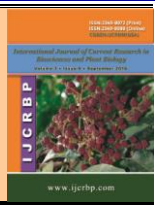
Int. J. Curr. Res. Biosci. Plant Biol. 2016, 3(9): 16-26

EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 (Online) • Volume 3 • Number 9 (September-2016)

Journal homepage: www.ijcrbp.com



Original Research Article

doi: http://dx.doi.org/10.20546/ijcrbp.2016.309.003

Comparative Investigation of the Biological Activity of the *Pterocarpus osun* and *Bosqueia angolensis* Seeds Protein Extracts

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Abstract

The study investigated the antioxidant, hemagglutinating and larvicidal activities of protein isolates from the seeds of two underutilized plant species (*Pterocarpus osun* and *Bosquiea angolensis*) in other to establish their medicinal value. The antioxidant power was assessed by employing DPPH radical scavenging assay, ferric reducing power assay and total antioxidant capacity (phosphomolybdenum method). Radical scavenging ability of the seeds protein extracts were found to be concentration dependent with IC_{50} value of 0.58 ± 0.05 mg mL^{-1} and 3.98 ± 0.28 mg mL^{-1} for *P. osun* and *B. angolensis* respectively. At 1 mg mL^{-1}, the reducing power potential of the proteins from *P. osun* and *B. angolensis* seed was 29.57± 0.25 and 2.67± 0.28 mg Ascorbic acid/g dry weight respectively. Total antioxidant capacity assay of the extract follows the same trend. The presence of lectin was detected by hemagglutinating activity assay which was carried out by serial dilution using trypinised and non-trypinised erythrocytes from four different species. *P. osun* seeds protein revealed a strong specificity for rabbit erythrocyte only and hemagglutinating activity was completely inhibited by mannose and its derivatives while *B. angolensis* seeds protein was galactose specific and agglutinated rabbit and human red blood cells with preference for rabbit erythrocytes. The antioxidant potential and hemagglutinating activity of the seeds extract were heat stable up to 50°C. The protein extracts from the two plants seeds did not show any larvicidal activity against the species of mosquito studied (*Culex quinquefasciatus*). These investigations conclude that the seeds protein extracts of *P. osun* and *B. angolensis* contain significant antioxidant and hemagglutinating activities which could contribute drastically to their medicinal value as well as their pharmaceutical applications.

Article Info

Accepted: 09 August 2016
Available Online: 06 September 2016

Keywords

Antioxidant
Bosquiea angolensis
Hemagglutinating
Larvicidal activity
Medicinal plants
Pterocarpus osun

Introduction

In customary medicine, various native plants are used alone or in combined form as decoction or infusion to treat different types of diseases associated with oxidative stress and some microbial infections (Iroanya et al., 2010; Sujatha and Shali, 2012). The medicinal potential of many plants have been attributed to the phytochemicals like steroids, saponins, phenolics, amino acid derivatives and alkaloids which have antioxidant potential (Martin et al., 2008; Kong et al., 2014; Liu et al., 2014). Studies have shown that some plants possess the ability to protect humans against oxidative damage when eaten (Eugenio-Perez et al., 2016). Fruits, leaf (vegetables), grains and seeds of some plants supply several phytochemicals that possess ability to scavenge free radicals to human diets and there are noticeable presences of anti-nutritional factors like lectins in the plants vegetative parts.

Chemicals used to lessen the adverse effect of reactive oxygen species produced by oxidative reaction in biological system are referred to as antioxidant. The large generation of free radicals, particularly reactive species and their high activity play important roles in the progression of a great number of pathological disorders like stroke, aging, Alzheimer's diseases, cataracts, cancer, heart disease, inflammation, atherosclerosis, diabetes mellitus and Parkinson's disease (Ozgen et al., 2012; Barbagallo et al., 2015; Phaniendra et al., 2015). For human, the potential health benefits of antioxidants from edible food include inhibiting lipid oxidation, scavenging radicals, reducing inflammation, suppressing tumor growth and prevention of obesity (Shirley et al., 2014; Bhangale and Acharya, 2016).

