



Research Article

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**Phytochemical examination, antioxidant potential and *in vitro* antibacterial studies of crude extracts of *Parthenium hysterophorus* Linn. leaves**

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**ABSTRACT**

The present research account is an investigation on the phytochemical content, antioxidant property and antibacterial activity of the whole plant crude extract of *Parthenium hysterophorus*. Plant extract were prepared in ethanol solvent. Phytochemical screening showed the presence of different bio-constituents such as Alkaloids, Phenols, Flavonoids, Steroids, Cardiac Glycosides and Carbohydrates was detected in ethanol solvent extract. Free radical scavenging assay against the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) proved the strong antioxidant property of the plant. A highest of  $89.23 \pm 5.40$  free radical inhibition was achieved with ethanol extract and the value was very close to that obtained with ascorbic acid standard ( $98.23 \pm 5.4$ ). The ethanol extract of whole plant was found to be exhibit *staphylococcus aureus*, *bacillus cereus*, *salmonella typhi*, and *klebsiella*. The extract and fractionates of fresh leaves of *Parthenium hysterophorus* showed a significant and remarkable activity against all the microorganisms. The findings of the present study provided a strong scientific background for recognizing this plant as potential medicinal herb used by many local tribes of South India.

**Keywords:** *Parthenium hysterophorus*, *Staphylococcus aureus*, Antibacterial studies, Phytochemicals, Antioxidant.

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**INTRODUCTION**

India is called the botanical garden of the world for its rich natural resources. Over 6,000 plants in India are used in traditional, folklore and herbal medicine. The Indian system of medicine has identified 1500 medicinal plants of which 500 are commonly used. Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, and turn over and metabolism, their natural distribution and their biological function.

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates). Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds.

Hence the present paper reports the Phytochemicals, Antioxidant property and antibacterial activity on the isolated essential oil of *Parthenium hysterophorus*.

## EXPERIMENTAL SECTION

### Collection of Plant Material

Fresh leaves of *Parthenium hysterophorus* were collected in December 2014 from Aruppukottai (Madurai – Tutucorin Road), India and nearby areas. The fresh leaves was thoroughly washed with tap water and then by distilled water. The leaves were dried for 20 days under low sun intensity. The dried materials were powdered mechanically and they were stored in polythene bag to be used as samples for the extraction.

### Juice Extraction

For the preparation of plant extract the concentration which was selected was 200 mg/ml, so 40 g leaf powder was mixed with 200 ml of ethanol. The extraction was carried out by reflux condenser apparatus method and followed by filtering using Whatman filter paper no.1. The supernatant liquid was collected and evaporated at 45°C to make the final volume 1/5<sup>th</sup> of the original volume. It was stored at 4°C in air tight bottles for further use.

### Procedure for the Phytochemicals Test

The extracts of the *Parthenium hysterophorus* were screened for Phytochemical constituents using standard chemical test with little modification (R. Sasi Kumar et al, 2014) and the values were tabulated in table 1.

#### Test for alkaloids:

##### Wagner's Test:

2ml of extract was treated with wagner's reagent (Iodine and Potassium iodide in 100ml water) and formation of reddish brown precipitate indicates of the presence of alkaloids.

#### Test for flavonoids:

##### NaOH Test:

2ml of extract was treated with few drops of aqueous NaOH and HCl and formation of yellow orange color indicates of the presence of flavonoids.

##### H<sub>2</sub>SO<sub>4</sub> Test:

A fraction of extract is treated with Conc. H<sub>2</sub>SO<sub>4</sub> and observed for the formation of orange color indicates of the presence of flavonoids.

#### Lead Acetate Test:

A small amount of extract was treated with lead acetate and observed the formation of white precipitate indicates of the presence of flavonoids.

#### Test for tannins:

##### Ferric Chloride Test:

Few ml of extract was added with alcohol and treated with neutral ferric chloride solution and observed for formation of blue or greenish color solution indicates of the presence of tannins.

#### Test for saponins:

##### Foam Test:

A small amount of extract was shaken vigorously with water and the formation of persistent foam indicates of the presence of saponins.

#### Test for Cardiac glycosides:

##### Kellar-Killani Test:

2ml of extract was treated with 1ml of glacial acetic acid, 1 drop of 5% FeCl<sub>3</sub> and Conc. H<sub>2</sub>SO<sub>4</sub> and the observed for the formation of reddish brown color appears at junction of the two liquid layers and upper appears bluish green indicates the presence of Cardiac glycosides.

#### Test for quinines:

A small amount of the extract was treated with Conc. HCl and the observed for the formation of yellow color precipitate indicates of the presence of quinones.

