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Research Article

ANTIOXIDANT, ANTIBACTERIAL AND DNA PROTECTING ACTIVITY OF SELECTED MEDICINALLY IMPORTANT *ASTERACEAE* PLANTS

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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. bosvallea* 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.

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

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, acetylenes and tannins. *Sphaeranthus indicus* is used in treating epileptic convulsions, mental illness and hemicranias[1]. Tribals of India use *Vicoa indica* (L.) DC., as a contraceptive and female anti-fertility drug. The infusion of whole plant is being used in abortion and roots as remedy to cough and jaundice[2]. *Wedelia* species have a long history of traditional use in revitalizing the liver and treating liver dysfunction and diseases[3]. *Vernonia cinerea* (L.) Less., has many therapeutic uses in malarial fever, worms, pain, infections, diuresis, cancer, abortion and various gastro-intestinal disorders[4,5]. *Psacalium decompositum* (A.Gray) H.Rob., *Psacalium peltatum* (Kunth) and *Acourtia thurberi* (A. Gray) of Asteraceae have the reducing capability of blood glucose level in mice[6]. The ethanolic extract of *Bidens alba* (Linnaeus), a medicinal plant used worldwide, has antimalarial activity[7]. Methanol extract of *Tricholepis glaberrima* DC[8], *Solidago gigantea* Ait., *Taraxacum officinale* [9], *Jurinea consanguinea* DC[10] and *Evax pygmaea*[11] are potential source of natural antioxidants in *Asteraceae* species.

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, xanthine oxidase activity, atmospheric pollutants, from transitional metal catalysts, drugs and xenobiotics[12]. However, the uncontrolled production of oxygen-derived free radicals is associated with the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis, arteriosclerosis and degenerative processes associated with aging. Exogenous chemical and endogenous metabolic processes in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Scavenging of reactive oxygen species (ROS) is important in preventing potential damage to cellular components such as DNA, proteins and lipids. Scientific evidence suggests that antioxidants reduce the risk of chronic diseases including cancer and heart disease. The human body system has number of mechanisms to eliminate free radicals formed. When the normal levels of antioxidant defense mechanism is not sufficient for the eradication of free radical induced injury, administration of antioxidant will have a protective role to play[5]. To protect biological targets from oxidative damage, the ROS should be scavenged by antioxidants before they react with other cellular components. The two most commonly used synthetic antioxidants; butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have restricted use because of their toxicity and DNA damaging properties. Natural antioxidants from plant extracts are safe and do not have any side effects.

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 bosvallea* (L. f.) DC., *Mikania micrantha* (L.) Kunth., *Spilanthes uliginosa* Sw., *Vernonia cinerea* (L.) Less., *Vicoa indica* (L.) DC., *Wedelia chinensis* (Osbeck) Merr and *Wedelia trilobata* (L.) Hitchc were selected. The total phenol content, antioxidant, antimicrobial and DNA protection activity were determined.

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-hydrazyl (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 calorimetric method[13]. 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 22º 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[14]. The $DPPH^{\cdot}$ 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 $DPPH^{\cdot}$ radical was carried out at 37ºC in dark 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 IC_{50} 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] x100

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[15] with slight modifications. ABTS radical cation ($ABTS^{\cdot+}$) 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 $ABTS^{\cdot+}$ 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 $ABTS^{\cdot+}$ 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] x100

Reducing Power estimation

This estimation of reducing power carried out as described previously[16] 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% $FeCl_3$. 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[17]. 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 choramphenicol. 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 pTZ57R/T plasmid DNA damage by *Asteraceae* plant extracts was tested as described[18] 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 H_2O_2, 50 µM ascorbic acid and 80 µM $FeCl_3$). 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 (R^2) and $p \leq 0.05$ 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[19]. 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 bosvallea* and *Wedelia trilobata* have the highest phenolic content of 218, 240 and 230 GAE (in µg), respectively. *Mikania micrantha, Vernonia cinerea* and *Wedelia chinensis* have 155, 195 and 197 GAE (in µg), respectively whereas *Artemisia cina, Eclipta alba, Spilanthes uliginosa* and *Vicoa indica* have lowest phenolic content. A significant linear correlation (R^2 = 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

Plantsin µg/ml)	DPPH assay(IC_{50} valuein µg/ml)	ABTS assay(IC_{50} value(1 mg/ml)	Total phenolic Content (GAE in µg)
Glossocardia bosvallea	80	70	240
Artimisia vulgaris	90	80	218
Wedelia trilobata	100	90	235
Wedelia chinensis	100	90	197
Mikania micrantha	130	100	195
Vernonia cinerea	120	110	155
Vicoa indica	210	180	135
Artemisia cina	250	120	135
Eclipta alba	220	170	115
Spilanthes uliginosa	290	200	100

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 (IC_{50} = 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 IC_{50} 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 IC_{50} 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.

