Functional properties and anti-microbial screening of *Blighia sapida* and *Picralima nitida* seed cake and protein concentrates

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Abstract
The functional properties and anti-microbial screening of Blighia sapida and Picralima nitida seed cake, protein concentrates and the effect of pH on these properties were investigated. The result of the phytochemical analysis showed the presence of terpenoid, flavonoid, tannin and saponin in P. nitida and B. sapida seed cake. The moisture content of the seed cake ranged between (10.33±0.58-11.67±0.29 %); crude protein (9.85±0.18-11.54±0.09 %); crude fibre (5.33±1.15-8.53±0.12 %); ash content (7.67±0.58-15.67±2.09 %) and carbohydrate (52.16±2.05%-56.28±2.05 %). Mineral element analysis showed that B. sapida and P. nitida cake contain high level of sodium (25,500-33,700 ppm), magnesium (44,900-37,000 ppm), potassium (24,000-34,700 ppm) and very low level of manganese (15.1-12.01 ppm); iron (34.00-14.01 ppm) and zinc (31.69-26.34 ppm). Water absorption capacity ranged between (2.30±0.06 to 3.60±0.12 g/ml) while oil absorption capacity was (2.30±0.12 to 2.73±0.21 g/ml). In both cake and protein concentrates, the protein solubility and emulsion stabilities were found to be high at a pH of 8 and 10. The ethanol extracts obtained from all the experimental seeds cake were active against the tested pathogens. Maximum antimicrobial activity was observed in B. sapida as compared to P. nitida within a concentration range of 25 mg/ml to 200 mg/ml. Antimicrobial activities observed in this study is an indication that the ethanol extract of these cake extracts showed potentials of serving as supplementary sources of essential nutrients to mankind and could probably serve as antimicrobial against pathogens.

Keywords: seed cake, protein concentrate, functional properties, Antimicrobial screening

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1. INTRODUCTION

The over dependence on only few plant species for food has made food security a challenge and a major concern in the world. This has led to several calls for investigation into alternative food sources to improve food security worldwide. Conservation, domestication and utilization of many indigenous wild fruit and vegetable species can contribute to hunger reduction and improved nutrition and health (Ekue *et al*., 2010). Proteins are important in food processing and food product development. They are found to possess some functional properties (both physical and chemical) that could influence the food products in the side of consumers. Protein solubility, water and oil absorption capacities, foaming capacity and stability, emulsion capacity and stability, viscosity and gelation potentials are some of the functional properties that influence protein quality. Amino acid composition and the ease with which digestive enzymes hydrolyze a food protein are also major determinants of food quality (Sze-Tao and Sathe, 2000; and Eunice *et al*., 2013).

Blighia sapida is a tree plant from the Sapidaceae family which is found mainly in some part of West Africa especially in Nigeria, Ghana and Ivory Coast. The tree is identified in Nigeria with different names like Ackee (English), "Ila" (Nupe), "Isin" (Yoruba), "Gwanja kusa" (Hausa) and "Okpu" (Igbo). It is used in traditional medicine for diabetes mellitus management and hypertension. Its fleshy aril fruit which is edible is known to contain saponins. Ackee (*Blighia sapida*) is a woody perennial multipurpose fruit tree species native to the Guinean forests of West Africa. It is well known for its unripe aril that contains hypoglycin A. They exert their blood glucose lowering effect through the inhibition of an enzyme such as salivary and pancreatic amylase (Frantz *et al.*, 2005). The stem bark of *B. sapida* was reported to show antidiarrheal potentials, Antwi *et al.* (2009). *B. sapida* is used traditionally for the treatment of dysentery, yellow fever, eye sore, burns,

wounds, skin sore (Etukudo, 2003). Various parts of *B. sapida* plants are locally used either alone or in combination for the treatment of psychosis, cancer, gonorrhea, stomach ache, hernia, backache, diarrhoea and constipation (Okogun, 1996 and Owonubi, 2006). The roots bark extract was found to exert significant hypoglycemic effect on the normoglycemic albino rats (Saidu *et al.*, 2012).

