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Preliminary phytochemical screening, antioxidant and antimicrobial activity of *Vernonia cinerea* (L.) Less. a member of 'Dashapushpa'

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Abstract
This plant belong to the family Asteraceae. In Malayalam, this plant is known as *Puvamkurunthal*. It is distributed throughout India, as a weed on roadsides and open places. An erect annual herb, 12-75 cm in height with cylindrical branched stem, leaves is variable in shape and flowers are many pinkish violet in small heads. The chief constituents are the triterpenes. This plant contains alkaloids, terpenes and flavanoid. The Ayurvedic Pharmacopoeia of India recommends the plant in intermittent fever, filariasis, blisters, boils, vaginal discharges andgin cases of psychoneurosis. Therefore, the main objectives of the present study are screening of various phytochemicals, antioxidant activity and antimicrobial activity of methanolic whole plant extract of *Vernonia cinerea*. Phytochemical screening showed the presence of alkaloids, terpenoids and flavonoids. Antioxidant activity of the methanolic extract of *Vernonia cinerea* was 88.27% for 1mg/ml and the antimicrobial activity was determined using well diffusion method. The results obtained in this study show high antioxidant potential of *Vernonia cinerea*.

Keywords: *Vernonia cinerea*, phytochemical screening, DPPH, spectrophotometric assay, well diffusion

Introduction
In India, for over three thousand years the Ayurvedic system of medicine has been in use. Charaka and Susruta, two of the earliest Indian authors had sufficient knowledge of the properties of the Indian medicinal plants. The medicinal form is governed by the laws of nature, which suggest that life is a combination of senses, mind, body and soul. This holistic approach gained worldwide acceptance to Ayurvedic treatments.

Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural products, organic chemistry and plant biochemistry. The Challenge of phytochemistry is to carry out the methods which are needed for separation, purification and identification of many different constituents present in plants through operations on small amount of material. When working with medicinal plant, the main goal is to identify constituents showing interesting biological properties.

The potential for developing antimicrobials from plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Bio molecules of plant origin appear to be one of the alternatives for the control of human pathogens.

Antioxidants are those substances which posses free radical chain reaction breaking properties. It has been established that oxidative stress is the major causative factors in the induction of many chronic and degenerative diseases like ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury.

Vernonia cinerea plant belong to the family Asteraceae. In Malayalam, this plant is known as *Puvamkurunthal*. It is distributed throughout India, as a weed on roadsides and open places. An erect annual herb, 12-75 cm in height with cylindrical branched stem, leaves is variable in shape and flowers are many pinkish violet in small heads.

The chief constituents are the triterpenes. This plant contains alkaloids, terpenes and flavanoid. *The Ayurvedic Pharmacopoeia of India* recommends the plant in intermittent fever, filariasis, blisters, boils+, vaginal discharges and in cases of psychoneurosis. The water soluble fraction of the methanol extract of the defatted dried ground whole plant of *Vernonia cinerea* showed significant diuretic activity in rats comparable to lasix a known diuretic. (Arun Raj *et al.*, 2013) [1].

Fig 1: Habit of *Vernonia cinerea* (L.) Less.

The present study was designated to investigate the preliminary phytochemical analysis, evaluation of the antimicrobial and antioxidant activities of the methanolic extract of the whole plant of *Vernonia cinerea*.

Materials and Methods

The plant was collected from naturally growing population of *Vernonia cinerea* Kerala University Campus, Kariavattom, Thiruvananthapuram. Phytochemical test were carried out using standard procedure to identify constituents. The plant was properly identified with the help of authentic literature and documented with their characteristic features and a voucher specimen has deposited in the Department herbaria (KUBH-10122).

Preparation of plant extract

The entire plant was used for the assay. The plant materials were washed and shade dried and chopped into small pieces for grinding. The plant material was powdered in an electric mixer. The powdered plant material was kept in air tight container with proper labeling for future use. The plant powder was extracted in a single solvent methanol. 10g of plant powder was extracted in 200ml of methanol for about 4-5 hours in Sohxlet apparatus at room temperature. The extract was collected and evaporated in an oven at a temperature of 55^0C. It was collected in a Petri dish, weighed and was stored in cold condition for further studies. The dried extract thus obtained was used for the phytochemical analysis, analysis of antimicrobial and antioxidant property using various standard procedures.

Qualitative preliminary phytochemical screening

Phytochemical screening

Chemical test were carried out using standard procedure by Harborne. J. B. 1998 to identify the constituents present in the plant.

