

## Antioxidant Activity Investigation on Various Extracts of *Parthenium hysterophorus* Aerial Parts

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### Abstract

In late year, the interest of plant-based medication, wellbeing items, drug, food supplements, nutraceuticals, cosmetic items, and so forth are expanding in both creating and created nations. Since plants contain antioxidant of natural origin like polyphenols, flavonoids, carotenoids, vitamin A, E and C. These antioxidants help to adjust chronic illnesses like diabetes, cardiovascular sicknesses, maturing, malignant growth and neurological infections. The free radicals are liable for the event of these infections. An enormous number of traditional plants are accessible with solid antioxidant profile, which can overcome such sicknesses that caused because of free extremists. *Parthenium hysterophorus* is one such conventional plants, which have cell reinforcement property and oversee different infections. It is a rich source of number of phytopharmaceuticals particularly phenolic mixes and flavonoids. Different samples of *Parthenium hysterophorus* aerial parts were arranged progressively arranged by expanding polarity using standard extraction strategies. Fundamental phytochemical screening indicated presence of bioactive classes of natural compounds such as triterpenoids, phenolic mixes and flavonoids in chloroform and methanol extracts of plant. The methanol extract of plant aerial parts exhibited maximum antioxidant activity with  $IC_{50}$  value of 76.07  $\mu\text{g/ml}$ , where as standard rutin showed  $IC_{50}$  value 4.93  $\mu\text{g/m}$ . The chloroform extract showed mild antioxidant activity with  $IC_{50}$  value of 318.72  $\mu\text{g/ml}$ . The bioactive methanol extract have shown presence of phenolic and flavonoid compounds, which have been reported to possess strong antioxidant activity.

**Keywords:** Antioxidant; Flavonoids; Herbal; *Parthenium hysterophorus*; Phenols

### Introduction

An intensive study of writing uncovers suggested that a natural medication wealthy in phenolic mixtures and flavonoids, for example, rutin, quercetin, kaempferol, naringenin and hesperidin has cell reinforcement or antioxidant properties [1]. These cell reinforcement specialists assume significant part in the administration of on-going infections, for example, diabetes, malignancy, hypertension, and so forth through different components. *Parthenium hysterophorus* weed is one of such plants which is wealthy in phenols and flavonoids.

In India around 20,000 therapeutic plant species have been recorded [2], yet in excess of 500 customary networks use around 800 plant species for relieving various illnesses [3]. Right now 80 % of the total populace relies upon plant-determined medication for the main line of essential medical services for human care since it has no side effects [4]. Plants are significant wellsprings of medications and recently about 25% of drug related solutions in the United States contain at least one plant based constituent. Somewhat recently, approximately 121 drug items were detailed dependent on the conventional information acquired from different sources [5].

## Review of Literature

The plant under investigation named *Parthenium hysterophorus* has been dispersed around 20 nations all through the world. This weed is fundamentally appropriated in West Indies, Southern North and focal South America, Mexico, China and India [6]. The *Parthenium hysterophorus* weed has been chiefly produced naturally in the various regions like wastelands, orchards, public gardens, forest field, agricultural places, playgrounds, roadsides, railway tracks and vacant plots. The *Parthenium hysterophorus* weed is ordinarily produced in the alkaline and loamy soils under dry season conditions [7].

## Objectives

Keeping in view the traditional, alternative and complementary medicinal uses, sporadic pharmacognostic reports are available on *Parthenium hysterophorus*. Therefore, it was considered worthwhile to establish a complete monograph of this clinically potential plant and implement the following plan of work to achieve the goal.

- The plant weed will be subjected to Soxhlet apparatus to prepare various crude extracts of in the increasing order of polarity.
- The prepared extracts of plant weeds will be subjected to preliminary phytochemical screening to detect presence of phytomolecules present there in.
- The prepared extracts of plant weeds will be evaluated for *in vitro* antioxidant activity using well established model named DPPH assay.

