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Examination of Phytochemicals, Antioxidant and Antibacterial Activity of Hemigraphisalternate

S. Agneeswari*, M. Jansi

Abstract: ***Plants as the source of medicine plays avital role in the health service around world. Hemigraphis alternata (Acanthaceae), exotic plants adapt to India, is versatile tropical lower-creeping perennial herbs that reachheight of 15 - 30 centimeters. The matured leaf of Hemigraphis alternata was collected from Kurunthankode , Kanyakumari district. Phytochemical screening of the H.alternata revealed the presence of some phytoconstituents such as carbohydrate, protein, alkaloid, flavonoid, saponin, terpenoid and tannin. Previous report shows that H.coloratacontains similar compounds like saponins, flavonoids and terpenoids. The antioxidant property of H.alternata extract were compared with standard ascorbic acid. The ethanolic extract of H.alternata leaf exhibit good scavenging activity with dose dependent manner. Antimicrobial activities of H.alternata leaf extract show the ethanol extract has the maximum activity against bacteria and fungi. This study revealed the presence of different Phytochemical, antioxidant and antibacterial activities of plant H.alternataand can be suggested that the bioactive components are promising natural antimicrobial agents.***

Keywords: H.alternata, antioxidant, antibacterial, medicine.

I. INTRODUCTION

Plant as source of medicines play an vital role in health service around world[1]. *Hemigraphis* , an exotic plant adaptin India, is a tropicalversatile, lower-creeping perennial herb that reach height of 15 - 30 centimeters. In Kerala, plant is also called as 'murianpachaor'murikootti'because of its fair good potency to heal wound. Hemigraphis mean 'half writing' because filaments of outer stamen bears brush[2]. The plant have different names like Metal leaf,Aluminiumemetaryplant,Red flame Ivy, Waffle plants, Java Ivy etc. *H. alternata* is known for its folk medicinal potency that plant has good wound healing activities[3] and it can cure anemia [4]. the leaftraditionally aretaken for gall stone, highlevelsof menstruations and as contraceptives.*H.alternata*for treating hemorrhoids, diarrhoea, excessive menstruations andkin disease [5]. The antidiabetics property of *H. alternata* were found for 1st time by using Wistar rat and Swiss albino mices byGatathri et al.[4]. Medicinal plantcan be focused by many workerwho found therapeutic benefitof traditional system of medicines in wound repairs[6, 7]. The medical property of plant differ in varying plant like roots, rhizomes, stems, flowers,eaf, fruits or seeds. So it is very essential to analyse pharmacognostic study in plants [8]. Some of the examples for pharmacognostic studies are *Tephrosia purpurea* root [9]; *Ferulasumbul* root [10]; rhizome of *Smilax domingensis*[11]; *Mangiferaindica* leaf [12]; *Diplaziumesculentum* leaf [13]; *Cissusquadrangularis* stem [14]; *Argyreiapilosa* stem [15]; fruit of *Helicteresisora*[16]; flowers of *Woodfordiafruticosa* [17] and *Aervalanata*[18].The antimicrobial action of plant on microorganisms are due to the presence of certain antimicrobial agents such as alkaloids, flavonoids, volatile oils, gums, tannins, saponins, steroids and some other secondary metabolites present in the plants [19-21].Therefore, the studyobjective was to assess pharmacological potentials like phytochemical constituents, antioxidant and antibacterial of the crude and solvent extracts of *H.alternata*leaves.

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* Correspondence Author (s)
*S. Agneeswari, Department of Zoology, Vivekananda College, Agasteeswaram, Kanyakumari,
M. Jansi, Department of Zoology, S.T. Hindu College, Nagercoil.
Corresponding Author mail: s.agneeswari@yahoo.com

II. MATERIALS AND METHODS

Sample collection and processing

The matured leaves of *Hemigraphis alternata* was collected from Kurunthankode of Kanyakumari district. The plant was taxonomically identified by Professor Dr. P. Nagendra Prasad, Head, Department of Biotechnology, Sri Paramakalyani College, Alwarkurichi, Tirunelveli. Freshly collected *H. alternata* leaves were washed in running tap water washed for 3 minutes. Then the plant parts were rinsed with sterile distilled water thoroughly to remove residues. Excess moisture was removed from the sterilized leaves .Then they were subjected to solvent and crude extraction.

Preparation of crude and solvent Extracts

About 10 g fresh leaves of *H. alternate* was macerated in mortarandpestle at room temperature and then filtered by muslin cloths under aseptic condition and the filtrate crude sample was collected in fresh sterilized glass tubes and stored at 4°C until use [22].Fresh leaves were cut into small pieces and to 5g of leaves, 10 ml of solvent like Ethanol,Petroleum Ether and Aqueous were added separately and grounded with motor and pestle. The extracts were boiled at 60°C for 3 hours, kept overnight at 37°C and then filterwith Whatman No. 1 filter papers. The extracts were dried and stored at 4°C.

Qualitative Phytochemical analysis

Phytochemical screening of leaf extracts of *H.alternata* subjected to qualitative phytochemical test for the presence of various classes of active chemical constitutes such as carbohydrate, protein, aminoacid, steroids, saponins, tannin, terpenoid, glycosides, alkaloid, flavanoid and phenol using standard procedure of Mukherjee[23].