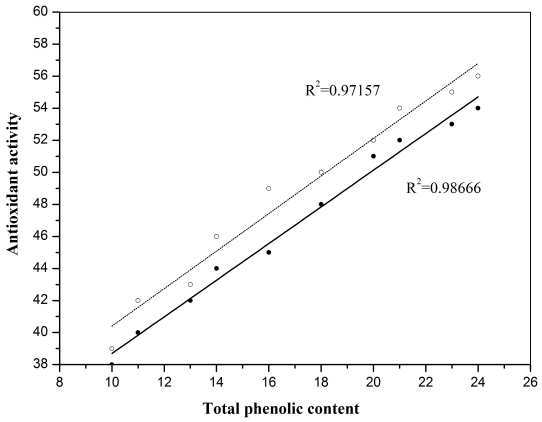

Fig. 1: Correlation between total phenolic content and antioxidant 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2'-azino-bis 3-thylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging potential of 10 *Asteraceae* plant methanolic extracts.

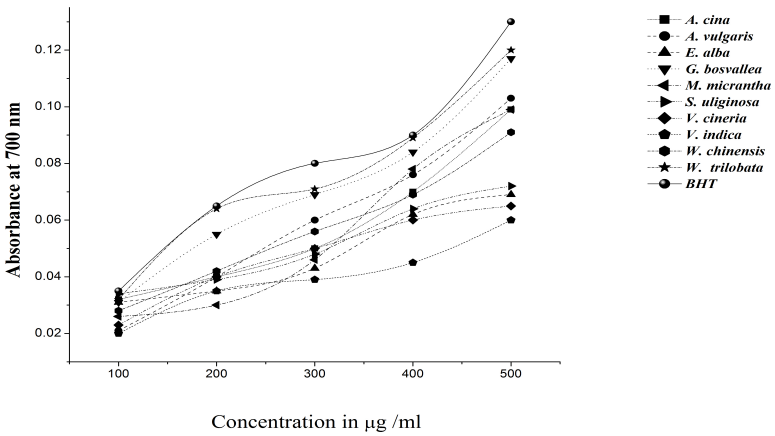

Fig. 2: Reducing power of 10 plant methanolic extracts at different concentrations compared to standard Butylated hydroxytoluene. Increased absorbance to 700 nm indicates stronger reducing power.

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*) = 0.985 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.

	Antibacterial zone of inhibition in mm					
Plants	***Bacilluscereus***	***Bacillussubtilis***	***Escherichiacoli***	***Klebsiellapneumonia***	***Staphylococcusaureus***	***Shigella flexneri***
	0	0	10	0	10	10
Artimisia vulgaris	0	0	12	12	10	12
Eclipta alba	0	12	10	0	14	10
Glossocardia bosvallea	10	10	10	10	10	10
Mikania micrantha	0	0	0	10	12	0
Spilanthes uliginosa	0	10	0	10	0	0
Vernonia cinerea	0	0	10	10	0	0
Vicoa indica	0	0	14	14	0	10
Wedelia chinensis	0	10	11	12	0	14
Wedelia trilobata	10	14	12	10	12	16
Chloramphenicol	10	18	20	18	19	25

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.

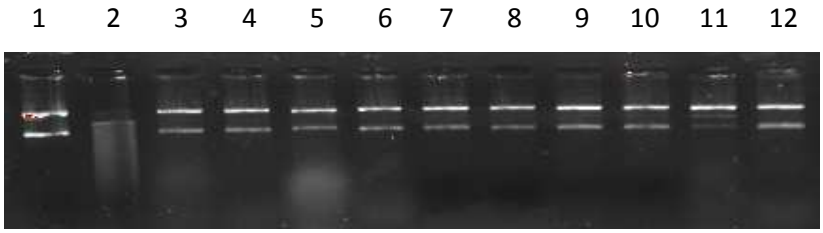

Fig. 3: Effect of methanolic extracts of ten *Asteraceae* plants against hydroxyl radical-mediated fragmentation. Lane 1: untreated DNA (control), lane 2: Fenton's reagent + DNA; lane 3 to 12, Fenton's reagent + DNA + methanolic extract sequentially represent *A. cina*, *A. vulgari, E. alba*, *G. bosvallea, 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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