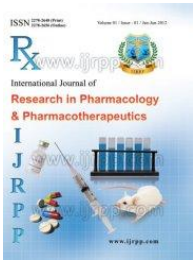
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 ***IJRPP |Vol.5 | Issue 2 | April- June - 2016***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Research article ***Open Access***

In vitro studies on phytochemical analysis and antioxidant activity of Heliotropium indicum linn.

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ABSTRACT

Objective

The *in vitro* studies on phytochemical analysis and antioxidant activity of *Heliotropium indicum Lin.* (Boraginaceous) was evaluated by different assaying.

Methods

The *in vitro* antioxidant activity was evaluated for total antioxidant activity, 1,1-diphenylhydrazyl (DPPH) radical scavenging and hydroxyl radical scavenging activity.

Results

The percentage of inhibition of the total antioxidant activity, DPPH radical scavenging and hydroxyl radical scavenging activity was varying from ethanol and aqueous extracts.

Conclusion

The results clearly indicate that the ethanol and aqueous leaf extracts of the study species is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

Keywords: Heliotropium Indicum, Antioxidant Activity, Phytochemicals, DPPH Activity.

INTRODUCTION

Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments such as arthritis, asthma, dementia, mongolism, arcinoma and Parkinson's disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources. [1]

Many oxidative stress related diseases are as an outcome of accumulation of free radicals in the body. Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and wenches of singlet oxygen formation Antioxidants are considered as a promising therapeutic approach as they may be playing neuroprotective (preventing apoptosis) and

urodegenerative roles. The main characteristic of an antioxidant is its ability to trap free radicals. [2]

Antioxidants are believed to play a very important key role in the body defence system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration. Many of the recent research have accepted that antioxidants are radical scavengers, Which producing the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration and ageing process. [3]

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine. Various medicinal plants have been used in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. [4] Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. [5]

Heliotropium indicum (Family: Boraginaceae) is an annual, hirsute plant that is a common weed native to Asia. In Bangladesh, H. indicum is known as Hatishur. This plant has great importance in traditional medicine. Traditionally, infusion of the leaves and young shoots are used to treat the nettle rash, infusion of the flowers taken in small doses regulates menstruation, where large doses are abortive, the juice of the leaves is antiseptic and anti-inflammation and applied to wounds, sores, boils, gumboils and pimples on the face. Many bioactive constituents were isolated from H. indicum as pyrrolizidine alkaloids named indicine, indicine-N-oxide, acetyl-indicine, indicinine, heleurine, heliotrine, supinine, supinidine and lindelofidine, all of them possess hepatoxic activity. Some aldehydes like phenylacetaldehyde (22.2%), (E)-2-nonenal (8.3%) and (E, Z)-2-nonadienal (6.1%), with a significant quantity of hexa hydro farnesyl acetone (8.4%) and another pyrrolizidine alkaloid named as helindicine were identified with moderate antioxidant activity. [6]

Most of the alkaloids are hepatoxic and therefore the internal use of these plants is not recommended. Biological and pharmacological activities of *heliotropium indicum* were evaluated and reported time to time. The methanolic extract of this herb showed significant wound healing activity in the wound infection model (with S. aureus and P. aeruginosa). The wound healing activity of the ethanolic extract of this plant was studied using excision and incision wound models in rats following topical application and showed better healing activity. The aqueous extract of H. indicum leaves was used in ulceration where dose dependent histo-gastro protective effects were observed. [7]

This herb showed significant activity in several experimental tumor systems. This herb produced a significant anti-inflammatory effect in both acute and subacute inflammation. The traditional medicinal uses such as relieving abdominal pain, hypertension and impotence and sexual weakness were explained by the receptor activity of H. indicum. The essential oil from the aerial parts of the herb showed significant antituberculosis activity. Significant antimicrobial, antioxidant, cytotoxic, thrombolytic and membrane stabilizing activity of this herb were reported. [8]

However, traditionally used medicinal plants await such screening. On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. [9] The present study is performed to characterize the antioxidant activity of *heliotropium indicum* extract to validate its medicinal potential. In me present study a preliminary attempt has been made to find out me *in vitro* antioxidant activity *of heliotropium indicum Linn*.

