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RESEARCH ARTICLE

Phytochemical screening and In vitro assessment of antimicrobial and antioxidant potential of *Andrographis serpyllifolia* - An endemic medicinal plant from South India.

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Abstract

The current study was aimed to investigate the phytochemical composition, antibacterial and antioxidant activity of A. serpyllifolia plant extracts. Phytochemical screening revealed the presence of alkaloid, tannins, steroids, terpenoids, phlobatannin, anthraquinones flavonoids, saponins and phenolic compounds. Quantitative analysis revealed the presence of saponins (4.2%) in high concentration followed by tannins (4.12%), phenolics (1.4%), alkaloids (1.2%) and flavonoids (0.98%). This study will provide phytochemical information for preparation of concentrated and effective extract of A. serpyllifolia. Petroleum ether, chloroform, benzene and methanol extracts of shade dried plant parts of A. serpyllifolia were tested for antibacterial activity against six strains of bacterial species, viz., Bacillus subtilis, *Escherichia coli*, *Proteus*, *Pseudomonas aeruginosa*, *Klebsiella* sps and *Staphylococcus aureus* using the standard agar disk diffusion method. All the extracts have shown significant activity against tested microbes with the inhibition zone ranged of 5.7-16.8mm. Among various solvent extracts studied petroleum ether extract showed a highest antibacterial activity followed by methanol, benzene, chloroform. Antioxidant potential of methanol extracts was determined by DPPH and ABTS free radical scavenging assay. The extracts showed a very good antioxidant property and the IC_{50} value was found to 268.12µg/ml for DPPH assay and 398.46µg/ml for ABTS assay. Ascorbic acid taken as control showed highest antioxidant power in the present study. The results suggest that A. serpyllifolia has promising antioxidant activity and could serve as potential source of natural antioxidants

1. Introduction

The herbal medicine represents one of the most significant fields of traditional medicine all over the world. Because increase in bacterial infections and their treatment due to indiscriminate use of commercial antibacterial drugs and development of multiple drug resistance in most of the microorganisms, the investigation of natural materials as sources of new antibacterial agents has been increased from the past 20 years. The source of the therapeutic effects to identify diverse medicinal plants have been tested, and to identify innovative substances that are active towards pathogens with high resistance, as a result novel antibacterial drugs have been accepted some natural products (Cragg *et al*., 1997**).** The natural products of higher plants may give possible new mechanisms of action with novel source of antimicrobial agents (Barbour *et al*., 2004**).** Each and every part of the plant is valuable in medicine and numerous preparations have been mentioned in indigenous system of medicine for several diseases anti-spasmodic, anti-periodic, stimulant, diaphoretic, bronchial catarrh, bronchitis, psoriasis, small pox, scabies and other ulcers and itchy skin eruptions (Behl *et al*., 1993). Plants are invaluable sources of pharmaceutical products and are recognized

for their ability to produce a wealth of secondary metabolites and mankind has used many plant species for centuries as antimicrobial, antiviral, antioxidant, antianalgesic and antidiabetic agents (Phillipson, 1994). India is known for its rich diversity of medicinal plants and hence called botanical garden of the World (Vedavathy *et al.,* 1997). Currently, despite the wide availability of clinically useful antibiotics, semisynthetic analogues, a continues search for new anti-infective agents remains indispensable because some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum and serious side effects. In addition, the emergence of resistant pathogens to many of the commonly used antibiotics has provided an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome the problems of resistance to currently available antimicrobial agents (Balandrin *et al*., 1985; Xu and Lee, 2001). Antibacterial alternatives for different pathogenic bacterial strains were always in focus because of their infectious nature and bacteria have ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Throughout the world the research work has been carried out on antimicrobial studies on different medicinal plants including *Andrographis Nees* (Chinnappan A, 2011), *Acacia nilotica* (Deshpande and Kadam, 2013), *Betula pendula* and *Ageratum houstonianum* (Bowers, 1976), *Calotrophis gigantean*, *Tinospora cordifolia*, *Withania somnifer*, *Adhatoda vasica* and *Nelumbo nucifera* (Hemraj Vashist and Anil Jinda, 2012). These studies have indicated the usefulness of the plants for control of resistant strains of bacteria. The drugs obtained from plants are less toxic; side effects are scanty and also cost effective.

Many indigenous and endemic plant species of India have been used in folk medicine to treat various ailments of man. Available reports tend to show that indigenous folk-medicinal plant preservation and study is vital because such plants are fully adapted to local environments (Qin and Xu, 1998). Pharmacologically active compounds and phytochemicals isolated from such endemic and indigenous plants used in folk medicine have been the center of interest during the past few decades (Farnsworth, 1994; Benzi & Ceci, 1997). Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. A number of phytochemicals isolated from plant material are used in the pharmaceutical drug industry today. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). These phytochemicals possess antioxidant activities, which prevent or can be used in the treatment of many diseases, including cancer. In the last few years, a number of studies have been conducted in different countries to demonstrate such efficacy (Benoit-Vical *et al.,* 2006). Being organisms devoid of mobility, plants have evolved elaborate alternative defense strategies, which involve an enormous variety of chemical metabolites as tools to overcome stress conditions (Hemalatha *et al.,* 2013).

