Assessment of Extracts Makeup of *Parkia Biglobosa* Fruit Husk: A Component of Indigenous Mud-Wall-PlasterTechnology in Ghana

Abstract

One important non-timber forest product derived from *Parkia biglobosa* fruit husk is the extract used in indigenous building technology. Therefore, water and ethanol extracts of the fruit husk were assessed. Methods used were Sohxlet extraction, phytochemical screening, HPLC, fractionation, and TLC, and the percentage yield of extracted mass of husk extract compared. Water was identified as a more efficient solvent for the extraction of the husk. Average percent yield of extract was 37.77% using water, 30.83% using ethanol and 8.53% using water after ethanol extraction. The storage period (after harvest) of husk did not influence the mass of extract obtained. The extract contained tannins, flavonoids, anthraquinones, saponins, anthraquinone glycosides and alkaloids. From the HPLC analyses, there were at least three classes of phenolic glycosides, one class of alkaloids, and two of the same or any two different classes of phenolic acids, flavonols and/or proanthocyanidins observed in the extract. The components of the extract are moderately polar, which associated together in minimum groups of two on TLC plates. A 2:1 mobile phase mixture of ethanol to water produced high R_f of 0.96 and 0.67 in the aqueous extract, and 1.0 in the strong acid fraction. 2:1 ethylacetate / chloroform mobile phase mixture produced the lowest retention factors (0.06) as well as separations. The strong acid fraction had at least three components; weak acid fraction had four; three in the basic fraction, and four in the neutral fraction. Some of the components have cementitious character and could be useful building technology.

Key words:
Parkia biglobosa/ extract/ phytochemicals/ phenolics/ indigenous building technology

Comment [1]: Place a sorting between the key words

INTRODUCTION

Parkia biglobosa (Jacq.) Benth belongs in the family *Leguminosae* and the subfamily *Mimosoideae*[1]. The tree (*fig. 1*) has a wide distribution ranging across the Sudan and Guinea savanna ecological zones. The range extends from the western coast of Africa in Senegal across to Sudan. Itis found in nineteen African countries [2].

Comment [2]: Only scientific names are written in italics

Figure 1:*Parkia biglobosa* tree **(Source:** Useful Tropical Plants Database [3]

Traditionally, village communities developed knowledge and skills concerning the management and utilization of such trees [4]. Investigations into the local perceptions of trees in West Africa had revealed *Parkia biglobosa* as one of the species preferred among fruit-bearing trees conserved in farmlands and forests by farmers [5]. Non-timber forest products derived from *Parkia biglobosa* are food, medicine, glazes, soil amendments, charcoal, and firewood. Various parts of the *Parkia biglobossa* tree are used to make tonics and ointments to treat many ailments [6], [7], [8]. In markets of some communities, it is common to find the seedpods and many different products derived or harvested from *Parkia biglobosa* and other trees being sold [9].

The most significant product from *Parkia biglobosa* is probably food. Throughout West Africa the pulp of the ripe fruit serves as food source in the dry season, and the processed seed is used in traditional soups [3], [9], [10], [11],[12], [13], [14]. After removing and utilizing the edible pulp and seed from the matured pods (*fig. 2*), the husk is reserved for various purposes [9].


Figure 2: Matured *Parkia biglobosa* pods (fruits) **(Source:** Useful Tropical Plants Database) [3]

Decoctions of the fruit husk (*fig. 3*) extracted by boiling in water are used to impart water resiliency to floors, walls, and ceramic pots.

Figure 3: Husk of *Parkia biglobosa* (**Source:** Personal fotos)

The 'sour water' produced from steeping and boiling the husks is mixed with mud to plaster walls of mud houses (*fig. 4 and 5*) in many rural communities. This extract is mixed into mud and used to strengthen and waterproof the plaster on the walls of living rooms, huts, barns and other parts of the houses built with mud and mud bricks.