Quantification of phytoconstituents

Phytoconstituents were estimated by various methods like carbohydrate [24], protein [25], saponins, alkaloid, flavonoid [26], tannin[27] and terpenoid [28].

Antioxidant activity determination of Ferrous ion chelating abilities

The ferrous ion chelating potentials of extract was analysed by Dinis et al.[29] methods. The reaction mixtures contained 1.0 milli litre ofdifferent concentration of extract (2-10 mg/ml) and 0.05 milli litre of 2 mM $FeCl_3$. The reactions were by adding 0.2 ml of 5 mM ferrozine. The reaction mixtures were taken vigorously and left standing at room temperatures for 10 minuteand absorbances of reaction mixtures were measured at 562 nm against blank. A lowabsorbance of reaction mixtures indicate high ferrous ion chelating abilities. The control contain all reagents except samples. Ascorbic acid was used as standard for comparisons.

% Inhibition = [(Control- Test)/control]×100

Nitric oxide radical scavenging assays

Nitric oxides from sodium nitroprusside interact with oxygen for producing nitrite ions which was measured by Griess reactions. This assay was done by proceduredin Green et al.[30]. The reaction mixtures containing 3ml of 10 mM sodium nitroprussides in phosphates buffered salines (pH 7.4) and different concentrations of (2-10 mg/ml) extract. The resulting solutions were then incubated at 25°C for 60 minute. To incubated samples 5.0 ml of Griess reagents (1% sulphanilamides, 0.1% NEDD in 2% H_3PO_4) were added and absorbances of chromophore formed was measured at 546 nm against a reagent blank. Percentage inhibition of the nitrite ions generated is observed. The standard ascorbic acid and BHT was used for comparison. The free radical scavenging activity was determined by evaluating % inhibition as above.

Antibacterial activity

Antibacterial activity of leaf extracts of *H. alternata* was determined by using agar disc diffusion method on Muller Hinton agar (MHA) medium [31]. The bacterial strains were first cultured in a nutrient broth for 18hours prior to use. Test organisms used are five Gram positive, five Gram negative and five fungi. 25 µl of sample with 100 µg concentration is used as test sample. Streptomycin 25µg was used as positive control and sterile disc (Hi-media) was used as negative control.

III. RESULTS

Phytochemical Screening

The phytochemical screening of *H.alternata* showed the presence of carbohydrate protein, alkaloid, flavonoid, saponin, tannin and terpenoid (Table 1).

Table 1: Phytochemical analysis of *H.alternata* leaf extracts

TEST NAME	Ethanol	Petroleum ether	Aqueous
Carbohydrate	+	-	+
Protein	-	+	-
Alkaloid	-	-	+
Flavonoid	+	-	-
Glycoside	-	-	-
Steroid	-	-	-
Saponins	+	+	-
Phenol	-	-	-
Tannin	+	+	-
Terpenoids	+	-	+

Quantitative estimation of phytoconstituents

The quantitative estimation of different extract were carried out and was tabulated in Table 2. Ethanol extract of leaves shows the presence of 55.6mg/G of flavonoids and 18.5mg/G of saponin. Maximum 67.5mg/G of carbohydrate and 52.1mg/G of terpenoid. Whereas, minimum amount of tannin 12.1mg/G. Petroleum ether contains 25.3mg/G of protein and 9.3 mg/G of saponin. The aqueous extract contains maximum 53.8mg/G of alkaloid, 36.9 mg/G of carbohydrate, 48mg/G of terpenoid and 26.1 mg/G of tannin.

Table 2: Quantitative estimation of phytoconstituents in *H. alternata*

Test	Ethanol	Petroleum ether	Aqueous
Carbohydrate mg/G	67.5	-	36.9
Protein mg/ G	-	25.3	-
Alkaloid mg/G	-	-	53.8
Flavanoid mg/G	55.6	-	-
Tannin mg/G	12.1	-	26.1
Saponin mg/G	18.5	9.3	-
Terpenoid mg/G	52.1	-	48

Antioxidant activity

Nitric oxide scavenging assay of all extracts was determined at 3 different concentrations (25, 50 and 100µg/ml) and results of scavenging efficiency of extracts are depicted in Table 3, 4 and Figure1, 2.

The results showed that scavenging is higher of 65.8% by the higher concentration of ethanol extract likewise the ferrous iron chelating assay was measured with three concentration of all extracts. The ferrous iron chelating activity increasewith increased concentrations. The chelating power was 56.9% by ethanol extract.

Table 3: Nitric Oxide Scavenging Activity of *H.alternata* leaf extracts

Percentage of Nitric oxide scavenged (%)				
Concentration	Ethanol	Petroleum ether	Aqueous	Standard (Ascorbic Acid)
25 µg/ml	33.1	21.4	31.6	56.3
50 µg/ml	48.1	34.9	45.4	69.5
100 µg/ml	65.8	41.8	54.6	92.5

Table 4: Ferrous Ion Chelating Activity of *H.alternata* leaf extracts

Percentage of Ferrous Ion Chelated(%)				
Concentration	Ethanol	Petroleum ether	Aqueous	Standard (GallicAcid)
25 µg/ml	25.3	15.5	22.4	55.4
50 µg/ml	45.8	36.1	41	74.9
100 µg/ml	56.9	47.5	53	93.4

Antimicrobial activity of *H.alternata*.

