

DOI: 10.21767/2472-0151.100033

Phytochemical Screening and *In-Vitro* Antioxidant Activity of *Peristrophe paniculata*

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Received date: March 27, 2018; Accepted date: April 06, 2018; Published date: April 12, 2018

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Citation: Srikanth M, Devi B, Kotirataiah K, Ramanjaneyulu M, Sulthana PN, et al. Phytochemical Screening and *In-Vitro* Antioxidant Activity of *Peristrophe paniculata*. Herb Med. 2018, Vol.4 No.1:1.

Abstract

Peristrophe paniculata is one of the traditional medicinal plant have been using in treatment of different diseases. The present study was carried out to provide scientific evidence about its medicinal use and phytochemical variation in different parts (stem, leaves and root) of it. Phytochemical studies were carried out for hexane, ethyl acetate and hydro alcoholic extracts using standard test procedures and antioxidant activity was carried on different free radicals i.e., superoxide, hydroxyl and 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Hydroalcoholic, ethyl acetate and hexane extracts of *P. paniculata* were found to possess concentration dependent free radical scavenging activity on superoxide, hydroxyl and DPPH free radicals. Qualitative phytochemical screening of *P. paniculata* extracts revealed the presence of diverse in phytochemical constituents like steroids, terpenoids, flavonoids, alkaloids, glycosides, phenols, tannins and carbohydrates. The extracts of different parts gave negative and positive results for the amino acids, oils and saponins. The selected plant extracts showed concentration dependent percentage of inhibition on tested free radicals along with the standard drug ascorbic acid. The hexane extract of all parts of *P. paniculata* showed lower activity compared to ethyl acetate and hydroalcoholic extracts. Hydroalcoholic extract showed better activity. The variation in the activity and phytochemical constituents in them maybe due to the compounds present in them either as individually or in mixtures. The further research is need to evaluate more pharmacological activities and in isolation of the bioactive compounds from *P. paniculata*.

Keywords: *P. paniculata*; Stem; Leaves; Roots; Phytochemical analysis; Antioxidant activity

Introduction

Medicinal plants play a central role in traditional medicines and are precursors for the modern pharmaceuticals/Allopathic medicines. Most of the natural products found in medicinal plants are the compounds biosynthetically derived from primary metabolites such as amino acids, carbohydrates and fatty acids and are generally categorized as secondary metabolites [1,2]. The high technology tests such as computer tomography scans, magnetic resonance imaging, endoscopies, radiotherapies and biopsies are expensive and often treatments frequently have side effects such as allergies, vomiting, headaches etc. whereas traditional remedies usually have none [3,4]. So, the traditional medicine is largely gaining popularity over allopathic medicine because of rising costs of medical care, free from side effects in several cases, easy availability of drugs from natural sources and etc. Different drugs from different parts of different plant sources shows various therapeutic activities and different biologically and pharmacologically active compounds were identified [5,6].

From earliest times and even today almost all drugs are intimately linked with plants and these plant products have extensive use in the ethno-medicine and traditional system of medicine. The untapped wealth of plant kingdom increased interest on medicinal plants enormously over last three decades. Thereby become target for the search of new drugs and lead molecules for treatment of different diseases [7-9]. In recent years, a comprehensive search on medicinal plants, pharmaceutical drugs recently published in clinical reports suggest the effectiveness of herbal remedies, their availability, low cost. Thus, the present study was carried out on evaluation of *In-vitro* antioxidant potential of different extracts of *Peristrophe paniculata*.

Peristrophe paniculata (*Peristrophe bicalyculata*) belongs to family *Acanthaceae*, is an erect, perennial plant with stems that can become more or less woody; it can grow up to 150 cm tall. Widespread through subtropical and tropical Africa, E. Asia-China, India, Nepal, Myanmar, through tropical Asia to Australia. Forest undergrowth, hedges, and wasteland. Weedy areas, roadsides at elevations from 600-2,200 meters [10]. The

plant *P. paniculata* can be used as a green manure and has medicinal potential. Leaf juice have been used for healing fractured bones and plant material maceration used as antidote to snake poison and the plant material was also used for treatment of hypertension, cardiovascular diseases [9-13]. There is very few phytochemical and biological works were reported on *P. paniculata* [14-17].

