INTRODUCTION

Phytomedicines play a major role in human health care system. There is a considerable interest in elucidating the mechanism of their action to develop better medicines. Plants contain many free radical scavenging molecules such as phenolic compounds, nitrogen compounds, vitamins, terpenoids etc.

*Asteraceae* is the largest family of flowering plants in terms of number of species. Several plants of this family are edible and are used as folk medicines. These plants produce sesquiterpenes, lactones, pentacyclic triterpene, alcohols, various alkaloids, used as folk medicines. These plants produce sesqui terpenes, nitrogen compounds, vitamins, terpenoids etc.

There is a considerable interest in elucidating the mechanism of action to develop better medicines. Plants contain many free radical scavenging molecules such as phenolic compounds, nitrogen compounds, vitamins, terpenoids etc.

*Asteraceae* is the largest family of flowering plants, traditionally known for its medicinal properties. In the present study antioxidant properties of 10 selected *Asteraceae* species were assessed by DPPH (1,1-diphenyl-2-picryl-hydrazyl), ABTS (2,2’-azino-bis(3- thylbenzthiazoline-6-sulphonic acid) method. The plants were extracted sequentially in soxhlet apparatus with petroleum ether, hexane, ethyl acetate, chloroform, methanol and water in the increasing order of polarity. These extracts were subjected to find its antioxidant activity and total phenolic contents. Antibacterial activity against some human pathogenic bacteria was tested by agar disk diffusion method. Among all the organic solvent extracts, methanol extracts had very good antioxidant and antibacterial activity. The extracts showed inhibition of human pathogenic bacteria in the order: *Escherichia coli* > *Klebsiella pneumonia* > *Shigella flexneri* > *Staphylococcus aureus* > *Bacillus subtilis* > *Bacillus cereus*. Minimum inhibitory concentration (MIC) was 100 µg/100 µl for many plant extracts, whereas MIC of *G. bosvallae* and *W. trilobata* was 70 µg/100 µl for *Bacillus subtilis*, *Klebsiella pneumonia* and *Shigella flexneri*. The extracts were tested for pTZ57R/T plasmid DNA protection against hydroxyl radicals as evidenced by DNA fragmentation assay. Significant and positive linear correlations ($R^2 = 0.9294$) were found between total antioxidant capacities and phenolic contents indicating that phenolics were the dominant antioxidant constituents in tested medicinal plants which are discussed in this manuscript. Our study clearly demonstrated that the selected plants have good antioxidant, antibacterial and DNA protecting properties.

Antioxidant activities are determined by DPPH and ABTS assays. Antimicrobial activity was performed against some human pathogenic bacteria like *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Shigella flexneri*. The Plant extracts were also tested for their DNA damage inhibition efficiency against oxidation of hydroxyl radicals.

Initially about 50 plant species of *Asteraceae* family were selected based on their common use in traditional systems of medicine and screened for bioactivity. Based on the antioxidant activity 10 species namely *Artemisia cina* O. Berg., *Artemisia vulgaris* L., *Eclipta alba* (L.) Hassk., *Glossocardia bosvallae* (L. f.) DC., *Mikania micrantha* (L.) Kunth., *Sphagneticola trilobata* (Osbeck) Merr., *Vicia indica* (L.) DC., *Wedelia chinensis* (Osbeck) Merr and *W. trilobata* (L.) Hitchc were selected. The total phenol content, antioxidant, antimicrobial and DNA protection activity were determined.

