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PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF VERNONIA ANTHELMINTICA

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ABSTRACT

Vernonia anthelmintica is a wild medicinal herb distributed throughout the India. It is used as a folk medicine against cough, skin diseases, diarrhea and fever. In the present study, ethanolic extract was obtained from seeds of *Vernonia anthelmintica* and phytochemical analysis was done to screen alkaloids, saponins, steroids, carbohydrates, flavonoids, glycosides, polyphenols and gums and mucilage. Antimicrobial activity was carried out by using four bacterial strains *Staphylococus aureus, E. coli, Pseudomonas aeruginosa, Bacillus subtilis* and two fungal strains such as *Aspergillus niger* and *Trichophyton rubrum.* This ethanolic extract showed good antibacterial activity against *Bacillus subtilis* (20.21±0.8mm) and *Pseudomonas aeruginosa* (19.25±0.6) whereas moderate activity against Staphylococcus aureus (15.34±0.5) and *E. coli* (14.56±0.8). Whereas ethanolic extract showed good anti-fungal activity against Aspergillus flavus (19.64±1.8) and showed moderate activity against Trycophyton rubrum (13.21±1.1) at highest concentration of ethanolic extract 80mg/ml concentration. *V.antihelmintica* seed ethanolic extract showed good free radical scavenging activity at all concentrations of ethanolic extract. Our results clearly saying that seed ethanolic extract showed high content of phytochemicals, highest antimicrobial and antioxidant activity and our results supported the usage of *Vernonia anthelmintica* in folk and traditional medicine.

Keywords: **Vernonia anthelmintica, phytochemicals, DPPH, Minimum Inhibitory concentration, antioxidant activity.**

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INTRODUCTION

Vernonia antihelmintica is a natural annual herb belongs to Asteraceae family and widely distributed throughout India. Microbial infections are an important health problem throughout the world and plants are possible sources of antimicrobial agents (Burapadaja & Bunchoo,1995). The widespread use of herbal remedies and health care preparation, such as those described in the ancient text like the Bible and the Vedas has been traced to the occurance of natural product with medicinal properties in fact; plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines (Nair et al., 2005). In recent times, attention has been reverted back to plants as sources of therapeutic agents due to their higher properties. These include among others reduced cost, relative lower includence of adverse reactions compared to modern conventional pharmaceuticals (Karachi, 2006), and ready availability. From ancient times plants have provided as source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being (Aiyelaagbe et al., 2000 and Nostro et al., 2000). With the rising prevalence of microorganisms developing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants (Jayashree and Maneemegalai, 2008). Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (Meretrk et al., 2006). Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. Diseases that have been managed traditionally using medicinal plants include malaria, epilepsy, infantile convulsion, and diarrhea, and dysentery, fungal and bacterial infections. The present study reported the phytochemical analysis, antimicrobial and antioxidant activities of *Vernonia anthelmintica.*

MATERIALS AND METHODS

Collection of plant material

The seeds of *V. antihelmintica* were collected from the seshachalam forest chittoor district of Andhra Pradesh and authenticated through the Hyderabad, India.

Extraction

The seeds of *V. antihelmintica* were shade dried at room temperature for seven days and pulverized to a coarse powder and extracted with ethanol by using sox let apparatus method. The filtrate was obtained and evaporated to dryness at 50-65^{0}C in a rotary vacuum evaporator to obtain a dark colored molten mass and then stored at 4^{0}C. All chemicals and reagents used were of analytical grade and obtained Sigma.

Phytochemical Analysis of Extract

The methods described by (Harborne, 1998) with slight modifications were used to screen the presence of the active ingredients in the seed ethanolic extract.

Test for Steroids

10ml of the ethanol extract was evaporated to dry mass and dissolved in 0.5 ml of solvent to this added 0.5ml of acetic anhydride and 2ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds (Harborne, 1998).

Test for Terpenoids

The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids (Harborne, 1998).

Test for Tannins

i) 1 cm^3 of freshly prepared 10%KOH was added to 1cm^3 of the extract. A dirty white

precipitate indicated the presence of tannins (Harborne, 1998).

ii) Ethanolic seed extract of the test plant (1.0gm) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% $FeCl_3$ were then added. Production of greenish precipitate indicated the presence of tannins (Harborne, 1998).

Test for Flavonoids
A small piece of magnesium ribbon was added to extract of the plant material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones (Harborne ,1998).

Test for Alkaloids
The extract of the plant sample (0.5g) was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract (Harborne, 1998).

Test for Saponins
Stem bark of the test plant was ground into powder form and 0.5g of the powdered stem bark was introduced into a tube containing 5.0ml of distilled water, the mixture was vigorously shaken for 2min., formation of froth indicated the presence of Saponins (Harborne, 1998).