Currently, the hunt for natural products with antioxidant potentials have been on the rise due to the risk of adverse effects encountered with the use of synthetic compounds and also the public's perception that natural and dietary antioxidant are safer than synthetic analogues (Manukumar and Madhu, 2013). The growing of medical interest also in the search for novel natural and dietary antioxidant had lead to the increasing reports of the existence and bioactivities of antioxidant proteins in plants (Prasad et al., 2010; Sivapriya et al., 2015; Ramadas et al., 2016). Proteins having significant antioxidant activity have been isolated from plants sources such as *Solanum torvum* seeds (Sivapriya et al., 2015), wheat germ (Mahmoud et al., 2015), *Cajanus indicus* leaves (Sarkar et al., 2006), *Cajanus cajan* seed coat (Manukumar and Madhu, 2013), *Lablab purpureous* seeds (Saha et al., 2014), *Curcuma species* (Boonmee et al., 2011; Angel et al., 2013), *Peganum harmala* seeds (Ahmed et al., 2013), *Terminalia chebula* fruit (Srivastava et al., 2012), *Moringa oleifera* seeds (Santos et al., 2005) and *Ginkgo biloba* seeds (Zhou et al., 2012). Some of these proteins were reported to have haemagglutinating properties (Saha et al., 2014).

Lectins are known to be proteins or glycoproteins binding to specific sugar moieties on the cell surfaces (Lam and Ng, 2011). The specificity of plants lectins concerning different sugars makes possible their application on pharmaceutical research such as cell growth and regulation (Wang et al., 2012), Cell induction (Yang et al., 2010), fertilization and agglutination of cells and bacteria (Santi-Gadelha et al., 2012) and immune recognition process (Larvie et al., 2012). They are considered strong candidates for therapeutic use, for they are macromolecules with noticeable resistance to unfavourable conditions like pH and temperature variations and isotonicity, with no significant alterations to their biological function (Coffey et al., 1993). Extensive studies discovered that some plant heamagglutinins have useful application in biotechnology (Naeem et al., 2007) and can also be used for prevention and/or treatment of some pathological disorders such as cancer (Liu et al., 2009) and malaria (Sá et al., 2009) among others.

Although various vegetative parts of many plants species have been investigated in the search for novel antioxidants and other bioactive components using different solvent for extraction, few reports refer to aqueous extract of seeds as sources for therapeutic agents. Yet, a large number of chemical compounds are present in seeds or seed coats, including alkaloids, lectins, and phenolic compounds. These compounds probably function in the protection of seeds from microbial degradation until conditions are favorable for germination (Komutarin et al., 2004). Hence, this study was carried out to investigate the possibility of utilizing the bioactive components of the aqueous extract of the seeds of two plants, *Pterocarpus osun* and *Bosqueia angolensis*, in the treatment and management of disorders linked with oxidative stress.

Pterocarpus osun Craib belongs to the *Dalbergieae* tribe of Papilionaceae subfamily. The crude extract of *P. osun* has also been found useful in the treatment of chicken pox in children in the eastern part of Nigeria (Ezeokonkwo and Okoro, 2012). The antioxidant potential and the attenuation of acetaminophen-induced redox imbalance by *P. osun* were reported recently (Ajiboye et al., 2010). Adewuyi et al. (2014) showed that the acetonides prepared from the seed oil of *P. osun* has no antibacterial activities but the leaves ethanolic extract of the plant does.

Bosqueia angolensis is a tropical rain forest tree and a member of the botanical family, *Moraceae.* There is little information regarding its general pharmacological activity apart from the reports of Nwosu (2011) and Nwamarah et al. (2015). Nwosu (2011) reported the presence of eleven antinutrients with alkaloids and total phenols predominating in the seeds subjected to different treatment. The nutrient, antinutrient and phytochemical compositions of roasted *B. angolensis* seeds were evaluated by Nwamarah et al. (2015). Though, the plant is lesser known and unconventional crop, it could be good sources of bioactive compounds that have high pharmacological potential which could be found useful in the treatment of various pathological disorders.

Consequently, this research was aimed to evaluate the possible beneficial biological potencies of the aqueous crude protein extract from these plant seeds.

Materials and methods

Collection and identification of plant material

Matured fruits of *P. osun* and *B. angolensis* were collected from the Botanical Garden of Obafemi Awolowo University, Ile-Ife, between January and March. The fruits were identified at Ife Herbarium, Botany Department, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of seed protein extract

Seeds were removed from the fruit and air-dried at room temperature until their weight were constant. The dried seeds were later ground into powder. 100 gram each of the plant powdered seeds was defatted using petroleum ether. The resulting fine powder was extensively extracted in Phosphate Buffer Saline (PBS) pH 7.2 on magnetic stirrer and centrifuged at 6,000xg for 15 minutes using cold centrifuge. The supernatant was subjected to 70% ammonium sulphate precipitation. The precipitate was collected after 24 hrs by centrifugation and extensively dialyzed against PBS. The dialysate was lyophilized into powder. The lyophilized powder was termed protein extract and used for the various bioassays (antioxidant, larvicidal and haemagglutinating activity).

