Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2015; 4(2): 74-80 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Trends for Antioxidant Power of Phytochemicals from *Pergularia tomentosa* L. (*Asclepiadacea)* Whole Plant

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Abstract: Traditional ethnomedicinal use of plant is recognized as an important potential source for compounds used in mainstream medicine. Herein, the *in-vitro* antioxidant property and phytochemical composition of *Pergularia tomentosa L*. plants widely used was evaluated. 1, 1-diphenyl-2-2-picrylhydryzyl (DPPH) radical scavenging effect of the crude extract and its fractions were determined between 0.1, 0.25, 0.5, 1.0 2.5, and 5.0mg/ml spectrophotometrically. While, qualitative phytochemical test revealed the presence of phenolics, flavonoids alkaloids, saponins, steroids and glycoside. A systemic activity trends analysis indicated that *P. tomentosa L*. whole plant has relative antioxidant activity ranged from crudes extract to basic, acidic, methanolic and hexane fractions in the order of increasing sequence (CRD>BSC>ACD>MET>HEX). The crude extract haven highest antioxidant activity, followed by the basic fraction (alkaloid) respectively compared to acidic and methanolic fraction. Hexane has the lowest activity. This studied medicinal plant have interesting antioxidant properties and a phytochemical composition that could provide scientific evidence for some folk uses in the treatment of diseases related to the production of ROS and oxidative stress. Thus, trends for activity flow of antioxidant properties demonstrated in this work will provide evidence for researcher to isolate active antioxidant compound from the fraction with highest activity (basic fraction i.e. alkaloid).
Keywords: *Pergularia tomentosa L. Asclepiadacea,* Antioxidant activity, and phytochemical screening.

INTRODUCTION

More and more chronic diseases are crippling the ageing population: cancers, arthritis and arthritis, cardiovascular and neurodegenerative diseases bring more people to hospitals and retirement boarding houses [1]. While free-radical contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [2-3]. Free-radicals are generated continuously in the body due to metabolism and disease [4]; oxidative stress, caused by reactive oxygen species (ROS), is known to result in the oxidation of biomolecules, there by leading to cellular damage and it plays a key pathogenic role in the ageing process [5]. Free-radicals attack the unsaturated fatty acids in the bio-membranes resulting in membrane fluidity [6]. However, natural products represent a rich source of biologically active compounds and are an example of molecular diversity. It recognized potential in drug discovery and development [7].

The plant kingdoms offer a wide range of phytochemical constituents as natural antioxidants. These phytochemicals, often secondary metabolites present in smaller quantities in both higher and lower plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins and many others. The phytochemicals isolated are then screened for different types of biological activity [8]. Alternatively, crude plant extracts can be first assayed for particular activities and the active fractions are then analyzed phytochemically [8]. A variety of bioassays are now available for the phytochemist to use in such work [9].

The milk weed family, *Asclepiadeceae*, comprises 200 genera and 2500 species of perennial shrubs and herbs distributed throughout the tropics and temperatures areas of the world mostly in the Sahara region extensively especially found across Nigeria and the genus *Pergularia* is represented in Saudi Arabia by two species, *P. tomentosa L. [Daemiacordat* (Forsck) R. Br. Ex. Schult] and *P. daemia* (Forsck) Choiv*{=Daemi a extensa* (Jacq) R. Br.][10]. *P. tomentosa L.*is reputed for diverse folk uses as an antirheumatic and in treatment of some skin diseases, as laxative, abortive and for treatment of asthma and bronchitis [10]. The plant was reported to have mullusical activity[11] and persistent hypoglycemic effects [12]; a potential antitumor agent [10]; antifugal effect against *Aspergillusniger*[13]; Anti-insecticidal activity [14] and antidermatophytes[15]. The presence

of ghalakinoside, calactin and pergularoside was reported in the roots of *P. tomentosa L.* ;three cardenolides, desglucouzarin coroglaucigenin and uzarigenin from the aerial parts of *P. tomentosa*[10] and two triterpenes o the taraxosterol skeleton were isolated; pergularine A and pergularine B.[16].

Antioxidants have been widely used in the food industry to prolong shelf life and in pharmaceuticals sector for curing, suppressing and prevention of diseases of human. Antioxidants can act by the following mechanisms in lipid per oxidation: (i) decreasing localized oxygen concentrations; (ii) preventing chain initiation by scavenging initiating radicals; (iii) binding catalysts, such as metal ions; and chain- breaking to prevent initiating radical generation; (iv) decomposing peroxides so they cannot be reconverted to initiating radicals and (v) chain-breaking to prevent continued abstraction by active radicals [17-18]. Thus, systematic trends for the flow of antioxidant activity ranging from crude extract, basic, acidic, methanolic and hexane fraction were evaluated from *P. tomentosa L.* whole plant.