Test for Glycosides
Coarsely powdered stem bark (1gm) was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5ml of water was added to the other beaker. The two beakers were heated for 3–5min and the contents filtered into labeled test tubes. The filtrate was made alkaline with 5%sodium hydroxide and heated with Fehling's solution for 3min. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides (Harborne, 1998).

Test for Gums and Mucilage
About 10 ml of various extracts were added separately to 25ml of absolute alcohol with constant stirring and then filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates (Harborne, 1998).

ANTIMICROBIAL ACTIVITY

Microorganisms for Antimicrobial activity
The species of bacterial strains such as *Staphylococcus aureus, E.coli, Pseudomonas auriginosa, Bacillus subtilis* and fungal strains such as *Aspergillus flavus* and *Trichophyton rubrum* were obtained from Institute of Microbial Technology (IMTECH), Chandigarh and tested on nutrient agar for bacteria and potato dextrose (PDA) for fungi (Cheesbrough, 1982; Cowan and Steel, 2004) and sub cultured on to nutrient broth for 24hrs prior to testing.

Antimicrobial activity
The agar disc diffusion method was used to determine the antimicrobial activity. The discs (6 mm diameter) impregnated with different concentrations of the extracts were placed on the surface of the petri plates containing 20ml of nutrient agar media for bacterial strains and potato dextrose agar media for fungal strains respectively, seeded with 100µl of microbial cultures (5x 10^5CFU/ml). The plates were incubated for 24hrs at 35 ± 2^0C for bacteria and for 72hrs. For fungi at 30^0C. The inhibition zones formed around the discs were measured and expressed in millimeter.

Minimum inhibitory concentration (MIC)
The minimum inhibitory concentration was determined, using a common broth micro dilution method in 96 well micro titer plates (Cruickshank, 1968 and NCCLS, 1999). From

the previously prepared different microbial suspensions, cultures (10^5CFU/mL) were added to each well. Plates were incubated for 18hrs, at 37^0C and then were examined with Elisa reader (TECAN, Sunrise, China) at 620nm and the lowest concentration of each extract showing no growth was taken as its minimum inhibitory concentrations (MIC). All the samples were tested in triplicate to confirm the activity and the values were noted.

Antioxidant Activity

Evaluation of antioxidant activity was done by using 2, 2'-diphenly-1-picrylhydrazyl (DPPH) method described by (Burits and Bucar, 1999). Antioxidants react with DPPH and convert it to α,α -diphenyl.-β-.picryl hydrazine. Various concentrations of 1 ml of plant extract were added to 3ml of 0.004% methanol solution of DPPH. After 20-30min incubation period at room temperature, the absorbance was read against blank at 517nm. Inhibition of free radical by DPPH in percent (1%) was calculated by using the following equation.The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity. % DPPH radical-scavenging = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] x 100

RESULTS

Phytochemical Analysis

S. No	Phyto chemicals	Ethanolic Extract
1.0	Steroids	+
2.0	Tri terpinoids	+
3.0	Tannins	+
4.0	Flavonoids	+
5.0	Alkaloids	+
6.0	Saponins	+
7.0	Glycosides	+
8.0	Gums and Mucilages	-
9.0	Carbohydrates	+
10	Phenolic compounds	+

Table-1 + Presence of phytochemicals - Absence of phytochemicals

Preliminary phytochemical screening of the seed ethanoic extract of *V.antihelmintica* showed for positive results for the presence of Steroids, Tri terpinoids, Tannins, Flavonoids, Alkaloids, Saponins, Glycosides, Carbohydrates and phenolic compounds, whereas Gums and mucilages was completely absent in ethanolic extract (Table-1).

Antimicrobial Activity

S.No	Organism Name	Inhibition Zone at 20 mgs/ml	Inhibition Zone at 40 mgs/ml	Inhibition Zone at 60 mgs/ml	Inhibition Zone at 80 mgs/ml	MIC
1.	*Pseudomonas aeruginosa*	09.24±3.2	13.76±0.2	17.43±2.6	19.25±0.6	11.21±1.5
2.	*E. coli*	05.16±0.6	08.95±0.1	12.58±1.4	14.56±0.8	16.64±0.5
3.	*Staphylococcus aureus*	03.61±0.8	07.55±0.6	11.74±0.2	15.34±0.5	19.41± 0.1
4.	*Bacillus subtilis*	07.26±1.5	11.46±1.2	14.26±2.1	20.21±0.8	13.42±0.4
5.	*Aspergillus flavus*	06.56±1.1	09.35±1.5	15.41±0.8	19.64±1.8	10.17±0.5
6.	*Trycophyton rubrum*	02.31±0.8	05.38±0.9	08.92±2.1	13.21±1.1	30.45±0.7