**Test for carbohydrates:**

**Molisch's Test:**

Few drops of molisch's reagent was added to each of the portion dissolved in distilled water, this was then followed by addition of 1ml of Conc. H<sub>2</sub>SO<sub>4</sub> by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5ml of distilled water. Formation of red or dull violet color at there was interphase of the two layers was a positive test indicates of the presence of carbohydrates.

**Fehling's Test:**

About 1ml of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5ml of equal volumes of fehling's solution A and B. Formation of red precipitate of cuprous oxide, indicates of the presence of reducing sugar which shows the presence of carbohydrates.

**Test for Terpenoids:**

**Liebermann – Burchard Test:**

1ml of extract was treated with chloroform, acetic anhydride and few drops of H<sub>2</sub>SO<sub>4</sub> was added and observed the formation of dark green color indicates of the presence of terpenoids.

**Test for Sterols:**

**Liebermann – Burchard Test:**

1ml of extract was treated with chloroform, acetic anhydride and few drops of H<sub>2</sub>SO<sub>4</sub> was added and observed the formation of dark pink or red color or reddish brown ring indicates of the presence of sterols.

**H<sub>2</sub>SO<sub>4</sub> Test:**

1ml of extract was treated with ethanol and H<sub>2</sub>SO<sub>4</sub> and observed the formation of violet or green color indicates of the presence of sterols.

**Test for Phenols**

**Liebermann Test:**

1ml of extract was heated with NaNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> and diluted with water and then add excess of dilute NaOH and observed the formation of deep red or green or blue color indicates of the presence of phenols.

**Test for Anthocyanin:**

**NaOH Test:**

2ml of extract was treated with 2M NaOH and observed the formation of blue green color indicates of the presence of anthocyanin.

**Antibacterial Activity**

The antibacterial activity for the leaves of *Parthenium hysterophorus* was determined by Disc-Diffusion method. The antibacterial activity studies in nutrient agar medium for the following organisms *staphylococcus aureus*, *bacillus cereus*, *salmonella typhi*, and *klebsiella* were carried out.

**Preparation of Nutrient Agar Medium**

Exactly 1g of peptone 0.5g of Beef extract and 0.5 g of sodium chloride were weighed and transferred into conical flask and dissolved in 100ml of distilled water after the pH range was checked for 7.0 – 7.2 finally added 1.5 g of agar into the conical flask. It was closely packed with cotton plug and placed in an autoclave for 15 minutes for sterilization. The Antibacterial assay was carried out using Agar well diffusion method. Amikacin is used as reference drug and corresponding solvent (Ethanol) is used as positive controls. About 20 ml of nutrient agar medium for bacteria poured sterilized Petri dishes and allow solidifying. The agar medium was spread was 24 hrs cultured 10<sup>8</sup> CFU/ml microbial sterilized rod. Wells of 6 mm diameter were made in the culture medium using sterile cork borers. About 30 µg and 50 µg of the plant extracts (1 µg/ml) was added to the wells. Plates were than incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the inhibition zone diameters in mm formed around the well. The assay was carried out in triplicates and the results thus obtained are taken as the mean of the three readings for each concentration and no statistical tools were used calculate the standard deviation. The Antibacterial activity of *Parthenium hysterophorus* L. leaves of ethanol extract against bacteria pathogens with reference to Amikacin is reported in table 3 and also represented as Figure 3.

**Screening for Antioxidant assay****Preparation of sample**

The different concentrations of plant extract (20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

**DPPH radical-scavenging activity**

DPPH radical-scavenging activity was determined by the method of *Bushra Sultana et al., (2009)*. To 2 ml aliquot of DPPH solution (25µg/ml) was to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

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

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

**Statistical analysis**

Tests were carried out in triplicate for 3 separate experiments. The scavenging activity of sample was expressed as 50% inhibition concentration ( $IC_{50}$ ), which represented the concentration of sample having 50% of radical scavenging effect. The amount of extract needed to inhibit free radicals concentration by 50%,  $IC_{50}$ , was graphically determined by a linear regression method using Ms - Windows based graph pad Instant (version 3) software. Results were expressed as graphically / Mean ± standard deviation.