Picralima nitida, from *Apocynaceae* family is primarily found in West Africa. It is a shrub or tree that grows up to 35 m tall with white latex in all parts. The bark is hard, brittle, pale to dark greyish black or brown, smooth to slightly rough or finely striped. The leaves are shown to be broad (3-10 cm) and oblong (6-20 cm long) with tough tiny lateral nerves of about 14 to 24 pairs (Burkill, 1985). The seeds are smooth, brown to orange, embedded in soft white to orange pulp. *P. nitida* was found to have many applications in folk medicine. Various parts of the plant such as the seeds, stem bark, leaves, and roots are used in the treatment of fever, hypertension, gastro-intestinal disorders, jaundice and malaria (Nwaogu, 2016). The dried seed is used in rheumatic fever in Nigeria (Ezeamuzie *et al.*, 1994) and as an anti-pyreticin in Ghana (Ansa-Asamoah *et al.*, 1990). Duwiejua *et al.* (2002) have reported that the dried powdered seeds of *P. nitida* are encapsulated and marketed in Ghana for the treatment of pain of various aetiologies and diarrhea. *P. nitida* has also been reported to exhibit a number of pharmacological actions including anti-inflammatory (Ezeamuzie *et al.*, 1994) analgesic (Ansa-Asamoah and Ampofo, 1986; Duwiejua *et al.*, 2002), hypoglycemic and anti-hyperglycaemic (Yessoufou *et al.*, 2013) and antiulcer (Mathew *et al.*, 2011).

There are variety of resources that contribute to the fundamental needs of human such as food, clothing and shelter in plants. Among plants of economic importance are medicinal plants. They have been utilized as therapeutic agents since time immemorial in both organized and unorganized forms (Girach *et al.*, 2003), both locally and internationally. Medicinal plants have been the mainstay of traditional herbal medicine for a long time ago to date. The availability of several organic compounds from living things, especially plants, has been the focus of the Natural Products Chemists for decades. However, there is a tremendous pressure on these plants probing their bioactive chemical constituents for a possible drug development and delivery. Many of these plants are utilized in herbal medicine in Nigeria for the treatment of enormous number of diseases and infections including typhoid fever and gastrointestinal disorders such as cholera, diarrhea and dysentery. Though many plants have being screened for antimicrobial properties, more pharmacological investigations are still necessary to be carried out. Thus this present research project was designed to evaluate the functional properties and anti-microbial screening of the seed cake and protein concentrate from *B. sapida* and *P. nitida* for better uses in in the treatment of various infections in humans.

2. MATERIALS AND METHODS

Collections, identification and preparation of the samples

The seeds used for this experiment were purchased from a local market in Ibadan, Oyo state, Nigeria between June and July. The plant seeds namely *B. sapida* and *P. nitida*, purchased from Oja Oba market in Oyo State, Nigeria; were manually cleaned to remove the foreign materials after which they were dehulled and sun-dried for a week to ensure proper drying. The dried seeds were then ground into powder, sieved and stored in clean polythene bags for analysis. Parts of the seed powder were defatted with n-hexane, following a small-scale hexane extraction method described by Tzeng *et al.* (1990). The seed cake were desolventized and stored in desiccators at room temperature for subsequent uses.

Preparation of seed protein concentrates

Seed protein concentrates (SPC) were obtained from seed cake as reported by El-Tinay *et al.* (1988). The seed cake of each of the sample was dispersed in distilled water at flour to water ratio of 1:10 (w/v). The pH was adjusted to 10 with 1

M NaOH and stirred for 3 hrs at room temperature. The extract obtained was separated from the residue by centrifugation at 4,300 x g for 20 min. The residues were extracted again twice as described above. The extracts obtained were combined at the end, pH was adjusted to 3.5 with 1 M HCl to precipitate the protein and then centrifuge at 4,300rpm for 20 min for the protein concentrate. The protein concentrates (precipitate) was washed twice with distilled water. It was then freeze-dried. The dried protein was stored in desiccators at room temperature for subsequent analyses. The protein content was determined by the kjeldahl method (AOAC, 2000).

The proximate analysis

The proximate composition of *Blighia sapida* and *Picralima nitida* seed cake were determined using the methods described by the Association of Official Analytical Chemists (2000). Carbohydrate content was determined by difference while percentage nitrogen was determined by the macro-Kjeldahl method (Person, 1976) and nitrogen was converted to crude protein by a factor of 6.25. Caloric value was estimated using the modified Atwater factor as follows: Caloric value (Kcal/100g) = [(lipid × 9) + (protein × 4) + (carbohydrates ×4)] as described by Hassan *et al*. (2008).

Mineral determination

Mineral element analysis of *B. sapida and P. nitida* seed cake were carried out to determine the presence of sodium, potassium, calcium, magnesium, manganese, iron, copper and zinc following the method used by Ajayi *et al*. (2015) and Idouraine *et al*. (1996). Sodium and Potassium were determined with flame photometry (Model, 405, Corning, UK) while other mineral were determined using atomic absorption spectrophotometer (Perkin–Elmer model 703, USA) All determinations were done in triplicate.

