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ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITIES OF *ANDROGRAPHIS PANICULATA* NEES. LEAVES

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ABSTRACT

Andrographis paniculata Nees. is an important medicinal plant in India which is used in traditional medicine. Although, its antibacterial property has been shown by some researchers, there are very few studies showing its antioxidant activity and there are no reports of its antifungal activity against dermatophytes. So, in this study we evaluate the antibacterial, antifungal and antioxidant activities of *A. paniculata* leaves in different solvent extracts. *A. paniculata* leaves were extracted with chloroform, hexane, methanol, ethanol and water separately and phenol and flavonoid contents were estimated in these extracts. The antibacterial and antifungal activity studies were carried out by using disc diffusion method and mycelial dry weight method respectively. Ferrous reducing antioxidant power assay, 1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging activity, lipid peroxidation inhibitory activity and superoxide scavenging activity were used for *in vitro* antioxidant activity studies. *A. panicualta* ethanol leaf extract showed the highest phenol and flavonoid contents of 64.82 mg/g and 0.87 mg/g respectively. The highest antibacterial activity was recorded in the ethanol extract with Minimum Inhibitory Concentration (MIC) values of 0.75 mg/ml for *Pseudomonas aeruginosa* and 1.0 mg/ml for *Staphylococcus aureus*. MIC values of 1.75 mg/ml and 3.0 mg/ml were recorded for *Epidermophyton floccosum* and *Trichophyton rubrum* in ethanol extract. Antioxidant activity was more in ethanol extract, which showed Inhibitory Concentration (IC_{50}) values of 0.5 mg/ml, 0.1 mg/ml and 0.9 mg/ml for DPPH scavenging activity, lipid peroxidation inhibitory activity and superoxide scavenging activity respectively. The result indicates that ethanolic leaf extract of *A. paniculata* shows potent antibacterial, antifungal and antioxidant activities.

INTRODUCTION: Plants have been an important source of medicine for thousands of years. Plants are considered not only as dietary supplement to living organism but also traditionally used for treating many health problems. The use of plants for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in the preparation of synthetic drugs, over 25% of prescribed medicines in industrialized countries are derived directly from plants [1]. The use of medicinal plants in developing countries is increasing, which offer a new source of antibacterial, antifungal and antioxidants agents. The higher plants are used to treat a number of infectious diseases around the world. The World Health Organization (WHO) also considers phytotherapy in its health programs and suggested basic procedures for validation of drugs from plant origin in developing countries [2]. The development of drug resistance as well as appearance of undesirable side effects of

certain drugs has led to the search of new antimicrobial agents in particular from medicinal plants. Literature indicates that medicinal plants are the backbone of traditional medicine. Indigenous plants are reservoirs of various metabolites and provide unlimited source of important chemicals that have diverse biological properties [3].

Almost all organisms are well protected against free radical damage by the presence of enzymes or compounds such as ascorbic acid, tocopherols and glutathione. When the mechanism of antioxidant protection becomes unbalanced by several internal and external factors, deterioration of physiological functions may occur requiring the system to depend on exogenous antioxidants from natural sources. Several synthetic antioxidants such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT) have restricted use in foods as they are suspected to be carcinogenic [4]. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years.

Andrographis paniculata Nees. belongs to the family Acanthaceae and is an important medicinal plant in India, China and Thailand. It is an annual, branched, erect herb whose aerial parts (stem and leaves) are used in traditional medicine. The plant is claimed to possess immunological, antibacterial, anti-inflammatory, antithrombotic, hepatoprotective, hypoglycaemic and hypotensive properties [5].

The importance of search and exploitation of natural antimicrobials and antioxidants of plant origin has greatly increased in recent years. Though, in traditional medicine *A. paniculata* has been claimed to possess antimicrobial and antioxidant properties, very few studies have shown the antibacterial and antioxidant activity and to our knowledge no study has been carried out to know its antifungal property against dermatophytes. In all the studies carried out till now on *A. paniculata*, the whole plant has been used. So, the aim of the present study was to investigate the antibacterial, antifungal and antioxidant activities of *A. paniculata* leaves in various solvent systems and to determine the solvent that best extracts the compounds responsible for these activities.

MATERIALS AND METHODS:

Plant Material: Fresh and healthy leaves of *A. paniculata* were obtained from local growers during 2009. The sample specimen was identified based on the taxonomical characteristics and deposited in the herbarium of department of Applied Botany, University of Mysore. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were subsequently dried in a hot air oven at 40^{o}C for 48 h, powdered and used for extraction.

