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Investigation of antioxidant potential of Petunia hybrida leaf extracts

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Abstract

The objective of the present study was to assess the antioxidant potential of different leaf extract of *Petunia hybrida* using the in vitro model. The extraction ability of different solvents for recovering extractable components from leaves followed the order: ethanol>chloroform>aqueous>pet ether. The findings of preliminary phytochemical analysis suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaf of the plant. The total phenolic content of pet ether, chloroform, ethanol and aqueous extracts of *Petunia hybrida* were 16.18±0.89, 23.97±0.61, 71.77±0.59 and 31.38±0.36 GAE mg/g, respectively. All the extracts were subjected to *in vitro* determination of antioxidant potential by DPPH method. The IC_{50} value of the DPPH scavenging potential for the chloroform, ethanol and aqueous extracts was found to be 163.3, 96.1 and 153.2 µg/mL respectively. The reducing potential was found to be dose dependent and the followed the order ethanolic>aqueous>chloroform. The IC_{50} value of the percent HRSA for the chloroform, ethanol and aqueous extracts was found to be 225.90, 172.02, and 177.7 µg/mL respectively. The IC_{50} value of the percent PRP for the chloroform, ethanol and aqueous extracts was found to be 226.17, 172.72, and 223.14 µg/mL respectively. It was found that the chloroform fraction was best able to inhibit the phosphomolybdenum complex.

Keywords: *Petunia hybrida,* DPPH, antioxidant, phosphomolybdenum, extract, hydroxyl, scavenging

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Introduction

An impressive number of modern drugs have been isolated from natural sources. One of the earliest records of the use of medicinal plants is in the treatment of leprosy using chaulmogra oil obtained from the seeds of *Hydnocarpa gaerta*[4]. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them. Antioxidants may be of great help in improving the quality of life as they can either prevent or postpone the onset of several degenerative diseases. Also, they have a potential for considerable savings in the cost of health care delivery.

Petunia hybrida is a hybrid of various species of petunia produced by hybridization *P. axillaris* and *P. integrifolia.*[11] The plant is known to contain some acylated anthocyanins which are cinnamic acid, coumaryl rutinoside glycosides and rutinoside glucosides acylated with caffeic acid. [11-15]

Owing to the presence of several anthocyanin flavonoids, *Petunia hybrida* could be explored a number of pharmacological actions. Several reports on the flavonoids presented the antioxidant potential and hence it was decided to explore the antioxidant activity of *Petunia hybrida* leaf extracts.

Material and Methods

The leaves and flowers of *P. hybrida* were procured from Shubham Nursery, Bhopal in the month of January and authenticated. The leaves of the authenticated plant were washed with distilled water and were dried under shade. The dried leaves were powdered using a hand blender at low speed. The powdered leaves were stored in air tight container until they were required for extraction purpose.

Extraction of leaves

500 g of the leaf powder was packed evenly in the extractor of the soxhlet apparatus and the phytoconstituents were extracted with various solvents of increasing polarity including petroleum ether, chloroform and ethanol (90%) by hot continuous extraction process for about 18 h. The aqueous extraction was carried out by cold maceration process after completion of the hot solvent extraction process. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extracts were concentrated by distillation to reduce the volume by 10 parts. The concentrated extracts were transferred to 100 ml beaker and the remaining solvents were evaporated on water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture**.** The dried extracts were stored in desiccators for further processing.

Preliminary phytochemical screening

The presence or absence of usual plant secondary metabolites was evaluated by qualitative reactions in all the extracts. The qualitative phytochemical screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as the criteria of confirmation to these tests.

Total Phenolic Content[28]

5 g dried powder of leaves was mixed with 80 mL of methanol and kept overnight. The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 100 mL with methanol. The solution was stored at 4°C in amber bottles and served as the stock solution (50 mg/mL) for subsequent analyses.

For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Determination of antioxidant potential[29]

Determination of DPPH radicals scavenging activity

Separately, 1mM solution of DPPH and extract solution (50-250 µg/mL) were prepared in ethanol. 1.5ml of the extract solution was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared using 3 mL ethanol. The control sample used was 3 mL of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

$$\text{DPPH scavenged (\%)} = \frac{(A_{con} - A_{test})}{A_{con}} \times 100$$

A_{con} - is the absorbance of the control reaction

A_{test} - is the absorbance in the presence of the sample of the extracts.