Phytochemical screening

B. sapida and *P. nitida* seed cake were screened for secondary metabolites which include cardiac glycoside, terpenoids, tannins, saponin, flavonoids, phenols, steroids and anthrquinone following Evans (2002) and Ajayi *et al*. (2011).

Evaluation of functional properties of the seed flours and their protein concentrates

B. sapida and *P. nitida* seed cake and their protein concentrates were analyzed for functional properties. The bulk density was determined following the method described by Okaka and Potter (1977). Emulsion capacity was determined using the procedure described by Kinsella (1979). The foam capacity and foam stability were determined following the method described by Narayana and Nara singa Rao (1982) and modified by Fagbemi and Oshodi (1991). The protein solubility was examined within a pH range (1-10) following a modified method described by Adeyeye *et al.* (1994). Water and oil absorption capacities and the least gelation concentration of flours and protein concentrates were determined by the method described by Adebowale *et al.* (2005) and Okaka and Potter (1977).

Antimicrobial Studies

Collection of microorganisms

A total of four clinical bacteria isolates and three fungi were obtained and used in this antimicrobial study. The clinical bacteria isolate include two gram-positive (*Staphylococcus aureus*, E*scherichia coli*), two gram-negative (*Pseudomonas aeruginosa* and *Bacillus subtilis*) while the fungi include (*Candida albicans, Penicillium notatum* and *Aspergillus niger*). The above organisms were pure isolate and obtained from culture unit of the Department of Pharmacological Microbiology, Faculty of Medicine, University of Ibadan, Oyo State, Nigeria.

Preparation of plant extracts for antimicrobial studies

A previously method described by Owolabi *et al*. (2007) was used with little modification in the preparation of the extracts from the various seed cakes. 50 g of each of the selected cake (powdered form) were soaked in 200 ml of ethanol and properly covered with cotton wool. The mixture was shaken and kept undisturbed at room temperature for 72 hours in a sterile flask

covered with aluminum foil to avoid evaporation. The sample (mixture) in the flask was subjected to filtration through sterilized Whatzman No.1 filter paper. The extract obtained after filtration was concentrated to dryness by means of rotary evaporator and stored in refrigerator for further analysis.

Determination of antimicrobial activity

The methods described by Ajayi and Ojelere, (2013) were implored in the determination of the antimicrobial activity of the extract obtained. Agar well diffusion method based on the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2002) was also considered. 20 ml of sterilized nutrient agar was poured into petri dishes and allowed for solidification. Twenty four hours nutrient broth grown pathogenic cultures were swabbed on the respective agar plates using sterilized cotton swabs after solidification. Wells of 6 mm diameter were punched over the agar plates using a sterile gel puncher and about 100 µl of different concentrations of plant solvent extracts were added using sterile syringe into the wells and allowed to diffuse at room temperature for 1hr. The plates were then incubated at 37°C for 18-24 hrs for bacterial pathogens and 28°C for 48 hrs for fungal pathogens respectively. The diameter of inhibition zones formed around each wells were measured after incubation, expressed in millimeter (mm) and recorded against the corresponding concentrations to evaluate the antimicrobial activity. Positive controls were set using standard antibiotics drugs (Gentamycin) while negative controls were set using ethanol.

Statistical analysis

The results obtained from this experimental work were analyzed and expressed as mean values ± standard deviation.

