INTRODUCTION

Medicinal plants are a source of diverse nutrients and bioactive compounds that play a significant main role in therapeutically application. Scientific interest in medicinal plants has increased due to phytochemicals which provide unlimited opportunities for new drug discoveries (Cosa et al., 2006). The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidant defence mechanisms. Antioxidants are substances which possess free radical reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re-antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad et al., 2006). Among the numerous naturally occurring antioxidants, ascorbic acid, carotenoids and phenolic compounds are more effective (Duh et al., 1999). The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Cao et al., 1996). On continuation of our experimental work for the search of antioxidant activity of medicinal plants, we studied extracts of six medicinal plants. The free radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) was evaluated during the course of work.

The ascorbic acid, carotenoids and total phenol contents with antioxidant activity were also determined. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants. As such, production offree radicals therapeutic oxygen species in the human body by numerous physiological and biochemical processes is reported (Halliwell, 1994). Antioxidants offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent the disease progression (Braughler et al., 1986). The most commonly used synthetic antioxidants at present are ButylatedHydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), Propyl Gallate and Tert-Butylhydroquinone. However, they are suspected of being responsible for liver damage and acting as carcinogens in laboratory animals (Anagnostopoulou et al., 2006). The search for new products with antioxidative properties and fewer side effects is very active domain of research. Therefore, the development and utilization of more effective antioxidants of natural origin is desirable (Sakagami et al., 1991). The plant A. mexicana cures leprosy, skin diseases, inflammation and bilious fevers. Roots are useful in guineaworm infestation and ets. The latex is useful in dropsy, jaundice, skin diseases, leprosy, blisters, conjunctivitis, inflammation, burning sensation and malarial fever. The oil is useful in indolent ulcers, wounds, leprosy and skin diseases, constipation, flatulences, colic and rheumatalgia.

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In Homeopathic system of medicine the drug prepared from this herb is used to treat the problem caused by tape worm. (Nacoulma, 1996; Rajyaidhya et al., 2012).

**MATERIALS AND METHODS**

**Plant material**

The leaf, stem and root parts of *A. mexicana* were collected by S. Pavithra from Maruthamalai tail of Western Ghats, during November 2015. The collected plant material was identified and authenticated by Botanical survey of India, Southern Circle, Coimbatore (NO.BSI/SRC/5/23/2015/Tech) and the voucher specimen has been deposited in Bharathiar University Herbarium, Department of Botany, and Coimbatore. The collected fresh plant for leaves, stem, and root were cleaned thoroughly with running tap water to remove dust and shade dried for a week at room temperature. The powers were in airtight container.

**Plant extracts preparation**

The powdered plant material was extracted in Soxhlet extractor successively with petroleum ether, chloroform, acetone and methanol. Each time before extracting with the next solvent, the thimble was dried in hot air oven below 40 °C. The different solvent extracts were concentrated by rotary vacuum evaporator and then air dried. The dried extract obtained with each solvent was weighed. The percentage yield was expressed in terms of air dried weight of plant material.

**Quantification of total phenolics, tannins and flavonoids**

**Quantification of total phenolics and tannin**

The total phenol content was determined according to the method described by (Makkar, 2003). 100 µL aliquots for plants extracts (5mg/mL) were taken in the test tubes and made up to the volume of 1mL with distilled water. Then 0.5mL of Folin Ciocalteau reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against blank. Reaction mixture without plant extract was taken as blank. The analysis was performed in triplicate and the results were expressed as Gallic acid equivalents. Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) 1mg of PVPP was weighed into a 2 mL eppendorf tube and to this 900 µL distilled water and then 750 µL of the sample extracts were added. The content was vortexed and kept in the test tube at 4 °C for 4hrs. Then the sample was centrifuged at 4000 for 10 minutes at room temperature and the supernatant was collected. This supernatant has only simple phenolics other than the tannins. The tannins would have been precipitated along with the PVPP. The phenolic content of the supernatant was measured and expressed as the content of non-tannin phenolics. From the above results, the tannin content of the sample was calculated as follows:

\[ \text{Tannin} \% = \frac{\text{Total phenolics} \% - \text{Non tanninphenolics} \%}{\text{Total phenolics} \%} \]

**Quantification of total flavonoids**

The flavonoid contents of all the extracts were quantified as it act as a major antioxidants in plants reducing oxidative stress. Estimated as per described by (Zhiishen et al., 1999).