Test Microorganisms: The bacteria used were clinical isolates causing urinary tract infections (UTI) namely, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella spp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterobacter aerogens*. The test fungi used were dermatophytes, namely, *Trichophyton rubrum and Epidermophyton floccosum*. Both the bacteria and the fungi were obtained from Department of Microbiology, JSS Medical College, Mysore. The bacterial and the fungal cultures were maintained on nutrient agar medium and saborauds dextrose agar (SDA) medium respectively.

Preparation of Aqueous Extract: Fifty grams of powdered leaves of *A. paniculata* were macerated with 100 ml sterile distilled water in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120^{o}C for 30 min. The extract was preserved aseptically in a brown bottle at 4^{o}C until further use.

Preparation of Solvent Extract: Fifty grams each of the powdered material was extracted initially with 300 ml of chloroform, hexane, methanol and ethanol separately for 24 h at 23±2^{o}C. The extract was filtered with sterile whatman filter paper into a clean conical flask. Second extraction was carried out with same amount of solvent for another 24 h at 23±2^{o}C and filtered. The extracts were later pooled and transferred into the sample holder of the rotary flash evaporator for the evaporation of the solvents.

The evaporated extract so obtained was weighed and preserved at 4^{o}C in airtight bottle until further use. 10

mg each of dried solvent extract was dissolved in 1 ml of respective solvent and used for the antibacterial assay.

Determination of Total Phenolic Content: Total soluble phenolic content was estimated by Folin-Ciocalteau method [6]. The extracts were oxidized with Folin Ciocalteau reagent and were neutralized with sodium carbonate. The absorbance of the blue color was measured at 650 nm in a spectrophotometer against a reagent blank. The concentrations of the total phenolic compounds in the extracts were obtained by extrapolating the absorbance of gallic acid on standard gallic acid graph. The concentration of total phenols was expressed as mg/g of dry extract.

Determination of Total Flavonoid Content: The total soluble flavonoid content was estimated by Spectrophotometeric method [7]. 0.5 ml of stock solution (1g/ml) of the extract, 1.5 ml methanol, 0.1 ml potassium acetate (1M) was added to reaction tubes and volume was made up to 5 ml with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Total flavonoid content was calculated by extrapolating the absorbance of reaction mixture on standard curve of rutin. The total flavonoid content was expressed as equivalent to rutin in mg/g of the extracts.

Antibacterial Screening: The antibacterial activity was carried out by disc diffusion method. Bacterial cultures (adjusted to 1 x 106 CFU/ml using spectrophotometer) were used to lawn nutrient agar plates evenly using a sterile swab. The plates were dried for 15 min and sterile discs (5 mm in diameter, Whatman No.1) impregnated with 10 µl of the plant extracts were placed on the nutrient agar surface. 10 µl of the respective solvent served as the negative control. Streptomycin standard antibiotic disc served as the positive control (10 µg/disc). The plates were then incubated at 37 ^{0}C for 18 – 24 h. After overnight incubation the plates were examined for the zone of inhibition.

Determination of Minimum Inhibitory Concentration (MIC): Broth dilution method was used to find out the MIC values. The test organisms were grown in nutrient broth medium to a concentration of 1 x 10^6 CFU/ml. 0.5 ml of extract (0.25 - 2 mg/ml) was mixed with 4 ml of nutrient broth inoculated with 0.5 ml of bacterial suspension. The tubes containing 4.5 ml of broth and 0.5 ml of bacterial suspension served as bacterial control and 5 ml of un-inoculated broth served as blank. The tubes were incubated at 37^{o}C for 18 h. Inhibition of bacterial growth was determined by measuring the absorbance at 600 nm in a colorimeter. The lowest concentration of the compound that inhibits the growth of the organism was determined as the MIC. The percentage of growth inhibition was calculated according to the formula:

$$\text{Percent growth inhibition} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Antifungal activity assay: The ethanol extract was selected for antifungal activity assay by mycelial dry weight method [8]. The dermatophytes grown on SDA medium for a week were flooded with 0.85% saline. After settling of the larger particles, conidia were counted with a haemocytometer and diluted in saborauds dextrose broth to a final spore concentration of 1x10^6 spores/ml.