Oxidative stress, a result of imbalance between the antioxidant defense system and the formation of ROS, may induce damage to cellular biomolecules such as DNA, RNA, proteins, enzymes, carbohydrates, and lipids through oxidative modification and contributing to the pathogenesis of human diseases (Prakash *et al.,* 2007). This imbalance leads to many diseases, such as brain dysfunction, cancer, heart diseases, agerelated degenerative conditions, declination of the immune system, cancer, coronary arteriosclerosis, ageing processes, carcinogenesis, gastric ulcer and DNA damage (Singh *et al.,* 2012; Venkata *et al.,* 2012). Antioxidants are the compounds that can delay or inhibit the oxidation of biomolecules by regulation of oxidative chain reactions (Arya and Yadav, 2011). The antioxidative effects are mainly due to secondary metabolite components like phenolic components. The phenolic compounds have antioxidative activity mainly due to their redox potential, which plays an important role in absorbing and neutralizing free radical**s** (Butkhup *et al.,* 2011; Coulidiati *et al*., 2011). Many researchers have investigated powerful antioxidants from natural herbal sources to prevent the reactive oxygen species related disorders in human as well as replace the synthetic compounds (Alam *et al*., 2012).

Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum, Piper cubeba Linn., Allium sativum Linn., Terminalia bellerica, Camellia sinensis Linn., Zingiber officinale* and several Indian and Chinese plants. The majority of the antioxidant activity is due to the Phenolics, flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Aqil *et al.,* 2006). Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Devasagayam *et al.,* 2004). Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability.

Acanthaceae are mostly herbs or shrubs comprising 250 genera and 2500 species. *Andrographis serpyllifolia* is an erect herb widely distributed in foot hills and moist forests of South India. *A. serpyllifolia* has been used in Indian traditional and Chinese herbal medicine for digestive problems, snake bites and infections ranging from malaria to dysentery. Therefore, the current phytochemical, antibacterial and antioxidant studies was aimed at validating the effects of endemic medicinal plant *A. serpyllifolia* (Vahl) Wight which belong to the family *Acanthaceae*. In this study we have evaluated the phytochemical profiles to correlated possible antibacterial and antioxidant properties of this plant.

2. Materials and methods

Collection of plant material

The plants Andrographis serpyllifolia were collected from local forest of Hassan district, Karnataka state, India during the month of October-November. The taxonomic identities of plant was confirmed by using flora of Hassan District and voucher specimen were deposited in National Ayurveda Dietetics Research Institute, Bangalore (Central Council for Research in Ayurvedic and Siddha, Department of AYUSH, Ministry of Health and F.W, Govt. of India, New Delhi) (No. RRCBI-5206 - *A. serpyllifolia*)

Preparation of plant extracts

Freshly collected plant materials were first washed with tap water and then thoroughly with distilled sterile water. Then it was shed dried for 5-7 days. Dried plant materials (10g) of the plant species were separately crushed and ground into fine powder using blender. Each powdered plant material was extracted separately using different organic solvents (100-150 ml); such as petroleum ether, chloroform, benzene and methanol at 40-60^{o} C in a Soxhlet apparatus for 5h.Concentrated plant extract were prepared by removing excess of solvent and used for further experiments (Vasu Kandati et al., 2012).

Phytochemical screening of *A. serpyllifolia*

Powdered specimens of leaves, twigs and roots of both plants were subjected to qualitative and quantitative phytochemical screening using standard procedures as described by Sofowara (1993). The plant extracts of A. serpyllifolia were analyzed for the presence of flavonoids, alkaloids, steroids, phenols, saponins, terpenoid, anthraquinones and tannins according to the standard methods (Mondal *et al.*, 2013).

Test for alkaloids

Alkaloids are basic nitrogenous compounds with definite, physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added (Siddiqui and Ali, 1997). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2 % hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent (Evans, 2002). Turbidity or yellow precipitation in the samples indicates the presence of alkaloids in the extracts.

Test for phenols

1 ml of methanol extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5 % ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds (Ramya *et al.*, 2012)

Test for Tannins

About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate. Development of brownish-green or blue-black color indicates the presence of tannins (Iyengar *et al.*, 1995).

Test for steroids

1 ml of methanolic extract was dissolved in 10 ml of the chloroform and equal volume of concentrated H2SO4 is carefully added by sides of the test tubes. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids (Siddiqui and Ali, 1997).

Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously; formation of emulsion indicates the presence of saponin (Kalita *et al.*, 2011).

Test for anthraquinones

0.5 gm of crude powder was shaken with 10 ml of benzene and was filtered. 0.5 ml of 10 % ammonia was added to the filtrate and was shaken well and the development of a pink, red or violet color in the ammonical (lower) phase indicated the presence of the anthraquinones (Sodipo *et al.*, 1990).

