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COMPARATIVE PHYTOCHEMICAL, PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF THE LEAF, FRUIT, STEM BARK AND ROOT OF *PAVETTA CRASSIPES* (K. SCHUM)

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ABSTRACT

Pavetta crassipes, a shrub distributed in Africa, is an important plant eaten as food and with therapeutic applications in traditional medicine. Reports on the medicinal, chemical and pharmacological properties are mainly on the leaves, with little or no investigation on the other plant parts. The study aimed to examine and compare the phytochemical, physicochemical, mineral element contents, and antimicrobial properties of the leaf, stem bark, root and fruit of *P. crassipes.* Standard methods were employed and elemental contents were determined by atomic absorption spectroscopy. The antimicrobial potentials were evaluated by the agar well diffusion method. Phytochemical screening detected alkaloids, terpenes, flavonoids, tannins and saponins in all the plant parts. The phenolic and flavonoid content was highest in the leaf with 77.26 mg GAE/g and 52.44 mg QE/g, respectively. Thin layer chromatography (TLC) analysis established the presence of rutin in the leaf only. The physicochemical properties ranged from 4.89–6.03 % for moisture, 3.67–13.55 % for total ash and 1.42–1.95 % for acid insoluble ash contents in the plant parts. Iron was the major constituent in all plant parts while sodium, lead and chromium were not detected. The extracts of the leaf, root and fruit inhibited the growth of *P. aeruginosa*, *B. subtilis* and *S. aureus*, respectively at 100 mg/mL, with diameters between 13 and 17 cm. The leaves, root and fruits are potential sources of drugs and thus, widens the scope of study on *P. crassipes* for further phytochemical and pharmacological studies on the plant.

KEYWORDS: ***Pavetta crassipes*; physicochemical; phytochemical; antimicrobial; thin layer chromatography; elemental analysis.**

INTRODUCTION

For centuries, medicinal plants have served as therapy for the treatment of diseases in many developing countries especially in sub-Saharan Africa. *Pavetta crassipes* (K. Schum) belonging to the family Rubiaceae, is one of such medicinal plants widely used in sub-Saharan Africa [1]. *P. crassipes* is a shrub distributed in the tropical African savanna of West and East Africa [2]. In West and Central Africa, *P. crassipes* leaves are eaten as food and used in the treatment of fever, hypertension, schistosomiasis, mental illness, convulsions, malaria, hookworms, onchocerciacis and gonorrhea [1, 3, 4]. It is also a widely used Nigerian ethnomedicine for the treatment of tuberculosis, and respiratory tract infections [5, 6]. Formulation studies on *P. crassipes* leaves as herbal tea have also been reported [7]. Phytochemical studies on the leaves of the plant revealed seven structurally diverse compounds; β-sitosterol, ursolic acid, methyl chlorogenate, ethyl chlorogenate, rutin, mannitol and

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ixoside, and also established the antimycobacterial activity of the leaf extracts of *P. crassipes* [3, 8]. The antimicrobial activities of the leaves have also been studied [9, 10]. Other pharmacological properties of the plant include antiprotozoal, antitumor, antiplasmodial, anti-inflammatory, antimycobacterial, neuropharmacological and gastrointestinal activities [11–13]. The toxicological studies of the plant on rodents reported on the safety of the aqueous leaf extract of *P. crassipes* with an LD_{50} greater than 5000 mg/kg [14]. Despite the aforementioned medicinal utilities of the plant which are mainly on the leaf, there is paucity of information on the rest of the plant parts and hence the need to widen the scope of study on *P. crassipes*. This study seeks therefore to establish some preliminary data to evaluate and compare the phytochemical composition, physicochemical properties, elemental content and antimicrobial activities of the leaf, root, bark and fruit of the plant.

MATERIALS AND METHODS

Plant Material

The leaves, roots, stem bark and fruits of *P. crassipes* were collected from Suleja, Niger State, Nigeria and were identified at the Herbarium unit of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, and assigned the voucher number NIPRD/H/6241. The plant materials were washed under running water, dried at room temperature and pulverized.

Reagents and Chemicals

Solvents, chemicals and reagents used for phytochemical and physicochemical studies were of analytical grade. Folin-Ciocalteu reagent was purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Metal element standards were purchased from Sigma-Aldrich Co. (Milwaukee, WI, USA). Quercetin, gallic acid and rutin reference standards were obtained from United States Pharmacopoeia, Rockville, Maryland, USA. The distilled water was laboratory prepared.

