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In vitro Assessment of Antioxidant potential of *Pergulariadaemia* (Forsk) Chiov Flower

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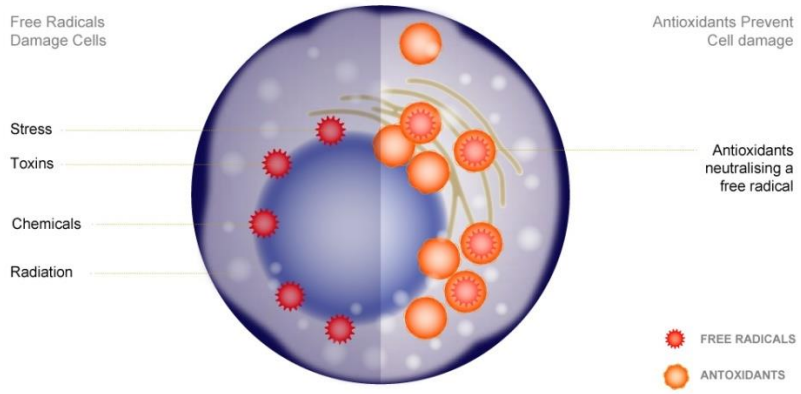

Abstract:
Pergulariadaemia(*Asclepiadaceae*)is traditionally used as medicinal agent to cure various ailments in human beings.It is a perennial herb widely distributed in the tropical and subtropical regions of the world. Naturally the plant has powerful antioxidants like phenols flavonoids and tannins.In the present study nitric oxide radical, metal chelating, superoxide radical, and hydrogen peroxide radical scavenging activities were investigated to assess the antioxidant potency of the methanol, petroleum ether and ethyl acetate extracts from flower of *Pergulariadaemia*.All the examined extracts were found to possess significant antioxidant activity ($P<0.05$).The results confirmed that *Pergulariadaemia*consistsof antioxidant activity and could serve as free radical inhibitor.

Keywords: *Pergulariadaemia*,antioxidant,superoxide radical, nitric oxide radical, metal chelating, hydrogen peroxide radical

Introduction:

Antioxidants are vital materials which at low concentrationcan significantly inhibit or delay the oxidative process, while often being oxidized them [1].Free radicals are important to any biochemical activities and play a significant role in aerobic life and metabolism [2]. Further, the most regular reactive oxygen species (ROS) include radicals like peroxyl ($ROO^{\cdot}$) & reactive hydroxyl ($OH^{\cdot}$) and superoxide (O_2^{-}) anion, or hydrogen peroxide (H_2O_2); similarly, the nitrogen derived free radicals are nitric oxide ($NO^{\cdot}$) and peroxynitrite anion ($ONOO^{\cdot}$). As we know free radicals are highly reactive and can attack any one of the molecules found in the living cells causing damage to the system which leads to various diseases in human beings such as arthritis,atherosclerosis, ischemia and reperfusion injury of many tissues;CNS injury, gastritis, diseases, cancer, aging, liver and AIDS [3]. Both exogenous and endogenous antioxidants either natural or synthetic can be effective in the prevention of the free radical formation by scavenging or by decomposition and suppression of such disorders [4]. So, in recent years the search for natural antioxidant has been widely increased.The *P.daemia* (Asclepiadaceae)plant is known as "Dushtupatige" in Telugu, "Uttaravaruni" in Sanskrit. Ethnopharmacological studies reported the plant *P.daemia* is used as anti helmintic, antipyretic, laxative, and expectorant; also used in treatmentof infantile diarrhoea and malarial intermittent fevers [5-7]. In continuation to our earlier study [8], in this investigation the author examined the *In Vitro*antioxidant activity of *P.daemia* flower in order ascertain the medicinal importance of the plant.

