Research Article

PHYTO CHEMICAL SCREENING AND ANTI- OXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *HELIOTROPIMUM INDICUM*

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ABSTRACT

The aim of the present study was to investigate phyto chemical screening and anti-oxidant activity of the ethanol extract of the whole plant of *Heliotropium indicum*. The ethanolic extract of the whole plant of *Heliotropium indicum* was studied for its anti-oxidant effect. During phytochemical screening flavonoids and phenols were found abundantly. Ethanolic extract of *Heliotropium indicum* was screened to evaluate free radical scavenging effect. The extract exhibited significant reducing power and free radical scavenging effect. This antioxidant activity was concentration dependant which was compared with standard antioxidant such as Ascorbic acid.

KEY WORDS: *Heliotropium indicum*, Ethanolic extract, Anti-oxidant activity, Reactive oxygen species, Flavonoids, phenols.

INTRODUCTION

Oxygen is an important element for life. All living systems have evolved to survive in the presence of molecular oxygen. Oxidative properties of oxygen play a vital role in many biological phenomena¹. It can also aggravate the damage within the cell by oxidative events. Free radicals are highly reactive compounds, they are chemical species associated with an odd or un paired electron and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. Free radicals generated by these reactions are capable of attacking the healthy cell of the body, causing rupture of their structure and function. Free radicals are capable of damaging a lot of cellular components such as proteins, lipids and DNA of cells. Numerous physiological and biochemical process in the human body may produce oxygen centered free radicals and other reactive oxygen species as byproducts. Over production of such free radicals can cause oxidative damage to biomolecules, eventually leading to many chronic diseases such as Cancer, Diabetes, and Aging etc. So intake of antioxidants is important for human. Plants are potential sources of natural antioxidants. In the present study we have aimed at investigating the antioxidant property of *Heliotropium indicum*. Several studies have been conducted to evaluate the correlation between phenolic compounds and antioxidant activity. Recently, there are numerous methods that have been developed to evaluate antioxidant activities of compounds and of complex mixtures such as plant extracts. The present work is about exploring *In Vitro* antioxidant activity for *Heliotropium Indicum*

Heliotropium Indicum is a coarse foetid herb distributed in the tropical and temperate regions of the World¹. The plant is a native of Asia and found in India, Bangladesh, Philippines. In India it is found sunny localities, on waste lands, and anthropogenic habitats, widely considered as a weed of fields.^{2,3,4} The botanical name of Indian heliotrope is *Heliotropium indicum* Family: *Boraginaceae*.⁵

It is used as local application for ulcers, sores, wounds, gum boils, skin infections, stings of insects and rheumatism. A decoction of the leaves is used in fevers and urticaria and that of roots in cough and fevers. It possess wound healing activity⁶, fertility control, antitumor activity⁷ and anti-inflammatory effect. Seeds are stomachic. The flowers are considered emmenagogue in small doses and abortifacient in large doses. Similarly whole herb is used for many other ailments^{8,9, 10}

MATERIALS AND METHODS

Dried whole plant was collected from Thirumala Hills Thirupathi, Andhra Pradesh, India, authenticated by Dr.Madhava chetty, Botanist, SV University Thirupathi, with the voucher number 939.

Extraction

Powdered drug was charged into Soxhlet apparatus and extraction was carried out with following solvents successively.

1) Petroleum ether, 2) Chloroform, 3) Ethanol, 4) Water.

Each time before employing the solvent of higher polarity marc was dried. Each extract was then concentrated using distillation method and dried residue was collected in an opaque glass bottles for further studies. Percentage practical yield of petroleum ether, chloroform, ethanol, water extracts were found to be 0.85, 0.92, 1.40, 1.29% respectively.

Phytochemical Screening Methods

Phyto chemical examinations were carried out for all the extracts as per the standard methods^{11, 12}

1.Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a)Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicate the presence of alkaloids.

b)Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c)Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d)Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthrol glycosides.

b) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

c) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponin glycosides.

4. Detection of Steroids

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates

5. Detection of Phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

b)Lead acetate test: Extracts were treated with lead acetate solution. Formation of white precipitation indicates the presence of phenols.

c)Acetic acid test: Extracts were treated with acetic acid solution. Formation of indicates the presence of phenols.

6. Detection of Flavonoids

a)1%KOH solution: Extracts were treated with KOH solution. Formation of yellow colour indicates the presence of flavonoids.