Materials and Methods

Chemicals and drugs

The chemicals and solvents used in the current study were analytical grade from S.D. Fine Chemicals Pvt. Ltd., Mumbai. 1,1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma Chemical Company, U.S.A., Riboflavin from Loba Chemic., Mumbai. Deoxyribose from Sisco Research Laboratories Pvt Ltd., Mumbai, Nitroblue tetrazolium from Sisco Research Laboratories Pvt Ltd., Mumbai.

Collection of plant material and preparation of extracts

The plant material was collected at Palnadu region, Andhra Pradesh, India, during the month November, 2017. The authentication of the plant was done by Rtd. Dr. Prayaga Murthy. Pragada, Department of Botany, Government Degree College, Yeleswaram, E. Godavari, Andhra Pradesh. Different parts of the plant were separated and shade dried. Then, plant materials were powdered and separately extracted using maceration process with hexane, ethyl acetate, and hydroalcoholic (ethanol (70% v/v)) were concentrated to dryness under vacuum using rotavapour.

Phytochemical analysis

Phytochemical studies were carried out for hexane, ethyl acetate and hydro alcoholic extracts of *P. paniculata* stem, leaves and roots separately to detect the presence of different phytochemical constituents like steroids, terpenoides, tannins, flavanoids, saponins, glycosides, amino acids etc. by using standard procedures [18-20].

In vitro antioxidant activity

For the assessment of antioxidant activity, the extract of *B. roxburghii* was dissolved in Dimethyl sulphoxide (DMSO). The results were showed in mean \pm SEM. The Percentage Inhibition and 50% Inhibition Concentration's (IC₅₀) were calculated [18,21]. The each values were studied against blank in every assay of scavenging activity.

Superoxide radical scavenging activity

Superoxide scavenging activity of the selected plant extract was evaluated as per method [22]. It is by absorption of light at 560 nm induction of superoxide free radical generation by

riboflavin and corresponding reduction by nitroblue tetrazolium.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity is measured as per established method. It was studied by the competition between deoxyribose and the extract's antioxidant molecules for hydroxyl radicals generated from the Fe²⁺/EDTA/H₂O₂ system [23].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was measured as per methods [24,25]. This method is based on measure of color absorbance of alcoholic DPPH solution (Blue color) after addition of antioxidant solution (Extract/Compound). If antioxidants present in the test compound blue color yellow color due to diphenyl-picrylhydrazine.

Calculation of percentage inhibition

The percentage inhibition of superoxide production by the extract was calculated using the formula: Inhibitory ratio=(A₀-A₁)/A₀+100

Where, A₀ is the absorbance of control; A₁ is the absorbance with addition of plant extract/ ascorbic acid.

Calculation of 50% inhibition concentration

The optical density obtained with each concentration of the extract/ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

Results

Qualitative phytochemical screening of *Peristrophe paniculata* stem extracts

Qualitative phytochemical screening of *P. paniculata* stem extracts revealed the presence of different phytochemical constituents. The divergence was found in the presence and absence phyto-compounds (Table 1). All the extracts revealed the presence of only carbohydrates and absence for quinones. The hexane extract gave positive results for the steroids, terpenoids, carbohydrates and oils but gave negative results for glycosides, saponins, flavanoids, alkaloids, tannins, phenols, amino acids and quinones. The ethyl acetate extract revealed the presence of terpenoids, glycosides, carbohydrates, flavanoids, alkaloids, tannins and amino acids but gave negative results for steroids, saponins, phenols, oils and quinones. The hydroalcoholic extract revealed the presence of steroids, glycosides, saponins, carbohydrates,

alkaloids, tannins, phenols and amino acids but gave negative results for terpenoids, flavanoids, oils and quinones.

Table 1 Nature of phytoconstituents in stem extract of *Peristrophe paniculata*. +, ++=Present, -=Absent.