Materials And Methods:

a) Plant sample collection

The *P.daemia*plant material was initially collected at Nallamalla forest, Prakasam district, Andhra Pradesh and its botanical identity was confirmed by senior Botanist of Depart of Botany, Acharya Nagarjuna University, Guntur, AP-India.

b) Preparation of the extract

The air dried powder of flower was extracted in a soxhlet extractor using selected petroleum ether, ethyl acetate and methanol solvents. Beforeextraction While extracting each time simultaneously with next solvent the material was dried using hot air oven at 40 degree Celsius then the material was softened by soaking (maceration) in hot water with rare stirring for 16 hours and water extract was filtered. Finally, all the three different extracts were evaporated to remove the final traces if any of the solvents used. The extract recoveries in the solvents were stated in percent of the plant sample dry matter.

c) Assessment of Nitric oxide radical scavenging activity

Nitric oxide revolutionary scavenging activity was dictated by sreejayan and Rao [8] strategy. Three mL of 10mM sodium nitroprusside in 0.2 M phosphate cradled saline (pH 7.4) was blended in with various focuses (40-200µg) of dissolvable concentrates and brooded at room temperature for 150 min. After brooding time, 0.5 mL of Griess reagent (1 rate sulfanilamide, 0.1

rate naphthylethylenediaminedihydrochloride in 2 rate H_3PO_4) was included. The absorbances of the chromophore shaped were estimated at 546 nm. BHA and similar blend of the response without test removes were utilized as certain and negative control. Rate revolutionary searching action of the example was determined asfollows:

Percentage NO radical scavenging activity = (control OD-sample OD/control OD) x 100

The investigation was done in triplicate. The sample concentration providing 50 percentage inhibition (IC_{50}) under the assay condition was calculate from the graph of inhibition percentage in opposition to sample concentration.

d) Assessment of superoxide radical scavenging activity

As per Beauchamp and Fridovich[9] Each 3 mL reaction mixture was contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin and 12 mM EDTA, 0.1 mg NBT and different concentrations of test samples(40 – 200µg). Reaction was begun by enlightening the response blend with test extricate for 90 seconds. Following light the absorbance was estimated at 590 nm. The whole reaction get together was encased in a container fixed with aluminum foil. the identical tubes with reaction mixture kept in dim filled in as clear. BHA was utilized as sure control. The rate restraint of superoxide anion age was determined as:

Percentage Inhibition = (control OD-sample OD / control OD) x 100

The investigation was done in triplicate. The sample concentration providing 50 percentage inhibition (IC_{50}) under the assay was calculate from the graph of inhibition percentage against sample concentration.

e) Assessment of Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was estimated by the protocol of Klein *et al.*,[10]. Various concentrations (40-20µg) of different solvent extracts were ransfered1mL of iron-EDTA solution (0.13 percentage ferrous ammonium sulfate and 0.26 percentage EDTA), 0.5mL of EDTA solution (0.018 percentage), and 1mL of dimethyl sulfoxide (0.85 percentage v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was startedwith adding 0.5 mL of ascorbic acid (0.22pecentage) and incuubated at 80-90°C for 15 min in a hot water bath. After incubation the reaction was stopped by the adding of 1mL of ice-cooled TCA (17.5 percentage w/v). Three mL of Nash agent (75.0g of ammonium acetate, 2 mL of acetyl acetone and 3 mL of glacial acetic acid, were clubbed and made up to 1 Litre with addition of distilled H_2O) and left at rt for 15 min. The BHA and the reaction mixture without sample were utilized as +ve and -Ve control. The strength of the color fomed was mesuredspectroscopically at 412 nm against reagent blank. The percentage hydroxyl radical scavenging activity was determined as follows:

Percentage HRSA = (control OD-sample OD / control OD) × 100

The investigation was done in triplicate. The sample concentration providing 50 percentage Inhibition (IC_{50}) assay circumstance was calculated from the graph of inhibition percentage in opposition to sample concentration.

f) Assessment of Metal chelating activity

The chelation of ferrous ions by the distinctive solventextract concentrates was assessed by the technique for Diniset al. [11]. Quickly, 50µL of 2mM $FeCl_2$ was added to test concentrates and standard EDTA (50 - 250µg). The response was started by the expansion of 0.2 mL of 5mM ferrozine arrangement. The blend was energetically shaken and permitted to remain at room temperature for 10 min. The absorbance of the arrangement was estimated at 562 nm.

The rate restraint of ferrozine-Fe^{+2} complex development was determined as

$[(A0-As)/As] \times 100,$

Where Ao was the absorbance of the control and As was the absorbance of the concentrate and EDTA was utilized as sure control.