Test for terpenoids

Five ml of each methanolic extract was mixed with 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to the mixture to form a layer. Formation of reddish brown coloration at the inter face shows positive results for the presence of terpenoids (Sanjay *et al.,* 2013)

Test for flavonoids

About 1 g of powdered sample was heated with 10 ml of methanol over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids (Edeoga *et al.,* 2005)

Test for phlobatannins

Deposition of red precipitate when an aqueous extract of each plant sample was boiled with 10 % aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins (Ajayi *et al.,* 2011)

Quantitative determination of the chemical constituency

Alkaloid determination

5g of the each sample material was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

Determination of total phenols

1g of Plant samples were boiled in 10 ml of 80% methanol for15 min at 70º C (Zieslin and Ben-Zaken, 1993). One ml of methanolic extract was added to 5 ml of distilled water and 250µl of F.C. reagent and incubated for 3 min. Then 1 ml of saturated Sodium carbonate (Na_2CO_3) was added and the mixture was again incubated for 1 h at 25^oC. The absorbance of the developed blue colour was measured using a spectrophotometer at 725nm. Phenol was used as the standard and the amount of phenolics was expressed mg / 100gm of dry weight.

Tannin determination

1g of the sample was weighed into a 100 ml of water and shaken for 1 h in a mechanical shaker. This was filtered into a 100 ml volumetric flask and made up to the mark. 5 ml of filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 NaCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10min (Van-Burden and Robinson, 1981). The total tannic acid content was expressed as mg of tannic acid equivalent per gram of extract.

Saponin determination

10 g of each sample were put into a conical flask and 100 ml of 20%aqueous ethanol was added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55° C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel containing 20 ml of diethyl ether and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated by adding 60 ml of n-butanol to aqueous part. The n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven, weighed expressed as mg per gram of dry weight and weight (Obadoni *et al.,* 2001).

Flavonoid determination

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42. The filtrate was later transferred into a crucible and evaporated into dryness over water bath and dried to a constant weight and expressed as percentage (Boham *et al.,* 1974).

Antimicrobial screening

Test culture

The pathogenic strains used for the screening antibacterial activity, such as *Bacillus subtilis*, *Escherichia coli*, *Proteus*, *Pseudomonas aeruginosa*, *Serratia marganni* and *Staphylococcus aureus* were obtained from Shekar Diagnostic Laboratory, Hassan, Karnataka, India. Cultures were maintained as nutrient agar slants in screw-capped bottles and stored at 4°C. All cultures were checked for viability and purity by regular plating and biochemical tests. Test cultures were prepared by transferring a loop full of bacteria from stock culture nutrient broth and incubated at 37°C for 24 h.

Antibacterial bioassay procedure

The antibacterial activities were determined using the agar disk diffusion method (Xu & Lee, 2001; Mahasneh, 2002). This method is highly effective for rapidly growing microorganisms, and the activities of the test extracts are expressed by measuring the diameter of the zone of inhibition. Sterilized filter paper disks (5mm in diameter) were impregnated in appropriate concentration of each plant extract. The disks (made from Whatman No.1) were allowed to absorb the plant extracts as described by Mahasneh (2002). Plates of Mueller-Hinton sensitivity agar were aseptically inoculated with broth cultures for the test organisms using sterile Pasteur pipette. The plates were allowed to evaporate. The disks containing the plant extract were transferred using sterilized forceps onto the surface of the seeded agar plates under aseptic condition. They were sufficiently spaced to prevent the resulting zones of clearing from overlapping. The extractive solvents were used as a negative control. The plates with the organisms were incubated for 24 h. After incubation, the growth inhibition rings were quantified by measuring the diameter for the zone of inhibition to the nearest millimeter from the lower surface of the Petri dishes. Inhibition zone values were corrected, that is, disk diameter was subtracted from the value of the inhibition zone. As the diameter of the disk was 5mm, inhibition zones of less than 6mm were not evaluated. Negative control disks contained only the solvent. A standard antibiotic Chloromycin (50 µg/ml) was used as positive control for comparison. The experiments were performed three-times to minimize errors and mean values are presented.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity

The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β- picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour.

The free radical scavenging capacity of the methanol extracts was determined using DPPH (Molyneux, 2004). The DPPH solution (0.006% w/v) was prepared in 95% methanol. Different concentrations of the test sample which is to be examined for antioxidant activity is prepared (50-300µg/ml). 3 ml of different concentration of test sample of *A. serpyllifolia* extracts were mixed with 1 ml of DPPH solution in dark. Ascorbic acid which is strong anti-oxidizing agent is taken as standard. 3 ml of different concentration of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and plant extracts samples was incubated for half an hour and then absorbance is taken with the help of U.V. Spectrophotometer at 517 nm. Methanol serves as a blank and the experiment was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula;

$$\textbf{DPPH radical scavenging activity (\%)} = \frac{\textbf{Control OD} - \textbf{Sample OD} \times 100}{\textbf{Control OD}}$$