## Material and Methods

### Plant material

The *Parthenium hysterophorus* aerial parts were collected from wild areas of Himachal Pradesh in the month of November, 2021. The authentication of the plant material was confirmed on the basis of literature reported and various photographic pictures available online.

### Preparation of various extracts of plant material

The aerial parts of plant material were dried in a hot air oven at a temperature 55°C for 48 hours to remove moisture present in plant material. The plant materials were powdered using mixer grinder. The 100 g of powdered aerial parts were successively

extracted with solvents (3×500 ml) in order of increasing polarity viz, n-hexane, chloroform and methanol through Soxhlet apparatus process at temperature of 80°C. The marc of aerial parts was extracted separately with distilled water (3×3 L) by decoction process at a constant temperature of 100°C. The solvent from crude extracts was recovered by distillation process to get n-hexane extract (HE), chloroform extract (CE), methanol extract (ME) and water extract (WE). The percentage yields (w/w) of crude extracts were recorded.

### Phytochemical screening of various extracts

All extracts of plant material were subjected to phytochemical screening, to detect different classes of phytoconstituents are present [8].

### *In vitro* antioxidant activity studies using DPPH method

#### Preparation of control

Free radical scavenging activity of sample was determined using DPPH assay [9]. A solution of 0.1 mM DPPH was freshly prepared in methanol. Then 5 ml of freshly prepared solution was added to 5 ml of methanol in a test tube. The test tube was kept in a dark for 30 min at room temperature. Then after 30 min absorbance was recorded at 517 nm using UV/Vis spectrophotometer against blank, i.e., methanol.

#### Preparation of standard solution

Accurately weighed 10 mg rutin was dissolved in methanol (100 ml) to make a stock solution (100 µg/ml), which was further diluted to get different concentrations (2, 4, 6, 8, or 10 µg/ml) with methanol. Then equal volume of different concentrations of standard were taken and added to methanolic solution of DPPH. The solution was kept in a dark for 30 min at room temperature. Then after 30 min absorbance was taken at 517 nm using UV/VIS spectrophotometer against blank, i.e., methanol.

#### Preparation of test sample

Accurately weighed 250 mg of each crude extract of plant was dissolved in methanol (25 ml) and made stock solutions (10,000 µg/ml). Then different concentrations of samples were prepared by diluting stock solution with methanol. The equal volume from different concentrations of various extracts were taken and added to methanolic solution of DPPH. The solution was kept in a dark for

30 min at room temperature. Then after 30 min absorbance was taken at 517 nm using UV/VIS spectrophotometer against blank, i.e., methanol.

**Determination of antioxidant activity**

Percentage radical scavenging activity was calculated by following formula:

$$\% \text{ Radical Scavenging Power} = [Ac - (As - Ao)] / Ac \times 100$$

Where Ac = Absorbance of control (DPPH); As = Absorbance of sample/ standard + DPPH; Ao = Absorbance of sample/standard without DPPH interaction.

The measurements were taken thrice, and scavenging effect was calculated based on the percentage of DPPH scavenged. IC<sub>50</sub> values of the samples for antioxidant activity were calculated using standard curve of rutin.

**Statistics**

The data were expressed as Mean ± S.D. The test groups were compared with the standard group using One way ANOVA followed by student Newman Keul’s test [10].

**Results and Discussion**

Various crude extracts of plant aerial parts prepared through successive extraction method using solvents viz., n-hexane, chloroform, methanol and water. The % yields of various crude extracts are shown in table 1.

| Extract            | Yield (% w/w) |
|--------------------|---------------|
| n-hexane extract   | 3.14          |
| Chloroform extract | 5.47          |
| Methanol extract   | 8.45          |
| Water extract      | 10.80         |