**Keywords:** *Asteraceae* plant species, Methanol extract, Antioxidant, Antimicrobial, DNA protection assay

**ABSTRACT**

*Asteraceae* is the largest family of flowering plants, traditionally known for its medicinal properties. In the present study antioxidant properties of 10 selected *Asteraceae* species were assessed by DPPH (1,1-diphenyl-2-picryl-hydrazyl), ABTS (2,2’-azino-bis(3-thylbenzthiazoline-6-sulphonic acid) method. The plants were extracted sequentially in soxhlet apparatus with petroleum ether, hexane, ethyl acetate, chloroform, methanol and water in the increasing order of polarity. These extracts were subjected to find its antioxidant activity and total phenolic contents. Antibacterial activity against some human pathogenic bacteria was tested by agar disk diffusion method. Among all the organic solvent extracts, methanol extracts had very good antioxidant and antibacterial activity. The extracts showed inhibition of human pathogenic bacteria in the order: *Escherichia coli* > *Klebsiella pneumonia* > *Shigella flexneri* > *Staphylococcus aureus* > *Bacillus subtilis* > *Bacillus cereus*. Minimum inhibitory concentration (MIC) was 100 µg/100 µl for many plant extracts, whereas MIC of *G. bosvallae* and *W. trilobata* was 70 µg/100 µl for *Bacillus subtilis*, *Klebsiella pneumonia* and *Shigella flexneri*. The extracts were tested for pTZ57R/T plasmid DNA protection against hydroxyl radicals as evidenced by DNA fragmentation assay. Significant and positive linear correlations ($R^2 = 0.9294$) were found between total antioxidant capacities and phenolic contents indicating that phenolics were the dominant antioxidant constituents in tested medicinal plants which are discussed in this manuscript. Our study clearly demonstrated that the selected plants have good antioxidant, antibacterial and DNA protecting properties.

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**Materials and Methods**

**Plant materials**

Ten traditional Indian medicinal plant species belonging to *Asteraceae* family were collected from Mysore district, Karnataka, India. Whole plant parts including root, shoot and leaf were used in this study. The voucher specimens of all species are maintained in the laboratory. Collected plant materials were washed...
thoroughly in tap water, dried in shade at room temperature for 10 days and used for further study. The whole plant parts including root, shoot and leaf was ground to a fine powder. About 50 g of coarsely powdered plant materials were extracted sequentially with petroleum ether, hexane, ethyl acetate, chloroform, methanol and water. The extracts obtained were then concentrated and finally dried to a constant weight.

Chemicals

1,1-diphenyl-2-picryl-hydrayl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), Folin-Ciocalteu reagent, ascorbic acid and gallic acid were purchased from Sigma Aldrich. All other chemicals and reagents used were of analytical grade.

Determination of total phenolics

The total phenolic content was estimated using Folin-Ciocalteu colorimetric method. Test sample (100 µl) was reacted with 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10 fold with distilled water) and allowed to stand at 22°C for 5 min. The reaction was neutralized with saturated sodium carbonate (60 g/l) and allowed to stand for 1.5 h in the dark at 22ºC. The absorbance of the resulting blue colour was measured at 725 nm (Hitachi U-3900 UV/visible spectrophotometer). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of known concentrations of gallic acid standard (25 to 250 µg/ml). The total phenolic contents were expressed as gallic acid equivalence (GAE) in µg.

DPPH radical scavenging assay

The free radical scavenging property of the methanol extracts of 10 plants were determined by DPPH method. The DPPH radical solution was prepared in methanol. The reaction mixture contained 5 µl of test samples and 95 µl of DPPH (300 µM) in methanol. The reaction for scavenging DPPH radical was carried out at 37ºC for 30 min and the absorbance was recorded at 517 nm (Spectra max 340, Molecular devises). Percent radical scavenging activity was determined by comparing with a solvent added control. The IC₅₀ values were determined, which denote the concentration of extracts required to scavenge 50% DPPH radicals. Ascorbic acid was used as positive control. Percent scavenging effect was determined by the following equation:

% inhibition = ([Absorbance of control – Absorbance of test sample]/Absorbance of control) x 100

ABTS radical scavenging assay

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) antioxidant activity was measured using Hitachi U-3900 UV/visible spectrophotometer according to the method described with slight modifications. ABTS radical cation (ABTS⁺) solution was prepared by reacting 7 mM ABTS and 2.45 mM potassium persulfate on incubating the mixture at room temperature in dark for 12 h. The resulting ABTS⁺ solution was then diluted with methanol to get an absorbance of 0.700 ± 0.005 at 734 nm. Different concentrations of test samples (50 µl) were added to 2.95 ml of ABTS⁺ 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 reference standard. The percent inhibition was calculated from the following equation:

% inhibition = ([Absorbance of control – Absorbance of test sample]/Absorbance of control) x 100

Reducing Power estimation

This estimation of reducing power carried out as described previously with slight modifications. About 0.75 ml of test sample solution (1 mg/ml) was mixed with equal volume of 0.2 M phosphate buffer (pH 6.6) and 0.75 ml of 1% potassium ferricyanide, followed by incubation at 50°C for 20 min. Trichloroacetic acid (10%, 0.75 ml) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.5 ml of the supernatant solution were mixed with equal volume of distilled water. Absorbance was measured at 700 nm (Hitachi U-3900 UV/visible spectrophotometer) after the addition of 0.5 ml of 0.1% FeCl₃. Ascorbic acid was used as standard and phosphate buffer was used as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power.

Antibacterial assay

Antibacterial activity of methanol extracts of 10 plants was determined by disc diffusion method on nutrient agar medium as described. Cultures of Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Shigella flexneri were spread on separate nutrient agar plates. Methanol extracts (50 µl, 50 µg) were loaded separately on sterile discs (6 mm diameter), allowed to dry and placed on the bacteria inoculated nutrient agar media. Negative control was prepared by loading the discs with solvents and positive control was by chloramphenicol. The plates were incubated at 37°C for 24 - 48 h and zone of inhibition around the disc were measured. The experiment was done with three replicates for consistency of the treatment. Minimum inhibitory concentration was determined as the lowest concentration of the plant extract needed to inhibit the growth of the organism.

DNA protection assay

Extent of protection against pTZ7Z7R/T plasmid DNA damage by Asteraceae plant extracts was tested as described with some modifications. Mixture of 5 µl of plant extract (1mg/ml) and 2 µl of plasmid was added to 5 µl of Fenton’s reagent (30 mM H₂O₂, 50 µM ascorbic acid and 80 µM FeCl₃). The final volume was made up to 15 µl with sterile water followed by incubating for 30 min at 37°C. The DNA was analyzed on 1% agarose gel using ethidium bromide staining.

Statistical Analysis

All determinations of antioxidant property of DPPH, ABTS and measurement of total phenolic contents were done in triplicates. The reported value for each sample was calculated as the mean of three measurements. The correlation coefficients (R), coefficient of determination (R²) and p ≤ 0.05 values were calculated using Microsoft Excel 2007.

RESULTS AND DISCUSSION

The hexane, ethyl acetate and chloroform extracts of the selected plants of Asteraceae exhibited very negligible or no antioxidant and antimicrobial activities. Only methanol extracts of these plants showed interesting and consistent results. This might be because of wide soluble properties of low molecular and polar substances including the antioxidant active phenolic compounds present in these plants. Hence, methanol extracts alone were selected to evaluate their total phenolic content, reducing power, antibacterial and protection against DNA damage. The methanol extracts exhibited concentration dependent inhibitory effects on all tested in vitro models.

Determination of total phenolic content

The phenolic content in 10 Asteraceae plant extracts varied from 135 to 240 GAE (Table 1). Artimisia vulgaris, Glossocardia bosvallae and Wedelia triolobata have the highest phenolic content of 218, 240 and 230 GAE (in µg), respectively. Mikania micrantha, Vernonnia cinerea and Wedelia chinensis have 155, 195 and 197 GAE (in µg), respectively whereas Artemisia cina, Eclipta alba, Splantaes uliginosa and Vicco indica have lowest phenolic content. A significant linear correlation (R² = 0.97 and 0.98) was observed between DPPH/ABTS radical scavenging activity and total phenolic content of all 10 plants. This positive correlation suggests that the antioxidant capacity of the methanol extracts could be attributed largely to the phenolic content of these extracts. Our results indicate that the phenolics play an important role in the antioxidant activity.
Table 1: DPPH and ABTS Antioxidant activity and total phenolic content of 10 Asteraceae plant methanolic extracts