Estimation of total soluble protein concentration

The total soluble protein content was estimated by Lowry's method (Lowry et al., 1951) using bovine serum albumin as a standard.

Evaluation of antioxidant potential of extracts

Antioxidant activity was determined by DPPH radical scavenging assay, ferric reducing power assay and total antioxidant capacity assay (Blois, 1958; Benzie and Strain, 1999; Prieto et al., 1999).

DPPH radical scavenging assay

The free radical scavenging activity of the extracts were measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay method of Blois (1958) with slight modification. DPPH reacts with an antioxidant compound that can donate hydrogen and thereby DPPH is reduced. Change in colour of the solution (from deep violet to light yellow) was measured. The intensity of the yellow colour depends on the amount and nature of radical scavenger present in the sample and standard compounds. Various concentrations of the extracts (1 ml) were mixed with 1 ml of 0.3 mM DPPH solution. The tubes were shaken properly and incubated for 30 min in the dark at room temperature. The changes in the absorbance of the samples were measured at 517 nm using a spectrophotometer. The radical scavenging activity of the extracts at different concentrations was determined and compared with that of Ascorbic acid which was used as the standard. DPPH solution without extract/standard formed the control. The DPPH radical scavenging activity was given as

$$\text{Percentage scavenging activity} = \{(Ac - As)/Ac\} \times 100$$

Where,
Ac is absorbance of control and *As* is absorbance of sample.

All samples were run in triplicate and radical scavenging activity was reported as mean ± SD. 50% inhibition concentration was obtained from a linear regression plot of percentage inhibition against concentration of the extract.

Ferric reducing antioxidant power (FRAP) Assay

FRAP reagents were freshly prepared by mixing 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml 2,4,6-Tris-(2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM/L HCl) and 2.5 ml $FeCl_3$ (20 mM) water solution. Each sample (150 µl) (0.5 mg/ml) dissolved in methanol was added to 1.5 ml of freshly prepared FRAP reagent and stirred and after 10 min, absorbance was measured at 593 nm, used FRAP working solution as blank (Benzie and Strain, 1999). All measurements were taken at room temperature with samples protected from direct sunlight. The ferric reducing antioxidant power was expressed as Ascorbic acid Equivalent (AAE)/g dried weight. The relative activity of the samples was compared to L-ascorbic acid.

Total antioxidant capacity

The total antioxidant capacity of various extracts was evaluated using the phosphomolybdenum method of Prieto et al. (1999) with slight modification. Extracts (0.5 mg) were dissolved in a mixture of 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate

and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the reaction mixture was measured at 695 nm against reagent blank containing only the respective solvents. The results were expressed as µM ascorbic acid equivalents/g of extract.

Hemagglutinating activity assay

The protein extracts of the seeds were assayed for the presence of lectin by hemagglutinating activity assay performed in 96-well U-shaped microtitre plates using glutaraldehyde-fixed and trypsinized erythrocytes. PBS (100 µl) was delivered sequentially into wells arranged in rows (each row contained 12 wells). The extract (100 µl) was added into the first well and a serial dilution was done by transferring 100 µl of the diluted sample in a particular well into the next well containing 100 µl PBS. Aliquots (50 µl) of the 4% glutaraldehyde-fixed erythrocyte suspension were added to each well and the microtitre plates were left undisturbed for 1 hr. The titre value was taken as the reciprocal of the highest dilution of the extract causing visible hemagglutination.