ABTS (2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

ABTS assay was performed according to the protocol Re *et al.*, 1999. The stock solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution followed by incubation for 12 h at room temperature in the dark to yield a dark-colored solution containing ABTS radicals. Working solution was prepared freshly before each assay by diluting the stock solution by mixing of stock solution to 50% methanol for an initial absorbance of about 0.700 (± 0.02) at 745 nm, with temperature control set at 30°C. Varying concentrations (50-3000µg/ml) of the plant methanolic extracts were allowed to react with 3 ml of the ABTS solution and the absorbance readings were recorded at 734 nm (Re *et al.*, 1999). Ascorbic acid was used as positive controls. The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula

$$\textbf{ABTS radical scavenging activity (\%)} = \frac{\textbf{Control OD} - \textbf{Sample OD}}{\textbf{Control OD}} \times 100$$

3. Statistical analysis

All the experiments were repeated for five independent observations. Data are presented as mean ± SEM. Data from laboratory studies were analyzed separately for each experiment and were subjected to analysis of Variance (ANOVA) (SPSS Software). All the experiments were performed in triplicate and analyzed according to DMRT ($P<0.05$).Values are expressed as mean± Standard error.

4. Results

Phytochemical screening

The phytochemical components of plant are summarized in **Table 1**. This reveals moderate concentration of alkaloids, tannins, saponins, phenols, flavonoids and phlobatannins were present in *A. serpyllifolia,* some of which chemical compounds have been associated to antibacterial activities and thus have curative properties against pathogens. Quantitative estimation of the percentage crude chemical constituents in this medicinal plant studied is shown in **Table 2.** The total alkaloid and phenol content was found to be 1.2 and 1.4 % respectively. Total tannin content was found to be higher (4.12 %) compared to flavonoids (0.98%).

Comparative antibacterial property evaluation

The results of the antimicrobial screening assay of the different solvent extracts of *A. serpyllifolia* against the tested strains are shown in **Table 3**. Results showed that the methanol, petroleum ether, and benzene extracts of both plants showed a broad spectrum of activity, being active on Gram +ve and Gram –ve organisms. The zones of inhibition ranged from 8.7-15 mm for petroleum ether extract, 10.7-16.8 mm for methanol extracts. In case of chloroform and benzene extracts the inhibition zones were ranged from 5.7-10 mm and 6-11 mm respectively. The methanolic and petroleum ether extracts were found to be most active against tested bacterial strains. The efficacy of the extract was however, directly proportional to its concentration, higher the level of its aqueous dilution and lesser the effective zone of bacterial inhibition.

In vitro Antioxidant activity

The free radical scavenging activity of methanolic extracts of was assessed by DPPH and ABTS assay. Figure 3 and 4 shows the significant decrease in the concentration of free radicals due to the scavenging ability of *A. serpyllifolia*. The scavenging activity was compared with that of Ascorbic acid. At a concentration of 300µg/ml the DPPH scavenging activity of extracts was 50%, while the standard ascorbic acid exhibited 80 % (**Fig. 1**). The IC_{50} value of DPPH assay was 268.12µg/ml

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants. The *A. serpyllifolia* extracts efficiently scavenged ABTS radicals generated by the reaction between 2, 2-azinobis (3-ethylbenothiazoline-6-sulfonic acid) and ammonium persulfate (**Fig. 2**). The activity was found to be increased in a dose dependent manner from 12-40% at a concentration of 50-300 µg/ml. the extract exhibited an IC_{50} value of ABTS assay was 398.46µg/ml.

Table-1: Qualitative analysis of the phytochemical components of *Andrographis serpyllifolia* leaves, twigs and roots.

Plant	***A. serpyllifolia***
Alkaloid	+
Tannin	+
Steroids	-
Phenols	+
Terpenoid	+
Phlobatannin	+
Flavonoid	+
Anthraquinones	+
Saponins	+

+, Presence of the compound; **-,** Absence of the compound;

Table 2: Percentage of crude alkaloids, phenols, tannin, flavonoids, and saponins on the medicinal plant investigated.

Phytochemicals	*A. serpyllifolia*
	Quantitative estimation (%)
Total Alkaloid	1.2
Total Tannin	4.12
Total Phenols	1.4
Total Flavonoid	0.98
Total Saponins	4.2

Data are means ± standard deviation of triplicate determinations. Means followed the same superscript in each column are not significant (P<0.05).

Table 3: Antibacterial activities of *Andrographis serpyllifolia* plant extracts against test bacteria.

Solvents	Test Bacteria	Zone of inhibition in diameter (mm) (Mean ± SE)
		A. serpyllifolia
Petroleum ether	*Escherichia coli*	12.3 ± 0.4^{ef}
	Proteus	11.2 ± 0.38^{de}
	Bacillus subtilis	15 ± 1.27^{fg}
	Staphylococcus aureus	8.7 ± 0.52^{bc}
	Klebsiella species	12 ± 0.78^{ef}
	Pseudomonas aerugenosa.	11 ± 0.57^{de}
Benzene	*Escherichia coli*	11 ± 0.62^{de}
	Proteus	-
	Bacillus subtilis	9.7 ± 0.34^{cd}
	Staphylococcus aureus	6 ± 0.5^{a}
	Klebsiella species	-
	Pseudomonas aerugenosa.	10 ± 0.78^{d}
Chloroform	*Escherichia coli*	-
	Proteus	5.7 ± 0.6^{a}
	Bacillus subtilis	7.7 ± 0.9^{bc}
	Staphylococcus aureus	-
	Klebsiella species	10 ± 1.0^{d}
	Pseudomonas aerugenosa.	-
Methanol	*Escherichia coli*	16.8 ± 1.67^{gh}
	Proteus	11.7 ± 0.79^{ef}
	Bacillus subtilis	15.7 ± 1.3^{g}
	Staphylococcus aureus	10.7 ± 0.9^{de}
	Klebsiella species	12 ± 1.1^{ef}
	Pseudomonas aerugenosa.	13 ± 0.9^{efg}

