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STUDIES ON ANTIOXIDANT ACTIVITY, PHENOL AND FLAVONOID CONTENT OF THE INDIAN MEDICINAL PLANT *HYGROPHILA AURICULATA*

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Abstract:

Antioxidant activity, total phenol and flavonoid content of the leaf of Hygrophila auriculata collected from three geographically distant regions of Tamil Nadu (Kanchipuram, Gummidipondi and Chengalpattu) were examined using extracts of aqueous, ethanol, acetone, chloroform and petroleum ether. Butylated Hydroxy Toluene (BHT), Gallic acid (GA) and Quercetin (Q) were taken as standard in case of antioxidant activity, phenol and flavonoid content respectively. The acetone leaf extract of Hygrophila auriculata collected from Gummidipoondi was found maximum in total phenol and flavonoid contents 20.83 mg Gallic Acid Equivalents (GAE)/g and 3.59 mg Quercetin Equivalents (QE) /g respectively. The leaf extracts were evaluated for antioxidant activities by DPPH (1, 1 – diphenyl -2- picryl-hydrazyl) radical scavenging assay. Among three accessions with different solvents used, maximum antioxidant activity was found in acetone leaf extract (88.1 %) from Gummidipoondi - accession followed by others. The powerful antioxidant effect is attributed to the greater amount of phenols and flavonoids compound in the acetone leaf extracts of Hygrophila auriculata.

Key words: *Hygrophila auriculata, antioxidant activity, DPPH, phenol, flavonoid.*

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INTRODUCTION:

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind [1].

Therapeutic benefits can be traced to specific plant compounds; many herbs contain dozens of active constituents that together combine to give the plant its therapeutic value [2]. A growing body of evidence indicates that secondary plant metabolites play important roles in human health and may be nutritionally important [3]. Phytochemical screening of various plants has been reported by many workers [4]. These studies have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [5]. A number of studies have focused on the biological activities of phenolic compounds which are antioxidants and free radical scavengers [6].

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA [7]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [8]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, either exogenous or endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders [9].

In addition, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. [10]. The crude extracts of herbs, spices and other plant materials, rich in phenolics and flavonoids are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [11].

Hygrophila auriculata (L.), a generally occurring wild herb belonging to Acanthaceae family has been advocated for the treatment of variety of diseases including most commonly diabetes and dysentery [12]. The plants are widely distributed throughout India, Sri Lanka, Burma, Malaysia and Nepal. Following various folk claims as a cure for numerous diseases, efforts have been made by researchers to verify the efficacy of the plant by scientific biological screening. The plant contains saponins, alkaloids, steroids, tannins, flavonoids and triterpenoids are the main phytoconstituents. The plant has been used in the treatment of jaundice, hepatic obstruction, rheumatism, inflammation, urinary infection, gout and malaria and revealed some notable pharmacological effects like antitumor, free radical scavenging, anthelmintic, antipyretic and antimotility activities [13]. The aim of the present work is to carry out the phytochemical screening, tannins content and antibacterial activity from leaf extracts of *Hygrophila auriculata*.

MATERIALS AND METHODS:

Collection of *Hygrophila auriculata*

The healthy plants of *Hygrophila auriculata* were collected from three different regions of Tamil Nadu namely Kanchipuram, Gummidipondi and Chengalpattu. The collected plants were brought to the laboratory and maintained at Dept. of Botany, Govt. Arts College, Thiruvannamalai, Tamil Nadu, India.

Preparation of the plant extract

Preparation of the extracts were done according to a combination of the methods used by Pizzale *et al.*, (2002) [14] [15]. About 15g of dried leaf fine powder of *Hygrophila auriculata* plant materials were extracted with 150 ml ethanol, acetone, chloroform, petroleum ether and aqueous extract for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40° C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10º C.

Qualitative analysis of Antioxidant activity of *Hygrophila auriculata*

The antioxidant activity of leaf extracts of *Hygrophila auriculata* was determined by following the method as described by George *et al.*, (1996) [16].

Fifty microliters of leaf extracts of *Hygrophila auriculata* were taken in the microtiter plate. 100µl of 0.1% methanolic DPPH (full form) was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of Free radical scavenging activity of *Hygrophila auriculata*

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Leaf extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control [17]. Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm.