H.alternata extracts exhibited variable inhibitory response against pathogenic bacteria and fungi. Here ethanol extract shows inhibition against most of the pathogens. Maximum inhibition zone of 38mm was observed against *Staphylococcus aureus* and 34mm against *Proteus mirabilis*. Antifungal activities of ethanol extracts of leaf shows 22mm zone of inhibition against *Pencillium notatum* and *Candida albicans*. The aqueous extract shows 18millimolar zones of inhibitions against *Proteus vulgaris* and moderate activity was recorded for other organisms, whereas petroleum ether extracts show negative results all bacteria and fungi (Table 5, 6 and Figure 3, 4 and 5).

Table 5: Antibacterial activity of *H.alternata* leaves extract against bacteria.

Samples	Strains									
	S. aureus	*B. subtilis*	*S. mutans*	*L. bacillus*	*E. faecalis*	*E. coli*	*K. pneumoniae*	P. mirabilis	*P. aeruginosa*	*P. vulgaris*
PE	-	-	-	-	-	-	-	-	-	-
Ag	10	10	10	10	10	9	11	12	9	18
E	38	26	12	23	13	24	31	34	22	24
PC	19	23	17	14	22	18	21	19	20	23
NC	-	-	-	-	-	-	-	-	-	-

Figure 3: Antibacterial activity of H. *alternata* leaf extract against Gram positive bacteria

Figure 4: Antibacterial activity of H. *alternata* leaf extracts against Gram negative bacteria.

Figure 5: Antibacterial activity of H. *alternata* leaf extracts against Gram negative bacteria.

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Table 6:Antifungal activities of*H. alternata* leaf extract against bacteria.

Samples	Strains				
	Aspergillus flavus	*Aspergillus niger*	*Pencillum notatum sp*	Rhizopus sp	Candida albicans
PE	-	-	-	-	-
Ag	9	-	-	10	9
E	17	-	22	13	22
PC	22	14	17	17	21
NC	-	-	-	-	-

IV. DISCUSSION

Phytochemical screening of the *H.alternata* revealed the presence of some phytoconstituents such as carbohydrate, protein, alkaloid, flavonoid, saponin, terpenoid and tannin. Previous report shows that *H.coloratac*ontains similar compounds like saponins, flavonoids and terpenoids [32]. The quantification of phytoconstituents revealed that ethanol extracts contains maximum amount of constituents like carbohydrate, flavonoid and terpenoid. It is supported by report of Priyanka et al.[33] that the ethanol extract of leaves shows the presence of steroid, proteins, amino acids, tannin, coumarins, alkaloids, diterpenes, phenol, tannin and flavonoids. Flavonoids are reported to possess many useful properties, including antiinflammatory, antimicrobial, enzyme inhibition, oestrogenic, antiallergic, antioxidant and anti-tumour activity. Plant contains polyphenolic compounds like flavonoids and they are common in leaves, woody parts, stem, bark and flowering tissues and they prevent the plant with defense against infection and injury [34].

The antioxidant property of *H.alternata* extract were compared with standard ascorbic acid. The ethanolic extract of *H.alternata* leaf exhibit good scavenging activity with dose dependent manner. Some effect was noted with ferrous ion activity of the ethanolic extract 56.9% in higher concentration and 65.8% in nitric oxide scavenging. Antioxidants provide protection to living organisms from damages caused by uncontrolled productions of reactive oxygen specieandconcomitant lipid peroxidations, proteins damages and DNA strand breakages [35].Some phytoconstituent in leaves extract act as chelating agent. Phenolic compound are efficient in hydrogen donors which makethemgood antioxidants. The phenolic acidlikecinnamate, chlorogenate,coumarates, gallates and ferulates in plant act as pro-oxidantand exhibitfree radical scavenging activities [36].

Antimicrobial activities of *H.alternata* leaf extract shows the ethanol extract has the maximum activity against bacteria and fungi. Zone of inhibition against *S.aureus* is more than positive control as it contains good antimicrobial agent. Benzene extracts of *H.colorata* leafshow its activities against *Streptococcus aureus and Acinetobacter*specieresponsible for activities. Antimicrobial activity of herbs is due to variety of secondary metabolites like phenols, unsaturated sterols, triterpenes saponins and phenolic terpenoids[38]. Therefore the phytoconstituents present in the leaf extract of *H.alternata*is responsible for the antimicrobial and antioxidant property.

V. CONCLUSION

This study analyse the presence of different phytochemical, antioxidant and antibacterial activities of plant *H.alternata*and can be suggested that the bioactive components are promising natural antimicrobial agents. Literature survey also showed that the plant has applications in both traditional and modern medical practices. Further, extensive studies are under process in identify and characterize the bioactive compounds responsible for antioxidant and antimicrobial activity.

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