MATERIALS AND METHODS

Collection of Plant materials

The plant species, namely *Heliotropium indicum Linn.* The leaves were collected during January 2016 from Vedarenyam, Nagapattinam (Dt), TamilNadu.

Preparation of Plant powder

The leaves were air dried under shade for 10 – 15 days. Then the dried material was grinded to a fine powder using an electric grinder and stored in an air tight bottle. The powder was used to screen the phytochemicals, and *in vitro* antioxidants.

Preparation of Plant extract

About 25g of the powder of *Heliotropium indicum Linn. The leaves* were exhaustively extracted with 100ml of ethanol, and aqueous using soxhlet apparatus.

Preliminary phytochemical screening

Qualitative phytochemical examinations were carried out for all the extracts, as per the standard methods**.** [10]

Test for Steroids

0.5g of the various solvent extract fraction of each plant was mixed with 2ml of acetic anhydride followed by 2ml of sulphuric acid. The colour changed from violet to blue (or) green in some samples indicates the presence of steroids.

Test for Phenols

1ml of various solvent extracts of sample, 2ml of distilled water, followed by a few drops of 10% aqueous ferric chloride solution were added formation of blue colour (or) green colour indicated the presence of phenols.

Test for Tannins

0.25g of the various solvent extract was dissolved in 10ml of distilled water and filtered 1% aqueous iron chloride (Fecl3) solution was added to the filtrate. The appearance of intense green, purple, blue or blank colour indicated the presence of tannins in the test sample.

Test for Flavonoids

One ml of extract, 5-10 drops of diluted HCL taken in a test tube. Kept in boiling water bath for a few minutes. Magenta color indicates the presence of Flavonoids.

Test for Carbohydrates

To 2ml Benedict`s reagent 5 drops of extract were added. It was boiled for 5 minutes and cooled. Formation of reddish yellow or green colour precipitated showed the presence of carbohydrates.

Test for Alkaloids

2 ml of extract was taken in a test tube and few ml of Wagner`s reagent to be added. Formation of reddish brown colour precipitated indicates the presence of alkaloids.

Test for Glycosides

2 ml of extract, 1ml of Chloroform taken in a test tube. Added 10% Ammonium solution. Pink colour not formed absence of Glycosides.

Test for Saponin

About 1ml of the extract was diluted to 5ml of water was and the tube was shaken vigorously. Formation of 1 cm layer of foam indicates the presence of saponin.

Test for Terpenoids

One ml of extract with 2ml Chloroform and concentrated sulphuric acid is added. Red brown colour shows the presence of Terpenoids.

Test for Anthraquinones

5ml of the extract solution was hydrolysed with diluted concentrated H_2SO_4 extracted with benzene. 1ml of diluted ammonia was added to it. Rose pink colouration suggested the positive response for Anthraquinones.

Test for Proteins: (Pitrowski Test)

2ml of the extract, 2 drops of 0.05 % $CuSo_4$ and 2ml of 10% NaoH was added. Appearance of violet/purple colour is considered as a positive test for Proteins.

Test for Anthocyanidins

5 ml of the extract, 5 ml of methanolic HCL was added. The Formation of red or purple colour considered as a positive test for Anthocyanidins.

Test for reducing sugars

5 ml of the extract, 5ml of Benedicts reagent was added. The test tube is incubated in boiling water bath for 10 – 30 minutes. The development of an orange red precipitate indicates presence of Reducing Sugars.

INVITRO ANTIOXIDANT ACTIVITY

DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of**.** [11]

Calculation

The DPPH radical scavenging activity was calculated according to the following equation.

$$\text{Radical scavenging activity (\%)} = \frac{[A_C - A_S]}{A_C} \times 100$$

Where A_C= control is the absorbance and A_S= Sample is the absorbance of reaction mixture (in the presence of sample).

