ISSN: 2348-8948
Vol: 2; Issue: 9

RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF WHOLE PLANT OF *PERISTROPHE PANICULATA FORSSK*

Israel raja johnley[1*] and Prabal joshi[2]

[1]Department of pharmacology, Mahatma Gandhi Medical college and Research Institute, Pondicherry, India
[2]Department of Physiology,SGT Medical college , Gurgaon, Haryana, India

Date Received:	Date of Accepted:	Date Published:
26- Aug -2014	05-Sep-2014	09-Sep-2014

Abstract:
The objective of this investigation was to evaluate the *in vitro* antioxidant activities of various extracts of whole plant of *Peristrophe paniculata* . All the three extracts were examined for DPPH (α,α-diphenyl-β-picrylhydrazyl) radical scavenging activity, Superoxide Anion Scavenging Activity with reference standard Rutin and Quercetin respectively through *in vitro* models and estimate the total flavonoids. The methanolic extract of *Peristrophe paniculata* was found to most active than that of other two extracts of *Peristrophe paniculata* scavenging DPPH radicals with Rutin was used as reference standard and an IC_{50} value was found to be 612µg/ml and 480µg/ml respectively. Both the method significantly showed the methanolic extract of *Peristrophe paniculata* was found to most effective than that of petroleum ether & ethyl acetate extract. The methanolic extract of *Peristrophe paniculata* was found higher content of flavonoids than that of petroleum ether and ethyl acetate. It is concluded that the methanolic extract of *Peristrophe paniculata* showed strong scavenging activity against free radical compared to all other extracts.

Keywords: Whole plant of *Peristrophe paniculata*, *In vitro* antioxidant, DPPH assay, Superoxide anion,flavonoids.

Introduction

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[1]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[2]. It is commonly recognized that antioxidants can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutations and therefore, help prevent cancer or heart disease. Recently there is a growing interest on the discovery of natural antioxidants, mainly for two reasons:(I) there are epidemical and clinical evidences suggesting that consumption of vegetables and fruits reduces the risk of developing chronic disease, e.g., Cancer; (II) phytochemicals are generally safer than synthetic chemicals[3]. Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same[4].

Peristrophe paniculata Forssk syn Peristrophe bicalyculata (Retz) Nees, Dianthera paniculata belongs to the family Acanthaceae is an herbaceous annual weed found in cultivated lands and waste wet lands. Herbs upto 1 meter tall , grey and hairy. Leaves are rounded to acute, apex gradually acute to acuminate. This plant used for fever[5]. The leaves are considered as antiseptic and are applied on wound[5].

The plant macerated in an infusion of rice is said to be antidote to snake poison[6]. Some aliphatic compounds[7] were obtained from *Peristrophe paniculata.* Based on the literature survey also revealed that lack of scientific report regarding antioxidant activity of the whole plant of *Peristrophe paniculata* . Hence the aim of the present study was to evaluate the antioxidant activity of various extracts of *Peristrophe paniculata* through various *in vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The whole plant of *Peristrophe paniculata Forssk* were collected from killikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Peristrophe paniculata* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[8] for 24 hrs. And the mark was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by in vitro Techniques:

DPPH photometric assay[9]

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[19]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging\ activity(\%) = \frac{A_{518}\,Control - A_{518}\,Sample}{A_{518}\,Control} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity[10]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[20]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Total flavonoids[11]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases[12].They are also involved in autoimmune disorders like rheumatoid arthritis etc[13].

DPPH scavenging activity

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Peristrophe paniculata* depicted in Table 1. The IC_{50} values of the petroleum ether extract of *Peristrophe paniculata* and Rutin were found to be 1020µg/ml and 480µg/ml respectively.

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Peristrophe paniculata* depicted in Table 2. The IC_{50} values of the ethyl acetate extract of *Peristrophe paniculata* and Rutin were found to be 830µg/ml and 480µg/ml respectively

The percentage of DPPH radical scavenging activity of methanolic extract of *Peristrophe paniculata* depicted in Table 3. The IC_{50} values of the methanolic extract of *Peristrophe paniculata* and Rutin was found to be 612µg/ml and 480µg/ml respectively.