Figure 4: Women plastering mud rooms using mud mixed with aqueous extract of *Parkia biglobosa* fruit husk(**Source:** Margaret Shao) [5]

Figure 5: Back view of Plastered mud walls of rooms using mud mixed with *Parkia biglobosa* husk extract (**Source:** Personal fotos)

The plaster is also used to paint tamped earthen floors. The extract of the husk act to bind the soil and render the surface impervious to water. Treatment of mud walls, pottery, floors and roofs with mucilage extract and decoction of the husk is believed to improve their quality and protect them against erosion by driving rain [5], [10].

The husk extract is also mixed with other components to make natural paintings or murals to decorate the walls (*fig. 6*). This tradition dates back to several decades when it was applied in the Navrongo Catholic Minor Basilica and other houses in the communities by the Sirigu Women in Pottery and Art (SWOPA) [10], [15], a non-governmental organization of women. The women developed their works from cultural remnants of laterite plastering and traditional wall painting and their works are replicated in most houses in the community and in other parts of Ghana [10].

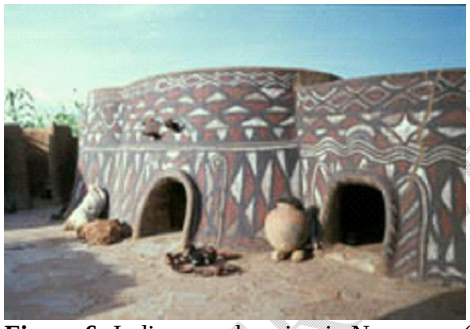

Figure 6: Indigenous housing in Navrongo (Ghana) plastered and coloured with aqueous extract of *Parkia biglobosa* fruit husk

The tannins in the bark and husks of the pods of *P. biglobosa* are also used for dyeing and curing leather as well as for dying sculpture [2], [12]. 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 [9].

The current chemical studies on the husk extract was therefore done to evaluate and validate traditional applications of the *P. biglobossa* husk. This work provides preliminary information required for possible industrialization of the local technologies and also contribute to validation steps of the indigenous applications, and promote agro forestry management of the tree.

MATERIALS AND METHODS

Materials

*P. biglobossa*husk, reagents, chemicals, doubly distilled water, chromatographic columns and HPLC equipment. Two forms of husk were used in the studies; husk from freshly harvested fruits, and husk from fruits collected from previous fruiting season.

Methods

Comment [3]: The type of experimental design used to conduct the research and the type of statistical analysis are mentioned

Sample Collection and Identification

The husk were collected, authenticated, extracted, and the extracts fractionated. The extracts and fractions were used for various analyses.

Husk from freshly harvested fruits were purchased from the Navrongo central market in the Upper East Region of Ghana from women who collected them from various farmlands in their communities, namely Pungu, Chiana, Natugunia, Vonania, Gongnia and Korania (New husk). Previous fruiting season husk were harvested in the preceding fruiting season and stored for the current purposes (Old husk). Random samples were picked out of the bulk husk and were authenticated at the KNUST Experimental Farms and also at the KNUST Botanical Gardens.

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.

Doubly distilled water in the laboratories of the Chemistry Department of Kwame Nkrumah University of Science and Technology (KNUST) was used for the preparation of all solutions.

Sample Processing

To eliminate traces of water, the dry husk were further air-dried for seven days in the laboratory to a constant weight. The moisture free husk were then pulverized in a mill at the Faculty of Agriculture - KNUST to obtain a coarsely powdered sample and bagged for extraction using the Soxhlet.

The extraction was done using ethanol and water separately as solvents, and the masses of extracts for both old and new husk were compared to evaluate for a better extracting solvent. 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. The extract was used in bulk and also fractionated for both quantitative and qualitative analyses. Thus, portions from this extract were taken for preliminary functional group determination, fractionation, TLC and High Power Liquid Chromatography (HPLC). The fractionated extract was also used in preliminary functional group determination and further TLC analyses.

Extraction of Sample

200.00g of air-dried coarsely powdered *Parkia biglobosa* husk sample was Soxhlet extracted using distilled water at boiling point until the solvent in the column of the

Soxhlet was colorless. The extract was concentrated under reduced pressure in the rotary evaporator, collected into a weighed empty beaker and dried in the oven to a constant weight at 105^{o}C. The process was repeated to obtain and record duplicate masses of husk extract.