Total antioxidant activity

The total antioxidant capacity was evaluated by the phosphor molybdenum method**.** [12]

Calculation

The total antioxidant activity was calculated according to the following equation.

$$\textbf{\% of the total antioxidant capacity} = \frac{[A_C - A_S]}{A_C} \times 100$$

A_c is the absorbance of the control

A_s is the absorbance of the sample in the reaction mixture

Hydrogen peroxide scavenging activity

Scavenging activity of extract was evaluated by hydrogen peroxide. [13]

Calculation

The hydrogen peroxide radicals scavenging activity was calculated according to the following equation.

$$\textbf{\% of hydrogen peroxide scavenging activity} = \frac{[A_C - A_S]}{A_C} \times 100$$

RESULTS

Table -1: phytochemical analysis of ethanol and aqueous extract of *heliotropium indicum* leaves

S.NO	PHYTOCHEMICALS	AQUEOUS EXTRACT	ETHANOL EXTRACT
1.	Steroids	+	+
2.	Phenols	+	+
3.	Tannins	+	+
4.	Flavonoids	+	+
5.	Carbohydrate	-	-
6.	Alkaloids	++	++
7.	Glycosides	-	-
8.	Saponins	++	++
9.	Terpenoids	-	-
10.	Anthraquinones	-	-
11.	Protein	+	+
12.	Anthocyanidins	-	-
13.	Reducing sugars	-	-

(++) Major
(+) Minor
(-) No phytochemical

Table 2: *In vitro* Antioxidant activity of aqueous and ethanol extract of heliotroium *indicum* leaves

S.No	Name Of The Extract	Percentage Of Activity		
		DPPH Radical Scavenging Activity	Total Antioxidant Activity	Hydrogen Peroxide Scavenging Activity
1.	Ethanol	55.2 ± 1.5	42.4 ± 0.05	23.5 ± 1.08
2.	Aqueous	35.3 ± 0.5	38.3 ± 0.1	26.9 ± 2.1

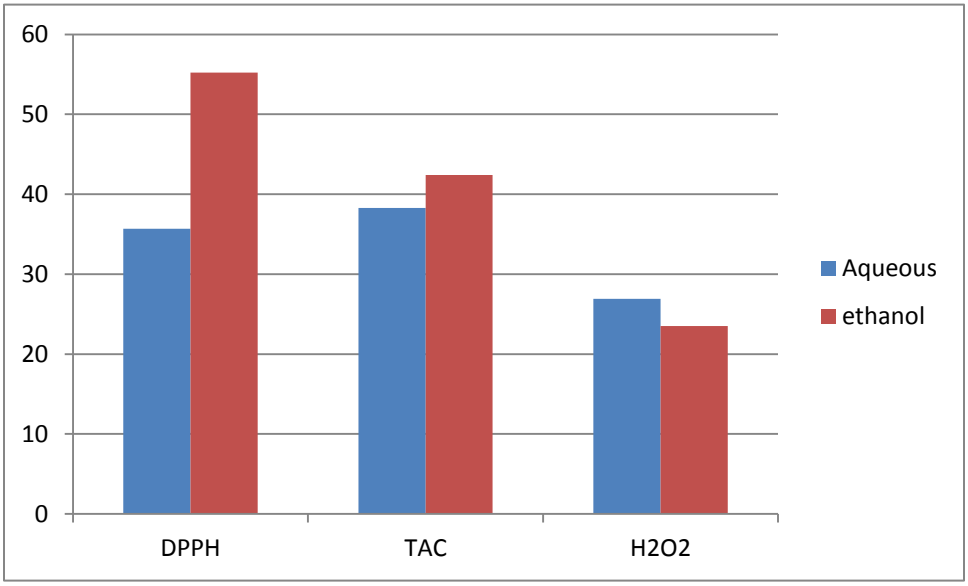

Figure 1: In Vitro Antioxidant activity of ethanol and aqueous extract of *heliotropium indicum* leaves