For anti-dermatophytic assay in broth, 5 ml of sterile saborauds dextrose broth medium taken in screw capped tubes were inoculated with 20 µl of fungal suspension and 1-5 mg/ml concentration of the extract. The tubes were incubated for a week at 30^{o}C. The visible mycelia growth in the tubes expressed the degree of activity of the extract. Fungal mycelia from the above tubes were separated by passing through Whatmann No. 1 filter paper. The filter paper was allowed to dry at 60 ^{0} C to reach a constant weight. Fungal growth inhibition was calculated by considering the control and sample mycelial dry weights. The percentage of growth inhibition was calculated according to the formula:

$$\textbf{\% growth inhibition} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Anti-oxidant Activity Assays:

Total Antioxidant Activity Assay: Total antioxidant activity was carried out by using the ferrous reducing antioxidant power (FRAP) assay [9]. The FRAP solution contained 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml 2, 4, 6-tripyridyl-s-tri-azine (10 mM solution in 40 mM HCl) and 2.5 ml Ferric Chloride (20 mM). The temperature of the solution was raised to 37 ^{0}C before

use. 0.15 ml of plant extracts were allowed to react with 2.85 ml of the FRAP solution for 30 min in the dark condition. Readings of coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Results were expressed in µM Fe (II)/g dry mass and compared with that of a synthetic antioxidant Butylated Hydroxytoluene (BHT).

DPPH radical scavenging activity: The free radical scavenging ability of the extracts was determined using DPPH assay [10]. Briefly, 0.1 ml of test sample at different concentration (0.1 - 0.9 mg/ml) was mixed with 0.9 ml of Tris-HCl buffer (pH 7.4) and 1 ml of DPPH (500 µM in ethanol). The mixture was shaken vigorously and left to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm in a spectrometer and compared with that of Butylated Hydroxyanisole (BHA). Radical scavenging potential was expressed as IC_{50} values, which represents the sample concentration at which 50% of the radicals are scavenged. The percentage of DPPH scavenging was calculated using the following formula:

Percent Scavenging = [($A_{control}$ – (A_{sample} - $A_{sample\ blank}$ / $A_{control}$] x 100

Lipid Peroxidation Inhibitory Activity: The lipid peroxidation inhibitory activity was determined in a liposome model [11]. In brief, egg lecithin (3 mg/ml phosphate buffer, pH 7.4) was sonicated in an ultrasonic sonicator for 10 min to ensure proper liposome formation. 0.1 ml of test samples of different concentrations (0.1- 0.9 mg/ml) was added to 1 ml of liposome mixture, the control was without test sample. Lipid peroxidation was induced by adding 10 µl of ferric chloride (400 mM) and 10 µl of L-ascorbic acid (200 mM).

After incubation for 1 h at 37^{o}C the reaction was stopped by adding 2 ml of hydrochloric acid containing 15% trichloroacetic acid and 0.375% of thiobarbutyric acid. The reaction mixture was subsequently boiled for 15 min, cooled, centrifuged at 1000 rpm for 15 min and the absorbance of the supernatant was measured at 532 nm and compared with that of BHA. Inhibitory activity was expressed as IC_{50} value, which represents the sample concentration at which 50% lipid peroxidation inhibition takes place. Percentage radical scavenging was calculated using the following formula:

Percent Inhibition = [($A_{control}$ – (A_{sample} - $A_{sample\ blank}$ / $A_{control}$] x 100

Superoxide radical scavenging activity: The superoxide scavenging ability was assessed according to the method of Nishikime *et al*. [12] with slight modifications. The reaction mixture contained **N**itroblue tetrazolium (0.1 mM) and Nicotinamide adenine dinucleotide (0.1 mM) with or without sample to be assayed in a total volume of 1 ml of Tris-HCl buffer (0.02 M, P^{H} 8.3). The reaction was started by adding Phenazine methosulphate (10 µM) to the mixture, and change in the absorbance was recorded at 560 nm every 30 sec for 2 min. The percent inhibition was calculated against a control without test sample. Radical scavenging potential was expressed as IC_{50} value, which represents the sample concentration at which 50% of the radicals are scavenged. The results were compared with that of quercetin. The percentage inhibition of superoxide anion generation was calculated using the following formula:

Percent Scavenging = [($A_{control}$ – (A_{sample} - $A_{sample\ blank}$ / $A_{control}$] x 100

Statistical Analysis: The experimental results are expressed as mean ± standard deviation (SD) of triplicate measurements. The data was subjected to One Way Analysis of Variance (ANOVA) and the significance of differences between the sample means was calculated by Turkey's post hoc test. Data was considered statistically significant at P value ≤ 0.001. Statistical analysis was performed using Graph Pad statistical software.

RESULTS AND DISCUSSION:

Total Phenol and Flavonoid Content: The result of total phenolic and flavonoid content of *A. paniculata* leaves in different solvent extracts is shown in **Table 1**. Ethanol extract had the highest phenol and flavonoid content of 64.82±1.35 mg/g and 0.87±0.06 mg/g respectively followed by methanol. The lowest phenol and flavonoid content was found in the chloroform extract. Polyphenols and flavonoids are the plant secondary metabolites and are very important by virtue of their antimicrobial [13] and antioxidant activity [14].

