μg/ml). All other extracts showed higher IC$_{50}$ value, indicating lesser antioxidant activity than standard and methanol leaf extract. Pet. Ether and chloroform leaf extract showed higher IC$_{50}$ value, 315.45 and 210.08 μg/ml respectively. IC$_{50}$ value of different extract has been shown in figure 2.

4 CONCLUSIONS
From the findings of this study, it can be concluded that Memecylon umbellatum leaf extracts, emerging as promising natural herbal sources of antioxidants and can be used in nutritional or pharmaceutical fields for the prevention of free radical-mediated perilous diseases (oxidative stresses). However, in-vivo assays are essential to characterise it as biological antioxidants. In addition to this, flavonoids, mainly responsible for antioxidant activity need to be investigated in details.

ACKNOWLEDGEMENT
Authors are thankful to Dr. S. R. Yadav, Department of Botany, Shivaji University, Kolhapur, India (M.S.) for their valuable guidance.

CONFLICT OF INTEREST:
We declare that we have no conflict of interest.
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