Review Article
ISSN:2249-1236
CODEN: IJRRBI
***Brahmaji Rao P et al., Int*. J. Res. Rev. Pharm. Appl. Sci.,*(2020) 10(1) 059-065**
International Journal of Research and Reviews in Pharmacy and Applied sciences

A Quantitative Study on Phytochemicals and Reduction Potential of Methanolic Extract of Flowerof *Pergulariadaemia* (Forsk) Chiov.

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Article received: 18.10.2018
Article Accepted: 29.02.2019

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Abstract:

Pergulariadaemia of the *Asclepiadaceae*family is a hispid perennial twinning herb distributed in the roadsides of tropical and subtropical regions.It has wide use in theethnomedicine.It was established thattraditionally whole plant possess pharmacological activity withpotential medicinal significance as an antifungal, antioxidant, anti-inflammatory, analgesic, fertility,hepatoprotective and anticancer agent.In the present study a quantitative estimation ofphytochemicals like phenols, tannins and flavonoids were determined in *Pergulariadaemia*flowers and antioxidant capacitywas also determined.The results confirms that the flower of*Pergulariadaemia*have significant phytocompounds and antioxidant capacity was significant in methanolic extract at 80 µg whichhighlights its reducing ability by donationg electrons and hydrogens for termination of radical chain reaction.

Keywords:*Pergulariadaemia, Flower, antioxidant, Medicinal plant, Asclepiadaceae*

Introduction:

Medicinal plants play a major role in preventing and treating many human diseases and are considered as gift to human beings [1]. According to WHO, approximately 80% of the population currently dependent on herbal medicine [2]. The major key role of medicinal plants is mainly because of the exploitation of natural plant resources as drugs, and drugs from plants are less harmful, with minimal side effects and also low cost [3].The *Pergulariadaemia*species also belong to this category and is a slender, hispid, fetid smelling lactiferous herb [4]. Belongs to the *Asclepiadaceae* family in which more than 2000 species were found. It is commonlycalled as "Dushtupatige" in Telugu and "Uttaravaruni" in Sanskrit and all parts of this plant contain various therapeutic activities. Traditionally, shoots parts are used for the treatment of whooping cough [5];the latex of the stem is used to cure muscular pain, venereal problems, asthma, arthritis, and rheumatism [6]. Similarly, the stembark of the is used to treat cold [7] and diarrhea in infants [8]. The *P. daemia*hasalsoantipyretic [9] and analgesic properties [10].

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 a senior taxonomist of Depart of Botany, Acharya Nagarjuna University, Guntur, AP-India.

b) Preparation of the extract:

The air dried powder of the flower was extracted in a soxhlet extractor using selected petroleum ether, ethyl acetate and methanol solvents. While extracting each time before extraction with next solvent the material was dried using a hot air oven at 40 degrees 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 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) Quantitative estimation of phytochemicals:

Estimation of total phenolic*:*

As per the protocol described by Siddhuraju and Becker, in 2003 [11], the total phenolic content was estimated and the analysis was performed in triplicate and the results were reported as tannic acid equivalents.

Estimation of total tannins:

According to the method described by Siddhuraju*et. al.*[12] the same extracts were determined for the quantity of tannins after treatment with polyvinyl polypyrrolidone (PVPP). The phenolic content of the supernatant was measured and articulated as the content of tannic

acid equivalents on the dry matter base. From the obtained results, the tannin content of each sample extract was estimated as

Tannin (percentage) = Total phenolics (percentage) – Non-tannin phenolics (percentage).

Estimation of total flavonoid content

The total flavonoid content was determined according to the method described byZhishen*et. al.*[13].Theestimations were performed in triplicate and outcomes were stated as routine equivalents.

d) Determination of antioxidant activity:

According to Siddhuraju*et. al.*[14] 20-100µg of different solvent extracts were taken in 1 mL of phosphate buffer. Then added 5 mL of 0.2M phosphate buffer (pH 6.6). To this solution, 5 mL of 1% potassium ferricyanide was added. The total mixture was incubated at 50°C for 20 min. Later, 5mL of 10% TCA was added. The contents werethen centrifuged at 1000 rpm for 10 min. the formed supernatant was collected and to this supernatant (5mL) was mixed using 5mL of double distilled water and then added 0.5mL of 0.1% ferric chloride. The absorbance of the reaction mixture was read spectroscopically at 700 nm.

Results and Discussion:

As per the above methods, the quantification of important phyto-compounds of the flowersobtained was summarized in Table 1. The flower shows the presence of a high amount of phenolics and they play a major role in the inhibition of growth of pathogens [15]. Also know that they have antibacterial properties against fungi and as well as in bacteria [16]. The antioxidant activity was determined by reducing power and summarized in Table 2.& Fig. 1. In which the maximum percentage of reducing power was found in methanol extract compared to stranded BHA. Epidemiological studies have reported the relationship between the plant antioxidants and the reduction of chronic diseases [17].

Table 1: Results of contents of phytochemicals present in flowers of *Pergulariadaemia*

Tests	**Petroleum ether**	**Ethylacetate**	**Methanol**
Flavonoids	9.31 ± 0.57	15.77 ± 0.37	7.11 ± 0.43
Phenols	59.66 ± 0.90	60.58 ± 0.82	140.69 ± 0.95
Tannins	19.78 ± 0.82	51.81 ± 0.37	14.79 ± 0.82

The above quantitativestudy on phytochemicals shown the presence of flavonoids, phenolics, Tannins, and alkaloids, (Table1). Moreover, it was also observed that the total content of flavonoids and tannins were found rich in ethylacetate extract,whereas phenolics were found very high in methanol extract.