Detection of alkaloids

Solvent free extract (50mg) was mixed with few drops of dil.HCl and was then filtered. Test for alkaloids was carried out in this filtrate.

Test 1- Mayer's Test: One or two drops of Mayers reagent (mercuric chloride 1.36g dissolved on 60ml distilled water and mixed in a solution of 5g of potassium iodide in 10ml distilled water) was added to the filtrate through the side of the test tube, formation of white creamy precipitate indicates the presence of alkaloids.

Test 2- Dragendroff's Test: The reagent (0.85g bismuth nitrate dissolved in 40ml distilled water and 10ml glacial acetic acid, followed by addition of 5g potassium iodide dissolved in 20ml distilled water) was added to filtrate. Formation of prominent yellow precipitate indicates the presence of alkaloids.

Test 3-Wagner's Test: Reagent (1.27g of iodine and 2g potassium iodide dissolved in 5ml distilled H_2O) was added to the filtrate. Formation of reddish brown precipitate indicates the presence of alkaloids.

Detection of glycosides

Molisch test: Two ml of the prepared filtrate were mixed with 0.2 ml of alcoholic solution of α-naphthol 10% and 2 ml of sulphuric acid, a reddish violet zone is formed, this indicates the presence of carbohydrates or glycosides**.**

Detection of terpenoids

Salkowski test: Five ml of extract was mixed with 2ml of chloroform and about 3ml of con.H_2SO_4 was carefully added. At the separation level of the two liquids, a reddish-brown ring forms, which indicates the presence of terpenoids.

Detection of carotenoids

About 0.02g of plant extract was mixed with chloroform, mixed well and then the mixture was filtered. To the filtrate, conc.H_2SO_4 was added, formation of a blue colour at the interface indicate the presence of carotenoids.

Detection of steroids

Libermann-Burchard test: One ml of extract was treated with 0.5ml of acetic anhydride and 1ml of H_2SO_4 carefully. A colour change from violet to blue or green indicates the presence of steroids.

Detection of saponin

Foam test: About 0.5gm of extract was mixed with 2ml of distilled water and heated for few minutes and filtered. The filtrate was vigorously shaken. The persistent froth was observed for 10 minutes, this indicates the presence of saponins.

Detection of flavanoids

The extract was shaken with 1ml of dilute ammonia solution and con. H_2SO_4. Formation of yellow colour indicates the presence of flavanoids.

Detection of phenol

To the plant extract, a few drops of 1% aqueous or alcoholic ferric chloride was added. The formation of bluish-black colour indicates the presence of phenol.

Detection of quinone

One ml of the plant extract was mixed with 5ml of con. HCl. The formation of yellow precipitate indicates the presence of quinone.

Detection of tannin

The sample was mixed with distilled water and boiled for 5 minutes and was filtered and was used for the test.

Two drops of 10% ferric chloride was added to 1ml of the filtrate. Formation of bluish or greenish or brownish black colour indicates the presence of tannins.

Spectrophotometric assay for the evaluation of antioxidant activity of methanolic plant extract using DPPH

Free radical scavenging activity by DPPH and spectrophotometric assay

DPPH radical scavenging activity of methanolic extract of the plants were tested for the antioxidant activity. The H-donor activity of the extract was estimated in this method. Different concentrations of methanol extract ranging from 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml was prepared. Hundred ml of DPPH radical solution in methanol was freshly prepared. One ml extract of different concentrations was added to 2ml of DPPH solution and the reaction mixture was incubated at 37ºC for 20 minute. The absorbance was read at 517nm against positive control which do not contain the extract. The assay was carried out in triplicate. A decrease in absorbance of DPPH solution indicates an increase in DPPH scavenging activity. The activity is given as percentage DPPH radical scavenging.

$$\% \text{ inhibition} = \left(\frac{Ab - As}{Ab}\right) X\ 100$$

Ab- absorbance of control
As- absorbance of sample

Determination of antimcrobial activity

Five samples of Multi Drug Resistant strains of clinical isolates were used for the study.

- *Pseudomonas aeruginosa*
- *Escherichia coli*
- *Proteus mirabilis*
- *Staphylococcus aureus*

All these samples were obtained from the Department of Microbiology, Pushpagiri Institute of Medical Sciences, Thiruvalla, Kerala, India. The cultures were grown on nutrient agar and stock cultures were maintained at 4^0C. In all the experiments, the evaluation of the antibacterial activity was screened with 18hrs nutrient broth and was incubated at 37^0C for 18hrs. Identification was done based on colony characteristics, gram staining, tube coagulase test etc.

