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Chemical analyses of aqueous extract of *Parkia biglobosa* fruit husk collected from Northern Ghana

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Water and ethanol extracts of *Parkia biglobosa* fruit husk were compared and analysed. Methods used were Sohxlet extraction, phytochemical screening, high power liquid chromatography (HPLC), fractionation and thin layer chromatography (TLC). Water was identified as a more efficient solvent than ethanol for the extraction of *P. biglobosa* fruit husk. The storage period (after harvest) of husk was not relevant to the mass of extract obtained. The extract contained tannins, flavonoids, anthraquinones, saponins, anthraquinone glycosides, and alkaloids. From the HPLC analyses, there was at least one class of alkaloids present in the aqueous extract, at least three classes of phenolic glycosides, and at least two of the same or any two different classes of phenolic acids, flavonols and/or proanthocyanidins observed. There were moderately polar components, which associated together in minimum groups of two on TLC plates. A 2:1 mixture of ethanol to water produced the high retention factors (Rf) of 0.96 and 0.67 in the extract, and 1.0 in the strong acid fraction. 2:1 ethylacetate-chloroform mobile phase mixture had the lowest Rf (0.06) as well as separation. The strong acid fraction had at least three components; the weak acid fraction had four; the basic fraction had three; while the neutral fraction had four components.

Key words: Phytochemicals, extraction, components, phenolics, fractionation.

INTRODUCTION

Parkia biglobosa (Jacq.) Benth. belongs to the family Leguminosae and the subfamily Mimosoideae (Hopkins, 1983). The tree has a wide distribution ranging across the Sudan and Guinea savanna ecological zones. It is found in 19 African countries (Hall et al., 1997). Investigations into the local perceptions on trees in West Africa revealed *P. biglobosa* as one of the species preferred among conserved fruit-bearing trees (Teklehaimanot, 2004). A survey on families in Burkina Faso on vegetable consumption and seasonality in two communities found that dawadawa (fermented seed of *P. biglobosa*) was consumed in 78 and 85% of all meals (Mertz et al., 2001). About 201,000 tonnes of *P. biglobosa* fruit is produced annually in Northern Nigeria alone (Sina and Traore, 2002). On markets of some Ghanaian communities, it is common to find the seedpods, dried bark, and many different products of *P. biglobosa* and other trees being sold (Margaret, 2002).

Various parts of *P. biglobosa* tree are used to make tonics and ointments to treat many ailments (Ajaiyeoba, 2002; Banwo et al., 2004; Irvine, 1961). The most significant product from *P. biglobosa* probably is food, but the husk is also sold in the market for various uses (Margaret, 2002), especially in the dry season (Becker, 1983; Ogbe et al., 1999). The seed is also fermented into a spice called “dawadawa”, used for seasoning traditional soups (Ajaiyeoba, 1998; Campbell-Platt, 1980; Margaret, 2002). Currently, the fermented seeds of *P. biglobosa* are produced commercially into cubes by industry for use in flavoring food (Alabi et al., 2004).

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On the other hand, extract of the husk has been used as a bonding agent between locally manufactured clay tiles and the soil beneath (Adama and Jimoh, 2011). Decoctions of the fruit husk are also used on floors, walls, and ceramic pots, on the walls of living rooms, huts, barns and other parts of houses to impart water resiliency, contribute to waterproof, improve their quality and/or protect them against erosion by driving rain (Abagale, 2008; Adwoa, 2006; Margaret, 2002). The husk extract is also mixed with other components to make natural paintings or murals to decorate walls (Abagale et al., 2013; Awindor, 2006; Lucas et al., 2004). The Sirigu Women in Pottery and Art (SWOPA) used *P. biglobosa* husk extract in mud wall plastering works in houses in their community (Lucas et al., 2004) and in other parts of Ghana (Adwoa, 2006). Also in Burkina Faso, the Karaboro and Gouin potters splash their pots with a vegetal solution made from the pods and husks which act as a sealant and creates a dark, mottled surface (Cookery, 2000). The extract of the bark and husks of the pods of *P. biglobosa* are also used for dyeing and curing leather as well as for dying sculpture (Campbell-Platt, 1980). Hall et al. (1997), and Sina and Traore (2002) have reported that the bark and husk contain tannin.