**Table 1:** Yield of various extracts of plant aerial parts.

The crude extract of the plant aerial parts were setup in expanding order of polarity by using a standard extraction technique. All unrefined extract of plant aerial parts were screened for presence of different classes of phytoconstituents. Phytochemical screening indicated presence of different gathering of phytoconstituents in different crude extract. n-hexane concentrate of the plant aerial

parts demonstrated presence of lipids and steroids. Chloroform extract contained triterpenoids. The methanol crude extract of plant aerial parts showing presence of flavonoids and tannins. The water extract indicated presence of proteins, and carbohydrates. The results of phytochemical screening are shown in table 2.

| Class of phytoconstituents | HE  | CE  | ME  | WE  |
|----------------------------|-----|-----|-----|-----|
| Carbohydrates              | -   | -   | -   | +   |
| Protein/Amino acids        | -   | -   | -   | +   |
| Alkaloids                  | -   | -   | -   | -   |
| Lipids                     | +   | -   | -   | -   |
| Anthraquinone glycosides   | -   | -   | -   | -   |
| Cardiac glycosides         | -   | -   | -   | -   |
| Flavonoids                 | -   | -   | +   | -   |
| Saponins                   | -   | -   | -   | -   |
| Tannins/Phenolic compounds | -   | -   | +   | -   |
| Steroids/Triterpenoids     | +/- | -/+ | -/- | -/- |
| Coumarins                  | -   | -   | -   | -   |

**Table 2:** Phytochemical screening of various extracts of plant aerial parts.

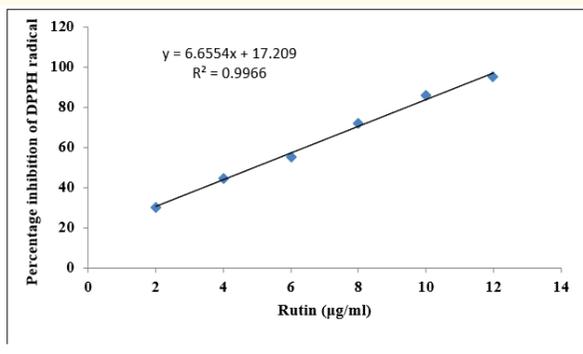
+: Present, -: Absent

**In vitro antioxidant activity of crude extract of plant aerial parts**

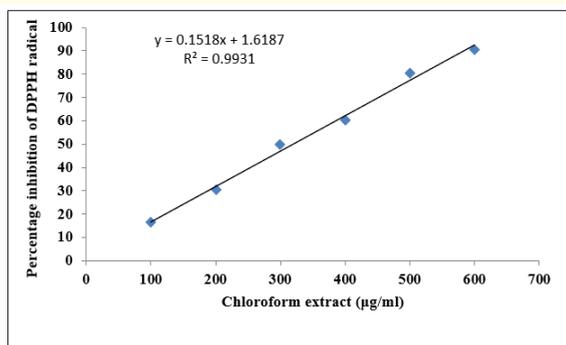
As phytochemical screening showed that only the chloroform and methanol extract contained bioactive classes of natural compounds. Thus only the chloroform extract and methanol extract of plant aerial parts were evaluated for *in vitro* antioxidant activity using DPPH assay. Rutin was used as standard antioxidant agent. Standard plots were prepared between % inhibition of DPPH and concentrations of samples (figure 1). Using regression equations of standard plots, IC<sub>50</sub> values of standard and samples were determined. It is clearly evident from table 3 that methanol extract of plant aerial parts exhibited maximum antioxidant activity with IC<sub>50</sub> value of 76.07 µg/ml, where as standard rutin showed IC<sub>50</sub> value 4.93 µg/m. The chloroform extract showed mild antioxidant activity with IC<sub>50</sub> value of 318.72 µg/ml.

