

Examination of Phytochemicals, Antioxidant and Antibacterial Activity of *Hemigraphis alternata*

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Abstract: Plants as the source of medicine plays a vital role in the health service around world. *Hemigraphis alternata* (Acanthaceae), exotic plants adapt to India, is a versatile tropical lower-creeping perennial herb that reaches a height of 15 - 30 centimeters. The matured leaf of *Hemigraphis alternata* was collected from Kurunthakode, 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 reports show that *H.colorata* contains similar compounds like saponins, flavonoids and terpenoids. The antioxidant property of *H.alternata* extract was compared with standard ascorbic acid. The ethanolic extract of *H.alternata* leaf exhibits good scavenging activity with a dose-dependent manner. Antimicrobial activities of *H.alternata* leaf extract show that the ethanol extract has the maximum activity against bacteria and fungi. This study revealed the presence of different phytochemicals, antioxidant and antibacterial activities of plant *H.alternata* and it 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 a vital role in health service around world [1]. *Hemigraphis*, an exotic plant adapted in India, is a tropical versatile, lower-creeping perennial herb that reaches a height of 15 - 30 centimeters. In Kerala, plant is also called as 'muriyapacha' or 'muriyokutti' because of its fair good potency to heal wound. *Hemigraphis* means 'half writing' because filaments of outer stamen bears brush [2]. The plant has different names like Metal leaf, Aluminium metal plant, 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 leaf traditionally is taken for gall stone, high level of menstruations and as contraceptives. *H.alternata* for treating hemorrhoids, diarrhoea, excessive menstruations and skin disease [5]. The antidiabetic property of *H. alternata* was found for 1st time by using Wistar rat and Swiss albino mice by Gatathri et al. [4]. Medicinal plant can be focused by many workers who found therapeutic benefit of traditional system of medicines in wound repairs [6, 7]. The medical property of plant differs in

varying plant like roots, rhizomes, stems, flowers, leaf, 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]; *Ferula sumbul* root [10]; rhizome of *Smilax domingensis* [11]; *Mangifera indica* leaf [12]; *Diplazium esculentum* leaf [13]; *Cissus quadrangularis* stem [14]; *Argyreiopilosa* stem [15]; fruit of *Helicteres isora* [16]; flowers of *Woodfordia fruticosa* [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 study objective was to assess pharmacological potentials like phytochemical constituents, antioxidant and antibacterial of the crude and solvent extracts of *H.alternata* leaves.

II. MATERIALS AND METHODS

Sample collection and processing

The matured leaves of *Hemigraphis alternata* were collected from Kurunthakode of Kanyakumari district. The plant was taxonomically identified by Professor Dr. P. Nagendra Prasad, Head, Department of Biotechnology, Sri Paramakalyani College, Alwarthur, 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. alternata* were macerated in mortar and pestle 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 filtered with Whatman No. 1 filter papers. The extracts were dried and stored at 4°C.

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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 constituents 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 of different concentration of extract (2-10 mg/ml) and 0.05 milli litre of 2 mM FeCl₃. 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 minute and absorbances of reaction mixtures were measured at 562 nm against blank. A low absorbance of reaction mixtures indicate high ferrous ion chelating abilities. The control contain all reagents except samples. Ascorbic acid was used as standard for comparisons.

$$\% \text{ Inhibition} = [(Control - Test) / control] \times 100$$

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 procedure in 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% H₃PO₄) 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 18 hours 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
Flavonoid 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 increases with 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 (Gallic Acid)
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	-	-	-	-	-	-	-	-	-	-
Aq.	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.

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

Samples	Strains				
	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Penicillium notatum</i> sp	<i>Rhizopus</i> sp	<i>Candida albicans</i>
PE	-	-	-	-	-
Ad	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.colorata* contains 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 specie and concomitant 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 make them good antioxidants. The phenolic acid like cinnamate, chlorogenate, coumarates, gallates and ferulates in plant act as pro-oxidant and exhibit free 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* leaf show its activities against *Streptococcus aureus* and *Acinetobacter* species responsible

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.