From the result indicated the IC_{50} values of methanolic extract of *Peristrophe paniculata* exhibits

Table 1: Effect of Petroleum ether extract of *Peristrophe paniculata* Forssk on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	12.14 ± 0.062	18.85 ± 0.076
2	250	23.93 ± 0.045	22.08 ± 0.054
3	500	39.25 ± 0.044	52.21 ± 0.022
4	1000	50.28 ± 0.029	69.83 ± 0.014
		IC_{50} = 1020 µg/ml	**IC_{50} = 480 µg/ml**

**All values are expressed as mean ± SEM for three determinations*

Table 2: Effect of Ethyl acetate extract of *Peristrophe paniculata* on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	19.44 ± 0.032	18.85 ± 0.076
2	250	29.90 ± 0.034	22.08 ± 0.054
3	500	43.24 ± 0.030	52.21 ± 0.022
4	1000	55.45 ± 0.067	69.83 ± 0.014
		IC_{50} = 830 µg/ml	**IC_{50} = 480 µg/ml**

**All values are expressed as mean ± SEM for three determinations*

Table 3: Effect of Methanolic extract of *Peristrophe paniculata* on DPPH assay:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	32.34 ± 0.042	18.85 ± 0.076
2	250	43.54 ± 0.024	22.08 ± 0.054
3	500	52.42 ± 0.045	52.21 ± 0.022
4	1000	63.15 ± 0.043	69.83 ± 0.014
		IC_{50} = 612 µg/ml	**IC_{50} = 480 µg/ml**

**All values are expressed as mean ± SEM for three determinations*

Table 4: Effect of Petroleum ether extract of *Peristrophe paniculata* on Superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	24.23 ±0 .028	73.81 ± 0.006
2	250	42.12 ± 0.036	91.31 ± 0.011
3	500	51.28 ± 0.047	92.99 ± 0.024
4	1000	59.62 ± 0.012	98.01 ± 0.012
		IC_{50} = 560 µg/ml	**IC_{50} = 60 µg/ml**

**All values are expressed as mean ± SEM for three determinations*

Table 5: Effect of Ethyl acetate extract of *Peristrophe paniculata Forssk* on Superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	17.87 ± 0.061	73.81 ± 0.006
2	250	35.54 ± 0.020	91.31 ± 0.011
3	500	57.23 ± 0.030	92.99 ± 0.024
4	1000	62.98 ± 0.020	98.01 ± 0.012
IC_{50} = 375 µg/ml			**IC_{50} = 60 µg/ml**

**All values are expressed as mean ± SEM for three determinations*

Table 6: Effect of Methanolic extract of *Peristrophe paniculata* on Superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	44.30 ± 0.054	73.81 ± 0.006
2	250	58.22 ± 0.034	91.31 ± 0.011
3	500	68.12 ± 0.043	92.99 ± 0.024
4	1000	75.54 ± 0.058	98.01 ± 0.012
		IC_{50} = 218 µg/ml	**IC_{50} = 60 µg/ml**

**All values are expressed as mean ± SEM for three determinations*

Table 7: The total flavonoids content of various extracts of whole plant of *Peristrophe paniculata*

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Petroleum ether extract of *Peristrophe paniculata*	0.034 ± 0.012
2	Ethyl acetate extract of *Peristrophe paniculata*	1.105 ± 0.018
3	Methanolic extract of *Peristrophe paniculata*	2.872 ± 0.024

**All values are expressed as mean ± SEM for three determinations*

significant antioxidant activity when compared to that standard Rutin. IC_{50} value of plant extract and Rutin was recorded as 612µg/ml and 480µg/ml respectively. But other two extracts showed lower activity when compared to that of methanolic extract of *Peristrophe paniculata* and standard Rutin.