Secondary metabolites are widely applied in medicine, food and the dye industry. Some of them have biological activities (Sparg et al., 2004), others have pharmacological effects (Ansel et al., 1995) and others have multiple uses. Anthraquinones are of fundamental importance both in dye industry and medicine. Anthraquinone glycosides, flavones, and tannin form important ingredients of several laxative medicines and in dyes. In the pharmaceutical industry, natural and synthetic derivatives of anthraquinones and flavonoids are beneficial to mammals and humans as they can display antibacterial, antitrypanosomal and antineoplastic activities (Heyman et al., 2009; Tarus et al., 2002; Velez-Cruz and Osheroff, 2004). In enhanced oil recovery, tracking the liquid removal through tracers is an important procedure. When inorganic tracers are use, the stratum is polluted and ions of SCN^- and SO_4^{2-} exist in many stratums (Dong et al., 2007). So the utilization of dyes has become a good idea. Valuable dye intermediates and blue dye stuffs, can be used as tracers and prepared by treating anthraquinones. Traditionally, the synthetical route of anthraquinone Cr^{3+} has been produced by oxidizing alkyl group with bichromate in industry and the problem of pollution is very serious (Dong et al., 2007). The synthesis of an anthraquinone dye generally involves a large number of steps (Kirk, 2012). The complexity of preparation has made the production costs of anthraquinone dyes higher even though they have excellent properties such as brilliancy of color, fastness, and excellent dyeing properties (leveling and dye bath stability). Highly toxic metals such as mercury or chromium (VI) are sometimes required in the synthetic production of anthraquinone dyes. Some of the processes also need to employ a large amount of organic solvent, and others involve a great quantity of waste acids. With the increasing demand for environmental protection, the regulation of pollutant effluents has also caused a sharp increase in the costs for waste-water treatment (Davood, 2010).

In general petroleum ether and hexane solvents are involved in the extraction of non polar compounds (for example, triterpenes and sterols), while alcohol (ethanol) is used for polar components like alkaloids and polar flavonoids (Harbone and Turner, 1984).

In recent times much research is geared towards possible ways of recycling agro wastes products for re-use to keep the environment clean and safe (Adama and Jimoh, 2011) and to find new bioactive agents from indigenous plants (Hook, 2011; Thomson, 1971). In view of the numerous traditional uses of the study material particularly in the building and tanning industry, it has become necessary to document the general chemical makeup of *P. biglobasa* fruit husk. The results will provide preliminary information for technological development and enhancement of the local uses of the husk and give a lead to their industrial development. It will also motivate agro-forestry management of the *P. biglobosa* tree.

MATERIALS AND METHODS

Two types of husk; freshly harvested dry husk (hereafter referred to as new husk) and husk harvested and stored for about a year (hereafter referred to as old husk) were collected, authenticated, extracted and the extracts fractionated. The extracts and fractions were used for various analyses. All reagents/chemicals used were of analytical reagent grade from the BDH Laboratory supplies Ltd, Poole, England and purchased from Revelation Products Ltd at Asafo in Kumasi, Ghana. The husks were purchased from women in the Navrongo central market in the Upper East Region of Ghana. These women reportly collected them from various farmlands in their communities; namely Pungu, Chiana, Natugunia, Vonania, Gongnia and Korania. The new husk looked shiny on the outer surface and the old husk looked dull and darker. The husks were collected separately into nylon sacks and transported to the laboratory for identification and processing.

Random samples were picked out of the bulk husk and were authenticated by Mr. Ben Yamba of the Kwame Nkrumah University of Science and Technology (KNUST) Experimental Farms and Mr. V. Sore, the Chief Technician at the KNUST Botanical Gardens.

The husk was further air-dried for seven days in the laboratory to a constant weight. The moisture-free husk was then pulverized in a mill at the Faculty of Agriculture at KNUST to obtain a coarsely powdered sample. Each was labeled for extraction using a Soxhlet apparatus. Phytochemical screening was also carried out on both aqueous and ethanol extracts. Based on the relative amounts of the extracts and the phytochemical results a selection of one extract for further analyses was made. The aqueous extract was selected for further analyses. Thus, portions from this extract were taken for preliminary functional group determination, fractionation, TLC and HPLC. The fractionated extract was also divided into portions for preliminary functional group determination and further TLC analyses.

Extraction

Three portions of approximately 200 g powdered *P. biglobosa* new husk were separately extracted, concentrated under reduced pressure and dried in the oven to a constant weight at 105°C. The process was repeated using the old husk and also using boiling 96% ethanol as solvent in a Soxhlet extractor. The ethanol extract was concentrated in the rotary extractor and dried in a weighed beaker at 80°C in the oven. The extract yield was calculated and

Table 1. Extraction efficiency (%) of the new husk of *P. biglobasa* fruit husk.