Phytochemical constituents	<i>Peristrophe paniculata</i> stem		
	Hexane extract	Ethyl acetate extract	Hydro alcoholic extract
Sterols	+	-	+
Terpenoids	+	+	-
Glycosides	-	+	++
Saponins	-	-	+
Flavonoids	-	++	-
Alkaloids	-	++	++
Tannins	-	+	+
Carbohydrates	+	+	+
Phenols	-	-	++
Oils	+	-	-
Amino acids	-	+	+
Quinones	-	-	-

Qualitative phytochemical screening of *Peristrophe paniculata* leaves extracts

Qualitative phytochemical screening of *P. paniculata* leaves extracts revealed the presence of different phytochemical constituents. The difference was found in the presence and absence phyto-compounds (Table 2). All the extracts revealed the presence of only sterols, carbohydrates. The hexane extract gave positive results for the steroids, terpenoids, glycosides, flavanoids, carbohydrates and oils but gave negative results for saponins, alkaloids, tannins, phenols, amino acids and quinones. The ethyl acetate extract revealed the presence of steroids, terpenoids, glycosides, carbohydrates, flavanoids, phenols, tannins, amino acids and quinones but gave negative results for alkaloids, saponins and oils. The hydroalcoholic extract revealed the presence of steroids, glycosides, saponins, carbohydrates, alkaloids, tannins and phenols but gave negative results for terpenoids, flavanoids, amino acids, oils and quinones.

Table 2 Nature of phytoconstituents in leaves' extract of *Peristrophe paniculata*. +, ++=Present, - =Absent (+=Less Intense; ++=More Intense).

Phytochemical constituents	<i>Peristrophe paniculata</i> leaves		
	Hexane extract	Ethyl acetate extract	Hydro alcoholic extract
Sterols	+	+	+
Terpenoids	+	+	-
Glycosides	+	+	+

Saponins	-	-	+
Flavonoids	+	+	-
Alkaloids	-	-	+
Tannins	-	+	+
Carbohydrates	+	+	+
Phenols	-	+	+
Oils	+	-	-
Amino acids	-	+	-
Quinones	-	+	-

Qualitative phytochemical screening of *Peristrophe paniculata* root extracts

Qualitative phytochemical screening of *P. paniculata* root extracts revealed the presence of different phytochemical constituents. The differentiation was found in the presence and absence of phyto-compounds (Table 3). All the extracts revealed the presence of terpenoids, alkaloids, phenols and absence for oils, amino acids, carbohydrates, quinones. The hexane extract gave positive results for the steroids, terpenoids, alkaloids, phenols but gave negative results for saponins, tannins, glycosides, flavanoids, carbohydrates, oils, amino acids, and quinones. The ethyl acetate extract revealed the presence of terpenoids, glycosides, flavanoids, alkaloids, phenols, but gave negative results for steroids, carbohydrates, tannins, saponins, oils, amino acids and quinones. The hydroalcoholic extract revealed the presence of steroids, terpenoids, glycosides, carbohydrates, alkaloids, tannins and

phenols but gave negative results for saponins, flavanoids, amino acids, oils and quinones.

Table 3 Nature of phytoconstituents in root extract of *Peristrophe paniculata*. +, ++=Present, - =Absent (+=Less Intense; ++=More Intense).

Phytochemical constituents	<i>Peristrophe paniculata</i> leaves		
	Hexane extract	Ethyl acetate extract	Hydro alcoholic extract
Sterols	+	-	+
Terpenoids	+	+	+
Glycosides	-	+	+
Saponins	-	-	-
Flavonoids	-	+	-
Alkaloids	+	+	+
Tannins	-	-	+
Carbohydrates	-	-	-
Phenols	+	+	+
Oils	-	-	-
Amino acids	-	-	-
Quinones	-	-	-

Antioxidant activity

In the present study, hydroalcoholic, ethyl acetate and hexane extracts of *P. paniculata* were found to possess concentration dependent free radical scavenging activity on superoxide, hydroxyl and DPPH free radicals.

Antioxidant activity of *Peristrophe paniculata* stem extracts

The stem extracts of *P. paniculata* were showed concentration dependent percentage inhibition on tested free

radicals (superoxide, hydroxyl and DPPH). The IC₅₀ values on superoxide radical of hydroalcoholic extract of *P. paniculata* stem and ascorbic acid were found to be 257 µg and 140 µg respectively (**Figure 1**). The IC₅₀ values on hydroxyl radical of hydroalcoholic and ascorbic acid were found to be 246 µg, 162 µg respectively. The IC₅₀ values for DPPH radical of hydroalcoholic, ethyl acetate and hexane extracts of *P. paniculata* were found to be 218 µg, 266 µg and for ascorbic acid was 120 µg. The *P. paniculata* stem extracts showed better scavenging activity on DPPH free radical than superoxide and hydroxyl free radicals. Among all *P. paniculata* stem extracts, the hydroalcoholic extract showed better activity. Hexane and ethyl acetate extracts at low concentration showed minimum inhibition on tested free radicals (**Table 4**).