Well diffusion method

Agar diffusion method was employed to evaluate the antimicrobial activity. Wells of standard size (6mm) were incised at specified distances in nutrient agar and 18hrs old nutrient broth cultures adjusted to 0.5 McFarland of the selected strains were swabbed on separate agar plates. Hundred µl each of the extracts prepared in 1ml (100mg extract mixed in 1ml DMSO) was added to separate wells. Hundred percentage DMSO was served as negative control. Antibiotic of standard quality was severed as positive control. After incubation at 37^0C for 24 hours, diameter of zone of inhibition was measured and consequently antimicrobial activity was assessed. Sensitivity of the strains used for the study against antibiotics was determined using Kirby-Bauer disc diffusion method as per CLSI guidelines.

Results

Phytochemical evaluation

Preliminary phytochemical analysis of methanolic extracts of the plants was done by using various preliminary analysis tests. Preliminary phytochemical analysis is done to identify the major groups of phytochemical present in the plant samples. Preliminary analysis results are shown in the table (Table.1).

Table 1: Preliminary screening of methanolic extract of whole plant of *Vernonia cinerea*

	Methanolic extract of *Vernonia cinerea*
Alkaloid	++
Glycosides	-
Terpenoid	+
Carotenoid	-
Steroid	-
Saponin	-
Flavanoid	+
Phenol	-
Quinone	-
Tannin	-

'+' sign indicate presence and **'-'** sign indicate absence. Number of '+' sign indicate the intensity

The methanolic extract of the plant showed the presence of different phytochemicals like alkaloids, terpernoids and flavanoids.

Antioxidant activity

Free radical scavenging activity by DDPH spectrophotometric assay

The DDPH assay was used to measure the antioxidant activity of the plant extract as it offers a rapid technique to screen the antioxidant property. The antioxidant values (percentage of inhibition) of the crude methanolic extract were examined.

The percentage of scavenging activity of DPPH radical was found to be concentration dependent i.e. concentration of the extract between 0.2-1mg/ml increasing the inhibition activity. Methanolic extract of whole plant show 46.91%, 76.24%, 87.06%, 87.21% and 88.27% of inhibition in 0.2, 0.4, 0.6, 0.8 and 1mg/ml concentration of extract respectively. From the result it is clear that highest scavenging was 88.27% at 1mg/ml concentration (Table.2)

Table 2: Antioxidant property of methanolic extract of whole plant of *Vernonia cinerea*

Conc.of sample (mg/ml)	**DPPH**	**Sample**	**Absorbance at 517 nm**	**% of inhibition**
Control	2ml	1ml methanol	0.665	0
Blank		3ml methanol	0	0
0.2		1ml sample	0.353	46.91
0.4			0.158	76.24
0.6			0.086	87.06
0.8			0.085	87.21
1			0.078	88.27

Antimcrobial activity

The *in vitro* antimicrobial activities of the methanolic extracts of the plant were investigated separately using well diffusion method against four Multi Drug Resisitant clinical isolates of bacterial strains including three gram negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*) and one gram positive bacteria (*Staphylococcus aureus*). The potency of extract was assessed by the presence or absence of inhibition zone and zone diameter. In the present study zone of inhibition was not obtained against any of the strain (Fig: 2).

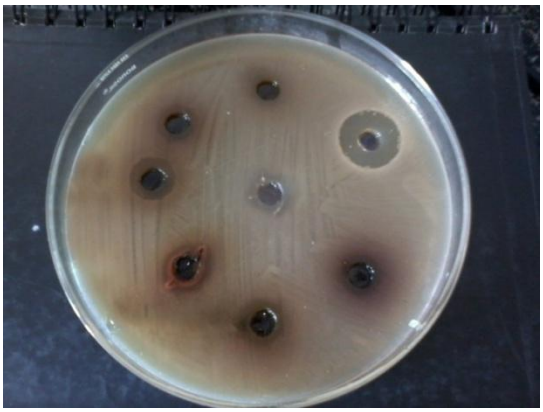

Fig 2: Well diffusion plate

Discussion

The medicinal plants are therapeutically active and they are used by the common people as a home remedy against several diseases like fever, cold, dysentery etc. Herbal drugs play an important role in healthcare programme especially in developing countries.