Table-2 Zone of Inhibition in (mm); Minimum inhibitory concentration (MIC) in (mgs)

The ethanolic seed extract of *V.anthelmintica* was tested for antimicrobial activity against four bacterial species (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Bacillus subtilis* and two fungal species (*Aspergillus flavus, Trycophyton rubrum*).The antimicrobial activity was evaluated from the Zone of Inhibition. With increasing concentration from 20, 40, 60 and 80mgs/ml of ethanolic extract of an apparent increase of antimicrobial activity was observed. The results revealed that seed ehanolic extract of the *V.anthelmintica* showed good anti-bacterial activity against *Bacillus subtilis* (20.21±0.8mm) and *Pseudomonas aeruginosa* (19.25±0.6) whereas moderate activity against *Staphylococcus aureus* (15.34±0.5) and *E.coli* (14.56±0.8). Whereas ethanolic extract showed good anti-fungal activity against *Aspergillus flavus* (19.64±1.8) and showed moderate activity against *Trycophyton rubrum* (13.21±1.1) at highest concentration of ethanolic extract 80mgs/ml concentration (Table-2).The higher levels of secondary metabolites such as Steroids, Tri terpinoids, Tannins, Flavonoids, Alkaloids, Saponins, Glycosides, Carbohydrates and phenolic compounds in seed ethanolic extract of *Vernonia anthelmintica* could be responsible for antimicrobial activity.

Antioxidant Activity

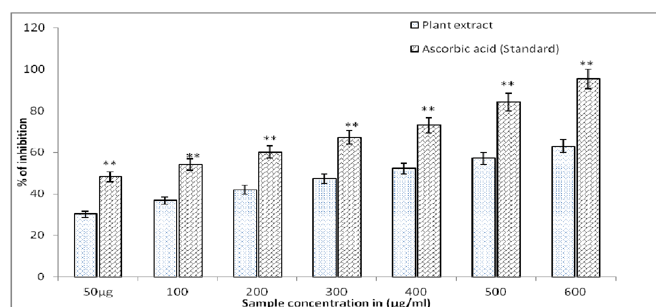

Figure 1
Hydroxyl radical scavenging assay

Hydroxy radical scavenging activities of *V.anthelmintica* seed ethanol extract and reference standard Ascorbic acid. The data represent the hydroxyl radical scavenging. The data mean ± S. D of five parallel measurements. **p<0.05. Seed ehanolic extract of *Vernonia anthelmintica* was checked for antioxidant activity by using DPPH method. With increasing concentration (50, 100, 200, 300, 400, 500 and 600µg/ml) of ethanolic extract and standard (Ascorbic acid) the anti-oxidant activity was also increased along with concentration. The standard Ascorbic acid showed good antioxidant activity than ethanolic extract at all concentrations (Fig-1).The presence of secondary metabolites such as polyphenol and flavonoids could be attributed to the highest antioxidant activity shown by the extract.

DISCUSSION

Our results were clearly revealed that the plant contained different bioactive compounds such as alkaloids, saponins, flavonoids, tannins, polyphenols, steroids; glycosides are connected with defense mechanism against many microorganisms. Similar results were also reported different plant parts such as the bark, roots and leafs extracts were shown good activity against, microorganisms, insects, and other herbivores of plants (Benjamin et al.,

1981, Duke and Wain 1981, Bonjar et al., 2004 and Ram Prasad et al., 2012). The crude extracts of Morinda tentoria and Abutilon indicum are used as a diagnostic tool for testing the adulteration (Atish et al., 2009 and Parekh et al., 2005). The medicinal plants such as *Azadirachta indica, Curcuma longa, Ocimum spp, Morinda citrifolia L, Zingiber officinale, Cassia auriculata* shown good antimicrobial activity (chopra et al., 1956, Chatterjee and Pakrashi, 1995, Nascimento et al., 2000, Ushimarul et al., 2007, Usha et al., 2010 and Maneemegalai and .Naveen, 2010).

CONCLUSION

The bioactive compounds such as of Steroids, Tri terpinoids, Tannins, Flavonoids, Alkaloids, Saponins, Glycosides, Carbohydrates and phenolic compounds were rich in the seeds ethanolic extract of *Vernonia anthelmintica*. Ethanolic extract showed good antimicrobial activity against tested bacterial and fungal strains such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Bacillus subtilis* and two fungal species (*Aspergillus flavus, Trycophyton rubrum*,) and seed ethanolic extract was also shown good antioxidant enzyme activity. Our results authenticate the usage of *Vernonia anthelmintica* by local people for treating skin diseases and against some bacterial and fungal infections. Our studies provide the basis for further isolation and evolution of major active principles present in the plant material and test their efficiency against various infections.

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