Preparation of Plant Extracts

Powdered plant material (100 g) from each sample was extracted with 1 L of methanol in a soxhlet apparatus. The resulting mixture was filtered and concentrated under reduced pressure at 40 °C on a rotary evaporator (Rotary Evaporator RE-201D, Henan, China) and further dried on a water bath (Karl Korb Scientific Technical Supplies D-6072, Dreieich, Germany).

Phytochemical Screening

The resulting methanol extract from each plant sample was qualitatively evaluated for secondary metabolites using standard procedures [15].

Determination of Total Phenolic Content

The phenolic content in the leaf, root, stem bark and fruit extracts of *P. crassipes* was estimated by the Folin-Ciocalteu method with slight modifications [16]. A concentration of the methanol extract (1 mg/mL) of each plant sample was prepared and 200 µL transferred to a 5 mL volumetric flask. Four hundred microliters (400 µL) of 2 N Folin-Ciocalteu reagent and 400 µL of Na_2CO_3 (20 % w/v) were added and the volume was made up with distilled water. The reaction was incubated for 2 hr. The absorbances of the samples were measured in triplicates at 760 nm on a UV-Vis spectrophotometer (Agilent Cary 60 UV spectrophotometer, Malaysia) against a reagent blank. Gallic acid was used as the standard and the phenolic contents of the plant samples were expressed as gallic acid equivalent per gram (GAE/g).

Determination of Flavonoid Content

The aluminum chloride colorimetric method was adopted with slight modifications [16]. Five hundred microliters (500 µL) of prepared concentration (1 mg/mL) of extracts of the plant samples was mixed with 100 µL of potassium acetate, 100 µL of 10% aluminium chloride $AlCl_3$ methanol solution and made up to 4 mL with distilled water. The mixture was incubated for 30 min. The absorbances of the samples were measured in triplicates at 415 nm using a UV-Vis spectrophotometer (Agilent Cary 60 UV spectrophotometer, Malaysia) against a reagent blank. Quercetin was used as standard and the total flavonoid content was expressed as quercetin equivalent per gram (QE/g).

Thin Layer Chromatography

The extracts of the plant samples were individually dissolved in methanol, and spotted along with rutin reference standard on TLC plates (Merck TLC aluminum sheets, silica gel-60 20 x 20 cm, 0.2 mm thickness) using micropipette tubes (20 µL). The TLC plates were then developed in a solvent system of toluene: ethyl acetate (5: 4). The developed plate was dried in the open and spots were detected by viewing under UV light at 254 nm.

Determination of Moisture Content

One gram of each plant sample was weighed on a tared aluminum pan and dried in an oven (Thermostat Oven DHG-9101-0SA, Zhejiang, China) at 105 °C for 180 min. The sample was then transferred to a desiccator to cool for 30 min, after which it was weighed again and the moisture content calculated [17].

Determination of Total Ash

Two grams of each plant sample was taken into a tared porcelain crucible and ignited at 550 °C in a furnace (Vecstar furnaces LF1, Chesterfield, United Kingdom) until the ash turned white in colour. The crucible was transferred to a desiccator to cool for 30 minutes, and the weight recorded [18].

Determination of Acid Insoluble Ash

The crucible containing the total ash was boiled with 25 mL of dilute hydrochloric acid for 5 min. The insoluble matter was collected on ashless filter paper and washed with hot water. The insoluble matter was transferred to the original crucible, and ignited at 500 °C in a furnace to constant weight. The crucible was allowed to cool in a desiccator for 30 min, and the weight recorded [18].

Determination of Mineral Elements

The method of Rashid *et al* was adopted and modified [19]

Digestion of Sample

A mixture of HNO_3 and $HClO_4$ (10 mL) in a ratio 7:3 was added to the resulting ash (total ash) and heated in a fume chamber until it became colourless. The mixture was allowed to cool, filtered (Whatman No. 42) and quantitatively transferred to a 50 mL volumetric flask and diluted to the mark with distilled water. A sample blank was similarly prepared.

Elemental Analysis

The mineral contents of the plant samples were determined with an atomic absorption spectrophotometer (AAS; GBC Avanta, version 2.0, GBC Scientific equipment, Hampshire, United Kingdom). Standard solutions of lead (Pb), copper (Cu), chromium (Cr), sodium (Na), zinc (Zn), manganese (Mn) and iron (Fe) were prepared from the stock solution (1000 mg/kg) of each metal. The blank (deionized water) was first aspirated into the AAS in order to zero the equipment, then the standard solutions of each metal were aspirated to get the calibration curve. The digested plant sample solutions were subsequently aspirated and the concentrations of the metals present were displayed by the instrument after extrapolation from the standard curves.