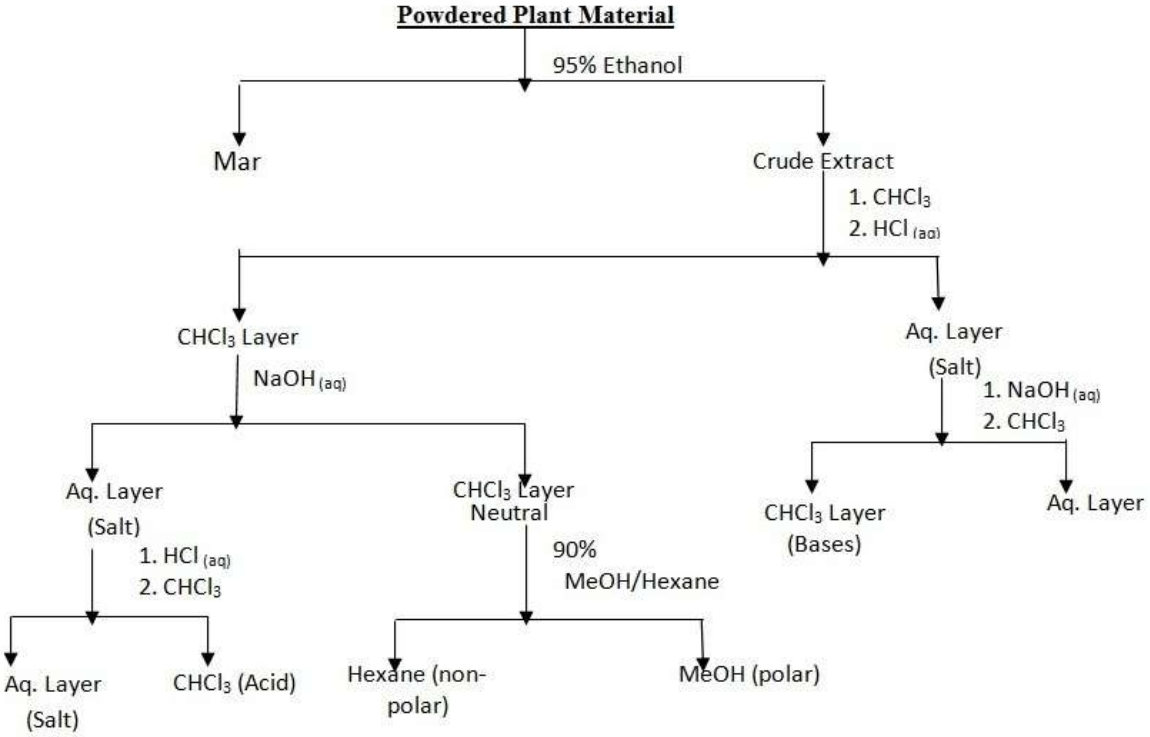
MATERIALS AND METHODS

Materials

Fresh plants of *Pergularia tomentosa L.* were collected at Nagazi around Federal College of Education, Kogi State, Nigeria. The plant was identified and confirmed at Ahmad Bello University, Zaria, Kaduna; ABU Herbarium (Botany Unit, Department of Biological Science) by Mr. Muhammad Musa, Voucher no. 645, specimens was deposited in the Herbarium. The plant materials (fresh whole plant) were air dried, pulverized into a fine powder using a commercial blender.

Extraction and Fractionation Procedure

Extraction and fractionation of the plant extract was carried out by bioassay guided fractionation protocol as shown below. The procedure was carried out using ethanol-water (95:5v/v) and different organic solvent in order of polarity (Hexane, chloroform and Methanol) using separatory funnel. These fractions were compared with the crude extract for effective antioxidant activity respectively.



Bioassay - guided fractionation protocol

One thousand grams of the powdered plant materials (20 mesh~1g) were extracted using percolation process in a mixture of 95ml of distilled ethanol and 5 ml of distilled water at ambient temperature overnight. The extract was filtered and re-extracted three times. The combined extract were filtered through a Whatman No. 1 paper and then concentrated in vacuo at 40^0C using a rotary evaporator. Model W2-100 SENCO® @ rpm of 100; Shanghai SENO technology Co, Ltd Japan. The various extracts was filtered, evaporated to dryness and residues were obtained in gram for crude extract, basic, acidic, polar and non-polar fraction as 97g, 7g, 6g, 35g, and 31g.

