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Nutritive Value, Phytochemical and Antifungal Properties of *Pergularia tomentosa* L. (Asclepiadaceae)

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Abstract: This study was aimed to assess the nutritive value, phytochemical constituents and antifungal activity of leaf, root and stem extracts of *Pergularia tomentosa.* Chemical composition of leaf, stem and root extracts of the plant were determined using standard methods. Aqueous and organic solvents extracts of the plant parts were screened for antifungal activity using agar dilution method. Phytochemicals detected in the leaf and stem extracts were alkaloids, cardiac glycosides, cyanogenic glycosides, saponins, flavonoids, tannins and anthraquinones. The roots contain trace amounts of cyanogenic glycosides, cardiac glycosides, saponins, tannins and anthraquinones. Mineral element composition of the plant showed higher amount of phosphorus and potassium in the root and stem and sodium, magnesium and calcium in the leaf extracts. All the plant parts used contain high percentages of carbohydrates and crude fibre ranging from 53.27±1.75 to 61.31±2.84% and 16.33±0.29 to 23.17±0.58%, respectively. Lipids (6.83±0.76%), ash (17.17±1.04%) and crude protein contents (6.39±0.17%) were higher in the leaf extracts while the stem was of higher moisture (10.67±0.76%) content. Hexane (HX), Petroleum Ether (PE) and chloroform (CHL) leaf, stem and root extracts were active against all the isolates tested with percentage inhibitions ranging from 41.90±5.63 to 97.52±0.28%. The organic solvent extracts demonstrated near complete inhibitions of the fungal isolates at 8.00 mg mL^{-1} while the aqueous (AQ) extracts of the plant parts inhibited the growth of the isolates at 27.17±7.79 to 97.45±0.21% with near complete inhibition of the tested isolates also at 8.00 mg mL^{-1}. The results showed that the leaf, root and stem extracts of *Pergularia tomentosa* have potential nutritional and antifungal uses.

Key words: *Pergularia tomentosa*, phytochemicals, proximate analysis, mineral elements, antifungal activity

INTRODUCTION

There is a current shift towards evaluating the chemical composition and nutritive value of tropical medicinal plants. In Northern Nigeria, wild plants are consumed as normal herb source to provide fairly good amounts of several nutrients. It is widely accepted that herbs are significant nutritional sources of minerals. Throughout the world, there is increasing interest in the importance of dietary minerals in the diet, even though they make up only 4-6% of the human body (Musa, 2005). Plants have been used to treat infectious diseases due to their antimicrobial properties. This is due to presence of various kinds of phytochemicals including phenolic compounds, alkaloids, terpenoids and essential oils (Lewis and Elvin-Lewis, 1995; Cowan, 1999).

Diseases caused by pathogenic bacteria and fungi represent a critical problem to human heath and they are one of the main causes of morbidity and mortality world wide (WHO, 1998). Resistance to antibiotics and the occurrence of toxicity during prolonged treatment with present day drugs have been the reasons for extended search for newer drugs to treat microbial infections (Fostel and Lartey, 2000).

Pergularia tomentosa (Milk weed) is a climbing to semi erect perennial herb. It is used in Northern Nigeria for tanning, treatment of skin diseases and making arrow poisons. The plant was reported to have molluscidal activity (Hussein *et al*., 1999), persistent hypoglycaemic effects (Shabana *et al.*, 1990) and its isolated cardenolides have been shown to cause apoptotic cell death of Kaposi's sarcoma cells (Hamed *et al*., 2006). The roots have found applications in the treatment of bronchitis, constipation and skin diseases (Hammiche and Maiza, 2006). The plant was also reported to contain beta-sitosterol glucoside, 3'-O-beta-D-glucopyranosyl calactin, 12-dihydroxy ghalakinoside and 6'-dihydroxy ghalakinoside (Gohar *et al*., 2000; Hamed *et al.*, 2006).

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The current shift from the use of synthetic chemicals in food processing necessitates a further evaluation of widely available but underutilized tropical medicinal plants like *P. tomentosa*. Information on potential food uses and antifungal activity of *P. tomentosa* is scanty. It is therefore important in this study to assess the antifungal activity, mineral composition, phytochemical and proximate analysis of *P. tomentosa*. The nutrient information and antifungal properties reported in this study would enhance efforts to promote wider use of the plant because of its nutritional benefits and medicinal properties.