Determination of Antimicrobial Activity

Test Organisms

Pure clinical isolates of *Bacillus subtilis* and *Kiebsiella pneumonia* were collected and biochemically confirmed from Diagnostic laboratory of NIPRD clinic. American typed cultures of *Escherichia coli* [ATCC 25952], *Staphylococcus aureus* [ATCC 25923], *Pseudomonas aeruginosa* [ATCC 27853] and *Salmonella paratyphi* [ATCC 9150] were also used in this study.

Antimicrobial Assay

The agar well diffusion method was adopted for the study [20]. One hundred microliter (100 µL) of the suspension of each standardized microorganism was incubated into sterile Moltel Mueller Hinton agar, swirled and poured into sterile petri dishes and allowed to solidify. Wells of 5 mm diameter were bored aseptically and the bottom of the holes were sealed using a drop of the agar. One hundred microliters (100 µL) of the extracts (100 mg/mL) and chloramphenicol (reference drug; 30 µg/mL) was dispensed into appropriately labeled wells. The extract concentration of 100 mg/mL was selected based on the pre-experiments. The plates were left to dry inside the biosafety cabinet and extracts were allowed to diffuse for 2 hr, and thereafter incubated at 37 °C for 24 hr. The antibacterial activity was carried out in duplicates and assessed by measuring the diameter of the zone of inhibition (in mm) surrounding the wells and taking the average of the readings.

Statistical and Data Analysis

All the experiments for determination of total phenols, total flavonoids, moisture content, total ash content and acid-insoluble ash content were conducted in triplicates. The data obtained were processed using statistical package for social sciences (SPSS) version 23. The values are expressed as the mean ± standard deviation (SD). Significance of difference was tested by one-way ANOVA. The correlation coefficients (r) and coefficients of determination (r^2) were calculated using Microsoft Excel 2010.

RESULTS

The appearance, yield, total ash, acid-insoluble ash and moisture contents of the plant parts of *P. crassipes* are as shown in Table 1. The highest and lowest extraction yields of 28.21 % and 8.64 % were obtained from the leaf and stem bark, respectively. The stem bark of the plant presented the highest total ash content (13.55 ± 0.28 %) while the fruit had the lowest (3.67 ± 0.23 %). The result from the acid insoluble ash content of the plant showed that the fruit had the highest value of 1.95 ± 0.18 % while the root had the lowest content of 1.42 ± 0.13 %. The moisture content of all parts of the plant was <10 % with the fruit presenting the highest value at 6.03 ± 0.03 % while the leaves was lowest at 4.89 ± 0.08 %. The results showed that there was significant difference in the moisture, ash and acid-insoluble ash contents of the plant samples ($p<0.5$). The TLC chromatogram showed spots with an R_f of 0.22 common to the leaf and reference standard, rutin (UV as detector). Qualitative phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, steroids/terpenes, and glycosides in all plant parts of *P. crassipes* except saponins which was detected in the leaf, root and stem bark but not in the fruit (Table 2). The TPC and TFC of the plant samples are summarized in Table 3. The leaves presented the highest TPC and TFC at 77.26 and 52.44 mg QE/g, respectively while the fruit and stembark were lowest at 14.66 and 0.63 mg QE/g, respectively. The differences in TPC and TFC amongst the plant samples were observed to be statistically significant at the threshold of 0.05. The results of the concentration levels of the elements are presented in Table 4. Iron presented the highest elemental concentration in all the plant samples while lead, chromium and sodium were not detected. The fruit had comparatively the lowest concentration of the elements anlaysed. The antimicrobial results on Table 5 report the activities of the plant extracts on *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, S. paratyphi and B. subtilis* at a concentration of 100 µg/mL. *P. aeruginosa, B. subtilis* and *S. aureus* were susceptible to the leaf, root and fruit extracts of *P. crassipes* with zones of inhibition at 17, 13 and 17 mm, respectively.