Initially 150 µL of all the plant extracts were taken in different test tubes. To each extracts 2 mL of distilled water was added. Then 150 µL of NaNO₂ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation 150 µL of AlCl₃ (10%) was added to all the test tubes. The test tubes were incubated for 6 minutes at room temperature. Then 2 mL of NaOH was added to all the test tubes which were made up to 5 mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids was read spectrophotometrically at 510 nm. The amount of flavonoids was calculated as rutin equivalents.

In vitro antioxidant studies

**Radical scavenging activity using DPPH method**

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method of (Prieto et al., 1999). Plants extracts at various concentrations (20 - 100 µL) was added to 5 mL of 0.1 m Methonolic solution of DPPH and allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm. Methanol was served as blank and solution without extract served as control. The mixture of methanol, DPPH and standard (ascorbic acid) served as positive control. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula. More significantly the IC₅₀ of the extracts were also calculated.

**Phosphomolybdenum assay**

The antioxidant power of the extracts has been assessed with the phosphomolybdenum reduction assay according to (14). The assay was based on the reduction of the extract and subsequent formation of a complex. 0.5 mL of extract combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was incubated at 95 °C for 90 minutes. The absorbance was taken at 695 nm using spectrophotometer. The results were calculated in ascorbic acid equivalents.

\[ \text{Percentage of Phosphomolybdenum} = \frac{\text{Control OD} - \text{Sample OD} / \text{Control OD}}{100} \times 100 \]

**Assay of superoxide radical (O₂⁻) scavenging activity**

The assay was based on the capacity of the sample extract to inhibit formazan formation by scavenging superoxide radicals generated in riboflavin- light-NBT system (Dinis et al., 1994). Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 2.33µg riboflavin and 12 mM EDTA, and 11.55 g NBT. Reaction was started by illuminating the reaction mixture with of sample extracts (100 µL) for 90 seconds. Reaction mixture with extract kept in dark served as negative control while the mixture without extract was taken as blank. Immediately after illumination, the absorbance was measured at 590 nm. The activity was compared to ascorbic acid. The percentage inhibition of superoxide anion generation was calculated using the following formula:

\[ \text{Percentage of inhibition} = \frac{\text{Control OD} - \text{Sample OD} / \text{Control OD}}{100} \times 100 \]
Metal chelating activity

Iron II chelating activity was measured by the inhibition of the formation of iron-(II)-ferrozine complex after preincubation of the sample. The Fe⁺ was monitored by measuring the formation of ferrous ion–ferrozine complex against methanol blank at 562nm. The chelating of ferrous ions by various extracts in plant was estimated by the method of (Nile and Khobragade, 2009). The chelating of ferrous ions by various extracts of A. mexicana was estimated. Initially, about 100µl of the extract samples were added to 50µl of 2 mM FeCl₂ solution. Then the reaction was initiated by the addition of 200μl of 5mM ferrozine and the test tubes were vortexes well and left standing at room temperature for 10 minutes. The reaction mixture containing deionized water in place of sample was considered as the negative control absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water). EDTA was against the standard metal chelating agent and the results were expressed as mg EDTA equivalents/g extract chelate the ferrous ion was calculated by, Percentage chelation = (1-(ABS sample/ABS control))×100. ECF value (mg extract/mL) is the effective concentration at which ferrous ions were chelated 50% by the extract.

Antibacterial activity

Antibacterial activity of A. mexicana leaf methanol extract determined using well diffusion method. The bacteria were cultured in nutrient broth at room temperature and kept in orbital shaking incubator (remi, India) at 200 rpm for 2-3 days. The mullerhinton agar plates (bacteriaculture) were prepared and poured into wells. The plants were incubated at 37°C for 24h (bacteria). After incubation the antibacterial activity was assessed. Each screening test was performed with three replicates and t the mean values are recorded.