The result from the present study indicates that the phenolic compounds and flavonoids are better extracted with ethanol than with other solvents.

Phenolic acids and flavonoids are generally extracted using alcohols, water or a mixture of water and alcohols [15].

The result obtained from our study may vary from the earlier report which has shown lower phenolic content in A. *paniculata* plant [16]. Significant differences between the plants are likely due to genotypic and environmental differences within species, choice of parts tested, time of taking samples and determination methods.

TABLE 1: POLYPHENOL AND FLAVONOID CONTENT OF *ANDROGRAPHIS PANICULATA* LEAVES IN DIFFERENT SOLVENT EXTRACTS

Solvents	Total phenols (mg/g)	Flavonoid content (mg/g)
Aqueous	17.53 ± 0.68^{a}	0.13 ± 0.01^{ab}
Ethanol	64.82 ± 1.35^{d}	0.87 ± 0.06^{d}
Methanol	47.84 ± 2.05^{c}	0.49 ± 0.02^{c}
Hexane	23.23 ± 2.27^{b}	0.21 ± 0.03^{b}
Chloroform	14.96 ± 1.19^{a}	0.11 ± 0.02^{a}

Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (α = 0.001)

Antibacterial activity of solvent extracts: The antibacterial activity of different solvent extracts against the pathogenic bacteria showed varied levels of inhibition. As shown in **Table 2**, among the solvent extracts tested, ethanol extract had a broad spectrum of activity and showed the highest zone of inhibition against *P. aeruginosa* (15.0±1.74 mm) and *S. aureus* (13.0±2.64 mm). The least zone of inhibition for all the tested bacteria was observed with the chloroform extract. The results obtained in the present study indicate that the ethanol extract is more active against the pathogenic bacteria and has a broad spectrum activity.

This indicates the involvement of more than one active principle of biological significance [17]. In the present study, we have shown the highest polyphenol and flavonoid content in ethanol leaf extract. As polyphenols and flavonoids are to known to exhibit antibacterial activity, the antibacterial activity of ethanol extract can be said to be due to the presence of these compounds [18]. The variation in the antimicrobial activity of different solvents can be rationalized in terms of the polarity of the solvents used, polarity of the compounds being extracted by each solvent and, in addition to their extrinsic bioactivity and by their ability to dissolve or diffuse in the media used in the assay [19].

TABLE 2: ZONE OF INHIBITORY ACTIVITY (IN MILLIMETER) OF DIFFERENT SOLVENT EXTRACTS OF *ANDROGRAPHIS PANICULATA* LEAVES AGAINST UTI CAUSING BACTERIA

Extract	*E. coli*	*E. aerogenes*	*Klebsiella sp.*	*P. vulgaris*	*S. aureus*	*P. aeruginosa*
Aqueous	2.0 ± 2.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.0 ± 1.0^{a}	3.0 ± 1.73^{a}	4.0 ± 1.72^{a}
Ethanol	11.0 ± 1.0^{bc}	10.0 ± 1.73^{c}	8.0 ± 3.0^{bc}	9.0 ± 1.70^{bc}	13.0 ± 2.64^{b}	15.0 ± 1.74^{c}
Methanol	8.0 ± 2.64^{b}	6.0 ± 1.73^{b}	5.0 ± 1.0^{b}	7.0 ± 2.0^{b}	9.0 ± 1.0^{b}	10.5 ± 1.80^{b}
Hexane	3.0 ± 1.0^{a}	1.0 ± 1.0^{a}	0.0 ± 0.0^{a}	2.0 ± 1.0^{a}	4.0 ± 1.73^{a}	4.5 ± 1.32^{a}
Chloroform	1.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.2 ± 0.26^{a}	3.0 ± 1.0^{a}
Streptomycin10µg/disc	15.0 ± 2.0^{c}	14.0 ± 1.73^{d}	11.0 ± 1.72^{c}	12.0 ± 1.73^{c}	11.0 ± 2.0^{b}	14.0 ± 1.70^{bc}

Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (α = 0.001)

Determination of MIC: As ethanol extract showed potent antibacterial activity against both gram positive and gram negative organisms, the determination of MIC values was carried out only with ethanol extract. MIC values for *P. aeruginosa* and *S.aureus* were found to be 0.75 mg/ml and 1.0 mg/ml respectively **(Table 3)**. MIC values for other bacteria tested were found to exceed 2.0 mg/ml. The result from the present study indicated that, *P. aeruginosa* was more susceptible to the ethanol extract followed by *S. aureus*. This is in contrary with the earlier reports, which have shown that, most antibacterial medicinal plants attack gram positive strains than gram negative strains because of their permeability differences [20, 21]. The possible mechanism for their broad spectrum activity against both gram positive and gram negative bacteria may due to their ability to complex with cell wall [22].