<table>
<thead>
<tr>
<th>Plants (µg/ml)</th>
<th>DPPH assay (IC50 value in µg/ml)</th>
<th>ABTS assay (IC50 value (1 mg/ml))</th>
<th>Total phenolic Content (GAE in µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glossocardia bosvallea</td>
<td>80</td>
<td>70</td>
<td>240</td>
</tr>
<tr>
<td>Artimisia vulgaris</td>
<td>90</td>
<td>80</td>
<td>218</td>
</tr>
<tr>
<td>Wedelia trilobata</td>
<td>100</td>
<td>90</td>
<td>235</td>
</tr>
<tr>
<td>Wedelia chinensis</td>
<td>100</td>
<td>90</td>
<td>197</td>
</tr>
<tr>
<td>Mikania micrantha</td>
<td>130</td>
<td>100</td>
<td>195</td>
</tr>
<tr>
<td>Vernonia cinerea</td>
<td>120</td>
<td>110</td>
<td>155</td>
</tr>
<tr>
<td>Vicia indica</td>
<td>210</td>
<td>180</td>
<td>135</td>
</tr>
<tr>
<td>Artemisia cina</td>
<td>250</td>
<td>120</td>
<td>135</td>
</tr>
<tr>
<td>Eclipta alba</td>
<td>220</td>
<td>170</td>
<td>115</td>
</tr>
<tr>
<td>Spilanthes uliginosa</td>
<td>290</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

Values represent the mean (n=3)

DPPH and ABTS radical scavenging assay

Our study clearly demonstrated that the plant extracts have good antioxidant properties when assessed by DPPH and ABTS models. The percentage of DPPH decolorization is attributed to hydrogen donating ability of test compounds. Variable DPPH activity was recorded for the 10 species. The extracts of *A. vulgaris*, *G. bosvallea*, *M. micrantha*, *V. cinerea*, *W. chinensis* and *W. trilobata* showed highest activity, whereas *A. cina*, *E. alba*, *S. uliginosa* and *V. indica* had shown moderate activity. *Glossocardia bosvallea* exhibited higher antioxidant activity (IC50 = 80 µg/ml) when compared to other species while *S. uliginosa* has the lowest DPPH activity. Reference standard ascorbic acid showed 50% inhibition at 70µg/ml. The IC50 for methanol extract of *G. bosvallea* is 70µg/ml in ABTS radical scavenging assay. The methanol extracts of *A. vulgaris*, *M. micrantha*, *V. cinerea*, *W. chinensis* and *W. trilobata* also had good activity, whereas *A. cina*, *E. alba*, *S. uliginosa* and *V. indica* showed moderate activity. Reference standard gallic acid showed 50% inhibition at 30µg/ml in ABTS model. Lower IC50 value implies higher antioxidant power.

Relationship among the estimates of total phenolic content with antioxidant DPPH and ABTS assays

Linear correlation between the amounts of total phenols and antioxidant capacity (DPPH and ABTS) is found in all ten plant species (Fig.1). The coefficient of determination ($R^2$) was 0.97157 and 0.98666 between total phenolic content and antioxidant DPPH and ABTS radical scavenging activity, respectively. This positive correlation suggests that the antioxidant capacity of the methanol extracts is greatly due to phenolic content.

**Fig. 1:** Correlation between total phenolic content and antioxidant 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2’-azino-bis-3-thylenbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging potential of 10 Asteraceae plant methanolic extracts.