Statistical analysis

The results were expressed as mean standard error (SE). Statistical analysis was carried out by analysis of variance (ANOVA) followed by Dun net's. $P<0.01$ and $p<0.05$ were considered as indicative of significance, as compared to the control group. All calculations were performed using SPSS (version 11.0; Chicago, IL, USA).

Results and Discussion:

In this investigation the author studied bioactivities such as nitric oxide radical scavenging activity, superoxide radical scavenging activity and metal chelating activity method and reported in following Tables 1,2 and 3.

Table 1: Results ofNitric oxide radical scavenging activity of *P. daemia*Flower in different solvent extracts.

Solvent extracts	**Percentage activity**				
	40(µg)	**80(µg)**	**120(µg)**	**160(µg)**	**200(µg)**
Petroleum ether	4.20 ± 0.31	8.54 ± 0.80	14.84 ± 0.98	20.16 ± 0.85	24.39 ± 0.64
Ethyl acetate	3.20 ± 0.27	7.29 ± 0.15	11.68 ± 0.41	15.23 ± 0.27	19.34 ± 0.57
Methanol	11.56±0.78	24.36 ± 0.38	35.43 ± 0.51	47.43 ± 0.33	59.54 ± 0.90

The percentage of scavenging activity in petroleum ether extract was determined between 4.20 ± 0.31 to 24.39 ± 0.64. The highest and lowest percentage activities were observed at 4.20 ± 0.31 at 40 µg and 24.39 ± 0.64 at 200 µg respectively, while moderate percentage activity was found

as 8.54 ± 0.80 at 80 µg, 14.84 ± 0.98 at 120 µg and 20.16 ± 0.85 at 160 µg concentrations. In respect of ethyl acetate extract the percentage of scavenging activity was determined between 3.20 ± 0.27 to 19.34 ± 0.57. The highest and lowest percentage activities were observed at3.20 ± 0.27 at 40 µg and 19.34 ± 0.57 at 200 µg respectively, while moderate percentage activity was determined as 7.29 ± 0.15 at 80 µg, 11.68 ± 0.41 at 120 µg and 15.23 ± 0.27 at 160 µg concentrations. Similarly, the percentage of scavenging activity in methanol extract was determined between11.56 ± 0.78 to 59.54 ± 0.90. The highest and lowest percentage activities were observed at 11.56 ± 0.78 at 40 µg and 59.54 ± 0.90 at 200 µg respectively, while moderate percentage activity was calculated as 24.36 ± 0.38 at 80 µg, 35.43 ± 0.51 at 120 µg and 47.43 ± 0.33 at 160 µg concentrations.

In this investigation comparative analysis between the extracts at different concentrations using bar diagrams (Fig.1).

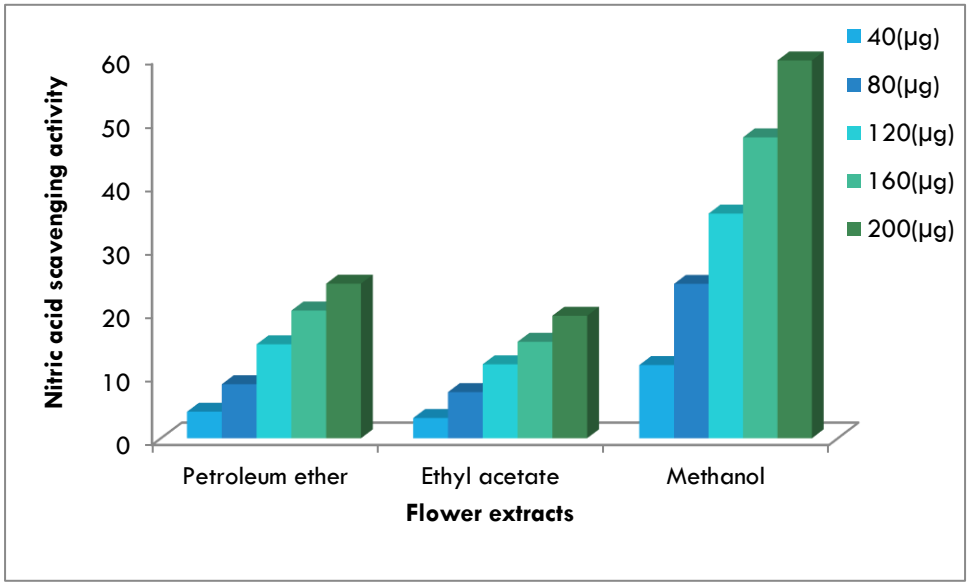

Fig. 1: Nitric oxide radical scavenging activity of *P. daemia*Flower in different solvent extracts.