TABLE 3. MINIMUM INHIBITORY CONCENTRATION (MIC) OF *ANDROGRAPHIS PANICULATA* LEAF ETHANOL EXTRACT USING BROTH DILUTION METHOD

Bacteria	Control	Concentration (mg/ml)				
		0.25	0.5	0.75	1.0	2.0
E. coli	0.27 ± 0.05^{c}	0.27 ± 0.01^{c}	0.17 ± 0.02^{b}	0.14 ± 0.03^{ab}	0.13 ± 0.00^{ab}	0.11 ± 0.00^{a}
E. aerogenes	0.30 ± 0.02^{d}	0.29 ± 0.01^{d}	0.24 ± 0.01^{c}	0.19 ± 0.02^{bc}	0.15 ± 0.01^{ab}	0.14 ± 0.01^{a}
Klebsiella sp.	0.25 ± 0.00^{c}	0.24 ± 0.01^{bc}	0.20 ± 0.02^{ab}	0.20 ± 0.02^{ab}	0.18 ± 0.02^{a}	0.16 ± 0.01^{a}
P. vulgaris	0.25 ± 0.01^{c}	0.24 ± 0.00^{c}	0.20 ± 0.00^{b}	0.19 ± 0.01^{b}	0.16 ± 0.00^{a}	0.15 ± 0.01^{a}
S. aureus	0.22 ± 0.04^{e}	0.21 ± 0.01^{de}	0.15 ± 0.00^{cd}	0.09 ± 0.02^{bc}	0.03 ± 0.01^{ab}	0.02 ± 0.00^{a}
P. aeruginosa	0.14 ± 0.03^{b}	0.13 ± 0.02^{b}	0.06 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.01 ± 0.01^{a}

Values are absorbance at 600 nm. Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (α = 0.001)

Antifungal activity assay by mycelial dry weight method: Table 4 represents the MIC values of *A. paniculata* leaf ethanol extract against two dermatophytes, *T. rubrum* and *E. floccosum*. Among the two fungi, *E. floccosum* was found to be more susceptible to the ethanol leaf extract with an MIC value of 1.75 ± 0.05mg/ml (74.6% growth inhibition) than *T. rubrum* which had an MIC value of 3.0 ± 0.04 mg/ml (70.9% growth inhibition). Aerial parts of *A. paniculata* have been known to possess diterpenoids, flavonoids and polyphenols [23]. In the present study, we have shown that the ethanol leaf extract of *A. paniculata* has the highest polyphenol and flavonoid content. As these polyphenols and flavonoids are known to possess antimicrobial activity, the antifungal activity of the extract could be related to the effect of these compounds. Based on our review of current literature, there are no previous reports showing the antifungal activity of *A. paniculata* leaf ethanol extract on dermatophytes.

TABLE 4: MINIMUM INHIBITORY CONCENTRATION (MIC) OF *ANDROGRAPHIS PANICULATA* LEAF ETHANOL EXTRACT AGAINST DERMATOPHYTES USING MYCELIAL DRY METHOD METHOD

	T. rubrum		*E. floccosum*	
	MIC (mg/ml)	% inhibition	MIC (mg/ml)	% inhibition
Ketaconazole	0.4 ± 0.01^{a}	90.3	0.25 ± 0.02^{a}	93.0
Extract	3.0 ± 0.04^{b}	70.9	1.75 ± 0.05^{b}	74.6

Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (α = 0.001)

Antioxidant Activity Assays: Ferrous reducing antioxidant power assay: The ferrous ion reducing ability of *A. paniculata* leaf extract in different solvents is summarised in **Table 5**. The ethanol extract showed a total antioxidant activity of 59.01 ± 1.06 µM Fe (II)/g which was higher than the other solvent extracts tested but lower than that of BHT (63.25 ± 0.35 µM Fe (II)/g).

From the results obtained, it was found that ethanol had the highest antioxidant activity which may be due to the increased concentration of polyphenols than the other solvent extracts. There was a direct correlation between the polyphenol content and total antioxidant activity. The results are in agreement with an earlier study which has shown the better efficiency of polyphenols in reducing ferric ions [24].