Another 200.00g portion of powdered husk was weighed and extracted using boiling 96% ethanol as solvent in a Soxhlet. The extract was concentrated in the rotary extractor and dried in a weighed beaker at 80 ^{o}C in the oven. The process was repeated to obtain duplicate masses of husk extract as well. The percent yield of extracts was calculated and the efficiency of the solvents to extract determined.

Phytochemical Screening

A portion each of both ethanol and water extract was taken for phytochemical screening. Solutions for the phytochemical screening were prepared using the distilled water. The tests were conducted on the moisture-free aqueous Soxhlet extracts and then repeated on the ethanol extracts. Standard tests were conducted for saponins, general glycosides, flavonoids, terpenes, tannins, alkaloids, anthraquinones and their glycosides and cyanogenitic glycosides [16], [17].

HPLC

For HPLC analyses 1.0 g/v of dry crude aqueous extract solution was prepared. The analysis was done using an HPLC equipment, manufactured by Kontron Instruments, fitted with Rheodyne injector with Pump 422 and a UV detector lamp 322 at various wavelengths in different columns. To determine the presence of phenolic acids, flavan-3-ols and flavonols or proanthocyanidin 20 μL of the prepared extract solution were injected, applying a SPHERI-5 RP-18 (5 µm) column at 35 °C and a flow rate of 1.0 ml min^{-1} on the HPCL equipment. The column was eluted by 82 % (v/v) acetonitrile (CH_3CN) to water and 4% (v/v) phosphoric acid / water mixtures. The peaks were detected at a wavelength of 280nm. The analysis of alkaloids was done using the methods of Hong-Xia *et. al.*, cited in Turkmen and Velioglu, [18]. The number of phenolic glycoside components in the extract was determined using the method validated by Muller *et. al*, [19].

Comment [4]: Remember the unit of measurement isg/ l or ml

Fractionation

The dried crude aqueous extract was fractionated using the Bulk Transfer methodology into separated portions depending on their polarity. The extract was shaken with 5% HCl and chloroform in a separatory funnel to obtain two layers. The aqueous layer, containing alkaline components in HCl was collected, neutralized with dilute NH_4OH and washed with chloroform again. The remaining chloroform layer contains the acidic and neutral components. To this layer, dilute NH_4OH was added to separate the strong acids into the aqueous medium. The aqueous layer was collected separately and 5% NaOH added to the organic layer to extract the weak acids into aqueous solution. The acid fraction in the aqueous layer was collected separately from the chloroform layer containing the neutral fraction. The various solvents were then evaporated to obtain dry weak and strong acidic,

basic and neutral fractions. Aqueous solutions of extracts and fractionated components were tested for their reaction with litmus paper [20].

Preliminary Functional Group Determination

For the preliminary functional group determination process, about 1.0 mL of distilled water was placed in a test tube and a few crystals of the extract were dropped from the end of a spatula directly into the solvent. The test tube was gently tapped with a finger to ensure mixing and then observed to see whether there was appearance of any mixing lines in the solution. The disappearance of the solid, or appearance of the mixing lines, indicates that solution is taking place. More drops of the solvent or a few more crystals of the extract were added to determine the extent of solubility. Aqueous solutions of extracts found to be soluble in water were tested for their acidity using litmus paper [20]. This was repeated using each of 5% NaOH, 5% $NaHCO_3$, 5% HCl and conc. H_2SO_4 solutions as solvents and separate portions of the extract. The entire process was repeated on the fractions of the extract.

Thin Layer Chromatography

Thin layer chromatography was performed on the crude water extract 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 for the analysis[21]. Mobile phase solvents used for the TLC were distilled water, 96% ethanol, methanol, ethylacetate, hexane, chloroform and various mixtures of the solvent systems [18].