The sugar specificity of the lectin was also investigated by determining the sugars that completely inhibited the agglutination of erythrocyte by lectins (Goldstein and Hayes, 1978). Lectin was diluted serially until the end-point dilution causing hemagglutination was obtained. The sugar solution (0.2 M) was added to each well at 50 µl per well while the control well contained PBS instead of sugar solution. Erythrocyte suspension (50 µl), was added to each well, and the titre of lectin activity was determined as described above. Inhibitory sugars caused a reduction in the titre of the lectin activity shown by the PBS-control experiment. The sugars tested include: D(+)-glucose, D(+)-mannose, D(+)-arabinose, D(+)-glucosamine hydrochloride, D(-)-sorbose, sorbitol, mannitol, maltose, sucrose, fructose, lactose, 1-O-methyl-α-glucopyranoside, rhamnose, raffinose, galactose, dulcitol, cellobiose, 2-deoxy-α-D-glucose, melibiose, L-fucose, melezitose and N-acetylglucosamine.

Effect of temperature on antioxidant and hemagglutinating activities

The protein solutions of the plant seeds were heated in a water bath at different temperatures ranging from 30–100°C for 20 minutes, cooled to room temperature and assayed for antioxidant activity by DPPH radical scavenging assay. The hemagglutinating activity assay was also carried out on the protein solutions after preheat at various temperature.

Larvicidal activity assay

Larvae of *Culex quinquefasciatus* were collected from the stock culture maintained at Entomology Research Unit of Zoology Department, Obafemi Awolowo University, Ile-Ife, Nigeria. The experiment was carried out at a temperature of 27±2°C, relative humidity (70±10%) and photoperiod of 12 hrs. Tests were performed by following the methodology described by WHO (WHO, 2005) with slight modification. A total of ten fourth instar larvae of *Culex quinquefasciatus* were collected with a Pasteur pipette, placed on absorbent paper to remove excess water and introduced with a tiny brush into 50ml disposable plastic cup containing various concentrations of the protein extracts. For each extract, three independent experiments were run in triplicate and distilled water was used as negative control. Mortality rate were registered after 24 hrs exposure period.

Statistical and data analysis

All data obtained from various experiment were subjected to descriptive statistical calculation using GraphPad^{R} Instat Statistical Package and expressed as mean values and standard error of mean (SEM) of multiple measurement.

Results

Extraction of the seeds soluble protein

Seeds of *P. osun* and *B. angolensis* were extracted in an aqueous buffer appropriate for proteins extraction and then precipitated with 70% ammonium sulphate saturation to concentrate the protein and remove small chemical impurities which may otherwise interfere with the antioxidant assays. A cloudy brown crude protein extract was obtained from the precipitation step and this was dialysed against double-distilled water extensively and the dialysate after lyophilization into powder was used for the various bioassays. Total protein content of the two extracts showed that the B. *angolensis* has the least protein concentration (Table 1).

Table 1. Protein concentration of the seeds extract of *P. osun* and *B.angolensis.*

Plants	Concentration (mg of protein/mL)
P. osun	20.7
B. angolensis	6.60

Antioxidant activity

The antioxidant activities of the protein extracts were determined by measuring the DPPH free radical scavenging activity and ferric reducing power. Fig. 1 shows the seeds aqueous protein extract scavenging activity. The DPPH activity was concentration dependent in both plants. The seeds protein extract of *P. osun* showed the highest activity with the least IC_{50} value of 0.58 ± 0.05 mg/ml compared with B. *angolensis* seeds extract with IC_{50} of 3.94 ± 0.28 mg/ml. The reducing antioxidant power of the seed protein extracts measured their reductive ability and was evaluated by the reduction of Fe^{3+} to Fe^{2+}. It was quantified based on linear regression equation obtained from Ascorbic acid standard curve. Thus the values were expressed as ascorbic acid equivalent. The reducing power of *P. osun* was higher than that of *B. angolensis* (Table 2). The total antioxidant capacity of the two seed extracts, which was expressed as above, showed that *B. angolensis* gave 1.58 ± 1.40 mg Ascorbic acid/g dried weight and is significantly difference from that of *P. osun* (38.93 ± 5.95 mg Ascorbic acid/g dried weight (Table 2).

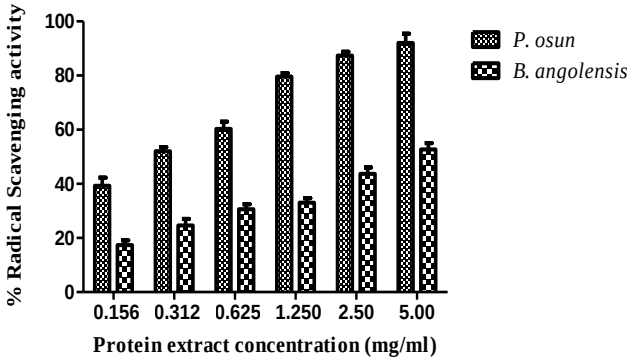

Fig. 1: DPPH radical scavenging activity of *P. osun* and *B. angolensis* seeds protein extract.