From the above results it was observed the maximum percentage of Nitric oxide radicalscavenging activitywas found for methanol extract forflower at 200 µg concentration, followed by same methanol extract at 160 µg concentration. However the authors also studied the IC50 values for the NO radical scavenging activity (Table 2).

Table 2: 50% inhibition of flower in different solvent extracts on NO radical scavenging activity.

Solvent	**50 percentage Inhibition (µg/mL)**	
Solvent extracts	**Flower**	**BHA**
Petroleum ether	154.86 ± 2.53	4.2 ± 2.1
Ethyl acetate	194.44 ± 2.20	
Methanol	64.85 ± 0.62	

The maximum value 194.44 ± 2.20 of flower was observed in ethylacetate extract and followed by petroleum ether extract 154.86 ± 2.53 and minimum value was recorded in methanol extract 64.85 ± 0.62.

The results of Superoxide radical scavenging activity of *P. daemia*Flower in different solvent extracts were reported in Table 3.

Table 3: Superoxide radical scavenging activity of *P. daemia*Flower in different solvent extracts

Solvent extracts	**percentage of Inhibition**				
	40(µg)	**80(µg)**	**120(µg)**	**160(µg)**	**200(µg)**
Petroleum ether	5.69 ± 0.35	9.76 ± 0.29	14.98 ± 0.49	20.04 ± 0.35	25.42 ± 0.41
Ethyl acetate	3.59 ± 0.17	6.32 ± 0.23	9.56 ± 0.29	12.56 ± 0.33	15.33 ± 0.23
Methanol	12.16 ± 0.28	24.67 ± 0.40	36.42 ± 0.46	48.43 ± 0.26	60.18 ± 0.34

The percentage of scavenging activity in petroleum ether extract was ranged from 5.69 ± 0.35 to 25.42 ± 0.41. Minimum and maximum percentage activity was observed as 5.69 ± 0.35 at 40 µg and 25.42 ± 0.41 at 200 µg respectively, while moderate percentage activity was observed as 9.76 ± 0.29 at 80 µg, 14.98 ± 0.49 at 120 µg and 20.04 ± 0.35 at 160 µg concentrations.Further, the percentage of scavenging activity in ethylacetate extract was ranged from 3.59 ± 0.17 to 15.33 ± 0.23. Minimum and maximum percentage activity was observed as 3.59 ± 0.17 at 40 µg and 15.33 ± 0.23 at 200 µg respectively, while moderate percentage activity was observed as 6.32 ± 0.23 at 80 µg, 9.56 ± 0.29 at 120 µg and 12.56 ± 0.33 at 160 µg concentrations. Similarly, the percentage of scavenging activity in methanol extract was ranged from 12.16 ± 0.28 to 60.18 ± 0.34. Minimum and maximum percentage activity was observed as 12.16 ± 0.28 at 40 µg and 60.18 ± 0.34 at 200 µg respectively, while moderate percentage activity was observed as 24.67 ± 0.40 at 80 µg, 36.42 ± 0.46 at 120 µg and 48.43 ± 0.26 at 160 µg concentrations. The results were also compared with a bar diagram as shown in Fig. 2 and the 50% inhibition study results were reported in Table 4.