Antioxidant activity of *Peristrophe paniculata* leaf extracts

The *P. paniculata* leaves extracts were produced concentration dependent percentage inhibition on tested free radicals, among the three extracts, hydroalcoholic extract showed better activity than remaining extracts. The hydroalcoholic, ethyl acetate and hexane extracts of *P. paniculata* leaves showed modest inhibition of free radicals at 340 µg and hydroalcoholic extract showed the better activity at 340 µg i.e., 38.4 ± 0.68. The extracts do not showed 50% inhibition on free radicals at tested concentrations, may they show 50% inhibition at higher concentration.

The IC₅₀ values for hydroalcoholic extract of *P. paniculata* leaves and ascorbic acid on hydroxyl radical of were found to be 291 µg and 162 µg, hexane extract showed less and ethyl acetate extract showed moderate activity. The IC₅₀ values for of hydroalcoholic and ethyl acetate extracts of *P. paniculata* leaves on DPPH radical were found to be 222 µg, 264 µg respectively (**Figure 2**). The IC₅₀ value of ascorbic acid was found to be 120 µg. Among all extracts hydroalcoholic extract at a concentration of 320 µg showed the better scavenging activity on DPPH free radical i.e., 58.2 ± 0.69 (**Table 5**).

Table 4 Concentration dependent percentage inhibition of different extracts of *P. paniculata* stem on superoxide, hydroxyl and DPPH free radicals.

Name of the extracts	% of inhibition on superoxide radical					% of inhibition on hydroxyl radical					% of inhibition on DPPH radical				
	20 µg	40 µg	80 µg	160 µg	32 µg	20 µg	40 µg	80 µg	160 µg	32 µg	20 µg	40 µg	80 µg	160 µg	32 µg
Hexane extract	0.0 0	6.5 ± 0.0 2	11 ± 0.3	22.4 ± 0.17	36. 2 ± 0.3	2.4 6 ± 0.3	5.6 ± 0.18	12. 3 ± 0.4	23.2 ± 0.82	42.3 ± 0.39	3.6 ± 0.3	8.2 ± 1.2	15.3 ± 0.4	28.6 ± 0.62	45.6 ± 0.2
Ethyl Acetate extract	3.4 ± 0.3 3	8.4 ± 0.0 6	15.2 7 ± 0.13	28.77 ± 0.68	48. 4 ± 0.3	0.0 0	3.5 ± 0.6	6.2 ± 0.4 3	12.9 ± 0.16	28.9 ± 0.63	6.2 ± 0.53	11.2 ± 0.35	20.9 ± 0.43	39.2 ± 0.6	56.2 ± 0.93

Hydro-alcoholic extract	8.5 ± 0.3	18.5 ± 1.0	21.4 ± 0.28	35.6 ± 0.34	59.2 ± 0.6	4.8 ± 0.29	9.5 ± 0.93	19.3 ± 0.28	40.3 ± 0.43	58.2 ± 0.82	6.3 ± 0.5	9.5 ± 0.93	19.3 ± 0.8	43.2 ± 0.8	60.48 ± 0.93
Ascorbic acid	5.8 ± 0.39	11.6 ± 0.89	25.7 ± 1.2	58.64 ± 0.18	75.36 ± 0.7	6.39 ± 0.84	11.93 ± 0.45	23.6 ± 0.71	47.89 ± 0.53	73.34 ± 0.62	10.26 ± 0.28	20.47 ± 0.75	36.9 ± 0.55	62.31 ± 1.26	78.08 ± 0.04