Secondary metabolites serve some role in plant defence mechanism. Solvent used in the extraction procedure helps in the successive isolation of botanical compound from plant material. Methanol was used for extraction in the present study. In Soxhlet extraction, the sample is continually exposed to fresh solvent. The traditional healers use water as the solvent but later it was clear that the plant extract by methanol provide more compounds (Jigna and Sumitra, 2007) [7].

Phytochemical evaluation

Qualitative preliminary phytochemical screening

The presence of constituents which are known to exhibit medicinal as well as physiological activities was revealed by phytochemical analysis. Preliminary analysis is much helpful for the identification of active compounds.

It has been reported that alkaloids and flavanoides are involved with antibacterial and antiviral activity while tannis and flavanoids are thought to be responsible for antidiarrheal acitivity (Enzo, 2007) [4], (Pithayanukul *et al*., 2007) [5]. The phytochemical analysis of some of the plants belonging to the group of *Dashapushpa* was reported (Deepan *et al*., 2012; Majumder *et al*., 2012) [2, 10]. The traditional uses of plants belonging to the group of *Dashapushpa* was reported (Jiny. V. K *et al*., 2010) [8].

The preliminary phytochemical screening of the present study revealed that the plant material possessed alkaloids, terpenoids and flavanoids. Flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants (Singh and Bhat, 2003) [17]. According to the results obtained in this study it is suggested that the identified phytochemical compounds may be the bioactive constituents.

Antioxidant evaluation

Different classes of phytochemicals and several plant extracts have been found to have quite prominent antioxidant activity (Tripathi *et al*., 1996; Rao, 1997; Vani *et al*., 1997) [18, 16, 19].

Among the secondary metabolites in the plant kingdom, flavanoids are groups of naturally occurring compounds which is widely distributed. These flavanoids have also been reported to possess antioxidant and antiradical properties (Nakayoma and Yamada, 1995) [13].

DPPH is one of the free radical widely used for testing preliminary radical scavenging activity of a compound or a plant extract. The DDPH test (Wagner, 1996) [20] provides information as the reactivity of test compounds with stable free radical. Because of its odd electron, 2, 2- diphenyl 1-picryl hydrazyl radical (DPPH) gives a strong absorption band at 517nm (Duh, 1999) [3]. DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour changes from purple to yellow after reduction which can be quantified by its decrease of absorbance.

Phenolic compound and flavanoids are widely distributed in plants, which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc (Miller, 1996) [11].

The antioxidant activities of the methanolic extract of the plants were carried out using DPPH (2, 2- diphenyl 1- picryl hydrazyl). The plant showed antioxidant activity above 50% due to the presence of flavanoid.

Antimicrobial evaluation

The methanoic extract of the plant were assessed separately against one gram positive and three gram negative bacteria to identify the antibacterial activity. The activity was assessed using Multi Drug Resistant strains. Majority of the bacteria that are pathogenic to human beings are gram negative in nature so the selection of the bacteria was based on this fact.

The antibiotic a patient takes during a bacterial infection might kill most of the bacteria, but few tenacious germs may survive by mutating or acquiring resistance genes from other bacteria. Such bacteria may multiply quickly creating antibiotic resistant strains. These strains get transmitted to others (Nordenberg, 1998) [14]. The indiscriminative use of antibiotics has resulted in the emergence of a number of resistant bacterial strains (Ramphile *et al*., 1991) [15]. The increasing resistance of antibiotics is seen as an ecological problem (Kummerer, 2004) [9]. The antimicrobial activity of ten plants, commonly known as '*Dashapushpam'* was reported (Mini. V. N *et al*., 2010) [12].

Antimicrobial activity were assessed by using four pathogenic bacterial species namely *Pseudomonas aeruginosa*, *Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus.* Well diffusion method was used in assessing the antibacterial studies. DMSO was used as negative control and Gentamycine was used as positive control in the present study. The present study showed the least activity of the plant extract i.e., no inhibiton zone was obtained against any of the strain used. This may be due to the absent of phenolic compound in the plant.

Conclusion

It is believed that plant based drug cause less or no side effects when compared with synthetic antibiotics. The presence of major phytochemicals like alkaloids, terpernoids, carotenoids, saponin and flavanoids can be identified by phytochemical analysis. The activity of the plant can be analyzed with the help of antioxidant and antibacterial studies. This helps in the development of new drugs for the treatment of various diseases. The results obtained from this study confirm high antioxidant activity and low antimicrobial activity of *Vernonia cinerea*.

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