Superoxide anion scavenging activity

Percentage scavenging of superoxide anion activity of petroleum ether extract of *Peristrophe paniculata* was depicted in table 4. The IC_{50} value of plant extract and Quercetin was recorded as 560µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion activity of ethyl acetate extract of *Peristrophe paniculata* was depicted in table 5. The IC_{50} value of plant extract and Quercetin was recorded as 375µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion activity of methanolic extract of *Peristrophe paniculata* was depicted in table 6. The IC_{50} value of plant extract and Quercetin was recorded as 50µg/ml and 60µg/ml respectively. Based on the above results the IC_{50} values and percentage scavenging capacity, it was found that methanolic extract of *Peristrophe paniculata* is more effective in scavenging superoxide radical than that of Quercetin (standard).

Total flavonoids

Flavonoids present in food of plant origin are also potential antioxidants[13,14]. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities [15]. The total amount of flavonoids content of various extract of whole plant of *Peristrophe paniculata* was present in Table 7.

Based on the result the methanolic extract of *Peristrophe paniculata* was found higher content of flavonoids than that of petroleum ether and ethyl acetate. Based on the above results indicated, the methanolic extract of *Peristrophe paniculata* exhibited significant antioxidant activity was comparable to that of petroleum ether & ethyl acetate extracts of *Peristrophe paniculata*.

CONCLUSION

In conclusion, the methanolic extract of *Peristrophe paniculata* was found the strongest antioxidant activity among the other two extracts. The prominent antioxidant activity may be due to presence of higher content of flavonoids. These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

REFERENCES

1. Andlauer, W. and Furst, P., (1998).Antioxidative power of phytochemicals with special reference to cereals. *Cereal Foods World*, 43, 356-359
2. Christensen Lars P, (1999). Tuliposides from Tulipa sylvestris and T. turkestanica, *Phytochemistry*, 51 (8), 969-974.
3. Dasmalchi K, Dorman H.J., and Kosar M (2007). "Chemical composition and in vitro antioxidant evaluation of a water soluble Moldavian blam extract". Food Sci. Technol.40: 239-248.

4. Jayaprakasha, G.K., Selvi, T. and Sakariah, 4. K.K.,(2003).Antibacterial and antioxidant activities of grape (*Vitis vinifera)* seed extract. *Food Res. Int*., 36,117-122.
5. Uniyal, (1999) Medicinal plants and raw drugs of india, published by purshotam kaushik and dhiman,415-416,
6. Selvanayaham Z.E., Gnanevethan S.G., Balakrishnan. K., Rao.R.B., (1994)Antisnake venom botanical from ethanomedicine,Jour.Herbs spices med. Plants 24,45-100
7. Singh R.S.,Pandey R.P., Singh B.K and Singh R.G.,(2000) Aliphatic compounds from peristrophe paniculata,Fitoterapia 71,1-80
8 Harborne J.B. (1984) Phytochemical methods 11 Edn. In Chapman &, Hall. New York: 4-5.
9 Mensor , L.L, Meneze, F.S., Leitao, G.G., Reis, A.S., Dos santor, J.C., Coube, C.S and Leitao,S.G (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother.Res.15, 127-130.
10 Winterbourne, C.C., Hawkins, R.E., Brain, M and Carrel, R.W (1975). The estimation of red cell superoxide dismutase activity. J. Lab.chem.Med. 85, 337-341.
8. Cameron GR, Milton RF and Allen JW (1943). Measurement of flavonoids in plant samples. Lancet. 179.
11 Roy, H. and Burdon, (1994). *Free Radical Damage and Its Control, E*lsevier Science b. V. Netherlands, p.125
12 Rao, M.S. and Raman, M.V., (2004). *Biochemical Systematics and Ecology*, 32, pp.447-448.
13 Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP and Rice Evans C, (1995) Polyphenolic flavonoids as scavengers of aqueous phase radicals and as chain-breaking antioxidants, *Arch Biochem Biophys,* , **322**(2), 339-346.
14 Van Acker SABE, Van den Vijgh WJF and Bast F, (1996) Stuctural aspects of antioxidant activity of flavonoids, *Free Rad Bio Med,* **20**(3), 331-342.
15 Hassig A, Liang WX, Shwabl K and Stampfl K,(1999)Flavonoids and tannins: plant based antioxidants with vitamin character, *Med Hypotheses,* **52**, 471-481.