Table 1 shows In our study, are observed in a phytochemical screening of the plant revealed the absence of glycosides, reducing sugar 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. [14]

From the result given in tables 2 and figure 1. It was inferred that, with respect to maximum percentage inhibition in DPPH models the aqueous extract of *heliotropium indicum* showed 35.7 percentage inhibitions whereas the ethanol extract of *heliotropium indicum* showed 55.2 percentage inhibitions respectively at 1.6 mg/100ml concentrations. The IC_{50} value of the ethanolic leaf extract of *heliotropium indicum* was determined to be109 µg/ML.

The result clearly showed that free radicals were scavenged by the test compound in a concentration dependent manner in all the models. From the result in table 2 and figure 1 it was inferred that with respect to maximum percentage inhibition in total antioxidant activity models the aqueous extract of *heliotropium indicum Linn* showed 38.7 percentage inhibition, whereas, the ethanol extract of

heliotropium indicun Linn showed 42.4 percentage inhibition respectively concentration at (200 µg/ ml).

Hydrogen peroxide scavenging activity assay is shown in table 2 and figure 1. It was observed that free radicals were scavenging by the test compounds in a concentration dependent manner in all the models. From the results given in table 4 figure 3 it was inferred that with respect to maximum percentage inhibition in hydrogen peroxide scavenging activity models the aqueous extract of *heliotropium indicum linn* showed 26.9 percentage inhibition whereas, the ethanol extract of *heliotropium indicum linn* showed 23.5 percentage inhibition respectively concentration at (200 µg/ml).

DISCUSSION

The above study clearly indicates the antioxidant activity of aqueous and ethanolic extract of *heliotropium indicum* in quenching the free radicals in a dose dependent manner which is similar to the antioxidant ascorbic acid that is used as the standard.

The free radicals are chemical species which contains one or more unpaired electron. They are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. In this study, it is evident that the extract of the study species, *heliotropium indicum Linn* possess effective antioxidant activity.

The antioxidant activity of ethanolic extract of *Heliotropium indicum Linn* can be attributed to the presence of active constituents such as flavonoids, phenols, tannins, steroids, alkaloids, and saponins. Phenolic compounds are known to have redox properties which help them act as hydrogen donators, reducing agents and singlet oxygen quenchers. In addition, they also exhibit potent metal chelation potential. Polyphenols on the other hand have oxidation-reduction properties that play an important role in neutralizing free radicals**.** [15] These properties of active constituents in plants could be responsible for their antioxidant activity. DPPH radical is considered to be a model of lipophilic radical. In the mode, scavenging activity is attributed to the hydrogen donating ability of antioxidants. Although the ethanolic extract of *heliotropium indicum Linn* leaves possesses good DPPH scavenging activity, it was evident that the extract could serve as free radical inhibitors or scavengers**.** [16] DPPH radical scavenging ability was increased with increasing concentration of the extract.

CONCLUSION

In the present study, it is found that ethanol and aqueous extracts of heliotropium indicum showed that free radical scavenging activity and this antioxidant effect may be due to the higher content of alkaloids, flavonoids, steroids, phenols, tannins and saponins, highly responsible secondary metabolite for antioxidant activities. Thus, the Heliotropium indicum leaf extract as promising natural sources of antioxidants can be used in nutritional or pharmaceutical fields for the prevention of free radical–mediated diseases. However, pharma-cognostical studies are suggested to confirm the antioxidant ability before going for commercialization.

ACKNOWLEDGEMENT

The authors are grateful thank to the principal, D.G.G.Arets College (W), Mayiladuthurai – 609001, Tamilnadu, India for providing necessary laboratory facilities to complete this manuscript and also to Dr. A. Malarvizhi (Head of the department) Department of Biochemistry, D.G.G.Arts College (W), Mayiladuthurai for supporting this research work.

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