3. RESULTS AND DISCUSSION

Proximate analysis

The proximate analysis of *B. sapida* and *P. nitida* seed cakes shows that they are good sources of carbohydrate and protein. They may therefore serve as sources of energy and nutrients in food formulation. Table 1 shows the results of the preliminary studies conducted to assess the major nutrient composition of *B. sapida* and *P. nitida* seed cake. These seed cake were analyzed for moisture, ash, fat, crude protein and carbohydrate. The results revealed that *B. sapida* and *P. nitida* seed cake are good sources of carbohydrate and protein; these may serve as source of energy and nutrients for the body metabolic activities. They could also be source of nutrition and natural energy for human around the world who lacks in many nutritional supplements as they were found to contain: fat content 1.23±0.12 % and 9.20±0.20 respectively. The crude protein is found to be 11.54±0.09 % (*B. sapida*) and 9.85±0.18 % (*P. nitida*). The crude proteins obtained are lower than 26.20±0.40 % (Jack bean); 24.46±0.32 % (Pigein pea) and 24.46±0.32 % (Cowpea) as reported by Arawande and Borokini, (2010). The moisture content of the samples was shown to be high in both seed cakes (10.33±0.58 and 11.67±0.29). The moisture contents obtained are higher than 7.6±0.6 % (*A. hybridus*) and 6.6±0.6 % (*T. occidentalis*) Adeyeye and Omolao, (2011). The carbohydrate values were relatively high in the entire samples with *B. sapida* (52.16±4.82 %) and *P. nitida* (56.28±2.05 %). The crude fibre values of 8.53±0.12% (*Blighia sapida*) and 5.33±1.15 % (*P. nitida*) obtained were found to be higher than 1.70±0.15 % and 1.6±0.08 respectively for *A. hybridus* and *T. occidentalis*, (Adeyeye and Omolao., 2011). Fiber has therapeutic effects in the prevention of heart diseases, colon cancer and diabetes and in the treatment of digestive disorders and constipation (Anderson *et al.*, 1994). This fiber can also be taken as part of diet to remove potential carcinogens and prevent the absorption of excess cholesterol from the body (El Somhaimy *et al.*, 2015). The low moisture content of the flour would enhance its storage stability by preventing mould growth and reducing moisture dependent biochemical reactions (Onimawo and Aklubor, 2012). Carbohydrates and lipid are the major sources of energy in food, therefore the seed cakes under study with high carbohydrate content will serve as good energy food and will have

their protein content improved by supplementation with other high protein content seed flour like soya bean, ground nut, *Momordica charantia* and melon seed flours among other. These defatted flours contain nutritional potentials that might find application in food ingredient, infant food formulation, food supplement and other food formulation.

Table 1: Proximate composition of the samples flours

Proximate	*B. sapida*	*P. nitida*
Moisture content	10.33 ± 0.58	11.67 ± 0.29
Ash content	15.67 ± 2.09	07.67 ± 0.58
Crude fibre content	08.53 ± 0.12	05.33 ± 1.15
Crude protein content	11.54 ± 0.09	09.85 ± 0.18
Fat content	01.23 ± 0.12	09.20 ± 0.20
Carbohydrate content	52.16 ± 4.82	56.28 ± 2.05

Values are expressed as mean ± standard deviation (n=3)

Mineral composition

The micro and macro-elements present in *Blighia sapida* and *Picralima nitida* seed cake are shown in Table 2. The elements such as potassium, calcium, magnesium, potassium and sodium found in reasonable amount in these seed cake are nutritionally and biochemically important for proper body function. For instance, calcium is known to play a significant role in muscle contraction, bone and teeth formation and blood clotting (Ahmed and Chaudhary, 2009). *P. nitida* has the highest value for sodium with the value 33,700 ppm, while *B. sapida* has 8,100 ppm. The potassium values are relatively high with *P. nitida* having 34,720 ppm, and *B. sapida* having least value of 24,000 ppm. Potassium works with sodium to maintain the water balance in the body and lowering the blood pressure. Sodium and potassium which are present in the intracellular and extracellular fluid can also help to maintain electrolyte balance and membrane fluidity (Ahmed and Chaudhary, 2009). The calcium values of 31,060 ppm, 11600 ppm, with manganese values of 15.10ppm and 12.01ppm were obtained respectively for *B. sapida*, *P. nitida* respectively. Iron values include 34.00 ppm and 14.01 ppm respectively. Magnesium is found to be important mineral in its healing effect on a wide range of diseases as well as in its ability to maintain healthy bones, calcium contributes in preventing cardiovascular diseases, regulates high blood pressure, migraines, insomnia and depression (Newsmax, 2011). Iron is very important element as a nucleus of hemoglobin that forms red blood cells in the body. Iron is also known to be a component of some metallo enzymes, myoglobin and heamoglobin (Ahmed and Chaudhary, 2009). Zinc can support the immune system and be useful for normal growth and development during pregnancy. Copper plays a role in the synthesis and maintenance of myelin and as a cofactor for the processes that neutralize the toxic free radicals. Manganese is very useful for activation of some enzymes that prevent tissue damage and used for digestion and utilization of foods (El Sohaimy *et al.*, 2015). Zinc present in the plant is beneficial to prevention and treatment of diarrheal episode, it is also involves in normal functioning of immune system. This result becomes so important when the usefulness of such mineral like Ca, Mg, Na, K in the body is considered and their usefulness in bone management.