RESULTS AND DISCUSSION

Extraction efficiency

From Table 1 and 2 below, using water to extract new and old husk respectively, the extraction efficiency of old husk (38.31%) was greater than that of the new husk (37.22%). On the other hand, the extraction efficiency of the old husk (30.70%) using ethanol as solvent was negligibly less than that of the new husk (30.95%). Using water to extract the samples after exhaustively extracting with ethanol offered the least values of extracts (8.65% from the old husk and 8.40% extract from the new husk). Though the percentages of the water soluble extractives were slightly higher for the old husk than for the new husk, the difference was insignificant.

Table 1: Extraction efficiency (%) of the new 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

Comment [5]: type of statistical analysis are mentioned

Table 2: Extraction efficiency (%) of the old 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

Generally polar solutes dissolve in polar solvents. Since water is more polar than ethanol and it will extract more solute than ethanol if the solute is very polar. Therefore, the extractable materials were likely made up of very polar substances.

On the average, the extract yield of *Parkia biglobosa* fruit husk was found to be 37.77% using water, 30.83% using ethanol and 8.53% for water (after ethanol). The average extract by water far outweighed the average extract by ethanol. Even after exhaustive ethanol extraction, a further extraction of the sample produced 8.53% extract using water. Therefore, water was identified as a preferable solvent for extracting the husk quantitatively since it was cheaper and yields more extract than ethanol.

The sum of total percentages of extracts using ethanol and that of using water after the ethanol extraction was realized to be higher than the mass of extract from only water. This further indicates the inability of ethanol to extract the components as much as water does. Water is more polar than ethanol and extracted more solute than ethanol, thus confirming that the extracts contained very polar compounds.

Phytochemical Composition of the Extract

The samples indicated the presence of five metabolites (Table 3). Tannins and polyphenols, and alkaloids were in appreciable amounts in the aqueous extract. Saponins and flavonoids were moderate in both extracts, anthraquinones were moderate in the ethanol extract but masked in the water extract, general glycosides were in trace amounts in both extracts whilst steroids and terpenes tested negative in both extracts. Earlier reports [2] Hall et al. suggested that the bark of *Parkia biglobosa* contains 12-14% tannin while the husk contains 27-44%.

Table 3: Secondary metabolites in the extracts

SECONDARY METABOLITE	EXTRACT	
	Water	**Ethanol**
Saponins (Test: Extract + distilled water froth test)	+++	++
General glycosides (Test: Extract & 20% NaOH & Benedicts solution)	+	+
Flavonoids (Test: Conc HCl + Mg turnings)	++	++
Steroids and Terpenes (Test: Acetic anhydride + conc H_2SO_4)	-	-
Tannins and polyphenols (Test: Extract + 0. 1% $FeCI_3$)	+++	+++
Alkaloids (Test: Extract + 10% HCl)		
Dragendoff's	+++	+++
Wagner's reagent	+++	+++
Anthraquinones (Extract, 12% H_2SO_4, $CHCI_3$, 10% NH_3 Solution)	-	+

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

The polarity of the extracts was further supported by these results. Tannins, alkaloids and saponins were generally intense; hence the extract is expected to be very polar. Since the phytochemical constitution in the water and ethanol extract differed only by slight margins, the quantitatively larger extract (the aqueous extract) was preserved for further analyses.

Chromatographic Components of the Extract

HPLC analyses determined the number of classes of alkaloids, phenolics glycosides, phenolic acids, flavonols and/or proanthocyanidins. Three peaks (fig. 1a.) in the extract indicated that it contained at least three classes of phenolic glycosides. Two absorption peaks (fig. 1b.) indicating the presence of at least two classes of phenolic acids, flavonols and/or proanthocyanidins were observed. Hence two classes of the same or the different groups of the prospected compounds may be present in the aqueous extract. One peak (fig. 1c.), representing one class of compounds, was indicated in prospecting for alkaloids. Hence there was also at least one class of alkaloids present in the extract.