Concentration of the extract used: 100mg/ml. **-,** Absence of zone of inhibition. The values are the average of three independent experiments. The means followed by same letter (s) are not significantly different according to DMRT ($P \leq 0.05$).

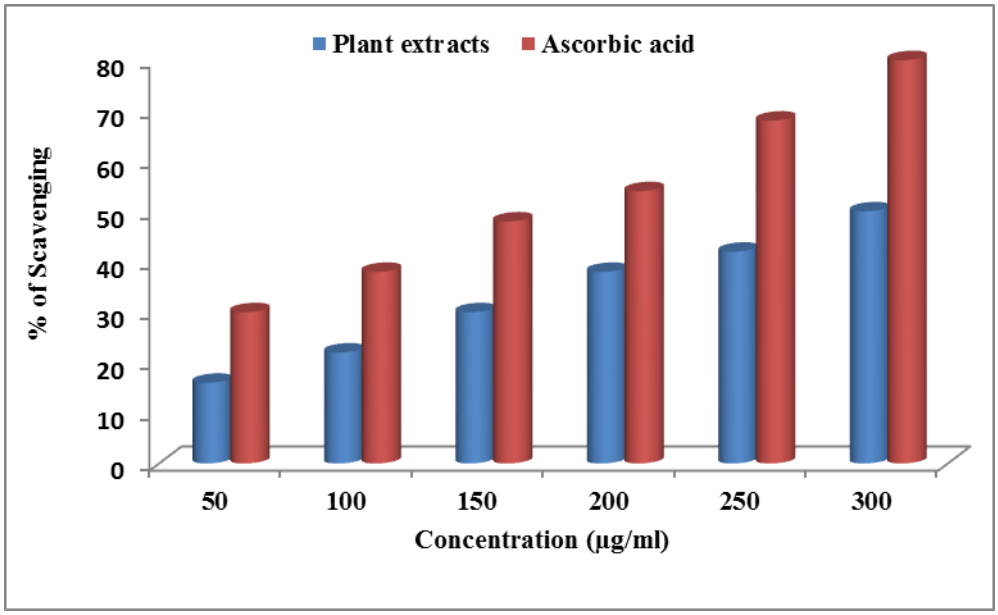

Figure.1 DPPH scavenging activities of various concentrations of methanolic extract of *Andrographis serpyllifolia* and Ascorbic acid.

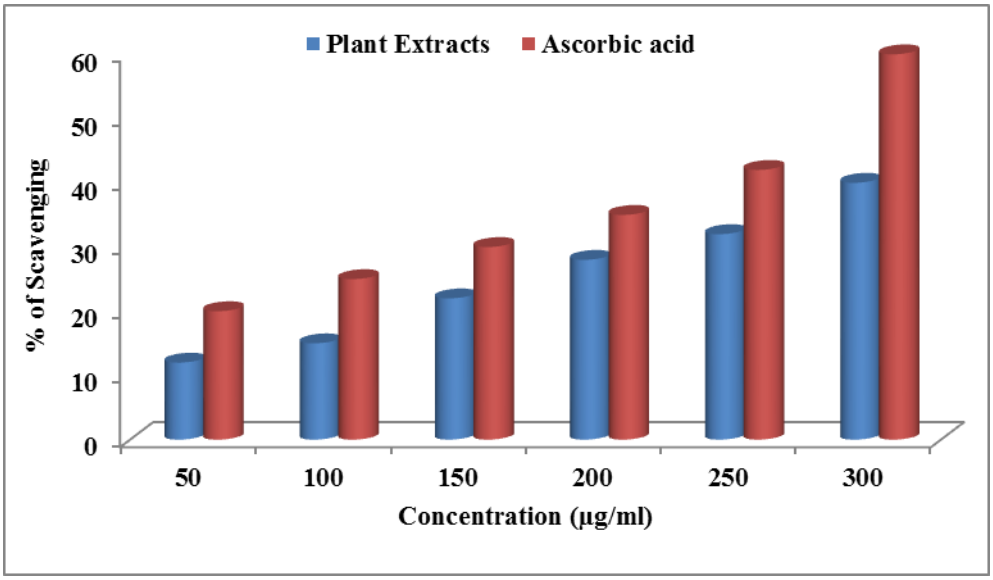

Figure.2 ABTS scavenging activities of various concentrations of methanolic extract of *Andrographis serpyllifolia* and Ascorbic acid