Phytochemical Screening of ethanolic extracts

Qualitative phytochemical analysis of different phytochemical compound (flavonoid, tannins, saponins alkaloid, glycosides and steroids were carried out in accordance with the procedure of [19].

Determination of antioxidant activity

The effect of the extracts and its fractions on DPPH radical was estimated using the method [20] with minor modifications. A solution of 0.135mM DPPH in methanol was prepared and 185µl of this solution was mixed with 15µl of varying concentrations of the crude extract/fractions (5, 2.5, 1, 0.5 and 0.25mglml) in 108-well plate the reaction mixture was vortexed and left in the dark for 30min (room temperature). The absorbance of the mixtures was determined at 570nm using a micro plate reader. Vitamin C was used as the reference antioxidant compound. The ability to scavenge the DPPH radical was calculated using the equation:

DPPH radical scavenging activity(%)=[(A control- A sample)/a Control] x 100Where A control is the absorbance of DPPH radical + methanol and A sample is the absorbance of DPPH radical + sample crude extract/fractions/standard.

Statistical analysis

For each crude extract and fractions, samples were analyzed and the assays were carried out in triplicate. The results were analyzed using one-way analysis of various (ANOVA) followed by Tukey's HSD test with $P<0.05$ (different letters mean significant differences; this treatment was carried out using Graph Pad Prism version 5.02 for Windows (1992-2009) Graph Pad Software Inc.

Chemicals

1, 1-Diphenyl-2-2picryhydrazyl (DPPH) and ethanol from Sigma-Aldrich Chemical Co. (St. Louis, USA), Vitamin C, Methanol and all others solvent.(Analytical grade) from Merck Co. (Darmstadt; Germany).

RESULT AND DISCUSSION

The phytochemical analysis of *P. tomentosa L.* whole plant was determined and revealed the presence of all the tested phytochemical compounds. It has been known that plant extracts which contain phenolic and flavonoid compounds have antioxidant and antibacterial effects [21-23]. Moreover, Plant metabolites like flavonoid, tannins, catechins and other phenolic compounds possess antioxidant activity [24]; Plant containing flavonoids have been reported to possess strong antioxidant properties [25, 26]; Findings from this work agreed with the work [27, 28] who reported that this plants contain flavonoids terpenoids and other phenolic compounds. Many herbal and plant infusions frequently used in folk medicine have anti-oxidative and pharmacological properties connected with the presence of phenolic compound, especially flavonoids [29].The antioxidant activity is conventionally used to indicate the ability of antioxidant to scavenge some radicals the more rapidly the absorbance decreases, the more potent are the antioxidant activity of the antioxidants in terms of hydrogen donating ability [30]. The rapid reduction of DPPH radical by antioxidants agents allows the evaluation of antioxidant power of different antioxidants.

However, the rate of free-radical scavenging and solvent polarity fractions activity was new parameters used in this work to evaluate the antioxidant activity for *P. tomentosa L.* The accessibility of the radical center of DPPH to each antioxidant could also influence the antioxidant power [31]. While, the activity of the compounds corresponds to the number of hydrogen atoms available for donation by hydroxyl groups [32]. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary [33-36]. Basic fractions of *P. tomentosa L.* whole plant showed good free-radical scavenging activity depending on the concentration and the rate of absorbance. The higher the concentration used, the higher the free radical-scavenging effect expressed in percentage inhibition asshown in(Fig 1a. &1b.). A reasonable inhibition above 50% was observed for both crude extract and basic fraction (alkaloid) at times (15 & 30 mins). Thus, increased intake of *P. tomentosa L.* whole plant extracts is good for our health.

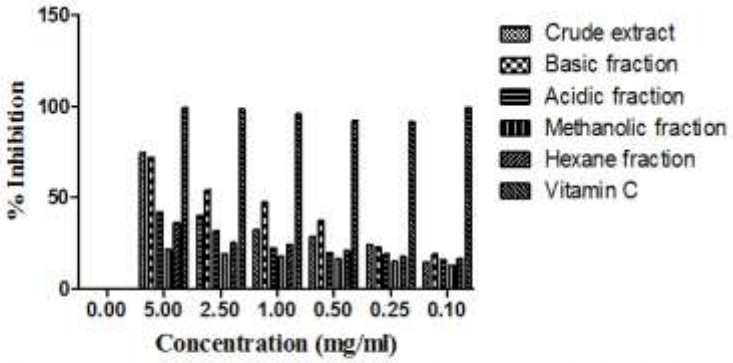


Fig. 1a: Concentration course of % inhibition of 2,2-diphenyl-1-picrylhydryzyl (DPPH) in methanol and on each tested sample (Vitamin C [vit. c], crude extract [CRD,) Basic fraction [BSC], Acidic fracion [ACD], Methanolic fracion [MET], and Hexane fracion [HEX] at time: 15 mins, by decolorization reaction of DPPH radical from purple to yellow of *P. tomentosa L.* whole plant