DISCUSSION

The highest yield of 28.21 % was obtained from the leaf extract of *P. crassipes*. A related study reported a similar trend with the leaf extract having the highest yield compared to the stem, root and fruit, with methanol and chloroform as extraction solvents [21]. The higher yield of the leaf extract may be attributed to a greater number and concentration of constituents in the leaf compared to the other plant parts. The TLC chromatogram qualitatively identified rutin as a constituent of the leaf extract of *P. crassipes* with an R_f of 0.22. Rutin is a flavonoid glucoside with a ring system moiety making the molecule a chromophore, hence the detection under ultraviolet wavelength of 256 nm. This confirms the earlier reports on the isolation of rutin from the leaves of *P. crassipes* [3, 9]. This finding also provides support for the use of rutin as a chemical marker for the identification and authentication of *P. crassipes* leaves by chromatographic methods. The percentage of total ash content in the different parts of *P. crassipes* were found to be in the increasing order fruit<leaf<root<stem bark. The purity and safety of a plant is useful for quality control purposes, and are determined by the ash value and moisture content. The moisture content determines the plant's susceptibility to microbial or hydrolytic spoilage, the ash content demonstrates the content of physiological and non-physiological ash while the acid-insoluble ash indicates the content of inorganic contaminants or silaceous material present in the plant. For all the plant parts, the moisture contents (< 10 %) and ash values (< 14 %) were considered permissible when compared to the range of values specified by United States Pharmacopoeia for articles of botanical origin. Qualitative phytochemical screening provides some preliminary information on the class of compounds present in the plant, and also a guide to the potential pharmacological activities which the plant may possess. The presence of flavonoids, steroids, terpenes and glycosides in the leaf extracts was confirmed in the study by Ibekwe *et al.,* where rutin, sitosterol, ursolic acid and mannitol were isolated [3]. The leaf and root extracts of *P. crassipes* presented moderate content of phenolic compounds and flavonoids compared to the stem bark and the fruit. Flavonoids and phenolics are known to have high antioxidant, anti-inflamatory, antimicrobial and anticarcinogenic capacties and also possess capacity to modulate key cellular enzyme functions [22, 23].

Seven elements; lead, copper, chromium, zinc, sodium, iron and manganese were assessed for their metallic content in the different plant parts of *P. crassipes* by atomic absorption spectroscopy (AAS). Elemental composition provides useful information on the nutritional benefits or otherwise of the plant. Sodium is classified as a macronutrient required in relatively large amounts in the body, while lead is termed as a heavy metal because of its relatively high density and is toxic or poisonous at low concentrations.

Table 1: Physicochemical parameters of the leaf, root, stembark and fruit of *P. crassipes*

Plant part	Appearance	Percentage yield (%)	Total ash (%)	Acid insoluble ash (%)	Moisture content (%)
Leaf	Dark paste	28.21	7.92 ± 0.25^{b}	1.65 ± 0.20^{b}	4.89 ± 0.08^{a}
Root	Dark paste	8.64	12.03 ± 0.24^{c}	1.42 ± 0.13^{a}	4.90 ± 0.20^{b}
Stem bark	Dark paste	9.02	13.55 ± 0.28^{d}	1.78 ± 0.15^{c}	5.95 ± 0.05^{c}
Fruit	Dark solid	12.40	3.67 ± 0.23^{a}	1.95 ± 0.18^{d}	6.03 ± 0.03^{d}
p value (calc)			0.00	0.00	0.00

Values followed by different superscript letters (a–d) in the same column are significantly different ($p < 0.05$).

Table 2: Phytochemical screening of methanol extracts of the leaf, root, stembark and fruit **of *P. crassipes***

Metabolite	Leaf	Root	Stem bark	Fruit
Flavonoids	+	+	+	+
Saponins	++	+	++	-
Tannins	++	+	+	+
Alkaloids	+	+	+	+
Steroids	++	+	+	++
Glycosides	+	+	+	+

Table 3: Total phenolic and flavonoid contents of the leaf, root, stembark and fruit *P. crassipes*

Plant part	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Leaf	77.26 ± 0.08^{d}	52.44 ± 0.05^{d}
Root	25.63 ± 0.38^{c}	21.31 ± 0.33^{c}
Stem bark	16.77 ± 0.15^{b}	0.63 ± 0.07^{a}
Fruit	14.66 ± 0.11^{a}	7.76 ± 0.10^{b}
p value (calc)	0.00	0.00

Values followed by different superscript letters (a–d) in the same column are significantly different ($p < 0.05$).