**RESULTS AND DISCUSSION****Phytochemical Analysis**Table 1: Phytochemical analysis of *Parthenium hysterophorus*

S. No	Secondary Metabolite Test & Reagents	Ethanol Extract
1	Alkaloids Wagner's Reagent	+
2	Flavonoids NaOH Test	-
	H <sub>2</sub> SO <sub>4</sub> Test	+
	Lead Acetate Test	+
3	Tannins Ferric Chloride Test	+
4	Saponins Foam Test	+
5	Cardiac Glycosides Keller-Killani Test	+
6	Quinones	+
7	Carbohydrates Molisch's Test	+
	Fehling's Test	+
8	Terpenoids Liebermann-Burchard Test	+
9	Sterols Liebermann-Burchard Test	-
	H <sub>2</sub> SO <sub>4</sub> Test	+
10	Phenols Liebermann Test	+
11	Anthocyanines NaOH Test	+

**Antibacterial Activity**Table 2: Antibacterial activity of *Parthenium hysterophorus* ethanol extract against bacteria pathogens with reference to Amikacin

Compound	Weight of the Compound (µg/ml)	Zone of inhibition (in mm)			
		<i>Salmonella Typhi</i>	<i>Staphylococcus Aureus</i>	<i>Bacillus cereus</i>	<i>Klebsiella</i>
<i>Parthenium hysterophorus</i>	10	12	5	5	5
<i>Parthenium hysterophorus</i>	30	17	9	12	9
<i>Parthenium hysterophorus</i>	50	23	14	20	12
Amikacin (Standard)	30	17	18	18	17

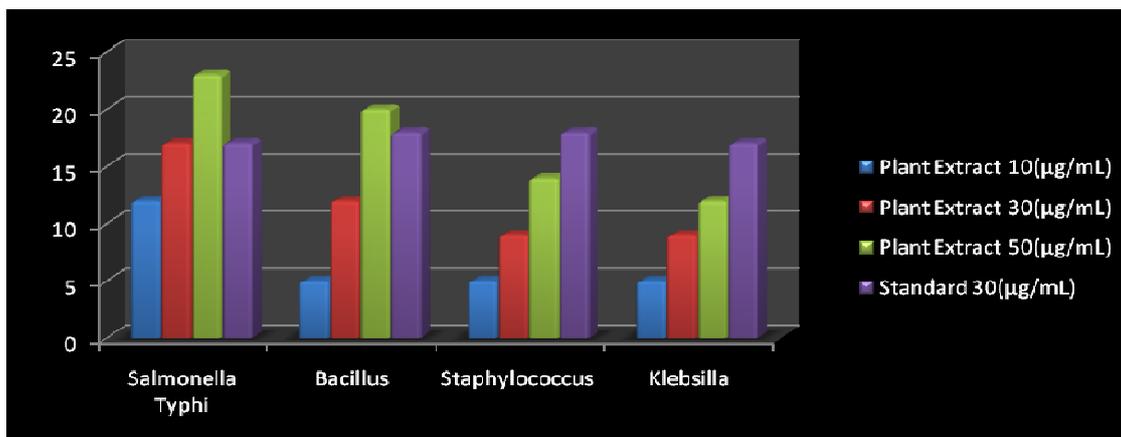


Figure 3: Comparison of Antibacterial activity of *Parthenium hysterophorus* and Standard Amikacin