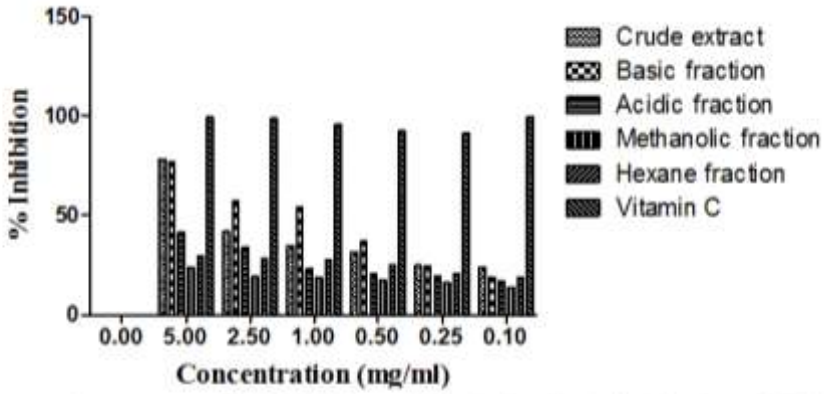


Fig. 1b: Concentration course of % inhibition of 2,2-diphenyl-1-picrylhydryzyl (DPPH) in methanol and on each tested sample (Vitamin C [vit. c], crude extract [CRD,) Basic fraction [BSC], Acidic fracion [ACD], Methanolic fracion [MET], and Hexane fracion [HEX] at time: 30mins, by decolorization reaction of DPPH radical from purple to yellow of *P. tomentosa L.* whole plant

There was no significant difference($P<0.05$) for crude extract with all the fractions except hexane fraction with vitamin C. These indicate that same activity in crude extract was present in the fraction with higher purity. Changes in antioxidant activity of the crude extract with the various fractions may also be attributable to unidentified substances or to synergistic interactions. The lower the rate of absorbance as expressed with concentration course of absorbance reduction in (Table 1) and demonstrated in (Fig. 2a& 2b). Thus, at a lower concentration of 0.1mg/ml, higher rate of absorbance was observed with variation for absorbance reduction at higher concentration of 5mg/ml. The absorbance of the DPPH radical was due to its reduction by different antioxidants.

Table 1: Comparison of absorbance reduction for *P. tomentosa L.* whole plant extracts and its fractions with Vitamin C expressed as (nm)

Concentration (mg/ml)	Vitamin C	Crude extract	Basic fraction	Acidic fraction	Methanolic fraction	Hexane
0.10	0.160	1.033	1.030	1.049	0.050	1.067
5.0	0.063	0.752	0.809	0.913	0.943	1.031

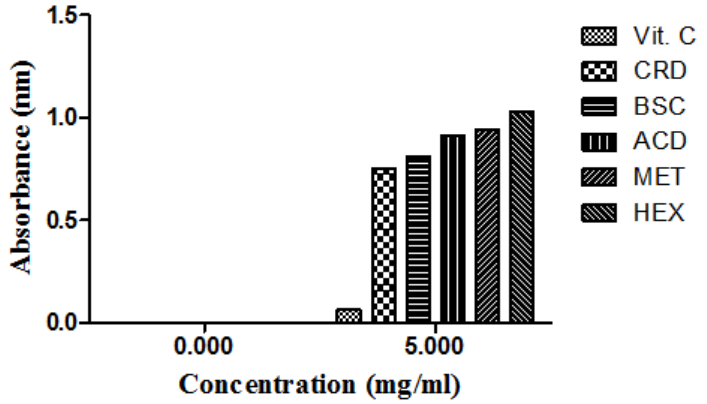


Fig. 2a: Concentration course of absorbance reduction of 2,2-diphenyl-1-picrylhydryzyl (DPPH) in methanol and on each tested sample (Vitamin C [vit. c], crude extract [CRD,) Basic fraction [BSC], Acidic fracion [ACD], Methanolic fracion [MET], and Hexane fracion [HEX] at concentration : 5mg/ml, by decolorization reaction of DPPH radical from purple to yellow of *P. tomentosa L.* whole plant