Extract	Mean mass of extracts (g)	Standard error of the mean (SEM)	Efficiency (%)
Water	74.440±0.06	0.0424	37.22
Ethanol	61.892±0.19	0.1344	30.95
Water (after ethanol)	16.790±0.59	0.4171	8.40

Table 2 Extraction efficiency (%) of the old husk of *P. biglobasa* fruit husk.

Extract	Mean mass of extracts (g)	Standard error of the mean (SEM)	Efficiency (%)
Water	76.62±0.98	0.69296	38.31
Ethanol	61.39±0.19	0.13435	30.70
Water (after ethanol)	17.29±1.54	1.08894	8.65

Table 3. Statistical analyses results of water extract of new husk.

Extract	Standard deviation (s) of the mass of extracts	Standard error of the mean (SEM) mass of extracts
Water	0.06	0.0424
Ethanol	0.19	0.1344
Water (after ethanol)	0.59	0.4171

used to determine the efficiency of the solvents to extract.

For purposes of further analyses another set of extractions were made and the extracts dried at room temperature.

Phytochemical screening

Phytochemical tests were done on both aqueous and ethanol extracts (dried under room temperature) using standard tests for saponins, general glycosides, flavonoids, terpenes, tannins, alkaloids, anthraquinones and their glycosides and cyanogenitic glycosides described in Herborne (1973) and Sofowara (1993).

High power liquid chromatography (HPLC)

For HPLC analyses 1.0 g/v solution of crude aqueous extract, dried under room temperature was used. Presence of phenolic acids, flavan-3-ols and flavonols or proanthocyanidin; alkaloids and phenolic glycosides was determined using the methods cited by Turkmen and Sedat (2007) and those validated by Andrea et al. (2006).

Fractionation and litmus tests

The crude aqueous extract, dried under room temperature, was fractionated using the Bulk Transfer methodology described in Pavia et al. (1995). The various solvents from the fractions were evaporated and the fractions dried under temperature for 72 h. Aqueous solutions of portions of the extracts and fractions were prepared and tested with litmus paper (Pavia et al., 1995). The remaining portions were used for further work.

Preliminary functional group determination

The preliminary functional group determination process was done According to methods described in Pavia et al. (1995), on the aqueous extract.

Thin layer chromatography (TLC)

TLC was performed on the crude water extract that was dried under room temperature as well as the acid, base and neutral fractions. Standard 5 x 10 cm silica pre-coated thin layer chromatography plates, from Macherey-Nagel GmbH & Co. KG, were used. Mobile phase solvents used for the TLC were distilled water, 96% ethanol, methanol, ethylacetate, hexane, chloroform and various mixtures applying methods in Pavia et al. (1995).

RESULTS

Extraction efficiencies

Extraction efficiencies results are shown in Tables 1 and 2. The standard deviation and standard error of the mean of the husk extracts were calculated on the mass of extract obtained and the summary presented in Tables 3 and 4.

Reliability was estimated at 95% confidence level and 1 degree of freedom (Bluman, 2006). The null hypothesis test, working at 95% confidence limit and probability (P), at $\alpha = 0.5$, is not rejected. The observed difference between the sample mean and the true values arise solely as a result of random errors (Bishop et al., 1992; Bluman, 2006).

Phytochemical screening of extracts

Secondary metabolites in water and ethanol extracts of *P. biglobasa* fruit husk is as shown in Table 5. Solubility

Table 4. Statistical analyses results of water extract of old husk.

Extract	Standard deviation(s) of the mass of extracts	Standard error of the mean (SEM) mass of extracts
Water	0.98	0.69296
Ethanol	0.19	0.13435
Water (after ethanol)	1.54	1.08894

Table 5. Secondary metabolites in water and ethanol extracts of *P. biglobasa* fruit husk.