Figure 4 Zone of inhibition – bacteria *Salmonella typhi*

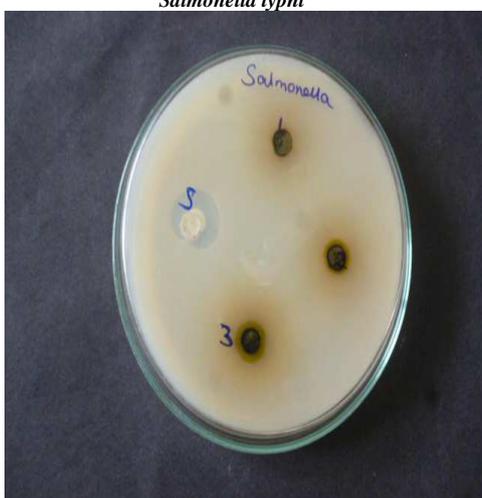


Figure 6 Zone of inhibition – bacteria *Bacillus cereus*

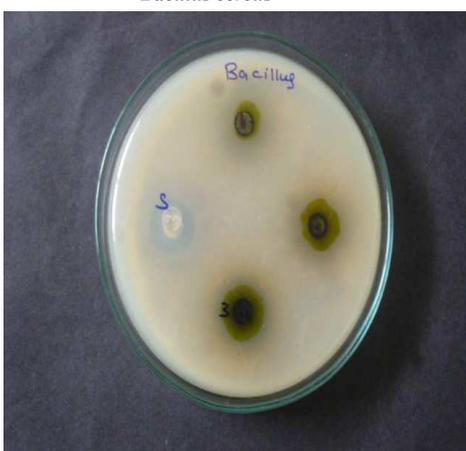


Figure 5 Zone of inhibition – bacteria *Staphylococcus aureus*

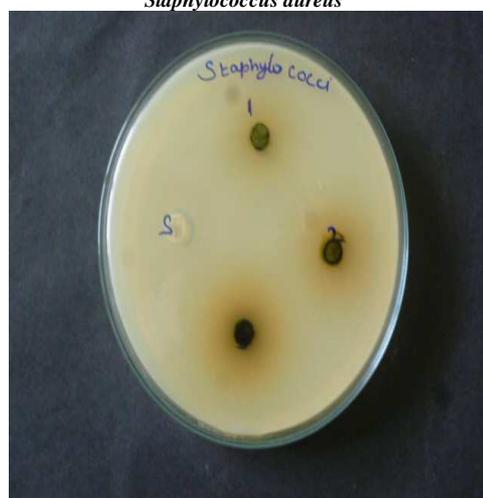


Figure 7 Zone of inhibition – bacteria *Klebsiella*

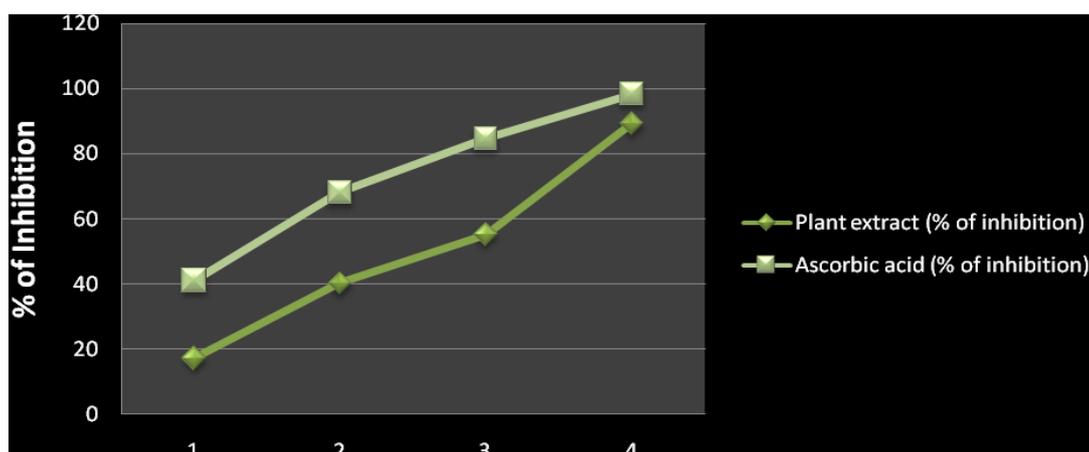


**DPPH radical scavenging activity of sample**

DPPH radical scavenging activity (%) can be calculated by using the formula as mentioned previously. The values obtained are tabulated in Table 4 and it is expressed as mean  $\pm$  standard deviation.

**Table 3: DPPH radical scavenging activity of plant extract and standard (Ascorbic acid)**

Concentrations ( $\mu\text{g/ml}$ )	Plant extract (% of inhibition)	Ascorbic acid (% of inhibition)
20	17.23 $\pm$ 1.27	41.00 $\pm$ 9.4
40	40.23 $\pm$ 2.54	68.10 $\pm$ 8.6
60	55.23 $\pm$ 3.78	84.64 $\pm$ 7.9
80	89.23 $\pm$ 5.40	98.23 $\pm$ 5.4
IC <sub>50</sub> ( $\mu\text{g/ml}$ )	58.45	25.57



**Figure 8: DPPH radical scavenging activity of plant extract and standard (Ascorbic acid)**

### CONCLUSION

Bioactive compounds such as alkaloids, flavonoids, terpenoids, sterols, carbohydrates, saponins and phenolic compounds were detected to be present in the leaves of *Parthenium hysterophorus* plant. Since this plant had been used in the treatment of different ailment such as malaria, cancer and skin burn etc., the medicinal roles of these plants could be related to such identify bioactive compounds. The present study portrays that the secondary metabolites in fresh *Parthenium hysterophorus* leaves may contribute in many significant ways for various studies in a truthful manner to the pharmaceutical activity of the plant. The antioxidant results showed that, a highest of 89.23 $\pm$ 5.40 free radical inhibition was achieved with ethanol extract and the value was very close to that obtained with ascorbic acid standard (98.23  $\pm$  5.4). The extract and fractionates of leaves of *Parthenium hysterophorus* showed a significant and remarkable activity against all the microorganisms.

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