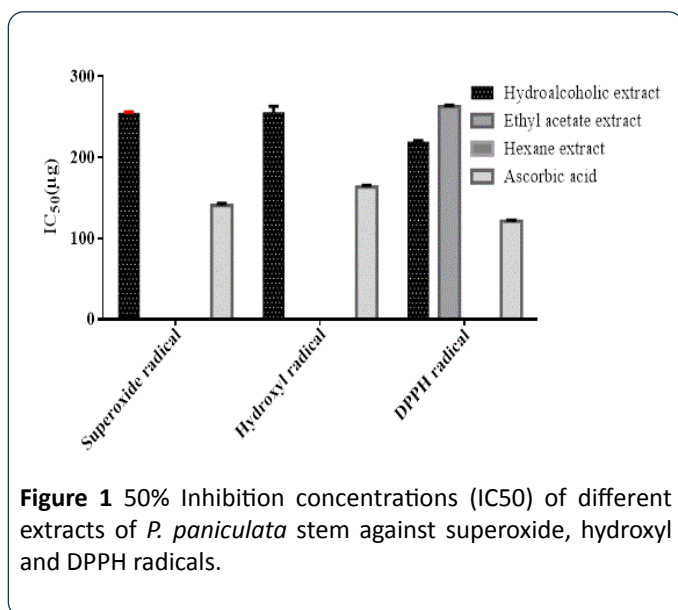


Figure 1 50% Inhibition concentrations (IC₅₀) of different extracts of *P. paniculata* stem against superoxide, hydroxyl and DPPH radicals.

Antioxidant activity of *Peristrophe paniculata* root extracts

The hydroalcoholic, ethyl acetate and hexane extracts of *P. paniculata* roots were found to possess concentration dependent scavenging activity. The extracts of *P. paniculata* root showed more percentage of inhibition at 320 µg. The IC₅₀ values for the root extracts were not able to detect because they showed minimal below 50% inhibition on free radicals production. The percentage of inhibition on superoxide radical

of hydroalcoholic, ethyl acetate and hexane extracts of *P. paniculata* root at 320 µg were found to be 19.4 ± 0.25, 38.3 ± 0.78 and 25.45 ± 0.75 respectively. The percentage of inhibition on hydroxyl radical of hydroalcoholic, ethyl acetate and hexane extracts of *P. paniculata* root at 320 µg were found to be 20.8 ± 0.6, 35.1 ± 0.8 and 39.3 ± 0.4 respectively. The percentage of inhibition on DPPH radical of hydroalcoholic, ethyl acetate and hexane extracts of *P. paniculata* root at 320 µg were found to be 12.28 ± 0.29, 14.35 ± 0.62 and 22.1 ± 0.13 respectively (Table 6). Among the samples, better DPPH free radical scavenging activity was found in hydroalcoholic root extract of *P. paniculata*.

Discussion

Free radicals are produced during metabolisms of the body. If they were produced in the normal level, the body produced antioxidants were sufficient to neutralize them without affecting the body [26]. Present day lifestyle are generating more oxidants in the body and the naturally available antioxidants in the body are not sufficient to neutralize excess amount [26,27]. The excess production of the oxidants in the body leading to unbalance physiological functions can causing different diseases like lipid peroxidation, DNA damage, atherosclerosis (oxidated LDL is more atherogenic), cancers, neurodegenerative and inflammatory bowel diseases and accelerated aging [28-30]. So, it is important to identify antioxidants without side effects and easily available and consumable [31].