Reducing power assay

Different concentrations of the extracts (50-250 µg/mL to the final concentration) in methanol (1.0 mL) were diluted with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and mixed with 2.5 mL 1% potassium ferricyanide. After incubation at 50°C for 20 minutes, 2.5 mL of 10% TCA were added to the mixture. 2.5 mL of the reaction mixture was diluted with an equal amount of distilled water and absorbance was measured at 700 nm after treatment with 0.5 mL of 0.1% $FeCl_3$. Increased absorbance of the reaction mixture indicates an increase in reduction capability.

Determination of hydroxyl radicalscavenging activity

Various concentrations of extract (50, 100, 150, 200and 250 µg) were taken and 1 mL of iron EDTA solution, 0.5mL of EDTA solution, 1 mL of DMSO and 0.5mL of ascorbic acid were added to it. The mixture was incubated in a boiling water bath at 80 to 90°C for 15 min. After incubation, 1 mL of ice cold TCA and 3mL of Nash reagent were added and the reaction mixture was incubated at room temperature for 15 min. The absorbance was read at 412 nm. The % hydroxyl radical scavenging activity is calculated by the following formula

$$\% HRSA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where, HRSA is the Hydroxyl Radical Scavenging Activity, Abs control is the absorbance of control and Abs sample is the absorbance of the extract.

Phosphomolybdenum assay

10mg of plant extract was dissolved in 1 mL of DMSO. 100µl of the sample was taken and 1 mL of the reagent solution was added to it. The mixture was incubated in a boiling water bath at 95°C for 90 min. After 90 min, the absorbance of the solution was read at 695 nm. Ascorbic acid (10 mg/mLin DMSO) was used as standard. The Phosphomolybdenum reduction potential (PRP) of the studied extracts were reported in percentage using the formula

% of inhibition = (control OD - sample OD/ Control OD) x 100.

Results and Discussion

The extraction abilitiy of different solvents for dissolving and extracting various components from leaves was in the order: ethanol>chloroform>water>pet ether.

The findings of the phytochemical analysis suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaves. The presence of anthocyanins has been reported by Griesbach et al[14].

Total Phenolic content

The pet ether, chloroform, ethanol and aqueous extract of *Petunia hybrida* were evaluated for quantifying the total phenolic content concentrations in extracts. The total phenolic content of pet ether, chloroform, ethanol and aqueous extract of *Petunia hybrida* were 16.18±0.89, 23.97±0.61, 71.77±0.59 and 31.38±0.36 GAE mg/g, respectively. The ethanolic extracts exhibited highest amount of total polyphenol content compared to all other solvent extracts.

Determination of Antioxidant Potential

The chloroform, ethanol and aqueous extracts rich in flavonoids and triterpenoids were subjected to *in vitro* determination of antioxidant potential.

DPPH radicals scavenging activity

The results obtained (figure 1) make it evident that that the extracts had the ability to donate hydrogen thereby stabilizing DPPH. The scavenging was found to dose dependent. The IC_{50} value of the DPPH

scavenging potential for the chloroform, ethanol and aqueous extracts was found to be 163.3, 96.1 and 153.2 µg/mL respectively.

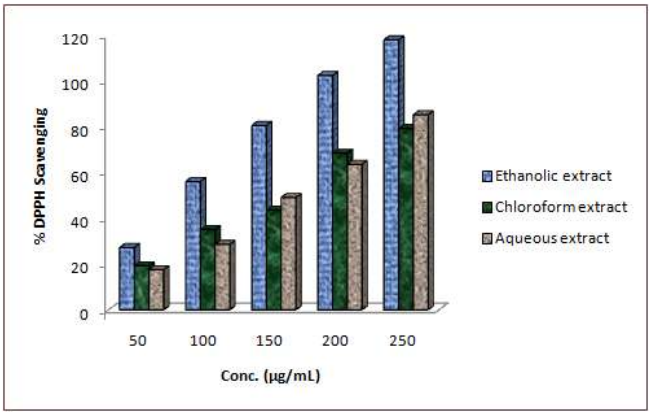

Figure 1 % DPPH scavenging potential of various extracts of *Petunia hybrida*

Reducing power assay

The reducing potential was found to be dose dependent and the followed the order ethanolic>aqueous>chloroform. The results are expressed in table 1-3.