The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100

Estimation of Total phenol content in *Hygrophila auriculata*

Total phenolic content in the acetone leaf extracts was determined by the Folin Ciocalteau colorimetric method [18]. For the analysis, 0.5 ml of dry powdered acetone leaf extracts were added to 0.1 ml of Folin-Ciocalteau reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (Na_2CO_3) was added and the mixture was allowed to stand for 30 min after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of Total Flavonoid Content in *Hygrophila auriculata*

Total flavonoids content in the acetone leaf extracts was determined by the aluminium chloride colorimetric method [19]. 0.5 ml of leaf extracts of *Hygrophila auriculata* at a concentration of 1mg/ ml were taken and the volume was made up to 3ml with ethanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

RESULTS AND DISCUSSION:

Scavenging activity for free radicals of DPPH (1, 1-Diphenyl-2-Picryl Hydrazyl) has widely used to evaluate the antioxidant activity of natural products from plant and natural sources. Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants [20].

Wild accessions of *Hygrophila auriculata* leaf samples were used for antioxidant studies. The Qualitative analysis on different extraction of ethanol, acetone, petroleum ether, chloroform and aqueous extract showed the presence of antioxidants. 50µl of leaf extracts (acetone, ethanol, petroleum ether, chloroform and aqueous extracts) of *Hygrophila auriculata* were estimated for free radical scavenging activity using Diphenyl-2-picryl hydrazyl (DPPH) assay. The samples observed for its bleaching from purple to yellow and pale pink were considered as strong positive and weak positive respectively (Table 1). And for Quantitative analysis, among the three wild accessions and five different solvent extracts of *Hygrophila auriculata*, the acetone leaf extract collected from Gummidipondi accession recorded the most effective DPPH radical scavenging activity (88.1 %) followed by Chengalpattu (85.0%) and Kanchipuram (70.8 %) accessions (Table 2, 3 & 4). Gummidipondi accession values being close to synthetic antioxidant (BHT) as positive control (98.4%). In each case, acetone leaf extracts recorded higher percentage of free radical scavenging activity than ethanol extractions followed by aqueous, petroleum ether and chloroform extract.

Table 1: Qualitative antioxidant activity of plant extracts of *Hygrophila auriculata*

S. No	Extracts	*Hygrophila auriculata*		
		Whole plant (Kanchipuram)	Whole plant (Gummidipoondi)	Whole plant (Chengalpattu)
1	BHT (standard)	++	++	++
2	Aqueous	+	+	+
3	Acetone	+	++	+
4	Ethanol	+	+	+
5	Chloroform	Semi positive	Semi positive	Semi positive
6	Petroleum ether	-	Semi positive	Semi positive

Table 2: Quantitative antioxidant activity of plant extracts of *Hygrophila auriculata* (Chengalpattu – accession)

Solvents	Minutes						
	0	5	10	15	20	25	30
Aqueous (OD)	0.36	0.33	0.32	0.31	0.31	0.30	0.30
(%)	71.6	74.0	74.8	75.5	75.5	76.3	76.3
Acetone (OD)	0.31	0.29	0.27	0.22	0.21	0.19	0.19
(%)	75.5	77.1	78.7	82.6	83.4	85.0	85.0
Ethanol (OD)	0.47	0.45	0.43	0.39	0.39	0.38	0.38
(%)	54.5	64.5	66.1	69.2	69.2	70.0	70.0
Chloroform (OD)	0.45	0.39	0.35	0.33	0.29	0.29	0.27
(%)	64.5	69.2	72.4	74.0	77.1	77.1	78.7
Petroleum ether (OD)	0.47	0.43	0.38	0.33	0.32	0.30	0.29
(%)	62.9	66.1	70.0	74.0	74.8	76.3	77.1
BHT (OD)	0.14	0.11	0.09	0.07	0.06	0.04	0.02
%	88.9	91.3	92.9	94.4	95.2	96.8	98.4
Conrol	1.27						

Table 3: Quantitative antioxidant activity of plant extracts of *Hygrophila auriculata* (Gummidipoondi - accession)