Table 2: Mineral element composition (ppm)

Minerals	*B. sapida*	*P. nitida*
Sodium	25,500	37,000
Potassium	24,000	34,720
Calcium	31,060	11,600
Magnesium	44,900	37,000
Manganese	15.1	12.01
Iron	34.00	14.01
Copper	492.92	146.07
Zinc	31.69	26.34

Phytochemical screening

B. sapida and *P. nitida* seed cake were screened for secondary metabolites such as

saponin, terpenoids, anthraquinone, flavonoids, cardiac glycosids, tannins steroids and phenolics. Both *B. sapida* and *P. nitida* have saponin, tannin, terpinoid and flavonoid present in them (Table 3). Cardiac glycosides, phenolics, steroids and free anthraquinones were absent in *B. sapida* while only steroid and free anthraquinone were absent in *P. nitida seed cake.* The result is in agreement with the report of Nkere *et al*. (2005) and also agrees with similar research done by Kela *et al*. (1999) and Menut *et al.* (2002). These phytochemical compounds have pharmacological effects and have been the basis of chemical synthesis of drugs used in modern medicine responsible for their medicinal use in traditional medicine (Sofowora, 2001). Flavonoids have been reported to possess some biological activities which include antibacterial, anti-inflammatory, anti-allergic, antiviral and anti-neoplastic activity among others. They act as antioxidants to neutralize free radicals which contribute to a variety of disease. Alkaloids have been reported to be powerful pain relievers, to exert an anti-pyretic, stimulating, anesthetic action and inhibiting activity against most bacteria. Alkaloids and saponin have been reported to be useful in hypertension treatment (Olaleye, 2007). Tannins are known to inhibit pathogenic fungi and for the treatment of wounds, sprains, bruises and arresting bleeding while phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc.

Functional properties

Protein solubility

The protein solubility profile (%) at different pH was expressed in Fig 1. It was observed that pH had significant effect on the solubility of both proteins concentrates and seed cake. The protein concentrates have high solubility values at pH 2 which decreases at pH 4. The protein solubility increased from pH 6 to a maximum protein solubility value up to pH 8 and 10. The solubility percentage for the protein concentrates ranged from 52.41-77.00 % for the protein concentrates while it is between 14.62- 17.58 % for the seed cake. The least protein solubility was observed around pH 4 which is around the isoelectric point of the protein from the samples. All the protein concentrates have highest solubility at pH 8. Among the functional properties of proteins, solubility is probably the most critical because it affects other properties such as emulsification, foaming and gelation (Kinsella, 1976). The nitrogen solubility characteristics are influenced by many factors such as origin, processing conditions, pH, ionic strength and the presence of other ingredients (Kinsella, 1976). The pH had a significant effect on the solubility and good protein solubility in both acidic and alkaline pH regions has also been reported (Idouraine *et al.*, 1991) and is considered as an important characteristic for food and non-food formulations. The high solubility of these cakes in the acidic pH range indicates that these seed cakes may be useful in the formulation of acidic food like protein rich carbonated beverages (Kinsella, 1979).

Table 3: Phytochemical screening of *B. sapida* and *P. nitida* seed cake

	B. sapida	***P. nitida***
Saponins	+	+
Terpenoids	+	+
Flavonoids	+	+
Tannins	+	+
Cardiac glycoside	-	+
Phenolics	-	+
Free anthraquinones	-	-
Steroids	-	-