Subjective chemical tests performed on different extracts plant aerial parts confirmed that n-hexane extract did not contained any content of flavonoids and phenolic compounds which have

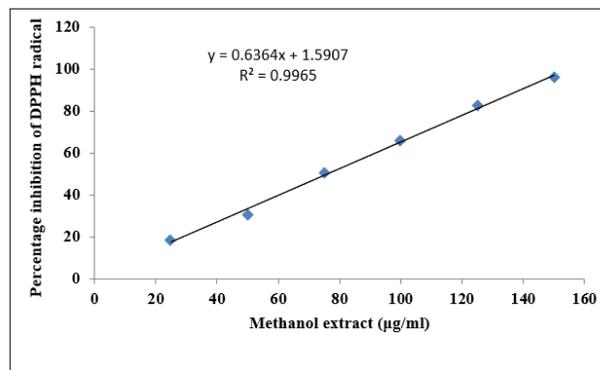
been viewed as primary constituents used as antioxidant. Similar preliminary phytochemical screening results were observed in water extract. Chloroform and methanol extracts indicated presence of triterpenoids, phenols and flavonoids in phytochemical screening examinations, therefore, these samples were exposed to in vitro antioxidant assessment. The natural antioxidants are commonly known as cancer preventive agents. In a new year, an expansion in intake of plant-based food materials has been noticed on the grounds that it is a decent wellspring of common cancer prevention agents. The impacts of cancer prevention agent on the human wellbeing are exceptionally critical. The natural antioxidants kill free radicals and keep safe from DNA harm. The DPPH measure is a simple, touchy technique to estimate antioxidant action of the plant samples. This protocol depends on the estimation of the deficiency of DPPH tone (colour) after response with test samples at 517 nm, and the response were seen with the assistance of spectrophotometer. The DPPH was stable free radicals of purple tone and within the sight of antioxidant agent it changed tone from purple to yellow. Shading change speaks to adequacy of the antioxidant. Change in the absorbance regarding control was determined as percent scavenging power. IC<sub>50</sub> is characterized as the value that causes a decline in the underlying grouping of DPPH by half.



(a)



(b)



(c)

**Figure 1:** Standard plots between % inhibition of DPPH vs. Concentration of test sample. (a), Rutin; (b), chloroform extract and (c), methanol extract of plant aerial parts.

| Treatment          | Concentration (µg/ml) | Mean <sup>n</sup> percentage inhibition of DPPH radical ± S.D. | IC <sub>50</sub> Values (µg/ml) |
|--------------------|-----------------------|--|---------------------------------|
| Rutin              | 2                     | 30.21 ± 0.325  | 4.93                            |
|                    | 4                     | 44.47 ± 0.220  |                                 |
|                    | 6                     | 55.48 ± 0.458  |                                 |
|                    | 8                     | 71.47 ± 0.417  |                                 |
|                    | 10                    | 85.70 ± 0.259  |                                 |
|                    | 12                    | 95.45 ± 0.896  |                                 |
| Chloroform extract | 100                   | 16.45 ± 0.314  | 318.72*                         |
|                    | 200                   | 30.47 ± 0.145  |                                 |
|                    | 300                   | 49.90 ± 0.325  |                                 |
|                    | 400                   | 60.48 ± 0.458  |                                 |
|                    | 500                   | 80.62 ± 0.321  |                                 |
|                    | 600                   | 90.47 ± 0.460  |                                 |
| Methanol extract   | 25                    | 18.47 ± 0.245  | 76.07*                          |
|                    | 50                    | 30.48 ± 0.450  |                                 |
|                    | 75                    | 50.78 ± 0.369  |                                 |
|                    | 100                   | 65.80 ± 0.489  |                                 |
|                    | 125                   | 82.46 ± 0.113  |                                 |
|                    | 150                   | 95.64 ± 0.879  |                                 |

**Table 3:** Antioxidant activity of chloroform and methanol extracts of plant aerial parts.

N = 3; \*P < 0.05 vs. Rutin; One way ANOVA followed by Student Newman Keul's test.

## Conclusion

The bioactive methanol extract have shown presence of phenolic and flavonoid compounds, which have been reported to possess strong antioxidant activity. These observations suggest that it is better to isolate main constituents responsible for antioxidant activity in future studies using column chromatography.

## Conflict of Interest

There is no conflict of interest.

## Financial Assistance

There is no any funding provided, granted and was taken for my study.

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