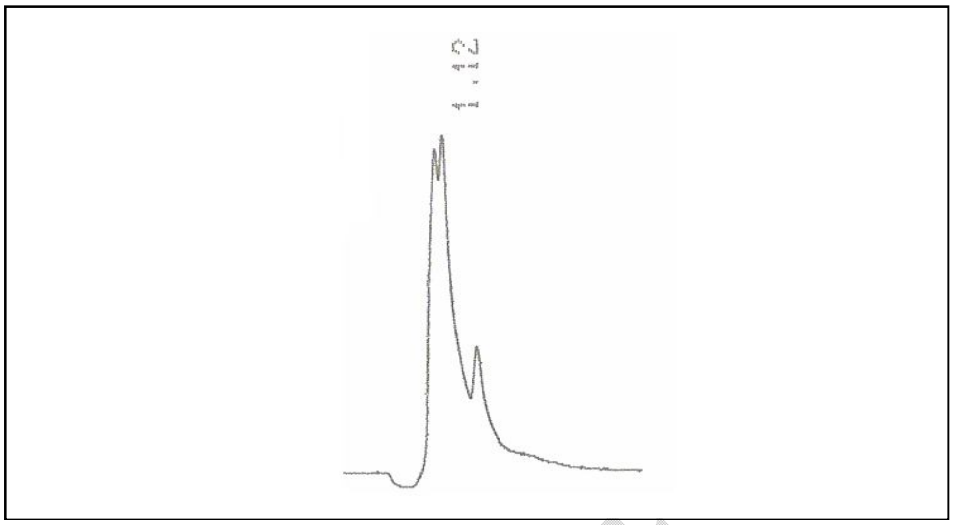

Figure 7a: Chromatogram of phenolic glycosides in the aqueous extract

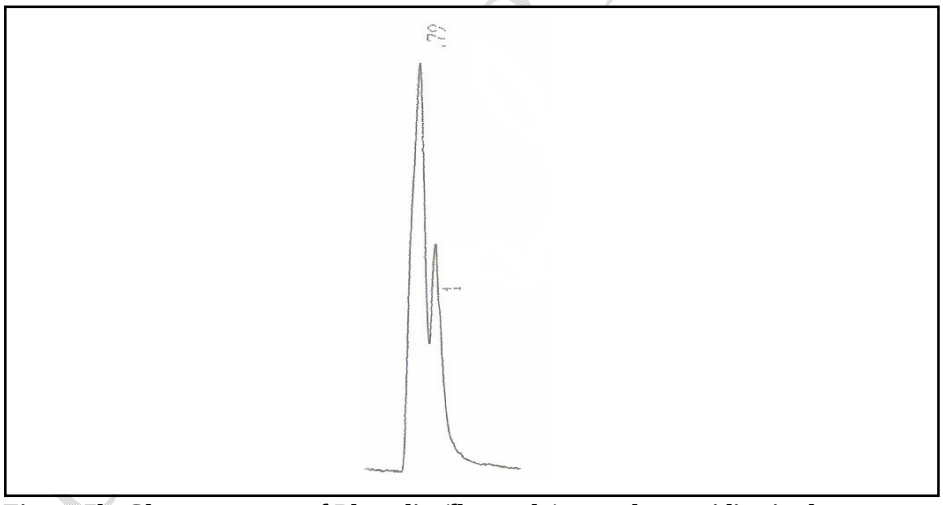

Figure 7b: Chromatogram of Phenolics/flavonols/proanthocyanidins in the aqueous extract

Figure 7c: Chromatogram of alkaloids in the aqueous extract

Fractions of the Extract

Basic, neutral, weak and strong acid fractions were obtained from the fractionation of the aqueous extract. The weak and strong acid fractions were in far larger quantities than the basic and neutral fractions. The fractions were all dried under laboratory conditions for 72 hours and stored in plastic containers for further work.

Using litmus test confirmation, 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. The larger quantities of acidic fractions pointed quite evidently to the larger presence of tannin products such as tannic acids, phenols and other such acidic components.