REFERENCES

- G. Thomson, Further consideration of Asian medicinal plants in treating common chronic diseases in the West, Journal of Medicinal Plants Research, 4 (2010) 125-130.
- D. Gledhill, The Names of Plants., Edn 4, Cambridge University Press, New York, (2008) 195.
- V. Silja, Varma, KS & Mohanan, K, Ethnomedicinal plant knowledge of the Mullu kuruma tribe of Wayanad., Indian Journal of Traditional Knowledge, 7 (2008) 604-612.
- V. Gayathri, Lekshmi, P & Padmanabhan, RN, Anti-Diabetes and Hypoglycaemic properties of Hemigraphis colorata in Rats, International Journal of Pharmacy and Pharmaceutical Sciences, 4 (2012) 224- 328.
- P. Mallikharjuna, Rajanna, LN, Seetharam, YN & Sharanabasappa, GK, Phytochemical studies of Strychnos potatorum L.F, A medicinal plant E-jour Chem, 4 (2007) 510-518.
- S. Nayak, Nalabothu, P, Sandiford, S, Bhogadi, V & Adogw, A, Evaluation of Wound healing activity of Allamanda cathartica. L. and Laurus nobilis. L. extracts on rats. , BioMed Central Complementary and Alternative Medicine, 6 (2006) 12.
- B. Rathi, Bodhankar, SL & Baheti, AM, Evaluation of aqueous leaves extract of Moringa oleifera Linn for wound healing in albino rats, Indian J Exp Biol., 44 (2006) 898-901.
- S. Chanda, Importance of pharmacognostic study of medicinal plants: An overview., J Phcog Phytochem, 2 (2014) 69-73.
- R. Shah, Shah, R & Chanda, S, Pharmacognostical and preliminary phytochemical investigation of Tephrosia purpurea (Linn.) Pers. root from Gujarat region, Int J Pharmaceutic Res., 3 (2011) 49-52.
- S. Batra, Kumar, A & Sharma, A, Pharmacognostic and phytochemical studies on Ferula sumbul Hook. Roots, J Phcog Phytochem, 4 (2017) 965-968.
- J. Yaque, Monan, M, Cuéllar, A, de Armas, T, Gómez, E & Dopico, E, Pharmacognostic and phytochemical studies of Smilax domingensis Willd. in Cuba., Am J Plant Sci., 8 (2017) 1462-1470.
- K.C. Rakholiya, S, Pharmacognostic, physicochemical and phytochemical investigation of Mangifera indica L. var. Kesar leaf. , Asian Pac J Trop Biomed., 2 (2012) S680-S684.
- G. Dash, Khadidi, SKJ & Shamsuddin, AF, Pharmacognostic studies on Diplazium esculentum (Retz.) Sw., Der Pharm Lett., 9 (2017) 113-120.
- K. Nagani, Kevalia, J & Chanda, S, Pharmacognostical and phytochemical evaluation of stem of Cissus quadrangularis L, Int J Pharmaceutic Sci Res., 2 (2011) 2856-2862.
- D. Prasanth, Srinivasa Rao, A & Rajendra Prasad, Y, Pharmacognostic study of Argyreia pilosa stem., Res J Phcog. , 4 (2017) 23-31.
- P.B. Kanthale, S, Pharmacognostic study of Helicteres isora L. , Pharmaceut and Biol Evaluat, 4 (2017) 47-51.