MATERIALS AND METHODS

Chemicals: All chemicals were of analytical grade.

Plant material: The leaves, roots and stems of *P. tomentosa* (Fatakko; Hausa language) were collected in May 2006, around Usmanu Danfodiyo University Campus, Sokoto, Nigeria. The voucher specimens of the plant were deposited in the departmental herbarium (Botany unit) for reference. The study was undertaken in June to July 2006. The portions collected were open air-dried under the shade, pulverized into a moderately coarse powder (using pestle and mortar) and stored in plastic containers until required for use.

Organisms: The fungal organisms used were *Trichophyton rubrum*, *Microsporum gypseum*, *Aspergillus niger* and *Aspergillus flavus*. They were clinical isolates obtained from Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. The organisms were maintained on Sabouraud Glucose Agar (SGA) medium and re-identified by microscopic examination of a portion of the colony for spores and characteristic hyphae (Cheesbrough, 1982).

Extraction and fractionation procedure: This was done by activity-guided fractionation using ethanol-water (1:1) and different (hexane, petroleum ether and chloroform) organic solvents as described by Morris and Aziz (1976) and Springfield and Weitz (2006). The powdered leaves of *P. tomentosa* (40 g) were extracted with ethanol-water (1:1, 400 mL) at room temperature overnight. The extract was filtered and partitioned in Hexane (HX) separately (250 mL) and clarified by further filtration. Evaporation of HX fraction to dryness in an oven at 45°C yielded residues (Table 1). The aqueous filtrate (ethanol-water) of the extract fraction was further partitioned (to obtain fractions of different polarities) with petroleum ether (250 mL) and chloroform (250 mL) separately. Evaporation of the Petroleum Ether (PE), Chloroform (CHL) and Last remaining water-ethanol fractions (LWEF) yielded residues (Table 1). The stems and roots were subjected to the same procedure. All procedures were repeated to obtain more residues. Another 40 g of each powdered leaf, root and stem were extracted with 400 mL of distilled water for 24 h and filtered. All the residues obtained were reconstituted in sterilized distilled water and screened for antifungal and phytochemical properties. This procedure enabled us remove the possible contributory effects of the organic solvents.

Table 1: Amount of residues obtained after extraction

Plant parts	Fractions	Amount recovered (%) (w/w)
Leaves	HX	8.50
	PE	9.00
	CHL	7.00
	LWEF	18.25
Stems	HX	7.00
	PE	6.25
	CHL	4.75
	LWEF	13.50
Roots	HX	3.80
	PE	3.25
	CHL	3.00
	LWEF	9.38

HX = Hexane, PE = Petroleum Ether, CHL = Chloroform, LWEF = Last remaining water-ethanol fractions

Antifungal activity: The antifungal activities of the aqueous and organic solvent extracts of *P. tomentosa* were evaluated according to reported procedures of Zacchino *et al.* (1999), using the agar dilution method with some modifications. The fungal species were maintained on Sabouraud Dextrose Agar (SDA) medium in 90 mm petridishes. Five milliliter of filter-sterilized reconstituted water solution of each plant extract (HX, PE and CHL) at concentrations of 10 to 120 mg mL^{-1} (stems, roots and leaves) were aseptically mixed with 15 mL of SDA (Liquefied and maintained at melting point in waterbath at 45°C) to give final concentrations ranging from 0.67 to 8.00 mg mL^{-1}. The crude water extracts of the plant parts (leaf, stem and root) at 10 to 120 mg mL^{-1} were also prepared using above procedure to give final concentrations of 0.67 to 8.00 mg mL^{-1}. The Petri-dishes (90 mm) were filled to 20 mL final volume with Sabouraud Dextrose Agar containing the requisite amounts of diluted extract solution. The Petri-dishes were then inoculated at their center with a disk (2×2 mm) cut from the periphery of a 14-day-old (*T. rubrum* and *M. gypseum*) and 7-day-old (*A. niger* and *A. flavus*.) fungal colonies. Griseofulvin (0.67 and 4.00 mg mL^{-1}), Clarion Medicals Ltd. Lagos, Nigeria, measured from the pulverized 500 mg tablet was also included to serve as positive control. Water (5 mL) in place of the extract and media (15 mL) were mixed together