Solubility of Extract and Fractions

The group of compounds indicated in the water extract compared well with groups identified in the phytochemical screening of the extract. The solubility revealed that there was presence of organic acids, nitrogen containing compounds, alcohols, alkenes and esters in the aqueous extract. These compounds could essentially be due to functional groups associated with phenolics such as tannins, flavonoids, alkanoids and glycosides. Presence of phenols and aromatic components could be due mainly to tannin with carboxylic acid groups resulting from hydrolysed tannins. The basic compounds

represent nitrogen containing compounds such as alkaloids whilst the alkanes, alkyl halides and aromatic compounds are in the neutral fraction.

Components and Polarity of the extract and fractions

Generally, the maximum number of observable TLC separations in all the crude extracts was 2. Thus, the components in the aqueous extract associated together in a minimum of two main groups.

The highest R_f values were produced by 2:1 mobile phase mixture of ethanol to water which produced R_f of 0.96 and 0.67 in the aqueous extract, and 1.0 in the strong acid fraction. A 2:1 mixture of ethanol to water is moderately polar. It is less polar than water but more polar than a 3:2 ethanol to water mixture. The components may be moderately polar since the highest separation was produced by a 2:1 mixture of ethanol to water. 2:1 ethylacetate : chloroform mobile phase is largely non-polar and gave the lowest retention factors as well as separations. On the basis of the general performance of the nonpolar solvents as mobile phases for TLC analyses, it was observed that the crude extract may not contain a good amount of nonpolar components.

TLC results of the fractions also showed that there were at least three components in the strong acid and basic fractions, four in the weak acid and neutral fractions. The highest R_f values recorded for the fractions were: 1.0 for 1:2 ethanol to water solvent mixture in the strong acid fraction, 0.87 for ethanol only in the weak acid fraction, 0.86 for ethanol only in the basic, and 0.92 for 1:2 ethylacetate to ethanol mixture in the neutral fraction.

The TLC results of the fractions conformed to results of the solubility tests of the fractions. The highest R_f value in all the fractions (1.0) registered for the strong acid fraction using a moderately polar solvent mixture of 1:2 ethanol to water. This indicated that the most polar components were contained in the strong acid fraction. The non-polar components were contained in the neutral fraction which registered the lowest R_f value (0.06) for a mobile phase mixture of 1:2 ethylacetate to water mixture.

From the foregoing chemical species identified, and the related characteristics, *Parkia biglobosa* fruit husk contains relevant components that could be harnessed industrially. The current findings indicate potential for enhanced indigenous utilization [21] of this parkia product, and that can further boost the interest [22] for conservation of the tree to enable continuous use.

CONCLUSION

From the results, it was found that the storage period, after harvesting the pods, did not influence the amount of extractable material at least for after a year of husk storage. For quantitative extraction of *Parkia biglobosa* husk, it was found that the optimum extraction is obtained using water as solvent compared to ethanol.

The husk contains large amounts of tannins and polyphenols as well as moderate levels of flavonoids, alkaloids and saponins, and trace amounts of anthraquinones and glycosides.

The components of the husk were weak and strong acids, basic and neutral compounds as observed from fractionation of the aqueous extract.
There were at least one class of alkaloids, three classes of phenolic glycosides, and two classes of any of phenolic acids, flavonols and/or proanthocyanidins in the extracts.
From the TLC, the components in the aqueous extract associated together in a minimum of two main groups with at least three components each in the strong acid and basic fractions, and four each in the weak acid and neutral fractions.
Among many mobile phases used for the TLC, the best separation was observed with 2:1 mixture of ethanol to water mobile phase which produced among others R_f of 0.96 and 0.67 in the aqueous extract, and 1.0 in the strong acid fraction. Also from the fractions, the highest R_f was 0.87 for a mobile phase of ethanol in the weak acid and 0.86 in the basic fraction, and 0.92 for 1:2 ethylacetate / ethanol mixture in the neutral fraction.
The results indicated that the husk has potential for many uses which must be researched into and exploited. Its indigenous application in mud-wall plasters, floor tamping and traditional wall paintings could be enhanced. It could be exploited as a component and used in the construction of modified bricks for building of simple low income houses with relative ease for local traditional adaptations.

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