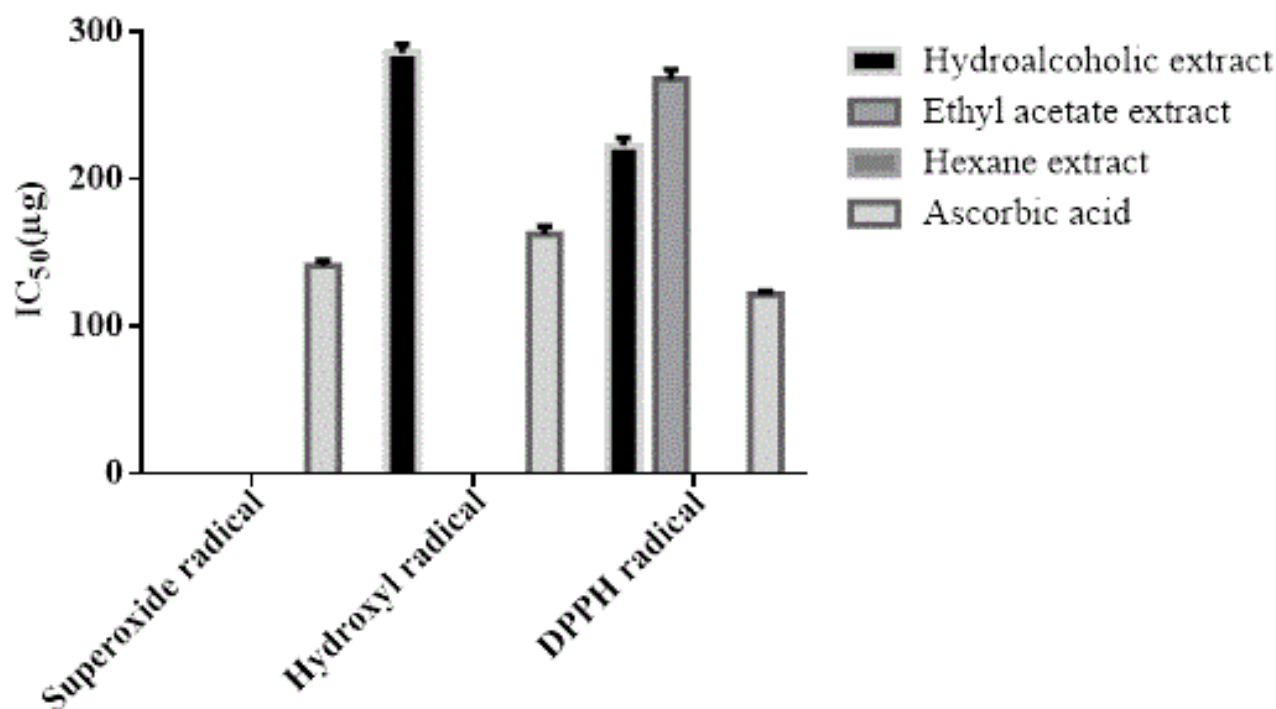


Figure 2 50% Inhibition concentrations (IC₅₀) of different extracts of *P. paniculata* leaves against Superoxide, Hydroxyl and DPPH radicals.

Table 5 Concentration dependent percentage inhibition of different extracts of *P. paniculata* leaves on superoxide, hydroxyl and DPPH free radicals.

Name of the extracts	% of inhibition on superoxide radical					% of inhibition on hydroxyl radical					% of inhibition on DPPH radical				
	20 µg	40 µg	80 µg	160 µg	320 µg	20 µg	40 µg	80 µg	160 µg	320 µg	20 µg	40 µg	80 µg	160 µg	320 µg
Hexane extract	0.00	3.88 ± 0.2	7.5 ± 0.3	13.8 ± 0.35	23.3 ± 0.42	2.32 ± 0.08	5.3 ± 0.28	11.3 ± 1.23	22.3 ± 0.63	38.25 ± 0.72	3.4 ± 0.3	6.3 ± 1.2	12.1 ± 0.83	21.82 ± 0.39	39.48 ± 0.39
Ethyl Acetate extract	0.00	2.4 ± 0.3	4.9 ± 0.13	9.3 ± 0.63	17.3 ± 0.8	0.0	1.45 ± 0.2	3.2 ± 0.15	6.8 ± 0.73	13.9 ± 0.53	2.3 ± 0.5	4.8 ± 0.9	10.2 ± 0.19	21.09 ± 0.63	36.4 ± 0.53
Hydro-alcoholic extract	2.2 ± 0.3	6.8 ± 0.1	12.4 ± 0.8	23.5 ± 0.2	38.4 ± 0.68	4.62 ± 1.08	9.2 ± 0.63	17.82 ± 0.83	34.6 ± 0.3	53.2 ± 0.61	5.23 ± 0.98	11.3 ± 0.19	21.23 ± 0.25	39.9 ± 0.93	58.2 ± 0.69
Ascorbic acid	5.82 ± 0.39	11.6 ± 0.89	25.7 ± 1.2	58.64 ± 0.18	75.36 ± 0.76	6.39 ± 0.84	11.93 ± 0.45	23.6 ± 0.71	47.89 ± 0.53	73.34 ± 0.62	10.26 ± 0.28	20.47 ± 0.75	36.9 ± 0.55	62.31 ± 1.26	78.08 ± 0.40

Table 6 Concentration dependent percentage inhibition of different extracts of *P. paniculata* roots on superoxide, hydroxyl and DPPH free radicals.