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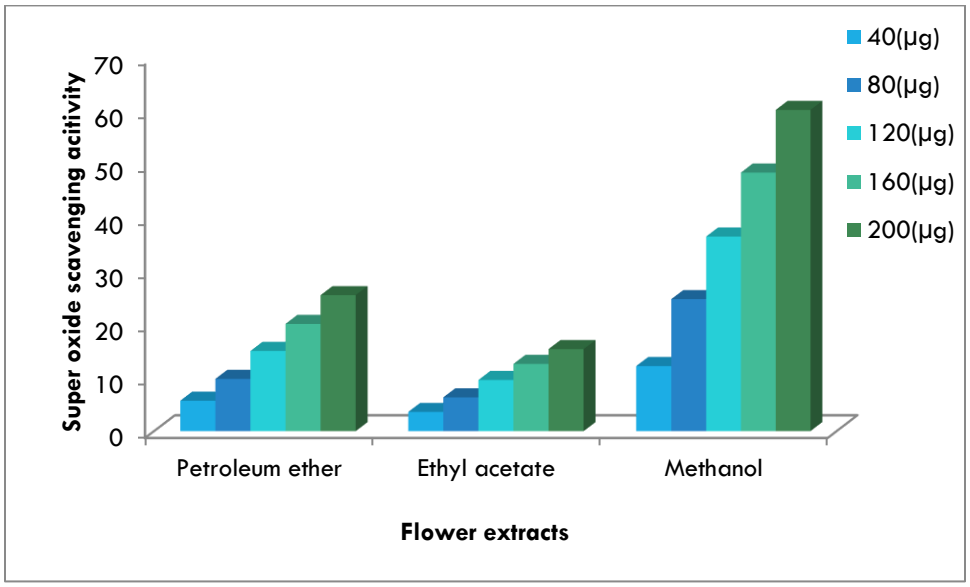

Fig. 2: Superoxide radical scavenging activity of *P. daemia*flower extracts in different solvents

Table 4: 50 % inhibition of flower extract in different solvents on superoxide radical scavenging activity.

Solvent extracts	**Flower**	**BHA**
Petroleum ether	131.61 ± 0.60	14.55 ± 0.16
Ethyl acetate	213.59 ± 1.59	
Methanol	54.23 ± 0.10	

The maximum value of flower was observed in ethylacetate extract 213.59 ± 1.59 and followed by petroleum ether extract 131.61 ± 0.60 and minimum value was observed in methanol extract 54.23 ± 0.10.

The results of Hydroxyl radical scavenging activity of *P. daemia*flower in different solvent extracts was reported in Table 5.

Table 5: Hydroxyl radical scavenging activity of *P. daemia*flower in different solvent extracts

Solvent extracts	**Percentage activity**				
	40(µg)	**80(µg)**	**120(µg)**	**160(µg)**	**200(µg)**
Petroleum ether	3.22 ± 0.51	8.69 ± 0.29	10.80 ± 0.79	16.80 ± 0.79	62 ± 0.51
Ethyl acetate	3.35 ± 0.47	5.72 ± 0.47	8.44 ± 0.26	11.49 ± 0.47	14.37 ± 0.47
Methanol	5.07 ± 0.76	11.37 ± 0.97	16.83 ± 1.68	22.92 ± 0.62	29.28 ± 0.41

The percentage of scavenging activity in petroleum ether extract was calculated to be between 3.22 ± 0.51 to 62 ± 0.51. Further, the low and high percentage activity was calculated to be at 3.22 ± 0.51 at 40 µg and 62 ± 0.51 at 200 µg respectively, while moderate percentage activity was found as 8.69 ± 0.29 at 80 µg, 10.80 ± 0.79 at 120 µg and 16.80 ± 0.79 at 160 µg concentrations. Further, the percentage of scavenging activity in ethylacetate extract was ranged between 3.35 ± 0.47 to 14.39 ± 0.47. Further, low and high percentage activity was found as 3.35 ± 0.47 at 40 µg and 14.37 ± 0.47 at 200 µg respectively, while moderate percentage activity was found as 5.72 ± 0.47 at 80 µg, 8.44 ± 0.26 at 120 µg and 11.49 ± 0.47 at 160 µg concentrations. Similarly, the percentage of scavenging activity in methanol extract was found between 5.07 ± 0.76 to 29.28 ± 0.41. The low and high percentage activities were observed at 5.07 ± 0.76 at 40 µg and 29.28 ± 0.41 at 200 µg respectively, while moderate percentage activity was foundat 11.37 ± 0.97 at 80 µg, 16.83 ± 1.68 at 120 µg and 22.92 ± 0.62 at 160 µg concentrations.