7. Detection of Proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Evaluation of *In-vitro* antioxidant activity

The anti-oxidant activity was evaluated by using two methods^{13,14}

1. DPPH RADICAL SCAVENGING METHOD:

Procedure:

STEP-1:

PREPARATION OF STOCK-1

100mg of Drug extract and Ascorbic acid in 100ml of ethanol

↓
1mg/1ml concentration solutions of Drug and Ascorbic acid were obtained

STEP-2:

0.06mM solution of DPPH in ethanol|taken as control.

↓
100, 250, 500, 750, 1000 μ g/ml concentration of test (drug extract) and standard (ascorbic acid) were prepared from stock 1 solution using ethanol as solvent.

↓
0.5ml of test of different concentrations+3.5ml of ethanolic DPPH solution (same for the standard)

↓
Keep a side for 30 min

↓
Now the absorbances were measured at 517nm using UV spectrophotometer

Ethanol was taken as blank and the samples were analysed in a triplicate manner and readings were noted.

Formula

Percentage DPPH radical scavenging activity = $1 - (A_s/A_c) \times 100$,

where A_s ; Absorbance of the DPPH solution containing samples. A_c ; absorbance of the control solution without sample but with DPPH.

2. HYDROGEN PEROXIDE SCAVENGING ACTIVITY

PREPARATION OF STOCK-1

100mg of Drug extract and Ascorbic acid in 100ml of ethanol

1mg/1ml concentration solutions of Drug and Ascorbic acid were obtained

STEP-2:

0.06mM solution of Hydrogen peroxide in Phosphate buffer as control.

100,250,500,750,1000 μ g/ml concentration of test (drug extract) and standard (ascorbic acid) were prepared from stock 1 solution using ethanol as solvent.

0.5ml of test of different concentrations+3.5ml of ethanolic DPPH solution(same for the standard)

Keep a side for 30 min

Now the absorbances were measured at 517nm using UV spectrophotometer

Ethanol was taken as blank and the samples were analysed in a triplicate manner and readings were noted.

Formula

% scavenging activity of H_2O_2 = $A_0 - A_T / A_0 \times 100$

Where; A_0 is the absorbance of control, A_T is the absorbance of test.

Figure 1 *Heliotropium Indicum* leaf with flowers

Figure 2: Soxhlet apparatus showing the extraction of *Heliotropium Indicum*

Figure 3: Anti-Oxidant activity by using DPPH Method

Figure 4: Anti-Oxidant activity by H_2O_2 Method

Figure 5 Percentage scavenging of test and standard by DPPH Method

Figure 6: Percentage scavenging activity of test and standard by using H_2O_2 method

Table 1 Percentage yield of *Heliotropium Indicum* by using different solvents

Solvents	Weight	B.P	Volume	Days	%yield
Petroleum ether	30gms	40-60	350ML	6days	0.85gm
Chloroform	30gms	61.2	350ML	4days	0.92gm
Ethanol	30gms	78.3	350ML	4days	1.40gm
Water	30gms	100	350ML	5days	1.29gm

Table 2 Preliminary Phytochemical screening of *Heliotropium Indicum*

Chemical constituents	Petroleum ether	Chloroform	Ethanol	Water
Flavonoids	-	+	+	+
Phenols and Tannins	-	+	+	+
Steroids	+	+	+	-
Alkaloids	-	+	+	+
Glycosides	-	-	-	-
Carbohydrates	-	-	-	-
Gums	-	-	-	-
proteins	-	-	+	+
Reducing sugars	-	-	-	-

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of the plants revealed the absence of glycosides, reducing sugars and gums and tested positive for flavonoids, phenols, tannins, saponins, alkaloids and steroids. The presence of flavonoids and tannins in the plant extract is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers.

DPPH Method

Antioxidant activity assay is based on the reduction of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Due to the presence of an odd electron it gives a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured

Table 3 and figure 5 shows the scavenging activity of *Heliotropium indicum* compared to standard Ascorbic acid by using DPPH Method. Its activity is significant when compared to the standard indicating that our extract has potent antioxidant activity.

Hydrogen peroxide method

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The decomposition of H_2O_2 by Poly herbal drug may at least partly result from its antioxidant and free radical scavenging activity. Figure 4 and Figure 6 shows the percentage scavenging activity of ethanolic extract of *Heliotropium indicum* when compared to the standard Ascorbic acid. Its activity is significant indicating that the test drug has potent antioxidant activity.