Table 2. Comparative analysis of FRAP and Total antioxidant capacity (TAC) in the seeds proteins.

Plants	FRAP (mg AAE/g)*	TAC (mg AAE/g)*
P. osun	29.57 ± 0.25	38.93 ± 5.95
B. angolensis	2.67 ± 0.28	1.58 ± 1.40

*mg of ascorbic acid equivalent per g of dried weight of the extract.

Hemagglutinating activity

Lectins are known for their hemagglutination properties. Hence, the seeds extracts were tested for the presence of lectin by determine the ability of the extracts to agglutinate the various blood group erythrocytes. Erythrocytes of human blood group A, B, and O, rabbit, bat and bovine were used. These blood cells were also trypsinized. The results showed presence of lectin in the seeds extract of the two plants. The lectin in the *P. osun* seeds extract was specific for rabbit erythrocytes and was unable to agglutinate all human blood group, bat and bovine erythrocytes. The seeds extract of B. *angolensis* agglutinated rabbit erythrocytes better than human red blood cells which were agglutinated non-specifically. Table 3 gives the summary of the result. The trypsinized blood showed higher hemagglutinating titre.

The sugar inhibition test showed (Table 4) that *P. osun* seeds extract contain mannose/glucose-specific lectin because the extract hemagglutinating activity was completely inhibited by mannose, α-methylmannoside and O-methylglucopyranose while glucose-containing sugars such as sucrose, maltose and N-acetylglucosamine slightly inhibited the activity. On the other hand, B. *angolensis* seeds extract hemagglutinating activity was completely inhibited by galactose, which classified the lectin as galactose-specific.

Temperature effect on the biological activity

P. osun seed protein antioxidant activity decreases with increase in temperature. It was stable up to 50°C at which 32% antioxidant was obtained. Hemagglutinating activity of *P. osun* was stable within the temperature range of 30-60°C after which the activity was completely lost (Fig. 2). *B. angolensis* seed protein antioxidant and hemagglutinating activity stability were better than that of *P. osun*. About 50% (27% inhibition) of radical scavenging ability was retained at 60°C compare to the control which gave 100% (56% inhibition) antioxidant activity. Significant amount of hemagglutinating activity was attained when the protein solution of *B. angolensis* seed was preheated up to 70°C. Though there was a gradual decline in the activity as the temperature increases, however, noticeable amount of hemagglutinating activity was recorded up to 80°C (Fig. 2).

Larvicidal activity

P. osun and *B. angolensis* seeds protein extracts were tested against fourth instar larvae of *Culex quinquefasciatus* at various protein concentrations (0-100 mg mL^{-1}). The protein extracts did not show any larvicidal activity against *Culex quinquefasciatus* as 0% mortality was observed in all concentration even after 48 hrs.

Table 3. Hemagglutinating activity of seeds protein extracts against human and animal erythrocytes.

Erythrocytes source	Hemagglutination titre*			
	P. osun		*B. angolensis*	
	Trypsinized	Non- trypsinized	Trypsinized	Non- trypsinized
Human blood group				
A	2^0	2^0	2^{15}	2^{12}
B	2^0	2^0	2^{12}	2^{12}
O	2^0	2^0	2^{11}	2^{10}
Rabbit	2^{12}	2^{10}	2^{17}	2^{14}
Bovine	2^0	2^0	2^0	2^0
Bat	2^0	2^0	2^0	2^0

*Hemagglutination titre is the reciprocal of the highest dilution of the extract causing visible agglutination of the erythrocytes.

Table 4. Sugar inhibition of hemagglutinating activity of the plant seeds protein extracts.