Table 2: Reducing power of *P. daemia*flowers in different solvents at different concentrations

Solvent extracts	Absorbance at 700nm				
	20(µg)	40(µg)	60(µg)	80(µg)	100(µg)
Petroleum ether	0.031 ± 0.001	0.087 ± 0.001	0.101 ± 0.001	0.152 ± 0.005	0.195 ± 0.002
Ethyl acetate	0.195 ± 0.010	0.224 ± 0.010	0.253 ± 0.010	0.286 ± 0.020	0.317 ± 0.045
Methanol	0.257 ± 0.024	0.347 ± 0.014	0.387 ± 0.031	0.473 ± 0.031	0.156 ± 0.015
BHA	0.385 ± 0.01	0.385 ± 0.01	0.385 ± 0.01	0.385 ± 0.01	0.385 ± 0.01

Values are means of three independent analysis of the extract ± standard deviation (n=3). BHA – Butylatadhydroxyanisole

The % of scavenging activity petroleum ether extract was found in the range of0.031 ± 0.001 to 0.195 ± 0.002. The highest and lowest percentage activities were observed at 0.031 ± 0.001at 20 µg and 0.195 ± 0.002 at 100 µg respectively. Further, the moderate percentage activity was observed at 0.087 ± 0.001 at 40 µg, 0.101 ± 0.001at 60µg and 0.152 ± 0.005at 80 µg concentrations.

The % of scavenging activity ethyl acetate extract was found in the range of 0.195 ± 0.010 to 0.317 ± 0.045. The highest and lowest percentage activities were observed at 0.195 ± 0.010 at 20 µg and 0.317 ± 0.045 at 100 µg respectively, while moderate % activity was found at 0.224 ± 0.010 at 40 µg, 0.253 ± 0.010 at 60µg and 0.286 ± 0.020 at 80 µg concentrations.

The % of scavenging activity methanol extract was found in the range of 0.257 ± 0.024 to 0.556 ± 0.015. The highest and lowest percentage activities were observed at 0.257 ± 0.024 at 20 µg and 0.556 ± 0.015 at 100 µg respectively, while moderate % activity was found at 0.347 ± 0.014 at 40 µg, 0.387 ± 0.031at 60µg and 0.473 ± 0.031at 80 µg concentrations.

In this investigation also the authors also made a comparative analysis between the flower extracts at different concentrations and with standard (BHA) using bar diagrams (Fig. 1).

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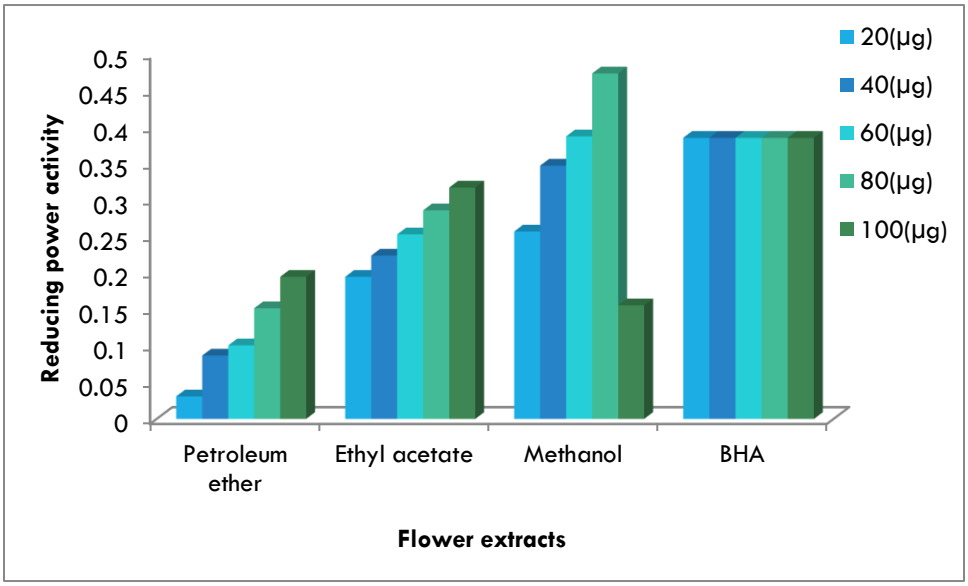

Fig. 1: Reducing power of *P. daemia*flowers in different solvent extracts

From Fig. 1, it was observed the maximum of reducing powerwas found in the methanolextract of flowerat 80µg concentration followed by the same methanol extract at 60 µg concentration compared to standard BHA. As we know that reducing power is associated with the existence of reducing agents. The antioxidant nature of reducing agents is based on the power of breaking of free radical chains by donation of atomic hydrogen or electrons. Reducing agents also sometimes react with certain precursors of peroxides, and preventsthe peroxide formation. The results of this study clearly demonstrate that the significant reducing activity of the methanol extract which maybe because of the rich polyphenols(Table 1) who have good electron and hydrogen atoms donating ability, andare responsible in the termination radical chain reaction. Above Figure 1 showed the concentration-dependent manner of reducing power of extracts which is supported with the reports of other researchers found in various plant extracts [18,19]. After methanol extract, the reducing ability was found good in ethylacetate extract than petroleum ether extract.

Conclusion:

The quantitative study shows that themethanolic extracts of*Pergulariadaemia*flower contain rich in phenolic phytoconstituents when compared with other extracts. While the antioxidant activity of *P. daemia* was observed maximum percentage of reducing power found from methanol extract compared to other extracts.So, this study reveals the existence of various phenolic components in the methanolic extract are responsible in radical chain breaking reactions and also found the antioxidant activity followed concentration dependant manner to some extent.

Acknowledgements: The authors are thankful to Acharya Nagarjuna University, Guntur, AP for providing facilities and Dr.Haribabu, Dept. of Chemistry, ANU for support and cooperation.

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