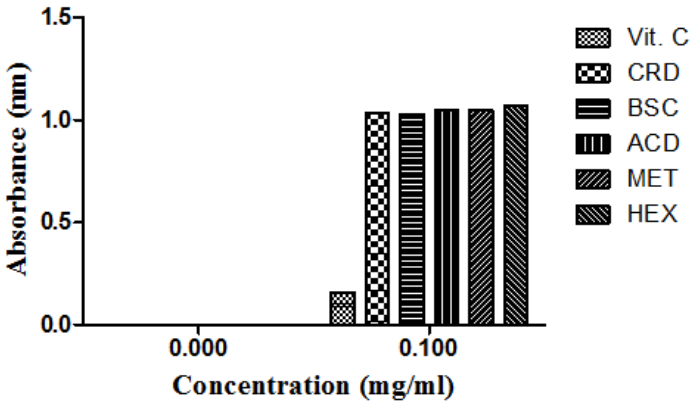


Fig. 2b: Concentration course of absorbance reduction of 2,2-diphenyl-1-picrylhydryzyl (DPPH) in methanol and on each tested sample (Vitamin C [vit. c], crude extract [CRD,) Basic fraction [BSC], Acidic fracion [ACD], Methanolic fracion [MET], and Hexane fracion [HEX] at concentration : 0.1mg/ml, by decolorization reaction of DPPH radical from purple to yellow of *P. tomentosa L.* whole plant

Absorbance decreases as a result of a color change from purple to yellow as the radical was scavenged by antioxidant through donation of hydrogen to form the stable DPPH-H. This indicates that DPPH solution was bleached with all the crude extract, basic, acidic, methanol and slight change for hexane fraction. However, differences could be observed through different fractions with their crude extract used considering various concentration. From(Table 2a) Vitamin C. showed higher percentage inhibition while crude extract, Basic, acidic fraction followed respectively, the bleaching ability of DPPH solution was nearly complete with higher concentrations of vitamin C as crude extract could do.

Table 2: Comparison of antioxidant activity of *P. tomentosa L.* whole plant extracts and its fractions with Vitamin C expressed as (%) inhibition

Concentration (mg/ml)	Vitamin C	Crude extract	Basic fraction	Acidic fraction	Methanolic fraction	Hexane
0.10	50.32	21.91	19.53	16.77	17.83	12.62
0.25	91.68	24.65	24.28	19.62	19.52	17.52
0.5	92.62	32.23	37.57	20.39	23.79	18.97
1.0	95.94	35.41	54.22	23.23	25.59	19.74
2.5	98.81	43.82	57.79	33.22	27.01	19.74
5.0	99.61	78.58	76.20	43.97	39.06	23.85

Vitamin C was substantially more active whatever the concentrations used as in (Table 2) in comparison with the crude extract and their fractions. With high concentration of vitamin C it was possible to observe nearly a 100% free radical scavenging effect crude extract and Basic fraction had nearly the same effect for similar concentrations used compared to crude extract. As for vitamin C and hexane fraction, a clear gradient activity was seen from the first to the last concentration used in (Table 2). Ascorbic acid and vitamin C were known to act as antioxidant [37] and different fractions from *P. tomentosa L* showed reasonable free- radical-scavenging activity depending on the concentration used. The present study showed that activity of crude extract for *P. tomentosa L*. whole plant are majorly reside on the basic fraction (alkaloid) to total antioxidant constituent was higher than other fractions. Moreover it can be seen from (Table 2) that crude extract, Basic fraction and acidic fraction exhibited higher antioxidant activity (expressed in terms of percentage inhibition) than methanolic fraction and hexane fractions for all the concentration tested.

CONCLUSION

The basic fraction (alkaloid) particularly showed higher antioxidant constituents from the crude extracts to the total antioxidant analyzed in the whole plant of *P. tomentosa L,* as alkaloid have been reported as the most important group of natural substance [38]and alkaloids extract of *P. tomentosa L.* produced showed against a significant increase in larval duration compared with the control. More so, it has been suggested that they constitute part of the plant defenses against phytophagous animals [39] together with terpenoids, phenols, flavonoid, and steroid. *P tomentosa L.* as such, the basic fraction (alkaloid) especially will be a centered area for further scientific research findings in isolating an active antioxidant component therein.

ACKNOWLEDGEMENTS

Authors are grateful to the staff of Chemistry, Integrated Science and Biological Department, Federal College of Education, Okene, Kogi state, Nigeria in persons of Mr, UsmanSuleiman, Adenoyi John, Anabe Idris Musa, and Yusuf Ibrahim Adinoyi for their laboratory assistant. Thank goes to Mr. Jubril of Biotechnology Sheda Science and Technology (SHETSCO) Abuja for statistical analysis.

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