Secondary metabolite	Extract	
	Water	Ethanol
Saponins (Test: Extract + distilled water froth test)	+++	++
General glycosides (Test: Extract and 20% NaOH and Benedicts solution)	+	+
Flavonoids (Test: ConcHCl + Mg turnings)	++	++
Steroids and Terpenes (Test: Acetic anhydride + conc H_2SO_4)	-	-
Tannins and polyphenols (Extract + 0.1% $FeCl_3$)	+++	+++
Alkaloids (Test: Extract + 10% HCl)		
Dragendoff's	+++	+++
Wagner's reagent	+++	+++
Anthraquinones (Extract, 12% H_2SO_4, $CHCl_3$, 10% NH_3 Solution)	-	+

+++ = appreciable amount; ++ = moderate amount; + = trace, - = complete absence.

Table 6. Solubility based suspected compounds in the aqueous extract and fractions.

Sample	Classes of compounds suspected present
Water extract	Low MW carboxylic acids, alkenes, alkynes, alcohols, ketones, aldehydes, nitro compounds, esters, ethers, amides
Strong acid fraction	Phenols, alkyl halides, aromatic compounds
Weak acid fraction	Alkynes, alcohols, carbonyls, nitro compounds, amides
Basic fraction	Alkenes, ketones, aldehydes, nitro compounds, esters, ethers
Neutral fraction	Low molecular weight neutral compounds; including alkanes, alkyl halides, aromatic compounds

based suspected compounds in the aqueous extract and fractions are shown in Table 6.

HPLC of the aqueous extract

Three absorption peaks (Figure 1a) were obtained under the analyses for phenolic glycosides; two for the presence of phenolic acids, flavonols and/or proanthocyanidins (Figure 1b); and one peak (Figure 1c) for the alkaloidal group of compounds.

Fractionation of aqueous extract, and litmus test its fractions

Basic, neutral, weak and strong acid fractions were obtained. The basic and neutral fractions were in smaller quantities compared with the weak and strong acid fractions. In the litmus test, red litmus paper remained unchanged in the weak acid, strong acid and neutral fractions but turned blue in the basic fraction. Blue litmus paper turned red in the strong and weak acid fractions, but remained unchanged in the neutral as well as basic fractions.

Thin layer chromatography of aqueous extract and fractions

The solvent systems used and the results of TLC analysis of the aqueous extract is as shown in Table 7. Also, the solvent systems used and the results of TLC analysis of the fractions is as shown Table 8.

DISCUSSION

The extraction efficiency of old husk in water was greater than that of the new husk while the extraction efficiency of the old husk in ethanol was rather lower than that of the new husk. The margin of difference in the extraction efficiency of old husk in water was greater than that of ethanol. Generally polar solutes dissolve in polar solvents

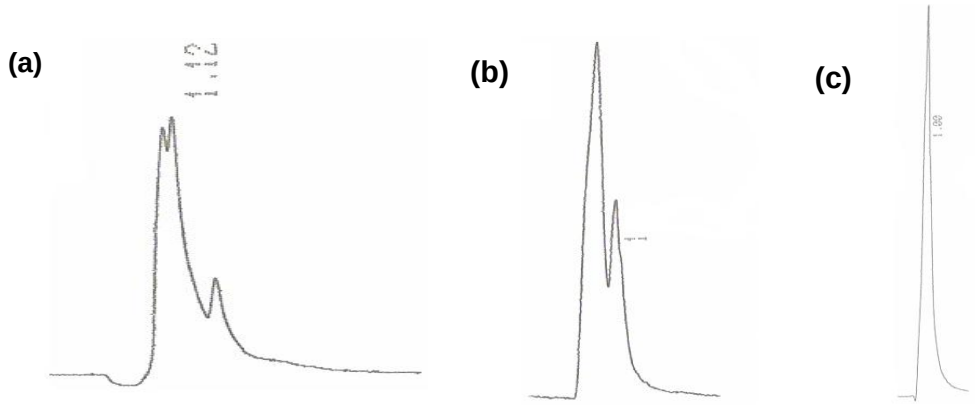

Figure 1. (a) HPLC of phenolic glycosides in 1.0 g/v solution aqueous extract of *P. biglobasa* fruit husk, **(b)** HPLC of phenolics/flavonols/proanthocyanidins in 1.0 g/v solution of the aqueous extract of *P. biglobasa* fruit husk, (**c)** HPLC of alkaloids in 1.0 g/v solution of aqueous extract of *P. biglobasa* fruit husk.

Table 7. Summary of TLC results of aqueous extract.