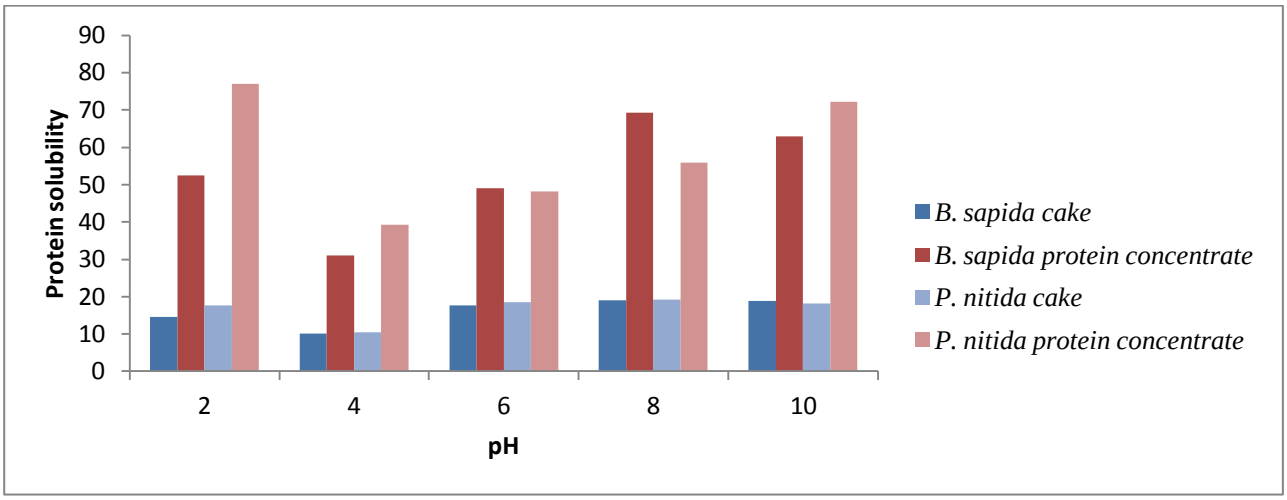

Fig. 1: Effect of pH on protein solubility of seed cake and protein concentrate

Water and oil adsorption capacity

Hydration or dehydration is perhaps the first most critical step in imparting desirable functional properties to protein in a food system. The water absorption capacity (g/ml) of the protein concentrates and seed caked are presented in Table (4). The water holding capacities observed were within the range of 2.93±0.75 and 2.3±0.75 ml/g in protein concentrate. Values which are lower than what was reported for protein concentrates of African yam bean (3.90 ml/g) (Adebowale *et. al.*, 2009). The oil holding capacity observed was comparable in both the seed cakes and protein concentrate. The result obtained in this report for these protein concentrates were observed to be lower than that observed by Fekria *et al.* (2012) which was about 2.93 and 2.86 ml/g for Barberton and Ashford respectively and the difference in this could be due to the presence of non -polar side chain which might bind the hydrocarbon chain of oil among the flour (Adebowale *et al.*, 2004).

Water and oil interact positively with flour and their effects on the flavor and texture of food are very important and need to be taking into consideration in food processes. Proteins and carbohydrates are major chemical constituents that enhance the water absorption capacity of flours because they contain hydrophilic parts, such as polar or charged side chains (Lawal and Adebowale, 2004). The difference in protein structure and the presence of different hydrophilic carbohydrates might be responsible for variation in the Water Absorption capacity (WAC) of the flours. Flours with high WAC have more hydrophilic constituents such as polysaccharides. The removal of fat from the samples exposes the water binding sites on the side chain groups of protein units previously blocked in a lipophilic environment thereby leading to an increase in WAC values in seed cake. Water absorption capacity is a critical function of protein various food products like soups, gravies, doughs and baked products (Sosulski *et al.*, 1976). The seed cake has high bulk density (ranging from 0.411-0.746 g/ml) than that of protein concentrates (within the range 0.38-0.397 g/ml), likewise least gelation concentration between 8.67-13.33 % while it is 6.00-8.00 % concentration in protein concentrates.

Table 4: Functional properties of *B. sapida* and *P. nitida* seed cake and Protein concentrate

	B. sapida	***P. nitida***
Seed cake		
Loose density (g/ml)	0.46 ± 0.02	0.56 ±0.01
Packed density (g/ml)	0.60 ± 0.04	0.75 ±0.08
Water holding capacity (ml/g)	3.60 ± 0.12	2.30 ±0.058
Oil holding capacity (ml/g)	2.73 ± 0.21	2.30 ±0.12
Least gelation (%)	8.67 ± 1.15	13.33 ±1.15

Protein concentrates		
Loose density(g/ml)	0.30 ± 0.08	0.34 ± 0.03
Packed density(g/ml)	0.42 ± 0.09	0.46 ± 0.04
Water holding capacity (ml/g)	2.40 ± 0.58	2.93 ± 0.75
Oil holding capacity (ml/g)	2.50 ± 0.38	2.40 ± 0.17
Least gelation (%)	6.67 ± 1.15	8.00 ± 0.00

Values are expressed as mean ± standard deviation (n=3)

Foam capacity

Low foam ability on the other hand can be related to highly order globular proteins, which resists surface denaturation. The success of whipping agents largely depends on how long the whip can be maintained. The foaming capacity (FC) of a protein refers to the amount of interfacial area that can be created by the protein and it is a function of the type of protein, pH, processing methods, viscosity and surface tension. The FC and FS of the groundnut seed cake flour of the cultivars Barberton and Ashford attained no significant differences. The foam produced by legume flours was relatively thick with low foam volume but high foam stability. In the results shown in Fig 2, the emulsion capacity versus pH profile suggested that emulsification was caused by the solubilized proteins. Dependence of emulsion capacity with pH was expected. This is because emulsion capacity of soluble proteins depends on the hydrophilic-lipophic balance which is affected by pH. Similar observation has been reported by Lin *et al.* (1997). The emulsifying properties are usually attributed to the flexibility of solutes and exposure of hydrophobic domains. The capacity of proteins to enhance the formation and stabilization of emulsion is important for many applications.