CONCLUSION

Heliotropium indicum has its applications in several traditional systems of medicine including Ayurveda and Siddha. From the

results obtained in the present study, it is concluded that the ethanolic extract of *Heliotropium indicum* shows anti oxidant activity by inhibiting DPPH and hydrogen peroxide radical activities. The preliminary phytochemical investigation indicates that the *Heliotropium indicum* –contains alkaloids, steroids, proteins, anthraquinones and high amounts of flavonoids, phenols, tannins, saponins. The presence of flavonoids and phenols in the plant plays a major role in anti-oxidant activity. It contains flavonoids groups which are responsible for higher anti-oxidant activity. The results of this study show that the ethanolic extract of *Heliotropium indicum* can be used as a easily accessible source of natural anti oxidants and as a possible food supplement or in pharmaceutical industry. However, the activity *Heliotropium indicum* shows higher activity in DPPH and lower in hydrogen peroxide when compared to standard. Antioxidant activity is the foundation of many actions which lead to its beneficial effects in majority of the diseases. Usually UV spectro photometric method can be used effectively for the qualitative and quantitative estimation of Flavonoids and phenolic acids for their therapeutical action. The results of the present study concluded that plant extract is a source of natural anti oxidants which might be helpful in preventing progress of various oxidative stress mediated diseases including ageing.

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REFERENCES

1. Sameena S. Reviewing the Protective Role of Antioxidants in Oxidative Stress Caused by Free Radicals .Asian Pacific Journal of Health Sciences 2014; 1(4): 401-406 .
2. Saguna D, Datta. PC. Pharmacognostic Study of the Leaf of *Heliotropium indicum* Linn. (Boraginaceae). Pharmaceutical Biology 1977; 15(3): 141-151.
3. Ashutosh M, Bikash kumar N, Anuj kumar A, Amiya Ranjan P, Promod kumar D. Study on Analgesic Activity of *Heliotropium Indicum*. Stem. Journal of Pharmaceutical Research and Development 2011; 3(10): 1 – 4.
4. Dash GK and Abdullah MS. A review on *Heliotropium Indicum* L. (Boraginaceae). International Journal of Pharmaceutical Sciences and Research 2013; 4(4): 1253-1258.
5. Image of *Heliotropium Indicum*(cited on 6.12.2014).Available from

- <http://www.flowersofindia.net/catalog/slides/Indian%20Heliotrope.html>
6. Reddy JS, Rao PR, Reddy MS. Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats. *Journal of Ethnopharmacology* 2002 ;79(2):249-251.
 7. Kugelmann M, Liu WC, Axelrod M, McBride TJ, Rao KV .Indicine-N-oxide: the antitumor principle of *Heliotropium indicum*. *Lloydia*.1976 ; 39(2suppl3):125-128.
 8. Theeraphan M, John K, Boonsom L, Saisunee L and Stephen G. Pyne.,Composition and antituberculosis activity of the volatile oil of *Heliotropium indicum* Linn. growing in Phitsanulok, Thailand. *Flavour and Fragrance Journal* 2006; 21(2); 265–267.
 9. Srinivas K. Anti-inflammatory activity of *Heliotropium Indicum* Linn. and *Leucas Aspera* Spreng. In albino rats. *Indian Journal of Pharmacology* 2000; 32: 37-38.
 10. Meenakshi P, Ritika K , Shukla LN . Critical review on pharmacological properties of *Heliotropium*. *International Journal of Indigenous Medicinal Plants* 2014;47(1): 1-8
 11. Khandelwal K, *Practical Pharmacognosy, Techniques and experiments*, Nirali Publication, 2nd edn, Nirali prakashan publishers 2000.
 12. Shoge Mansurat O, Ndukwe G, Amupitan J. Phytochemical and antimicrobial studies on the aerial parts of *Heliotropium indicum*. *Annals of Biological Research* 2011; 2 (2):129-136.
 13. Yasmin Begum, Antibacterial, Antioxidant and Cytotoxic activities of *Heliotropium Indicum* , *The Experiment* 2014 ;23 (1):1564-1569.
 14. Sanjay Parihar, Kartik D. Virani, E. A. Pithawala, M. D. Shukla, S. K. Lahiri, N. K. Jain and H. A. Modi. Phytochemical screening, total phenolic content, antibacterial and antioxidant activity of wild edible mushroom *Pleurotus ostreatus*. *Int. Res. J. Pharm.* 2015; 6(1):65-69 <http://dx.doi.org/10.7897/2230-8407.06115>

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