**Fig. 2:** Reducing power of 10 plant methanolic extracts at different concentrations compared to standard Butylated hydroxytoluene. Increased absorbance to 700 nm indicates stronger reducing power.
Reducing power estimation
The reducing capacity of the 10 plant methanol extracts was compared to standard Butylated hydroxytoluene (BHT) (Fig. 2). An increase in absorbance at 700 nm indicates the reducing power of the extracts. *Glossocardia bosvallea* and *W. trilobata* showed significantly (p<0.05) higher reducing power than other species. The extract of *A. cina*, *A. vulgaris*, *M. micrantha* and *W. chinensis* also showed good reducing power, whereas *E. alba*, *S. uliginosa*, *V. cinerea* and *V. indica* showed moderate reducing power. Reducing power showed significant correlation (p<0.05) with phenolic content for all extracts. \[ R^2 (A. cina) = 0.908, R^2 (A. vulgaris) = 0.991, R^2 (E. alba) = 0.929, R^2 (G. bosvallea) = 0.966, R^2 (M. micrantha) = 0.918, R^2 (S. uliginosa) = 0.960, R^2 (V. cinerea) = 0.948, R^2 (V. indica) = 0.936, R^2 (W. chinensis) = 0.905 and R^2 (W. trilobata) = 0.947 \].

Antibacterial assay
The antibacterial activity of methanol extracts of ten selected plants against human pathogenic bacteria is presented in Table 2. Plant extracts inhibited the growth of major test organisms with some exceptions. *Glossocardia bosvallea* and *W. trilobata* showed significant antibacterial activity against all bacterial species. Pathogenic *E. coli* and *K. pneumonia* were also inhibited by most of the plant extracts. Minimum inhibitory concentration (MIC) was 100 µg/100 µl for all the plant extracts, whereas MIC of *G. bosvallea* and *W. trilobata* was 70 µg/100 µl for *Bacillus subtilis*, *Klebsiella pneumonia* and *Shigella flexneri*. These primary extracts open up the possibility of existing new clinically effective antibacterial compounds. Further research is necessary to identify the specific antibacterial compounds from these plants and to determine their full spectrum of efficacy.

Table 2: Antibacterial zone of inhibition on exposure to 100 µG/100 µL concentration of plant extracts.

<table>
<thead>
<tr>
<th>Plants</th>
<th><em>Bacillus subtilis</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Klebsiella pneumonia</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Shigella flexneri</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artimisia vulgaris</em></td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><em>Eclipta alba</em></td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td><em>Glossocardia bosvallea</em></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><em>Mikania micrantha</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Spilanthes uliginosa</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Vernonia cinerea</em></td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Vicia indica</em></td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td><em>Wedelia chinensis</em></td>
<td>0</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td><em>Wedelia trilobata</em></td>
<td>10</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><em>Chloramphenicol</em></td>
<td>10</td>
<td>18</td>
<td>20</td>
<td>18</td>
<td>19</td>
</tr>
</tbody>
</table>

DNA protection assay
Normal pTZ57R/T plasmid (lane 1) showed two bands on agarose gel electrophoresis. The plasmid DNA when fragmented using Fenton’s reagent, showed two thin bands with smear as shown in lane 2 of Fig. 3. The role of plant extracts in preventing DNA damage was assessed here. All methanol extracts showed stronger protective effect against hydroxyl radical released by Fenton’s reaction. *W. chinensis* showed partial protection of DNA as shown in lane 11.

Fig. 3: Effect of methanolic extracts of ten Asteraceae plants against hydroxyl radical-mediated fragmentation. Lane 1: untreated DNA (control), lane 2: Fenton’s reagent + DNA; lane 3 to 12, Fenton’s reagent + DNA + methanolic extract sequentially represent *A. cina*, *A. vulgaris*, *E. alba*, *G. bosvallea*, *M. micrantha*, *S. uliginosa*, *V. cinerea*, *V. indica*, *W. chinensis* and *W. trilobata*.

CONCLUSION
In conclusion, Asteraceae plants have a definite role to play in the health care system. Our in vitro antioxidant studies provide scientific evidence to prove the traditional claims of Asteraceae plants. The methanol extracts of all 10 selected medicinal plants bear potent antioxidant property. Their constituents scavenge different free radicals and exert protective effects against oxidative damage to biological macromolecules such as DNA. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to potent chemical entities for clinical use. Identification and separation of antioxidant compounds from these plant extracts will go a long way in developing new drugs.

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