5. Discussion

Antimicrobial activities of medicinal plants are obviously related to differences in their contents of active compounds (Boakye *et al.*, 1975). Available reports tend to show that alkaloids and flavonoids are the major responsible compounds for the antimicrobial activities in higher plants (Cordell *et al*., 2001). Moreover, it is also claimed that secondary metabolites such as tannins and other compounds of phenolic nature are classified as active antimicrobial compounds (Rojas *et al*., 1992). Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by this compound (Prasad *et al*., 2008). The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the studied plant showed that the leaves, stems and roots were rich in alkaloids, flavonoids, tannins, phenols and saponins. The presence of these secondary metabolites in *A. serpyllifolia* confirms their antibiotic properties and usefulness by the traditional medicine practitioners. Flavonoids are also known to have a wide array of therapeutic activities as antihypertensive, anti-rheumatism, antimicrobial,

diuretic and antioxidants (Trease and Evans, 2002). Search for plants exhibiting antimicrobial activity has increased in recent years, and several reports on this subject have been reported (Bagghi, 2000). In this present work the plant showed significant antibacterial activity. The differences observed in the antimicrobial activities assays suggest the susceptibility of the test microorganisms to various secondary metabolites present in these endemic plants. The composition of these secondary metabolites in turn varies from species to species, climatic conditions, and the physiological state of developments of the endemic plants (Hussain & Deeni, 1991). Also, in view of the fact that prevalence of *S. aureus* resistant strains to conventional antibiotics has increased to high levels of some hospitals (Shalit *et al*., 1989; Usman *et al*., 2007) and that *S. aureus* is a pyogenic bacterium known to play significant role in invasive skin diseases including superficial and deep follicular lesion (Srinivasan *et al*., 2007). The extract could serve as a remedy to such resistance bacterium. The extract has also showed the same level of activity against *E. coli* which is the commonest cause of urinary tract infection and accounts for approximately 90% of urinary tract infection in young women. This study also provides some validity for the use of the plant parts in Indian traditional medicine and as a source of chemotherapeutic agents of grate novelty if studied well to harness the potential in the plant.

Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants of free radical scavengers (Khan and Sultana, 2006). The DPPH antioxidant assay provides information on the reactivity of the test compounds with a stable free radical. The degree of reduction in absorbance measurement is indicative of the radical scavenging potential of the extract. The present study shows that *A. serpyllifolia* methanolic extract is a rich source of natural antioxidants that can be important in disease prevention. Oyedemi *et al*., (2010) attributed the antioxidant activity observed in the aqueous stem bark extract of *S. henningsii* to the presence of flavonoids, phenols and proanthocyanidins. These compounds have been reported in several studies of medicinal plants to quench free radicals or decompose formation of peroxides owing to the presence of conjugated rings or carboxylic acid (Oyedemi SO, Afolayan, 2011). Hence scavenging activity of ABTS and DPPH radicals by the *A. serpyllifolia* plant extracts was found to be considerable; which implies that it may be useful for treating radical related pathological damage especially at higher concentrations.

6. Conclusion:

The plant studied here contains potential antimicrobial components that may be used for development of pharmaceutical for therapy of infections. The methanols, petroleum ether, chloroform and benzene extracts of *A. serpyllifolia* possess significant inhibitory effect against the tested pathogens. The results of the study support the folkfore claim of this plant. The *in vitro* antioxidant assays indicate that these plant extracts are a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

References

Ajayi, I. A., Ajibade, O. and Oderinde, R. A. (2011): Preliminary Phytochemical Analysis of some Plant Seeds. Research Journal of Chemical Sciences. 1(3): 58-62.

Alam, B.M., M.S. Rahman, M. Hasan, M.M. Khan, K. Nahar and S. Sultana (2012): Antinociceptive and Antioxidant activities of the *Dillenia indica* bark. Int. J. Pharm., 8:243-251.

Aqil, F. I. Ahmed, Z. Mehmood, Turk. (2006): J. Biol., 30:177-183.

Arya, V. and J.P. Yadav (2011): Antioxidant properties of the methanol extracts of the leaves, seeds and stem of *Cassia occidentalis*. Res. J. Med. Plant., 5: 547-556.

Bagghi, A.K. (2000): Alternative medicine- Old wine in a new bottle. Indian Medicine Association. 98: 332-333.

Balandrin, M.F., Kjocke, A.J. and Wurtele, E. (1985): Natural plant chemicals: sources of industrial and mechanical materials. Science. 228:1154-1160.

Barbour, E.K., Al Sharif, M., Sagherian, V.K., Habre, et al., (2004): Screening of selected indigenous plants of Lebanon for antimicrobial acitivity. J. Ethnopharmacol. 93:1-7.

Behl, P.N., Arora, R.B., Srivastava, G. and Malhotia. (1993): Herbs Useful in Dermatological therapy. CBS Publishers and Distributor, Delhi.

Benoit-Vical, F., Grellier, P., Abdoulaye, A., Moussa, I., Ousmane, A. and Berry, A. (2006). *In vitro and* in *vivo* Antiplasmodial Activity of *Momordica balsamina* Alone or in a Traditional Mixture. Chemotherapy, 52:6:288-292.