as negative control. The treated and the control Petri-dishes were incubated at room temperature for 14 days for superficial mycosis (dermatophytes) and 48 h for *A. niger* and *A. flavus*. Growth was observed each day to the last day. From these, the percentage inhibitions were calculated using the following formula:

$$I(\%) = \frac{dc - dt}{dc} \times 100$$

Where:
dc = Diameter of colony of control culture
dt = Diameter of colony of treated culture

Phytochemical screening: This was done using standard procedures of Wall *et al.* (1954), Harborne (1973), Trease and Evans (1978) and El-Olemyl *et al.* (1994).

Proximate analysis and nutrients composition: The extracts were analysed for moisture content using the method of Oyeleke (1984) and crude protein by modified Kjeldhal method. The method of Yawas and Obi (2001) was employed in the analysis of carbohydrates. Ash content was determined by the method of Samuel *et al.* (1997). Crude lipids and fibre content were determined by the procedures of Association of Official Analytical Chemists (AOAC, 1980).

RESULTS AND DISCUSSION

The nutritive value, antifungal and phytochemical properties of leaf, stem and root extracts of *P. tomentosa* are shown in Table 2-7. The leaf and stem extracts revealed the presence of alkaloids, cardiac glycosides, cyanogenic glycosides, saponins, flavonoids, tannins and anthraquinones while the roots contain trace amount of these phytochemicals with absence of alkaloids and flavonoids (Table 2).

Higher amount of phosphorus and potassium were present in root and stem with sodium, magnesium and calcium in the leaf extracts (Table 6). Higher contents of carbohydrates (53.27±1.75 to 61.31±2.84%) and crude fibre (16.33±0.29 to 23.17±0.58%) were observed in all the plant parts but lipids, ash and crude protein were higher in the leaf compared to the other extracts. The moisture (10.67±0.76%) content was higher in stem extracts with leaves having the lowest (0.69±0.29%) amount (Table 7). All the organic solvents extracts of the plant parts were active against the tested fungal isolates with percentage inhibition of 41.90±5.63 to 97.52±0.28% (Table 3-5) with near complete inhibitions at 8.00 mg mL^{-1}. All the aqueous extracts of the plant parts at this concentration had also shown near complete inhibition (97.45±0.21%) of the isolates.

The present study presents systematic report on the antifungal properties of *P. tomentosa* against multiple drug resistant common pathogenic fungi and its nutritive value. This supports the view that *P. tomentosa* is a potent antifungal agent with valuable nutritional information. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of conventional antimicrobial drugs commonly used in the treatment of infectious diseases (Subramanian *et al.*, 2006). This forced scientific researchers to find alternative antimicrobial drugs from other sources including medicinal plants. The mechanism of action of the AQ, HX, PE and CHL extracts of *P. tomentosa* against the pathogenic fungal isolates may be due to inhibition of fungal cell wall (due to pore formation in the cell and leakage of cytoplasmic

Table 2: Phytochemical analysis of water and organic solvent extracts of *P. tomentosa*

Plant parts	Extract fractions	ALK	GLY	CG	CYG	SAP	FLV	TA	ATG
Leaves	I	+++	+++	+++	+++	+++	+++	+++	+++
	II	+++	-	-	-	+++	+++	+++	-
	III	+++	-	-	-	+++	+++	+++	-
	IV	+++	-	-	-	+++	+++	+++	-
	V	+++	+++	+	+	+++	+++	+++	+++
Stems	I	+++	+++	+++	+++	+++	+++	+++	+++
	II	+++	-	-	-	+++	+++	+++	-
	III	+++	-	-	-	+++	+++	+++	-
	IV	+++	-	-	-	+++	+++	+++	-
	V	+++	+	+	+	+++	+++	+++	+++
Roots	I	-	+	+	+	+	-	+	+
	II	-	-	-	-	+	-	+	-
	III	-	-	-	-	+	-	+	-
	IV	-	-	-	-	+	-	+	-
	V	-	+	+	+	+	-	+	+