Table 1. Total phenolic, tannins and flavonoid content of leaf and stem extract of A. mexicana.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Plant material</th>
<th>Total phenol (GAE mg/100g)</th>
<th>Tannin (GAE mg/100g)</th>
<th>Flavonoid (RE mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pet ether</td>
<td>28.38 ± 2.27</td>
<td>3.12 ± 0.46</td>
<td>9.16 ± 1.10</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>22.26 ± 3.17</td>
<td>2.31 ± 0.22</td>
<td>12.42 ± 0.65</td>
</tr>
<tr>
<td>3.</td>
<td>A.m leaves</td>
<td>32.26 ± 3.17</td>
<td>5.01 ± 0.44</td>
<td>13.60 ± 1.31</td>
</tr>
<tr>
<td>4.</td>
<td>Methanol</td>
<td>49.93 ± 5.30</td>
<td>5.32 ± 0.46*</td>
<td>14.76 ± 0.65*</td>
</tr>
<tr>
<td>1.</td>
<td>Pet ether</td>
<td>20.14 ± 1.02</td>
<td>2.31 ± 0.22</td>
<td>10.49 ± 1.65</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>21.14 ± 0.22</td>
<td>3.05 ± 0.33</td>
<td>8.04 ± 1.25</td>
</tr>
<tr>
<td>3.</td>
<td>Acetone</td>
<td>37.63 ± 5.62</td>
<td>4.25 ± 0.46</td>
<td>11.09 ± 1.51</td>
</tr>
<tr>
<td>4.</td>
<td>A.m stem</td>
<td>Methanol</td>
<td>39.81 ± 2.73*</td>
<td>4.88 ± 0.58*</td>
</tr>
<tr>
<td>1.</td>
<td>Pet ether</td>
<td>36.78 ± 1.23</td>
<td>0.12 ± 0.71</td>
<td>10.71 ± 2.37</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>17.9 ± 2.30</td>
<td>1.25 ± 0.33</td>
<td>10.93 ± 1.00</td>
</tr>
<tr>
<td>3.</td>
<td>Acetone</td>
<td>38.69 ± 2.62</td>
<td>4.23 ± 0.67</td>
<td>11.02 ± 0.65</td>
</tr>
<tr>
<td>4.</td>
<td>A.m root</td>
<td>Methanol</td>
<td>34.22 ± 1.25*</td>
<td>4.55 ± 0.33*</td>
</tr>
</tbody>
</table>

Values are mean of replicate determination (n=3) ±standard deviation. GAE-Gallic acid equivalence, RE-Rutin equivalence.

Table 2. Antibacterial activity of A. mexicana leaf methanol extract

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Control (100µg/ml)</th>
<th>Zone of inhibition in diameter(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25(µg/ml) 50(µg/ml)</td>
<td>75(µg/ml) 100(µg/ml)</td>
</tr>
<tr>
<td>staphylococcus pyogens</td>
<td>35±0.5</td>
<td>28±0.05 30±1.03 33±0.05 34±0.07</td>
</tr>
<tr>
<td>staphylococcus aureus</td>
<td>38±0.5</td>
<td>35±0.05 29±0.03 32±0.07 36±0.03</td>
</tr>
<tr>
<td>E.coli</td>
<td>36±0.4</td>
<td>21±0.05 25±0.05 32±0.03 34±0.01</td>
</tr>
<tr>
<td>Pseudomonas Aeruginosa</td>
<td>33±0.1</td>
<td>26±0.5 25±0.03 30±1.02 31±0.06</td>
</tr>
<tr>
<td>klebsiella pneumonia</td>
<td>25±1.03</td>
<td>15±0.03 17±0.04 19±0.6 22±0.03</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Estimation of total phenolic and tannins

The phenolic, tannin and flavonoid content of A. mexicana were estimated shows the table 1. Leafmethanolic extract has maximum phenolic content and was found to be 49.93 ±5.30mg GAE/100g of extract, when compared to other extract. Tannin content determination of plant extract showed that the leafmethanolic extract possess the higher tannin content (5.32 ±0.46 mg GAE/100g) of extract. The estimation of total flavonoids content in leaves, stems and roots revealed and the methanol leaf extract of possess the maximum (14.76 ± 0.65mg RE /100g) of extract. Phenolic and flavonoids are common groups of polyphenolic compounds and have important roles in stabilizing lipid per oxidation due to their anti - oxidative activities. Many studies have indicated that the antioxidant capacities of flavonoids are due to the number and position of hydroxyl groups in their structures (Hau et al., 2009). These studies strongly support that A. mexicana undoubtedly can have antioxidant another medicinal from oxidative damage by possessing antioxidants such as polyphenolics compounds.