Name of the extracts	% of inhibition on superoxide radical					% of inhibition on hydroxyl radical					% of inhibition on DPPH radical				
	20 µg	40 µg	80 µg	160 µg	320 µg	20 µg	40 µg	80 µg	160 µg	320 µg	20 µg	40 µg	80 µg	160 µg	320 µg

Hexane extract	0.00	1.85 ± 0.6	4.3 ± 0.2	8.9 ± 0.53	19.4 ± 0.25	0.00	2.23 ± 0.53	5.1 ± 0.29	11.23 ± 1.89	20.8 ± 0.6	0.00	0.00	1.83 ± 0.28	5.23 ± 0.5	12.28 ± 0.24
Ethyl Acetate extract	0.00	5.2 ± 0.06	11.2 ± 0.3	20.8 ± 0.69	38.3 ± 0.78	0.00	3.46 ± 0.52	7.8 ± 0.91	18.3 ± 0.35	35.1 ± 0.8	0.00	0.00	2.5 ± 0.63	6.8 ± 0.39	14.35 ± 0.62
Hydro-alcoholic extract	0.00	3.2 ± 0.52	5.8 ± 0.25	13.2 ± 0.35	25.4 ± 0.95	2.2 ± 0.78	5.3 ± 0.28	12.6 ± 0.73	25.2 ± 0.13	39.3 ± 0.4	0.00	2.63 ± 0.05	5.8 ± 0.32	10.92 ± 0.43	25.19 ± 0.13
Ascorbic acid	5.82 ± 0.39	11.6 ± 0.89	25.7 ± 1.2	58.64 ± 0.18	75.36 ± 0.76	6.39 ± 0.84	11.93 ± 0.45	23.6 ± 0.71	47.89 ± 0.53	73.34 ± 0.62	10.26 ± 0.28	20.47 ± 0.75	36.9 ± 0.55	62.31 ± 1.26	78.08 ± 0.40

Medicinal plants have attracted great attention for their potent biological activities, no side effects and economic viability over the past years. The biological activities (properties) of medicinal plants may be due to the presence of diverse group of chemical compounds like steroids, glycosides, phenolics, glycosides, anthocyanins, flavonoids etc. [32-34]. The search of biologically active compounds from medicinal plants has always been of great interest to scientists looking for new sources of useful drugs against different hazardous diseases. Many plant species have been investigated in the search for antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species [18,21,35,36]. Many studies have shown that natural antioxidants in medicinal plants closely related to their biofunctionalities, such as the prevention or suppression of aging and many diseases associated with oxidative stress, cancer, cardiovascular diseases, rheumatoid arthritis, autoimmune diseases and AIDS [37].

The present work carried out to identify variability in the presence of phytochemical compounds in different parts and antioxidant capacity of different parts of traditional medicinal plant, *P. paniculata* and we succeeded in identification of the medicinal value of *P. paniculata* scientifically. The phytochemical analysis of different parts extracts showed presence of different phytochemical compounds in them (Tables 1-3). The hexane extracts of *P. paniculata* showed mainly presences of steroids, terpenoids, oils and carbohydrates. Ethyl acetate extracts showed mainly presences of glycosides, flavanoids, alkaloids, phenols, tannins etc. Hydralcoholic extracts showed the presences of maximum compounds in them but have little variability compared to hexane and ethyl acetate extracts. The variation in the presence of phytochemical compounds may be due to their solubility in the solvents used for their extraction [38]. The presence of compounds may also varies from one region to other regions, because of their geographical variation.

The concentration dependent percentage inhibition of tested plants extracts varies from one free radical to other on tested free radicals. The hydralcoholic extract showed more activity on superoxide free radical and DPPH free radicals, hexane extract showed less activity. Among three parts extracts of *P. paniculata*, stem extracts showed more percentage inhibition on free radicals and the phytochemical

analysis of different parts extracts showed presence of different phytochemical compounds in them. In recent decades, there were many scientific reports on medicinal plants about their antioxidant activities [25,38]. So, the results of the present study provide scientific evidence for its traditional medicinal uses.

Acknowledgements

The authors thanking to authorities of MAM College of Pharmacy for providing the necessary laboratory chemicals and equipments.

Conflict of Interest

The authors have none to declare.

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