- = Absence, + = Trace amounts, +++ = Presence, I = Water, II = Hexane, III = Petroleum ether, IV = Chloroform, V = Last remaining water-ethanol extract, ALK = Alkaloids, GLY = Glycosides, CG = Cardiac Glycosides, CYG = Cyanogenic Glycosides, SAP = Saponins, FLV = Flavonoids, TA = Tannins, ATG = Anthraquinone Glycosides

Table 3: Percentage inhibitions of fungal isolates by aqueous and organic solvent leaf extracts of *P. tomentosa*

Extract conc. (mg mL^{-1})	*T. rubrum*	*M. gypseum*	*A. niger*	*A. flavus*
0.67 HX	58.07±4.42	57.08±4.69	70.61±8.22	63.30±3.53
2.00 HX	71.70±3.15	63.20±3.18	84.52±5.45	69.07±3.98
4.00 HX	76.52±5.09	79.23±1.35	86.93±4.46	74.50±2.43
8.00 HX	97.40±0.14	96.87±0.18	96.80±0.50	97.40±0.18
0.67 PE	60.88±3.96	60.18±3.49	69.74±5.26	66.29±5.15
2.00 PE	76.39±2.97	71.49±3.82	85.74±3.38	77.04±3.82
4.00 PE	86.16±2.52	87.02±3.50	93.29±2.64	94.04±4.16
8.00 PE	97.36±0.13	96.89±0.35	96.84±0.23	97.53±0.07
0.67 CHL	55.22±5.18	40.04±2.80	68.29±7.15	57.24±4.73
2.00 CHL	66.25±4.72	44.44±3.67	82.11±4.41	60.79±5.47
4.00 CHL	70.00±3.18	69.44±2.78	84.99±5.81	71.63±5.59
8.00 CHL	97.40±0.03	96.85±0.21	97.30±0.37	97.17±0.26
0.67 AQ	27.17±7.79	34.72±6.06	35.25±4.56	65.79±6.58
2.00 AQ	40.76±8.43	48.15±5.61	43.73±7.30	71.79±5.11
4.00 AQ	53.55±6.09	53.79±2.59	66.45±6.67	81.23±3.62
8.00 AQ	93.14±3.89	76.16±2.44	96.36±1.21	96.05±2.28
0.67 GS	64.66±3.59	66.81±4.59	71.05±5.62	70.17±4.75
4.00 GS	96.48±0.13	96.46±0.48	88.51±3.26	85.00±4.20

Diameter of water control petridishes of all the organisms used is 90 mm, Values are mean±standard deviation of percentage inhibitions, 0-25% = None or little inhibition; 25-50% = Average inhibition; 50-75% = Strong inhibition; 75-100% = Very strong inhibition. HX = Hexane, PE = Petroleum ether, CHL = Chloroform, AQ = Aqueous, GS = Griseofulvin, n = 3

Table 4: Percentage inhibitions of fungal isolates by organic solvent stem extracts of *P. tomentosa*

Extract conc. (mg mL^{-1})	*T. rubrum*	*M. gypseum*	*A. niger*	*A. flavus*
0.67 HX	58.07±4.42	56.08±3.39	69.40±8.140	61.39±3.65
2.00 HX	69.60±1.82	62.54±2.47	83.28±5.280	68.74±4.16
4.00 HX	76.52±5.08	77.58±2.30	84.68±4.670	71.54±1.68
8.00 HX	97.27±0.36	97.52±0.28	97.19±0.440	97.13±0.66
0.67 PE	60.88±3.96	61.85±3.49	64.55±13.00	65.21±4.71
2.00 PE	76.39±2.97	74.96±2.85	83.91±3.380	74.04±7.19
4.00 PE	86.16±2.52	86.18±3.49	84.86±11.82	93.45±4.50
8.00 PE	97.32±0.07	97.42±0.07	97.32±0.230	96.43±2.07
0.67 CHL	55.23±5.18	45.28±3.76	67.19±7.630	59.05±1.93
2.00 CHL	66.25±4.72	50.12±3.39	80.72±3.640	59.65±5.01
4.00 CHL	69.98±3.15	75.52±2.54	84.38±5.300	71.40±4.73
8.00 CHL	96.36±1.64	90.52±3.65	97.36±0.080	96.43±1.76
0.67 AQ	56.81±4.85	40.01±4.36	35.42±5.860	64.94±6.96
2.00 AQ	69.89±3.34	52.58±5.35	42.59±7.280	71.28±4.89
4.00 AQ	74.17±4.69	57.34±2.42	65.93±6.160	81.36±3.79
8.00 AQ	96.65±0.72	76.49±2.30	96.45±1.160	97.02±0.74
0.67 GS	64.66±3.59	66.81±4.59	71.05±5.620	70.17±4.75
4.00 GS	96.48±0.13	96.46±0.48	88.51±3.260	85.00±4.20