Benzi, G. and Ceci, A. (1997): Herbal medicines in European regulation. Phamacolog. Res. 35: 355-362.

Boakye-Yiadom, K. and G.H. Konning, (1975): Incidence of antibacterial activity in the Connaraceae. Planta Med., 28: 397-400.

Boham, B.A., Kocipai, A.C. (1974): Flavonoids and condensed tannins from leaves of Hawaiian *vaccinium vaticulatum* and *V. calycinium*. Pacific Sci., 48: 458-463.

Bowers, W.S., Ohta, T., Cleere, J.S. & Marsella, P.A. (1976): Discovery of insect anti-juvenile hormone in plants. Science. 193:542-547.

Butkhup, L. and S. Samappito (2011): *In vitro* free radical scavenging and antimicrobial activity of some selected Thai medicinal plants. Res. J. Med. Plant, 5: 254-265.

Chinnappan, A. (2011): Antimicrobial Potential and Phytochemical Screening of *Andrographis Affinis Nees* - An Endemic Medicinal Plant From India. International Journal of Pharmacy And Pharmaceutical Sciences. 3 (2):157-159.

Coulidiati, T.H., H. Millogo-Kone, A. Lamien-Meda, M. Yougbare-Ziebrou, J. Millogo-Rasolodimby and Nacoulma, O.G. (2011): Antioxidant and antibacterial activities of two Combretum species from burkina faso. Res. J. Med. Plant., 5: 42-53.

Cordell, G. A., Quinn-Beattie, M. L. & Farnsworth, N. R. (2001): The Potential of Alkaloids in Drug Discovery. Phytotherapy Research., 15:183-205.

Cragg, G.M., Newman, D.J., Suader, K.M. (1997): Natural Products in drugs discovery and development. Journal of Natural Products. (60):52-60.

Deshpande, S.N., Kadam, D.G.(2013): Phytochemical analysis and antibacterial activity of *Acacia nilotica* Against *Streptococcus Mutans*. International Journal of Pharmacy and Pharmaceutical Sciences. 5 (1):236-238.

Devasagayam, T. P. A., Tilak, J.C., Boloor, K.K. (2004). Review: Free radicals and antioxidants in human health: Curr Stat Fut Prosp. JAPI, 52: 794-804.

Edeoga, H.O., D. E. Okwu, and B.O Mbaebie. (2005): Phytochemical constituents of some Nigerian medicinal Plants. African Journal of Biotechnology. 4 (7):685-688.

Evans, W.C. (2002): Treasure and Evan's Pharmacognosy. Haarcourh Brace and Company. Gamble, J.S. 1915-1936. Flora of the Presidency of the Madras. London: Adlard & Sons Ltd.

Harborne, J.B. (1973). Phytochemical methods, London. Chapman and Hall, Ltd. 49-188.

Hemraj, V. and Anil, J. (2012): Antimicrobial activities of medicinal plants – review. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3 (1): 222-230

Hill, A. F. (1952): Economic Botany. A textbook of useful plants and plant products. 2nd edn. McGraw Hill Book Company Inc. New York 205.

Hussain, H.S., Deeni, Y.Y. (1991): Plants in Kano ethnomedicine; screening for antimicrobial activity and alkaloids. Int. J. Pharmacognosy., 29: 51- 56.

Iyengar, M. A. (1995): Study of Crude Drugs, Manipal Power Press, Manipal, India.8*th*edition, 2.

Farnsworth, N.R. (1994): Ethnopharmacology and drug development. Ciba Foundation Symposium. 185: 42-59.

Khan, T.H. and Sultana, S. (2006): Apigenin induces apoptosis in Hep G2 cells: possible role of TNF-alpha and IFN-gamma. Toxicology. 217:206–212.

Mahasneh, A.M (2002): Screening of some indigenous Qatari medicinal plants for antimicrobial activity. Phytotherapy Rese. 16(8): 751-753.

Molyneux, P. (2004): The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 26:211–219.

Mondal, S., Marouthu.I.P.Pushyami and P. Suresh. (2013): Toxicity studies of ethanol extract from *Ixora pavetta andrews* leaf. World Journal of Pharmacy and Pharmaceutical Sciences. 3(1):350-360.

Hemalatha, M., Thirunavukkarasu Thirumalai, Rajamani Saranya, Erusan Kuppan Elumalai, Ernest David. (2013): A review on antimicrobial efficacy of some traditional medicinal plants in Tamilnadu. Journal of Acute Disease. 99-105. doi: 10.1016/S2221-6189(13)60107-9.

Obdoni, B.O., Ochuko. P.O. (2001): Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci. 8b:203-208.

Oyedemi, S.O., G. Bradley and A.J. Afolayan (2010): *In vivo* and *in vitro* antioxidant activities of aqueous stem bark extract of *Strychnos henningsii* (Gilg). Afr. J. Pharm. Pharmacol., 4: 70-78.