Solvents	Minutes						
	0	5	10	15	20	25	30
Aqueous (OD)	0.53	0.47	0.43	0.39	0.36	0.32	0.32
(%)	58.2	62.9	66.1	69.2	71.6	74.8	74.8
Acetone (OD)	0.35	0.33	0.27	0.23	0.19	0.17	0.15
(%)	72.4	74.0	78.7	81.8	85.0	86.6	88.1
Ethanol (OD)	0.45	0.43	0.37	0.35	0.33	0.29	0.26
(%)	64.5	66.1	70.8	72.4	74.0	77.1	79.5
Chloroform (OD)	0.49	0.45	0.41	0.39	0.37	0.34	0.34
(%)	58.2	62.9	66.1	69.2	70.8	73.2	73.2
Petroleum ether (OD)	0.46	0.42	0.37	0.36	0.36	0.36	0.36
(%)	63.7	66.9	70.8	71.6	71.6	71.6	71.6
BHT (OD)	0.14	0.11	0.09	0.07	0.06	0.04	0.02
%	88.9	91.3	92.9	94.4	95.2	96.8	98.4
Control	1.27						

Table 4: Quantitative antioxidant activity of plant extracts of *Hygrophila auriculata* (Kanchipuram - accession)

Solvents	Minutes						
	0	5	10	15	20	25	30
Aqueous (OD)	0.62	0.59	0.57	0.53	0.51	0.49	0.46
(%)	51.1	53.5	55.1	58.2	59.8	61.4	63.7
Acetone (OD)	0.52	0.49	0.43	0.42	0.39	0.37	0.37
(%)	59.0	61.4	66.1	66.9	69.2	70.8	70.8
Ethanol (OD)	0.65	0.63	0.57	0.54	0.51	0.49	0.49
(%)	48.8	50.3	55.1	57.4	59.8	61.4	61.4
Chloroform (OD)	0.76	0.69	0.65	0.59	0.57	0.56	0.54
(%)	40.1	45.5	48.8	53.5	55.1	55.9	57.4
Petroleum ether (OD)	0.79	0.74	0.67	0.63	0.59	0.57	0.56
(%)	37.7	41.7	47.2	50.3	53.5	55.1	55.9
BHT (OD)	0.14	0.11	0.09	0.07	0.06	0.04	0.02
%	88.9	91.3	92.9	94.4	95.2	96.8	98.4
Control	1.27						

Phenolics are the most widespread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as radical scavengers. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [21]. In present study, total phenol content (TPC) of *Hygrophila auriculata* leaf extract was estimated by using Folin-Ciocalteau colorimetric method and represented in terms of gallic acid equivalent (GAE). The result of the present study showed that the phenol contents of *Hygrophila auriculata* (Gummidipondi accession) acetone leaf extract was found to be maximum in (20.83 mg GAE/g) (Table 5). It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [22]. Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds [23]. Phenolic compounds are effective hydrogen donors, making them good antioxidants [22]. Similarly, Shahidi and Naczk reported that naturally occurring phenolics exhibit antioxidant activity [24].

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content varied from plant to plant. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [25]

In our study, total flavonoid content of *Hygrophila auriculata* leaf extract was estimated by using aluminium chloride method and represented in terms of quercetin equivalent (QE). The result showed that the flavanoids contents of *Hygrophila auriculata* (Gummidipondi accession) acetone leaf extract was found to be maximum in (3.59 mg QE/g) (Table 5).

Table 5: Estimation of Total phenol content from acetone leaf extract of *Hygrophila auriculata*

Sample	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)
Hygrophila auriculata (Gummidipoondi - accession)	20.83	3.59
Hygrophila auriculata (Chengalpattu - accession)	16.97	2.13
Hygrophila auriculata (Kancheepuram - accession)	11.76	2.73

CONCLUSION:

In conclusion, antioxidant activity, total phenol and total flavonoid content of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent steps for screening of plants for secondary metabolites. The present research work attempts to assess the importance of antioxidant activity, total phenol and total flavonoid properties in leaves of *Hygrophila auriculata* to improve the health status of people and also to use in nutraceutical products of commercial importance. The results indicate that the plant material may become an important source of compounds with health protective potential.