Antioxidant assays

DPPH radical scavenging activity

The free radical-scavenging activities of leaf, stem and root of the plants A. mexicana extract investigated antioxidant activity of DPPH and the results are shown in figure 1. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. The colour change from purple to yellow is visually evident. A lower value of IC₅₀ (inhibitory concentration at 50%) indicates a higher antioxidant activity. Generally, the acetone and methanol extracts of all the parts showed significant reduction of DPPH radical. However the highest was free A. mexicana radical scavenging activity was exerted by methanol extract of leaf, stem and root (IC₅₀ value were 10.25µg/ml;11.22µg/ml; 11.89µg/ml).
Figure 1. DPPH scavenging activity of *Aregmone mexicana* leaf, stem and root extract

Figure 2. Phosphomolybdenum assay leaf, stem, and roots extracts of *A.mexicana*

Figure 3. Superoxide radical scavenging activity of *A.mexicana* leaf, stem, and root extracts
Phosphomolybdenum assay

The antioxidant activity of phosphomolybdenum assay analysed and the results are show figure 2. The phosphomolybdenum reduction assay was based on the reduction of Mo(VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature. Among A. Mexicana stem showed higher activity in compared to other solvents extracts of leaf and root. Methanol extract of stem (48.13 mg/g extract) have highest phosphomolybdenum reduction compared to other solvent extracts.

Superoxide radical scavenging activity: The results of superoxide anion scavenging of different extracts of leaf stem and root were analysed. A.mexicana root methanolic extract was higher (76.07%), Then the plant stem and leaf methanol extract (64.15%; 66.15%). A.mexicana low significant activity was shown stem as compared to leaf and stem. These results were compared with natural (Ascorbic acid) and synthetics (BHT) antioxidants.

Metal chelating activity

Ability of antioxidants to form insoluble metal complexes with ferrous ion or to generate stearic hindrance that prevent interaction between metal and lipid is evaluated using the ion chelating capacity (Elmastas et al., 2005). The activity is measured by monitoring the decrease in absorbance of lead Ferricferrozine complex as antioxidants compete with ferrozine in chelating ferrous ion (Soler-Rivas et al., 2000). The Fe$^+$ chelating capacity of different solvent extracts of leaf and stem of A.mexicana were analyzed, in plant maximum activity were observed for the methanolic extract (47.56 mg EDTAE/100g; 44.25 mg EDTAE/100g) extract as compared to other extract.

Antibacterial activity

The antibacterial activity of the A.mexicana methanol extract. The zone of inhibition was maximum at the concentration (100µg/ml) of given sample the highest zone of inhibition was observed in staphylococcus aureus (36±0.03mm) at concentration of 100µg/ml. Similarly the lowest zone of
inhibition was observed in *klebsiella pneumonia* (22±0.03mm) at concentration of 25µg/ml. The results confirm that the given sample shows excellent antibacterial activity.

**Statistical analysis**

Total phenolic, tannin and total flavonoids contents analyses were carried out in triplicates, the results of the contents were performed from the averages of all samples reading Mean ± SD used Excel 2003. The antioxidant data were statistically analyzed using one way ANOVA followed by Duncan’s test for antioxidant studies Mean value were considered statistically significant when P<0.05.

**Conclusion**

Numerous phytochemical screening studies have been carried out in different parts of the world using antioxidant studies. In the present study, *A. mexicana* extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. In addition to this, the results of the antioxidant profile can be used as pharmacological tool for the medicinal purposes of this plant.

**Acknowledgements**

The authors would like to thank Dr.T.Sekar, Assistant Professor, Department of Botany, Bharathiar University, Coimbatore, for providing technical assistance during the research work.

**REFERENCES**


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