In this investigation a comparative analysis between the extracts at different concentrations using bar diagrams as shown in Fig.3 and IC_{50} results were given in Table 6.

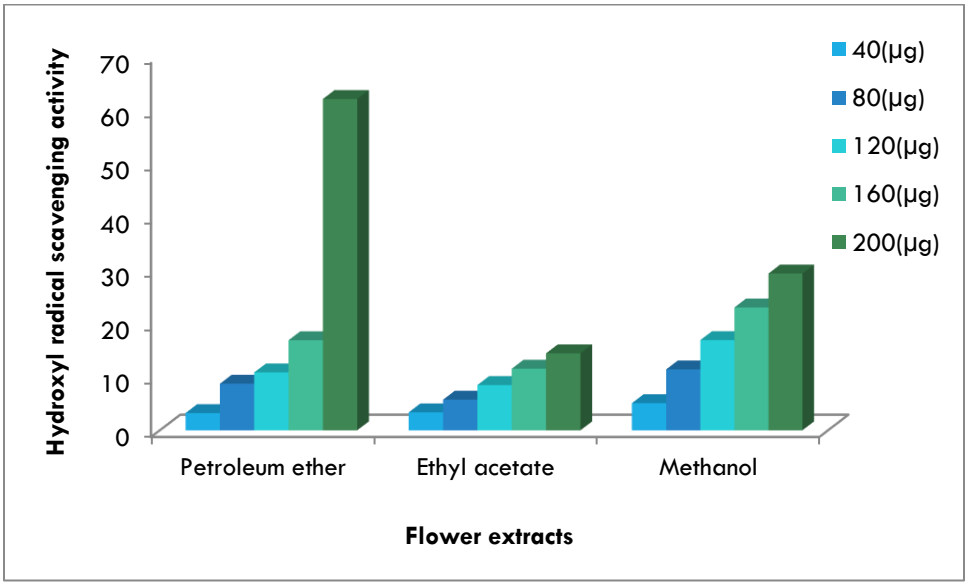

Fig. 3: Hydroxyl radical scavenging activity of *P. daemia*flower in different solvent extracts

From the above results it was observed the maximum percentage of hydroxyl radical scavenging activity was found for petroleum ether extract of flowers at 200 µg concentration and while minimum is reported for same extract at 40 µg concentrion. The results were compared with standard BHA.

Table 6: 50% inhibition of *P. daemia*flower in different solvent extracts on Hydroxyl radical scavenging activity

Solvent extracts	Flower	BHA
Petroleum ether	134.20 ± 1.95	7.92 ± 1.09
Ethyl acetate	182.19 ± 4.00	
Methanol	94.04 ± 2.01	

The maximum value in flower, was reported in ethylacetate extract 182.19 ± 4.01followed by petroleum ether extract 134.20 ± 1.95, minimum value was recorded in methanol extract 94.04 ± 2.01.

The results of Metal chelating activity of *P. daemia*flower in different solvent extracts was reported in Table 7.

Table 7: Results of Metal chelating activity of *P. daemia*in different solvent extracts

Solvents	Fe^{+2} Metal chelating effect (percentage)
	Flower
Petroleum ether	7.32 ± 0.39
Ethyl acetate	13.18 ± 0.49
Methanol	96.36 ± 0.29

The maximum value in flower was observed in methanol extract as 96.36 ± 0.29 moderate value was recorded in ethyl acetate extract with 13.18 ± 0.49 percentage, minimum value was recorded in petroleum ether extract with 7.32 ± 0.39 percentage.

Various studies have suggested that flavonoids commonly function as antioxidants and may protect plants against oxidative stress caused by suboptimal environmental conditions [12]. The antioxidant capacity of flavonoids are attributed to have the high reactivity of the hydroxyl substituent, with the number of hydroxyl groups on the B-ring being correlated with ROS scavenging capability [13].

Conclusion:

The outcome got in the current examination demonstrates that the extracts from the blossom of P. daemia display antioxidant agent activities which might be ascribed to the presence of poly phenolics and different phytochemicals constituents. The flower of P.daemia could be potential source of natural antioxidant that could have incredible significance as remedial operators in forestalling different liver issues and oxidative stress related degenerative ailments.

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