Diameter of water control petridishes of all the organisms used is 90 mm, Values are mean±standard deviation of percentage inhibitions, 0-25% = None or little inhibition; 25-50% = Average inhibition; 50-75% = Strong inhibition; 75-100% = Very strong inhibition. HX = Hexane, PE = Petroleum Ether, CHL = Chloroform, AQ = Aqueous, GS = Griseofulvin, n = 3

Table 5: Percentage inhibitions of fungal isolates by organic solvent root extracts of *P. tomentosa*

Extract conc. (mg mL^{-1})	*T. rubrum*	*M. gypseum*	*A. niger*	*A. flavus*
0.67 HX	41.90±5.63	47.58±2.38	72.23±7.09	62.790±4.42
2.00 HX	51.03±6.85	49.11±3.83	75.36±5.80	67.210±5.77
4.00 HX	53.60±4.73	49.34±2.23	78.79±5.08	77.030±5.56
8.00 HX	60.54±4.61	62.87±6.35	78.33±4.19	73.233±6.05
0.67 PE	49.08±5.42	44.97±3.71	68.93±6.18	64.800±5.44
2.00 PE	53.69±3.42	45.43±4.14	72.36±4.39	66.380±5.00
4.00 PE	58.70±5.29	50.04±5.93	74.25±3.86	67.870±4.58
8.00 PE	64.89±4.99	53.02±4.89	74.04±4.20	71.500±4.05
0.67 CHL	48.05±4.79	38.34±4.29	69.82±5.87	56.870±4.89
2.00 CHL	51.24±4.99	41.08±4.37	70.37±6.01	59.890±5.74
4.00 CHL	58.50±5.61	41.49±1.76	80.13±5.80	69.240±6.01
8.00 CHL	61.01±5.62	47.39±3.62	80.56±4.59	69.770±6.05
0.67 AQ	59.54±5.32	57.43±2.05	34.39±5.13	62.760±4.55
2.00 AQ	61.87±5.87	57.67±3.26	41.72±7.84	70.400±6.04
4.00 AQ	72.07±3.79	77.35±4.26	64.29±7.52	79.400±4.86
8.00 AQ	97.09±0.72	97.45±0.21	67.11±7.32	80.260±5.16
0.67 GS	64.66±3.59	66.81±4.59	71.05±5.62	70.170±4.75
4.00 GS	96.48±0.13	96.46±0.48	88.51±3.26	85.000±4.20

Diameter of water control petridishes of all the organisms used is 90 mm, Values are mean±standard deviation of percentage inhibitions, 0-25% = None or little inhibition; 25-50% = Average inhibition; 50-75% = Strong inhibition; 75-100% = Very strong inhibition, HX = Hexane, PE = Petroleum Ether, CHL = Chloroform, AQ = Aqueous, GS = Griseofulvin, n = 3

Table 6: Mineral element content of extracts of *P. tomentosa*

Plant parts	Phosphorus (ppm)	Potassium (ppm)	Sodium (ppm)	Magnesium (%)	Calcium (%)
Leaves	1.85±0.05	2.97±0.210	4.13±0.31	0.32±0.060	0.25±0.010
Roots	8.13±0.06	167.30±5.030	2.33±0.15	0.25±0.008	0.08±0.003
Stems	7.07±0.06	215.00±10.00	2.03±0.15	0.15±0.030	0.16±0.010