Table 4: Elemental concentrations of methanol extracts of the leaf, root, stembark and fruit of *P. crassipes*

	Concentration (mg/kg)			
Element	**Leaf**	**Root**	**Stem bark**	**Fruit**
Lead	0.00	0.00	0.00	0.00
Copper	5.28	2.78	2.8	4.28
Chromium	0.00	0.00	0.00	0.00
Zinc	4.03	7.58	0.00	0.20
Sodium	0.00	0.00	0.00	0.00
Iron	104.75	589.43	338.50	24.50
Manganese	17.00	6.45	11.18	0.00

Table 5: Antimicrobial assay of methanol extracts of the leaf, root, stembark and fruit of *P. crassipes*

	Zone of inhibition (mm) at 100 mg/mL				
Tested micro-organisms	**Leaf**	**Root**	**Stem bark**	**Fruit**	**Chloramphenicol****
S. aureus	NA*	NA	NA	17	18
E. coli	NA	NA	NA	NA	25
K. pneumonia	NA	NA	NA	NA	22
P. aeruginosa	17	NA	NA	NA	25
S. paratyphi	NA	NA	NA	NA	23
B. subtilis	NA	13	NA	NA	20

* no activity, **the concentration of chloramphenicol was 30 µg/mL.

Trace elements which include copper, zinc, iron, manganese and chromium are essential micronutrients that are present in living tissues in small amounts, accounting for less than 0.01 % of the total body weight [24]. Sodium, lead and chromium were not detected in any of the plant parts. The absence of lead in the plant samples was an indication that there was no contamination of the plant from sources such as the soil, industrial emissions, harvesting processes, amongst other factors. The highest concentration of manganese was found in the leaves of *P. crassipes* at 17 mg/kg but was not detected in the fruit. Manganese acts as cofactor for many enzymes, however the deficiency of Mn in humans often leads to immunodeficiency disorder, rheumatic arthritis in adults as well as myocardial infraction and other cardiovascular diseases [25, 26]. The highest concentration of zinc was found in the root of *P. crassipes* at 7.58 mg/kg, followed by the leaf at 4.03 mg/kg while the element was not detected in the stem bark. Zinc is necessary for proper growth, blood clotting, thyroid function and protein and DNA synthesis [27]. Deficiency of zinc is reported to clinically affect the epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems [28]. The copper (Cu) concentration ranged from 2.78–5.28 mg/kg for the different parts of *P. crassipes*, with the leaf and root having the highest and lowest concentrations, respectively. Copper is essential for some physiological processes in the human body including iron utilization, elimination of free radical, bone and connective tissue development [25]. Its deficiency may be a risk factor for cardiovascular disease and symptoms such as neuropenia, cardiac disorders, osteoporosis and anemia may occur [29]. Copper could also be toxic depending on the dose and duration of exposure [30]. The concentration levels of iron (Fe) ranged from 24.50–589.44 mg/kg with the highest content in the root and the lowest in the fruit. Iron has several key functions in human body including oxygen supply, energy production and immunity. Deficiency of Fe is associated with anaemia and impairment in the immune system [31]. The high iron content of Fe in all the plant parts may be the reason it is consumed as food [3]. The leaf, root and fruit extracts of *P. crassipes* inhibited the growth of *P. aeruginosa*. *B. subtilis* and *S. aureus* only at a concentration of l00 mg/mL with diameters ranging between 13–17 mm. The stem bark did not exhibit activity against any of the organisms at the concentrations tested. It has been postulated that a plant extract with an inhibition zone of 14 mm or more possesses significant antimicrobial activity [32]. *S. aureus* and *P. aeruginosa* are two bacteria commonly found together in human polymicrobial infections causing chronic infections in individuals with a variety of comorbidities, including cystic fibrosis [33]. *B. subtilis* is a bacterium responsible for diseases like pneumonia and endocarditis in humans. The infections caused by these bacteria are known to be very difficult to treat as they are mostly opportunistic and highly resistant to antibiotics [34, 35]. The activities displayed by the leaf and fruit of the plant corroborate the findings of Bello *et al* [9] and Balde *et al* [10] who reported activities of the plant against *P. aeruginosa* and *S. aureus*, respectively.

CONCLUSION

Preliminary phytochemical, physicochemical, mineral element content, and antimicrobial studies were carried out on the leaves, stem bark, roots and fruits of *P. crassipes*. The physicochemical properties would be useful as quality characteristics which would enable idenitification and authentication of the plant. The phytochemical and antimicrobial results from this study predispose the plant to futher chemical and pharmacological studies, towards drug discovery and development.

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