TABLE 5: TOTAL ANTIOXIDANT ACTIVITY OF *ANDROGRAPHIS PANICULATA* SOLVENT EXTRACTS

Solvents	Total antioxidant activity (µM Fe(II) / g)
Aqueous	15.71 ± 0.59^{a}
Ethanol	59.01 ± 1.06^{d}
Methanol	43.70 ± 1.71^{c}
Hexane	21.83 ± 0.77^{b}
Chloroform	16.10 ± 1.49^{a}
BHT	63.25 ± 0.35^{e}

Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (α = 0.001)

DPPH Scavenging Activity: Figure 1 represents the DPPH radical scavenging activity of *A. paniculata* leaf extracts in different solvents. Ethanol extract showed the highest scavenging ability of 53.16% at 0.5 mg/ml (IC_{50} value). Methanol extract showed a scavenging ability of 53.86% at a higher concentration of 0.9 mg/ml (IC_{50} value).

The synthetic antioxidant BHA had an IC_{50} value of 0.0053 mg/ml. IC_{50} values could not be achieved with other solvent extracts tested in this assay. The underlying principle in this assay is the relatively stable DPPH radical is reduced in an alcoholic solution by the presence of hydrogen donating antioxidants. The ethanol extract recorded the highest phenolic and flavonoid content and also had the highest scavenging activity. There was a linear correlation between the antioxidant activity and total phenolic and flavonoid content of *A. paniculata* leaves. This result was consistent with the earlier study which reported such positive correlations between total phenolic content and antioxidant activity [25].

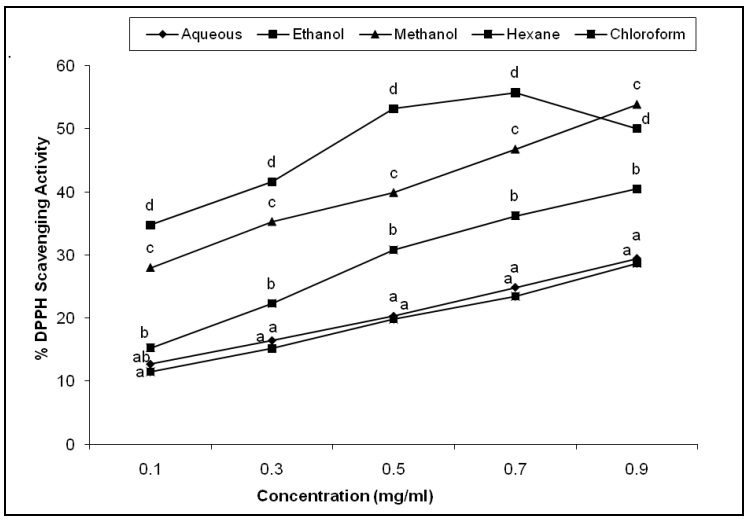

FIG. 1: DPPH SCAVENGING ACTIVITY OF *A. PANICULATA* LEAF EXTRACTS AT DIFFERENT CONCENTRATIONS (MG/ML)

Lipid Peroxidation Inhibitory Activity: The lipid peroxidation inhibitory activity of *A. paniculata* leaf extracts in the liposome model is shown in **Figure 2**. Ethanol extract had an IC_{50} value as low as 0.1 mg/ml and showed a lipid peroxidation inhibition of 58.73% which was followed by methanol with an IC_{50} value of 0.7 mg/ml. BHA showed a very strong lipid peroxidation inhibitory activity with an IC_{50} of 0.012 mg/ml. As with other extracts tested 50% inhibition could not be reached even at higher concentrations.

Antioxidant effect of flavonoids on lipid peroxidation is the result of scavenging of hydroxyl radicals at the stage of initiation and termination of peroxyl radicals. It is reported that lipid peroxidation can be inhibited by flavonoids, possibly through their activity as strong oxygen scavengers and singlet oxygen quenchers [26]. The inhibitory activity of the ethanol extract in this study was better than other solvent extracts tested even at lower concentration. This indicates that, the phenols and flavonoids in the ethanol extract were responsible for this inhibitory activity. There was a gradual decrease in the inhibition of lipid peroxidation even with the increase in the concentration of the extract which may be due to degradation or peroxidation of the source itself [27].