Oyedemi, S.O. and A.J. Afolayan, (2011): *In vitro* and *in vivo* antioxidant activity of aqueous leaves extract of *Leonotis leonurus* (L.) R. Br. Int. J. Pharmacol., 7: 248-256.

Phillipson, J.D. (1994): Natural products as drugs. Trans R Soc Trop Med Hyg. 88: 17-19.

Vedavathy, S., Mrudula, V. and Sudhakar, A. (1997): Tribal medicine in Chitoor district, Andhra Pradesh, India, Vedams e Books (P) Ltd.

Prakash, D., Upadhyay, G., Singh, B.N., Singh, H.B., (2007): Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). Food Chem. 104:783–790.

Prasad, N. R. et al., (2008): Preliminary phytochemical screening and antibacterial activity of *Samanea saman*. Journal of Medicinal Plants Research. 2: 268-270.

Qin, G.W., Xu, R.S. (1998): Recent advances on bioactive natural products from Chinese medicinal plants. 18(6):375-82.

Ramya, S., Gopinath, K., Ramaraj, J., Nagoorgani, P., Aruna, D. (2012): Bioprospecting *Solanum nigrum* Linn. (*Solanaceae*) as a potential source of Anti-Microbial agents against selected Bacterial strains. Asian J. of Biomed and Pharma Sciences. 2(12):65-68.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans,C. (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 26:1231-1237.

Rojas, A., Hernandez, Pereda-Miranda, R. and Mata, R. (1992): Screening for antimicrobical of crude drug extracts and pure natural products from Mexican medicinal plants. Journal of Ethnopharmacology. 35: 275-285.

Sanjeeb Kalita, Gaurav Kumar, Loganathan Karthik, Kokati Venkata Bhaskara Rao. (2011): Phytochemical Composition and *In Vitro* Hemolytic Activity of *Lantana Camara* L. (*Verbenaceae*) Leaves. Pharmacologyonline: 59-67

Sanjay, S., Kharat, Pradeep, B., Kumkar Siddhesh, R. Rajpure and Kishor, S. Sonawane. (2013): Qualitative phytochemical screening of *Gnidia glauca* (Fresen) Gilg. Plant Extract. Int J. Pharm Bio Sci. 4(4): 144 – 148.

Siddiqui, A.A., Ali, M. (1997): Practical Pharmaceutical chemistry. 1st edn, CBS Publishers and Distributors, New Delhi, 126-131.

Singh, S., Carg, V., Yadav, D., Beg, M.N., Sharma, N. (2012): *In-vitro* antioxidative and antibacterial activities of various parts of Stevia rebaudiana (bertoni). Int J Pharmacy and Pharm Sci. 4(3):468-73.

Sodipo, O.A, Mohammad, S.L., Nigeria. (1990): Journal of Basic and Applied Science, 4(182) 41-52.

Sofowora, A. (1993): Recent trends in research into African medicinal plants. J Ethnopharmacol. 38(2-3): 209-214.

Srinivasan, R.S., Dillard, M.E., Lagutin, O, V, Lin, F.J., Tsai, S., Tsai, M.J., Samokhvalov, I.M., Oliver, G. (2007): Lineage tracing demonstrates the venous origin of the mammalian lymphatic vasculature. Genes Dev. 21:2422–2432,

Trease, G.E., Evans, W.E. (2002): *Pharmacognosy.* 15th edn. W.B. Saunders Company Limited, London. 585.

Usman, H., F.I. Abdulrahman, and A.A. Ladan. (2007): Phytochemical and Antimicrobial Evaluation of *Tribulus terrestris* L.(Zygophylaceae) Growing in Nigeria. Res. J. Bio. Sci. Medwell Journals. 2(3): 244-247.

Venkata, S.P., Raju, K., Murali, M.C., Hara, S.S, Praneth, D. V.S. (2012): Antioxidant activity and phytochemical analysis of Scolopia *crenata* (*Flacourtiaceae*) stem bark. Int J Pharmacy and Pharm Sci. 4(3):315-19.

Van-Burden, T.P., Robinson, W.C. (1981). Formation of complexes between protein and Tannin acid. J. Agric. Food Chem. 1: 77.

Vasu Kandati, P Govardhan , Ch Siva Reddy, A Ravinder Nath , R. R. Reddy. (2012): *In-vitro* and *in-vivo* anti-inflammatory activity of Andrographis serpyllifolia (Rottl. Ex Vahl.) Wt. International Current Pharmaceutical Journal, 1(8): 199-204.

Vasu, K, Govardhan., Ch. Siva Reddy, A. Ravinder Nath and R. R. Reddy. (2013): *In-vitro* and *in-vivo* anti-inflammatory activity of *Syzygium alternifolium* (wt) Walp. Journal of Medicinal Plants Research. 6(36), 4995-5001, DOI: 10.5897/JMPR12.431.

Xu, H.X., & Lee, S. (2001): Activity of plant flavonoids against antibiotics resistant bacteria. Phytotherapy Research. 15: 39-43.

Zieslin, N. and Ben–Zaken, R. (1993): Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiol. Biochem., 31: 333–339.