Least gelation

The least gelation obtained for *B. sapida* and *P. nitida* seed cake and protein concentrates are shown in table 4. The values obtained ranged from 6.67±1.15 to13.33±1.15. The least gelation values obtained are higher in the seed cake compared to that of protein concentrate. Gelation properties are interrelated to water absorption capacities hence the low water absorption capacity recorded by the seed cake could explain the deficient gel formation capacity. It was reported that foam ability is related to the rate of decrease of the surface tension of the air/water interface caused by absorption of protein molecules (Sathe *et al.*, 1982). The lower least gelation concentration, the better is the gelating ability of the protein ingredient (Akintayo *et al.*, 1999). Variations in gelling properties may be ascribed to the ratio of different constituents, such as proteins, carbohydrates and lipids (Sathe *et al.*, 1982).

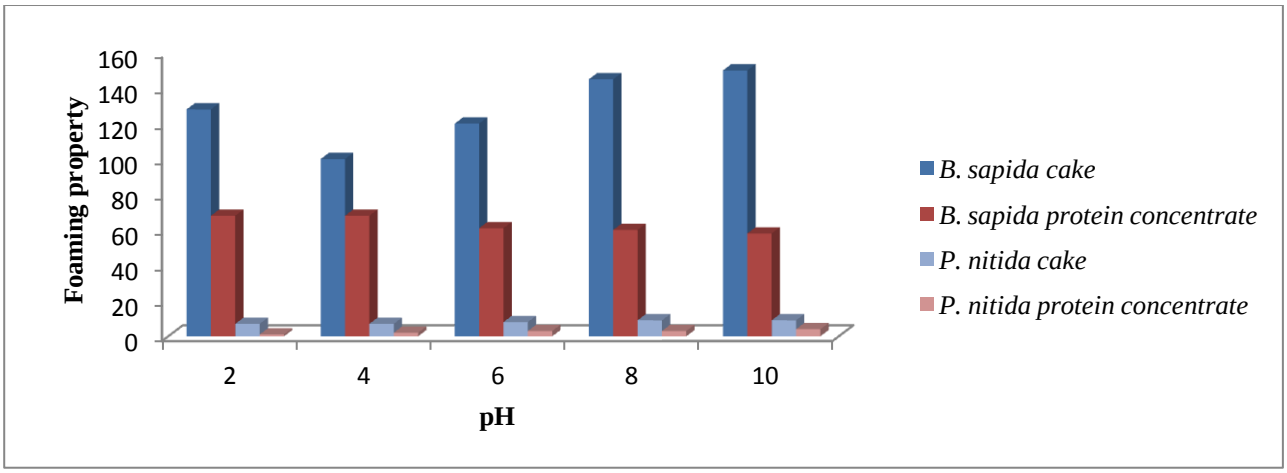

Fig. 2: Effect of pH on foaming properties of seed cake and protein concentrate

Table 4: Functional properties of *B. sapida* and *P. nitida* seed cake and Protein concentrate

	B. sapida	*P. nitida*
Seed cake		
Loose density (g/ml)	0.46 ± 0.02	0.56 ±0.01
Packed density (g/ml)	0.60 ± 0.04	0.75 ±0.08
Water holding capacity (ml/g)	3.60 ± 0.12	2.30 ±0.058
Oil holding capacity (ml/g)	2.73 ± 0.21	2.30 ±0.12
Least gelation (%)	8.67 ± 1.15	13.33 ±1.15
Protein concentrates		
Loose density(g/ml)	0.30 ± 0.08	0.34 ± 0.03
Packed density(g/ml)	0.42 ± 0.09	0.46 ± 0.04
Water holding capacity (ml/g)	2.40 ± 0.58	2.93 ± 0.75
Oil holding capacity (ml/g)	2.50 ± 0.38	2.40 ± 0.17
Least gelation (%)	6.67 ± 1.15	8.00 ± 0.00