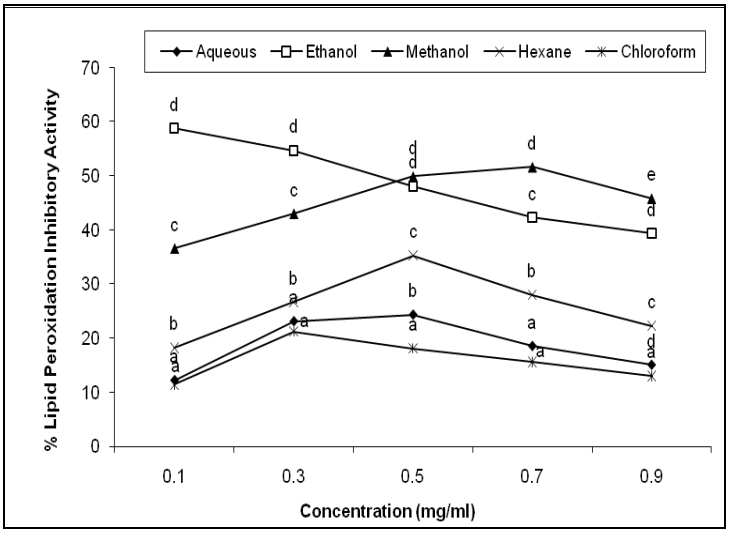

FIG. 2: LIPID PEROXIDATION INHIBITORY ACTIVITY OF *A. PANICULATA* LEAF EXTRACTS AT DIFFERENT CONCENTRATIONS (MG/ML)

Superoxide Scavenging Activity: The superoxide radical scavenging ability of *A. paniculata* leaf extracts in different solvents is depicted in **Figure 3**. There was a scavenging of 51.06% of superoxide anions with ethanol extract at a concentration of 0.9 mg/ml (IC_{50} value). Quercetin was found to be more potent having an IC_{50} value 0.155 mg/ml.

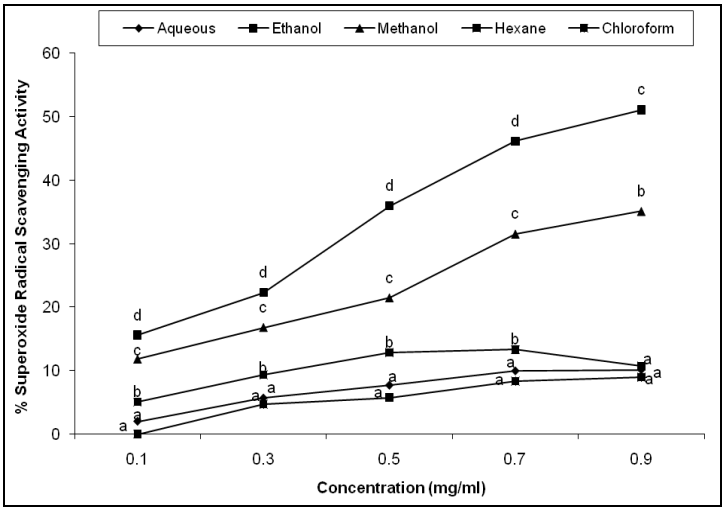

FIG. 3: SUPEROXIDE SCAVENGING ACTIVITY OF *A. PANICULATA* LEAF EXTRACTS AT DIFFERENT CONCENTRATIONS (MG/ML).

Even though other solvent extracts showed superoxide scavenging, IC_{50} values could not be achieved with the concentration used in this assay. Superoxide radicals are generated during the normal physiological process mainly in mitochondria. Although, superoxide anion is by itself a weak oxidant, it gives rise to the powerful and dangerous hydroxyl radicals as well as singlet oxygen both of which contribute to oxidative stress [28]. Therefore, superoxide radical scavenging by antioxidants has physiological implications.

From the present study, it was shown that IC_{50} values could be reached only with ethanol extract at a higher concentration. But there was a positive correlation with the flavonoid content and superoxide scavenging activity of the extract which may be due to the presence of flavonoids [29].

CONCLUSION: In the present study, ethanolic extract of *A. paniculata* leaves gave the highest phenolic and flavonoid contents and showed potent antibacterial and antifungal activity against UTI causing bacteria and dermatophytes respectively. This explains the use of *A. paniculata* in folk medicine for treatment of infectious diseases. It was evident from the present study that, ethanolic extract of *A. paniculata* leaves showed strong lipid peroxidation inhibitory activity, DPPH scavenging activity and superoxide scavenging activity. On the basis of the results obtained, it can be concluded that ethanol is the best solvent for extracting antimicrobial and antioxidant bioactive compounds (phenols and flavonoids). Further research is needed to identify the nature and number of individual phenolic compounds and the existence of possible synergism if any, among these compounds.