17. Y. Baravalia, Nagani, K & Chanda, S, Evaluation of pharmacognostic and physicochemical parameters of *Woodfordia fruticosa* Kurz. Flowers. , Phcog J, 2 (2011) 13-18.
18. N. Silvia, Rajeswari, CH, Mounica, D, Manasa, R & Prasanth, DSNBK, Pharmacognostic and phytochemical studies on flowers of *Aerva lanata* [L.] Juss. ex. Schult. , Phcog J, 6 (2014) 29-32.
19. S. Kochlar, Tropical Crops. In: A Textbook of Economic Botany. , Macmillan Publishers Ltd, London and Basingstoke. , (1986) 21-25, 33-34.
20. E. Sofowora, Medicinal Plants and Traditional Medicine in Africa., John Wiley and sons, U.S.A., (1982) 10-40.
21. J. Oyagade, Awotoye, OO, Adewumi, JT & Thorpe, HT, Antimicrobial Activities of Some Nigerian Medicinal Plants. Screening for Antimicrobial Activity. , Bioscience Research Communication, 11 (1999) 193-197.
22. P. Goyal, Khanna, A, Chauhan, A, Chauhan, G & Kaushik, P, In vitro evaluation of crude extracts of *Catharanthus roseus* for potential antibacterial activity, International Journal of Green Pharmacy, 2 (2008) 176-181.
23. P.c.o.h.d. Mukherjee, business horizons pharmaceutical publishers, , Quality control of herbal drugs, business horizons pharmaceutical publishers., 356 - 358., New Delhi, 356 - 358. (2002).
24. J. Roe, The determination of sugar in blood and spinal fluid with anthrone reagent, The Journal of biological chemistry, 212 (1955) 335-343.
25. O. Lowry, Rosenberg, NJ, Farr, AL & Randal, RJ, Protein measurement with the Folin-Phenol reagent, The journal Biological Chemistry, 193 (1951) 265-275.
26. W. Evans, Trease Evans Pharmacognosy, 14th ed., London: WB Saunders Ltd.1966.
27. E. Robert, Method for estimation of tannin in grain sorghum, Agro J, 63 (1971) 511.
28. N. Ghorai, Chakraborty, S, Guchait, S, Saha, SK & Biswas, S, Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent., scientific protocols, (2012).
29. T. Dinis, Madeira, VMC & Almeida, LM, Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers, Arch Biochem Biophys, 315 (1994) 161-169.
30. L. Green, Wagner, DA, Glogowski, J, Skipper, PL & Wishnok JS, Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids, Anal Biochem, 126 (1982) 131-138.
31. K. Salah Alhashimi, Khaleel Rashid, I, Ghoson Saleh, S, Alea Abdulhadi, M & Tara Taher, A, The antimicrobial activity of leaves and callus extracts of *Thevetia peruviana* In vitro, Journal of Biotechnology Research Center, 7 (2013) 74-80.
32. J. Sheu, Jayakumar, T, Chang, C, Chen, Y, Priya, S, Ong, E, Chiou, H & Elizebeth, AR, Pharmacological actions of an ethanolic extracts of the leaves *Hemigraphis colorata* and *Clerodendron phlomoides*., Clin Mol Med 4(2012) 1-3.
33. S. Priyanka, Anupama, D, Misna, M, Nisha Jayan, Reshma, J, Reshma, PR, Sana, PA Saranya, KG, Vidya, PV & Thomas, L, Phytochemical screening and biosynthesis of silver nanoparticles of selected medicinal plants used in Traditional Medicine, Journal of Medicinal Plants Studies, 4 (2016) 01-05.
34. S. Ghosal, Tripathi, VK & Chauhan, S, Active constituents of *Emblica officinalis*. Part I. The chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B., Ind J Chem, 35 (1996) 941-948.
35. R. de Sousa, Queiroz, KC, Souza, AC, Gurgueira, SA, Augusto, AC, Miranda, MA, Peppelenbosch, MP, Ferreira, CV & Aoyama, H, Phosphoprotein levels, MAPK activities and NFκB expression are affected by fisetin, Journal of Enzyme Inhib Med Chem, 22 (2007) 439-444.
36. R. Deepak, Renjima, V & Murugan, K, Antioxidant Potential of *Hemigraphis colorata* (Blume) H.G.Hallier and *Rhinacanthus nasutus* (Linn). Kurz, A Search. In Proceedings of the 2007 Kerala Science Congress (03-40), Kannur, Kerala., (2007) 1-4.
37. V. Anitha, Antonisamy, JM & Jeeva, S, Anti-bacterial studies on *Hemigraphis colorata* (Blume) H.G. Hallier and *Elephantopus scaber* L, Asian Pac J Trop Med 5(A2012) 52-57.
38. T. Fujiwara, Sugishita, EY, Takeda, Y, Shimizu, M, Nomura, T & Tromita, Y, Further studies on the structures of polysachharides from the bark of *Melia azadirachta*. , Chemical and Pharmacology Bulletin., 32 (1984) 1385-1391.