Extract	Solvent system	Separations	Retention factors
Water	Distilled Water	2	0.77, 0.42
	96% ethanol	2	0.86, 0.62
	2:1, Ethanol:Water	2	0.96, 0.67
	5:1, Ethanol:Water	2	0.80, 0.41
	3:2, Ethanol:Water	2	0.94, 0.26
	1:2, Ethylacetate:Ethanol	2	0.94, 0.22
	2:1, Ethylacetate:Ethanol	2	0.11, 0.07

Table 8. Summary of TLC of the fractions of the aqueous extract.

Fraction	Solvent system	Separations	Retention factors
Strong acid	Distilled water only	2	0.14, 0.60
	1:2, Ethanol : Water	3	0.17, 0.60, 1.0
Weak acid	76% Ethanol only	4	0.07, 0.37, 0.78, 0.87
	1:2, Ethanol : Water	4	0.12, 0.31, 0.45, 0.687
Basic	Ethanol only	2	0.64, 0.86
	2:1, Ethanol : Water	3	0.44, 0.61, 0.76
	2:1, Ethylacetate : Ethanol	3	0.06, 0.55, 0.64
Neutral	Water only	3	0.06, 0.56, 0.82
	Ethanol only	3	0.035, 0.37, 0.70
	1:1, Ethanol : Water	4	0.14, 0.41, 0.56, 0.81
	1:2,Ethylacetate : Ethanol	4	0.06, 0.47, 0.68, 0.92

(Harbone and Turner, 1984). Since water is more polar than ethanol it will extract more solute than ethanol if the solute is very polar. However, using ethanol, and followed by water to extract the same sample produced a greater percentage of extract than the use of water alone. This indicated that a larger proportion of the components were mildly polar hence got extracted by ethanol (for example, anthraquinones) more than by water. The mild polarity of the extracts was further supported by the phytochemical results. Tannins, alkaloids and saponins were generally intense; hence the extracts would be moderately polar. Also, the average extract by water far outweighed the average extract by ethanol. Therefore water was identified as a preferable solvent for extracting the husk quantitatively since it was cheaper and yields more extract than ethanol. For its chemical makeup, the

phytochemical constituents in the extract were alkaloids, tannins, general glycosides, flavonoids, saponins and anthraquinones. These results compare well with those of Hall et al. (1997), and Sina and Traore (2002). All the metabolites identified in the phytochemical screening verified the solubility results. The suspected compounds found in the solubility results could essentially be due to functional groups associated with the secondary metabolites. At least three classes of phenolic glycosides, two classes of phenolic acids, flavonols and/or proanthocyanidins and one class of alkaloids were confirmed by HPLC to be in the extract. The colour changes of the litmus tests confirmed the pH ranges of the fractions. Thus the acid, base and neutral fractions were present as the fractionation sort to achieve. Further fractionation of the acid portion into weak and strong acids has also been shown to be possible. Two TLC separations in the aqueous extract showed that the components in the extract were associated in a minimum of two groups. Also they may be moderately polar since the widest separation (0.96) was produced by a 2:1 mixture of ethanol to water which is moderately polar. 2:1 ethylacetate: chloroform is largely non-polar and performed poorly as the retention factors and separations were least. Therefore, the crude extract may not contain a good amount of non-polar components. The TLC results of the fractions also conformed to results of the solubility tests of the fractions. The highest Rf value in all the fractions (1.0) was found in the strong acid fraction, using a moderately polar solvent mixture of 1:2 ethanol to water. This is a strong indication that the most polar components were contained in the strong acid fraction and moved along with the mobile phase. The non-polar components were contained in the neutral fraction having Rf value of 0.96 for a mobile phase mixture of 1:2 ethylacetate : water. Also there were at least three groups of compounds in the strong acid and basic fractions; and four in the weak acid and neutral fractions as indicated by the number of separations in the TLC of the fractions. Therefore the extract is capable of fractionating with relative ease, and compounds in the fractions have shown the possibility of being isolated for use.

Conclusion

Water was identified as the preferred extraction solvent compared to ethanol for quantitative extraction of *P. biglobosa* husk, and the length of storage time (after harvesting the pod), did not influence the amount of extractable amount of material at least for a year. The husk contains tannins and polyphenols, flavonoids, alkaloids and saponins, anthraquinones and glycosides. Steroids and terpenes may not be present. The aqueous extract of the husk can easily be fractionated into weak and strong acids, as well as basic and neutral compounds. Further studies are required to verify the possible use of the husk extract in molding modified mud bricks for building of simple low income houses especially in rural African communities; formulation into organic tracers in oil exploration; or the natural dye making.

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