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REFERENCES

1. Newman DJ, Cragg GM and Snader KM. The influence of natural products upon drug discovery. Natural Product Reports 2000; 17(3): 215-234.
2. Anushia C, Sampathkumar P and Ramkumar L. Antibacterial and antioxidant activities in *Cassia auriculata*. Global Journal of Pharmacology 2009; 3(3): 127-130.
3. Tomoko N, Takashi A, Hiromi T, Yuka I, Hiroko M, Munekazu I, Totshiyuki T, Tetsuro I, Fujio A, Iriya I, Tsotomu N and Kazuhito W. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. Journal of Health Sciences 2002; 48: 273-276.
4. Basniwal PK, Sutha, M, Rathore GS, Gupta R, Kumar V, Pareek A and Jain D. *In-vitro* antioxidant activity of hot aqueous extract of *Helicteres isora Linn*. Fruits. Natural Product Radiance 2009; 8(5): 483-487.
5. Mishra KS, Sangwan SN and Sangwan SR. *Andrographis paniculata* (kalmegh): A review. Pharmacognosy Reviews 2007; 1(2): 283-298.
6. Malick CP and Singh MB. Plant Enzymology and Histo Enzymology. New Delhi: Kalyani Publishers; 1980.
7. Woisky R and Salatino A. Analysis of Propils: Some parameters and procedure for chemical quality control. Journal of Agricultural Research 1998; 37: 99-105.
8. Rasooli I and Abyaneh R. Inhibitory effects of thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. Food Control 2004; 15: 479-483.
9. Benzie IFF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 1996; 239: 70-76.
10. Moon JH and Terao J. Antioxidant activity of caffeic acid and dihydrocafeic acid in lard and human low density protein. Journal of Agricultural and Food Chemistry1998; 46: 5062-5065.
11. Duh PD and Yen GH. Antioxidative activity of three herbal water extracts. Food Chemistry 1997; 60: 639-645.
12. Nishikimi M, Rao NA and Yagi K. The occurrence of superoxide anion in the reduced phenazine methosulphate and molecular oxygen. Biochemical and Biophysical Research Communication 1972; 46: 849-864.
13. Igbinosa OO, Igbinosa EO and Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcad* (Linn). African Journal of Pharmacy and Pharmacology 2009; 3(2): 58-62.
14. Annegowda HV, Ween Nee C, Mordi MN, Ramanathan S and Mansor SM. Evaluation of phenolic content and antioxidant property of hydrolysed extracts of *Terminalia catappa* L. leaf. Asian Journal of Plant Sciences 2010; 9: 479-485.
15. Tsao R and Deng S. Separation procedures for naturally occurring antioxidant phytochemicals. Journal of Chromatography B 2004; 812: 85-99.
16. Tanwer BS, Choudhary R and Rekha V. *In vivo* and *In vitro* comparative study of primary metabolites and antioxidant activity of *Andrograhphis paniculata*. Journal of Chemical and Pharmaceutical Research 2010; 2(2): 489-495.
17. Vaghasiya Y and Chanda VS. Screening of methanol and acetone extracts of 14 Indian medicinal plants for antimicrobial activity. Turk Journal of Biology 2007; 31: 243-248.
18. Kathad HK, Shah RM, Sheth NR and Patel KN. *In vitro* antioxidant activity of leaves of *Garuga pinnata* Roxb. International Journal of Pharmaceutical Research 2010; 2(3): 9-13.
19. Anjana S, Rani V and Padmini R. Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens. World Applied Science Journal 2009; 7(3): 332-339.
20. Priscilla IU, Mariama TNS, Luiz CDS, Luciano B and Ary FJ. Antibacterial activity of medicinal plant extracts, Brazilian Journal of Microbiology 2007; 38: 717-719.
21. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Srisha P and Rama SV. Antibacterial activity of plants used in Indian herbal medicine. International Journal of Green Pharmacy 2010; 4(1): 22-28.

22. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999; 12: 564-582.
23. Rao YK, Vimalamma G, Rao CV and Tzeng Y. Flavonoids and andrographolides from *Andrographis paniculata*. Phytochemistry 2004; 65: 2317-2321.
24. Wong SP, Leong LP and Koh JHW. Antioxidant activities of aqueous extracts of selected plants. Food Chemistry 2005; 99(4), 775-783.
25. Tawaha K, Alali FQ, Gharaibeh M, Mohammad M and El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chemistry 2007; 104: 1372-1378.
26. Bergman M, Perelman A, Dubinsky Z and Grossman S. Scavenging of reactive oxygen species by a novel glucurinated flavonoid antioxidant isolated and purified from spinach. Phytochemistry 2003; 62: 753-762.
27. Bendich A, Machlin LJ, Scandurra O, Burton GW and Wayner DDM. The antioxidant role of vitamin C. Adv. Free Radical Biology and Medicine 1986; 2: 419-444.
28. Pietta PG. 2000. Flavonoids as antioxidants. Journal of Natural Products 2000; 63: 1035-1042.
29. Robak J and Gryglewski IR. 1988. Flavonoids are scavengers of superoxide anions. Biochemical Pharmacology 1988; 37: 837-841.

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