Values are mean±standard deviation, n = 3

Table 7: Proximate composition of extracts of *P. tomentosa*

Plant parts	Carbohydrates (%)	Lipids (%)	Crude protein (%)	Ash (%)	Crude fibre (%)	Moisture (%)
Leaves	53.27±1.75	6.83±0.76	6.39±0.17	17.17±1.04	16.33±0.29	0.69±0.29
Roots	61.31±2.84	2.67±0.29	3.35±0.48	11.83±0.29	20.83±2.47	8.67±0.58
Stems	56.92±1.27	2.17±0.76	4.74±0.14	13.00±2.18	23.17±0.58	10.67±0.76

Values are mean±standard deviation, n = 3

constituents by the active components such as alkaloids), protein, amino acid and sphingolipid biosynthesis and electron transport chain (Shelton, 1991; Lartey and Moehle, 1997; Ueki and Taniguchi, 1997; Dominguez and Martin, 1998). The present study highlights the use of *P. tomentosa* in folk medicine for the treatment of fungal diseases and underscores the importance of the ethnobotanical approach for the selection of *P. tomentosa* in the discovery of new bioactive compounds.

The presence of alkaloids, cardiac glycosides, cyanogenic glycosides, saponins, flavonoids, tannins and antraquinones (Table 2) in the extracts may be attributed to the antifungal actions of *P. tomentosa*. The antifungal actions of these phytochemicals have been associated with antimicrobial properties (Scalbert, 1991; Favel *et al*., 1994; Cook and Samman, 1996; Oyagade *et al*., 1999; Shale *et al*., 1999).

The level of dietary fibre is quite high (23.17±0.58%) in stem when compared to root and leaf extracts. The presence of high fibre levels in diet can cause intestinal irritation, lower digestibility and overall decreased nutrient utilization (Oyenuga and Fetuga, 1975). The low crude protein content of *P. tomentosa* (3.35±0.48 to 6.39±0.17%) coupled with the fact that it is abundant in northern Nigeria and with antifungal properties may still contribute to the sources of proteins. Higher carbohydrates content of the extracts (53.27±1.75 to 61.31±2.84%) may encourage its use as high carbohydrates sources in some food formulations and as fodder for animals. The higher moisture content of the root and stem extracts (8.67±0.58 to 10.67±0.76%) implies that their shelf life will be longer than that of leaves. The ash content of *P. tomentosa* was higher than that of the medicinal plants *P. thonningii* (3.50%) and *S. bicolar* (5.34%) (Jimoh and Oladiji, 2005; Adetuyi, 2004).

The levels of calcium and magnesium were low in the extracts. Magnesium is an antioxidant micronutrient (Talwar *et al*., 1989) and its presence may boost the immune system and aid in removing magnesium deficiencies which could lead to severe metabolic disorders and compromise the health of the organism. Higher contents of phosphorus were observed in stem (7.07±0.06 ppm) and root (8.13±0.06 ppm) compared to leaves extracts. However, it is worth noting that calcium in conjunction with magnesium, chlorine and proteins are involved in the formation of bone (Abulude, 2001). It also plays an important role in blood clotting, coordination of inorganic elements present in the body and balancing of calcium and phosphorus. The root (167.30±5.03 ppm) and stem (215.00±10.00 ppm) of *P. tomentosa* are also good sources of potassium. Higher potassium with low sodium as presented in this study is protective against excessive sodium intake. The higher potassium content in the stem and root extracts may be an added advantage over the leaf samples for therapy and are vital for bone (Dzomeku *et al*., 2006). Increasing dietary potassium lowers blood pressure in humans and reduces the risk of stroke. Thus, maintaining a high potassium intake may be achieved by consuming the stems and roots of *P. tomentosa*.

In view of the present study, the leaf, root and stem extracts of *P. tomentosa* could be utilized as a cheap source of nutrients and antifungal agent(s). Further studies on isolation of the active antifungal agent(s) and toxicological evaluations of the leaf root and stem extracts of *P. tomentosa* are recommended.

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