Table 1 Reducing Power of Ethanolic extract

Conc (µg/mL)	Absorbance at 700 nm		
	10 min	20 min	30 min
50	0.056	0.051	0.042
100	0.097	0.089	0.078
150	0.139	0.127	0.11
200	0.176	0.168	0.153
250	0.221	0.201	0.191

Values are mean ± SEM of six determinations

Table 2 Reducing Power of Chloroform extract

Conc (µg/mL)	Absorbance at 700 nm		
	10 min	20 min	30 min
50	0.036	0.031	0.028
100	0.068	0.057	0.049
150	0.105	0.101	0.089
200	0.153	0.142	0.103
250	0.189	0.174	0.135

Values are mean ± SEM of six determinations

Table 3 Reducing Power of Aqueous extract

Conc (µg/mL)	Absorbance at 700 nm		
	10 min	20 min	30 min
50	0.042	0.037	0.029
100	0.089	0.07	0.056
150	0.128	0.121	0.105
200	0.165	0.153	0.141
250	0.205	0.191	0.176

Values are mean ± SEM of six determinations

Hydroxy radical scavenging assay

The results indicate a dose dependent hydroxy radical scavenging activity (HRSA) in the extracts and also reveal that the aqueous extract was able to inhibit the hydroxy radical more than the ethanolic and the chloroform extract (figure 2). At concentration of 250 µg/mL, the aqueous and the ethanolic extracts were able to scavenge the hydroxy radical at par with ascorbic acid (the standard solution).

The IC_{50} value of the percent HRSA for the chloroform, ethanol and aqueous extracts was found to be 225.90, 172.02, and 177.7 µg/mL respectively.

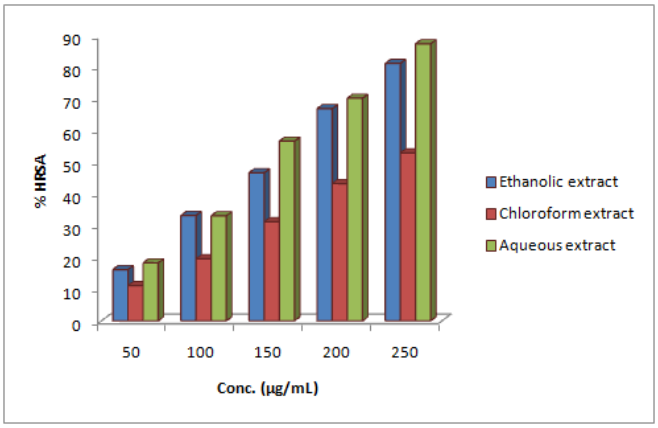

Figure 2 % HRSA of various extracts of *Petunia hybrida*

Phosphomolybdenum assay

The IC_{50} value of the percent PRP for the chloroform, ethanol and aqueous extracts was found to be 226.17, 172.72, and 223.14 µg/mL respectively. It was found that the chloroform fraction was best able to inhibit the phosphomolybdenum complex (figure 3).

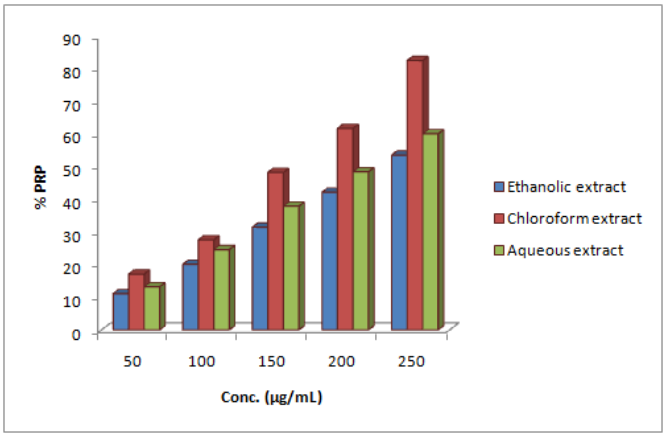

Figure 3 % PRP of various extracts of *Petunia hybrida*

Conclusion

The objective of the present study was to assess the antioxidant potential of different leaf extract of *Petunia hybrida* using the in vitro model. The results obtained led to the conclusion that *Petunia hybrida* leaves are a rich source of potential antioxidants. Despite being extracted at high temperature, the inhibition of the oxidation species suggest the presence of phytochemical that are heat stable and possess high antioxidant potential. The wide availability of the plant and no specific agro-climatic conditions for its growth make the evergreen plant a good source of natural antioxidant that may be converted as nutraceutical supplements.

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