Values are expressed as mean ± standard deviation (n=3)

Antimicrobial activity

The results obtained for the antimicrobial activity is represented on Table 5. The antimicrobial activity of *B. sapida* and *P. nitida* ethanol extracts were investigated against some selected pathogens such as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella typhi*. Others are *Klebsiella pneumoniae, Candida albicans, Aspergillus niger, Penicillium notatum and Rhizopus stolonifer.* The agar well diffusion method was used and all the examined seed cake ethanol extract showed varying degrees of antimicrobial activities against the clinical pathogens tested and the results observed were expressed in the tables. It was observed that the antimicrobial activity of the extract was dosage-dependent and the activities varied with concentration against the tested pathogens as shown on Table 5. The extract of *Monodora myristica* showed maximum zone of inhibition against *Staphylococcus aureus* (20 mm), followed by *Escherichia coli* and *Bacillus subtilis* (18 mm), *Pseudomonas aeruginosa (*16 mm) *and Klebsiella pneumonia, Salmonella typhi, Candida albicans, Aspergillusniger and Rhizopusstolonifer* (14 mm) and no inhibition was observed for *Penicillium notatum* across the concentration. *Blighia sapida* ethanol extract was observed to show the least antimicrobial activity across the concentration of all the seed ethanol extract investigated. It showed activity against all the ten pathogens including *Penicillium notatum* which seemed not to have inhibition to both *Monodora tenuifolia* and *Monodora myristca* with *Staphylococcus aureus having inhibition* of 18 mm, and *Escherichia coli* and *Salmonella typhi* following it with inhibition of 16 mm, least inhibition was observed in *Candida albicans* that has inhibition of (12 mm), the rest of the pathogens has inhibition of (14 mm) zone. The maximum zone of inhibition (26 mm) for *P. nitida* extract was observed against *Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa* followed by *Escherichia coli* (24 mm), *Klebsiella pneumoniae* (22 mm), *Aspergillus niger and Salmonella typhi* (20 mm), *Candida albicans* (18 mm) and *Rhizopus stolonifer* (16 mm). Minimum inhibitory zone of (10 mm) was shown against *Penicillium notatum.*

Table 5: Antimicrobial activity of the ethanol extracts of *B. sapida* and *P. nitida* seed cake on isolated pathogens

Isolated bacteria and fungi	**Concentration**	**in (mg/ml)**				**Gentamycin**
Microorganism	200	100	50	25	12.5	10µg/ml
	***B. sapida* seed**	**cake extract**	**zone of**	**inhibition**	**in (mm)**	
Bacillus subtilis	14	12	10	---	---	23
Staphylococcus aureus	18	14	12	10	---	21
Pseudomonas aeruginosa	14	12	10	---	---	20
Escherichia coli	16	14	12	10	---	23

Aspergillus niger	14	10	10	---	---	19
Penicillium notatum	14	12	10	---	---	20
Candida albicans	12	10	---	---	---	19
S. typhimurium	16	12	10	---	---	
K. pneumonia	14	10	---	---	---	
R. stolonifer	16	12	10	---	---	
	***P. nitida* seed**	**cake extract**	**zone of**	**inhibition**	**in (mm)**	
Bacillus subtilis	26	20	18	14	12	23
Staphylococcus aureus	26	22	18	14	10	21
Pseudomonas aeruginosa	20	20	18	14	10	20
Escherichia coli	24	20	18	14	12	23
Aspergillus niger	20	18	14	12	10	19
Penicillium notatum	10	14	12	10	---	20
Candida albicans	18	16	14	12	10	19
S. typhimurium	20	18	18	14	10	
K. pneumonia	22	18	16	14	10	
R. stolonifer	16	14	12	10	---	

4. CONCLUSION

This study has characterized the chemical composition, functional properties and anti-microbial screening of *B. sapida* and *P. nitida* seed cake and protein concentrates. The effects of pH on these properties were also investigated. The results obtained showed that all the selected plant seeds have appreciable amount of nutrients such as carbohydrate, protein, fibre, and minerals in amount that might be beneficial to health, as such, these seeds could be incorporated to livestock feed while the protein concentrates showing good functional properties might be considered as food supplement. The pharmacological effect of the phytochemicals and antimicrobial activity observed in this work is an indication that *B. sapida* and *P. nitida* seed cake might have medicinal characteristics. The outcome of this research work suggests that their use as nutritional supplement would add to their economic value.

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