REFERENCES:

1. Momin RK, Kadam VB. Determination of ash values of some medicinal plants of genus *Sesbania* of marathwada region in maharashtra. J Phytol, 2011; 3: 52-54.
2. Helen PA, Aswathy MR, Deepthi KG, Mol R, Joseph RJ, Jaya Sree S. Phytochemical analysis and anticancer activity of leaf extract of *Mangifera indica* (Kotttukonam Varika). Int J Pharma Sci Res, 2013; 4: 819-824.
3. Jeeva S, Sheela JD, Shamila RMI, Lekshmi PJ, Brindha RJ. Antimicrobial activity and phytochemical analysis of *Sanseiveria roxburghiana* leaf. Asian J Plant Sci Res, 2012; 2: 41-44.
4. Parekh J, Chanda S. Phytochemicals screening of some plants from western region of India. Plant Arch. 2008; 8: 657- 662.
5. Hagerman AN, Rield KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plat polyphenolics (tannins) as biological antioxidants. J Agric Food Chem, 1998; 46:1887-1892.
6. Cespedes CL, El-Hafidi M, Pavon N, Alarcon J. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis* (Elaeocarpaceae), Maqui. Food Chem, 2008; 107: 820-829.
7. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem, 1995; 41: 1819-1828.
8. Gulcin I, Oktay M, Kufrevioglu OI, Aslan A. Determination of antioxidant activity of lichen *Cetraria islandica* (L.). Ach J Ethnopharmacol, 2002; 79: 325-329.
9. Tiwari A. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidant therapy. Curr Sci, 2001; 81: 1179.
10. Miller AL. Antioxidant flavonoids: structure, function and clinical usage. Altern. Med. Rev, 1996; 1:103-111.
11. Chu YH, Chang CL, Hsu HF. Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agricul, 2000; 80: 561–566.
12. Neharkar, Sunil K, Ramdas P. Acute Toxicity Study of *Hygrophila auriculata* L. Leaves Methanolic Extract in Albino Rats Vishnu S. J Pharm Chem Biol Sci, 2015; 3(3): 388-395.

13. Esther CJ, Saraswathi R, Dhanasekar S. In vitro antibacterial and antifungal activities along with x-ray irradiation studies of medicinal plant *Hygrophila auriculata*, Int J Pharm Pharm Sci, 2012; 4(4): 352-358.
14. Pizzale L, Bortolomeazzi R, Vichi S, Conte LS. Antioxidant activity of sage and oregano extracts related to their phenolic compound content. J Sci Food Agricul, 2002; 82: 1645–1651.
15. Lu Y, Foo Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chem, 2001; 75: 197-202.
16. George H, Teng CM, Wu CL, Ko FN. Marchantin has a natural antioxidant and free radical scavenger. Arch Biochem Biophys, 1996; 334:18- 26.
17. Lee SE, Hwang HJ, Ha JS. Screening of medicinal plant extracts for Antioxidant activity. Life Sci, 2003; 73: 167-179.
18. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual Methods. Am J Enol Vitic, 1977, 28: 49-55.
19. Mervat MM, Far El, Hanan A, Taie A. Antioxidant Activities, Total Anthocyanins, Phenolics and Flavonoids Contents of Some Sweet potato Genotypes under Stress of Different Concentrations of Sucrose and Sorbitol. Aust J Basic Appl Sci, 2009; 3: 3609-3616.
20. Mammadov R, Ili P, Vaizogullar H. Antioxidant activity and total phenolic content of *Gagea fibrosa* and *Romulea ramiflora* . Iran J Chem Chem Eng, 2011; 30: 57-62.
21. Shahidi F, Wanasundara PK. Phenolic antioxidants. Crit Rev Food Sci Nutr, 1992; 32: 67-103.
22. Evans RC, Miller NJ, Bolwell GP, Bramley PM, Pridham JB. The relative antioxidants activities of plant-derived polyphenolic flavonoids. Free Rad Res, 1995; 22: 375-383.
23. Huang Z, Wang B, Eaves DH, Shikany JM, Pace RD. Total phenolics and antioxidant capacity of indigenous vegetables in the Southeast Unites States: Alabama Colloboration for cardiovascular equity project. Int J Food Sci Nutr, 2009; 60: 100-108.
24. Shahidi F, Naczk M. Method of Analysis and Quantification of Phenolic Compounds. Technomic Publishing Company. Lanchester, 1995; 287-293.
25. Kessler M, Ubeaud G, Jung L. Anti- and prooxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol, 2003; 55: 131- 142.