Sugars	Hemagglutination titre*	
	P. osun	*B. angolensis*
Lactose	2^9	**2^2**
Sucrose	2^4	2^{14}
Maltose	2^2	2^{14}
Galactose	2^9	**2^0**
Mannose	**2^0**	2^{16}
Sorbose	2^6	2^{15}
Glucose	2^3	2^{14}
Fructose	2^5	2^{14}
Arabinose	2^{10}	2^{14}
Mannitol	2^{12}	2^{15}
N-acetyl mannosamine	2^7	2^{16}
α-methyl mannoside	**2^0**	2^{14}
O-methyl glucopyranose	**2^0**	2^{15}
N-acetyl glucosamine	2^3	2^{15}
*Positive control	2^{12}	2^{16}
*Negative control	2^0	2^0

* Each experiment contained of 100 µl of serially diluted seeds extract in a U-shaped microlitre well. 50 µl 0.2 M sugar solution and 50 µl of 4% suspension of erythrocytes (Rabbit erythrocyte) were added to each well. Positive control was without sugar while negative control did not contain extract and sugar.

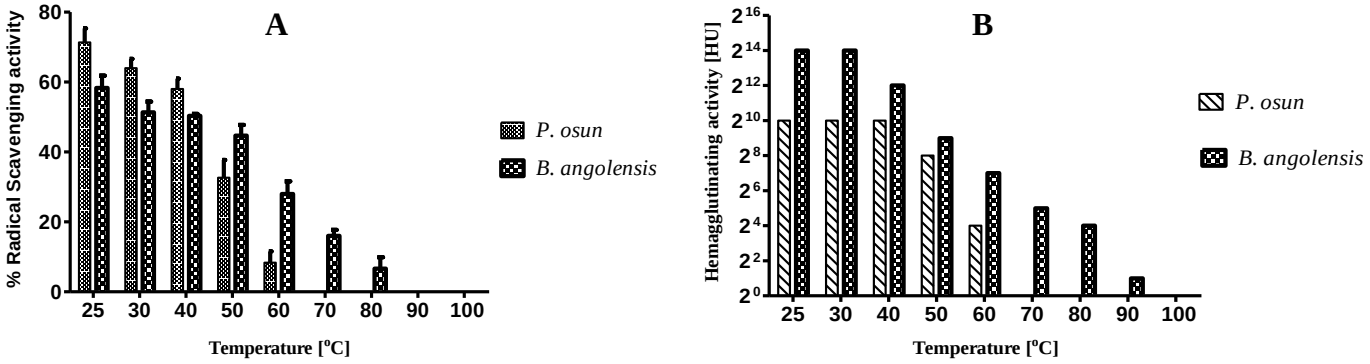

Fig. 2A and 2B: Effect of temperature on the antioxidant and hemagglutinating activity of the seeds protein extract.

Discussion

Our investigation showed that soluble proteins were present in the seeds extract of *P. osun* and *B. angolensis* with *P. osun* having the highest amount of protein. The method used in extracting the bioactive component may likely responsible for good amount of protein obtained. The same method was employed to extract the following bioactive proteins: antifungal protein from *Curcuma longa* (Petnual et al., 2010), haemagglutinating protein from *Curcuma ammarissima* (Kheeree et al., 2010), anticarcinogenic protein from soybean seed coat (Sessa and Wolf, 2001), and antioxidant protein from Red gram seed coat (Manukumar and Madhu, 2013).

Oxidative stress is defined as the interruption between the prooxidant and antioxidant equilibrium in favor of the increased level of the former in organs, tissues and cells. This *in vivo* pathophysiological condition arises as a result of either the increased production of reactive oxygen species (ROS) or the decreased level of the antioxidant defence (Sarkar et al., 2009). Mitochondria converts certain amount of cellular oxygen consumed into free radical such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). Under normal physiological conditions, these free radicals, in turn, react with the cellular macromolecules and cause peroxidative tissue damage (Dalle-Donne et al., 2005). It is the function of the natural antioxidative defense mechanism to scavenge the ROS in order to prevent the oxidative trauma. Nevertheless, if the rate of production of ROS rises above the rate of their consumption, the body needs the supplementation of antioxidants from outside, to deal with the surplus ROS. Currently, the hunt for natural products with antioxidant potentials have been on the rise due to the risk of adverse effects encountered with the use of synthetic compounds and also the public's perception that natural and dietary antioxidant are safer than synthetic analogues (Manukumar and Madhu, 2013). Search into the detailed protective mechanism of various medicinal plants in organ pathophysiology are urgently required. So, isolation of the biologically active components with essential antioxidant activity from the various plant extracts will therefore, contribute a lot to the reduction in the level of oxidative stress. Various antioxidant assays have been developed for the estimation of antioxidative properties of plants. Ideally, a combination of assays, incorporating different mechanisms of action is useful in order to provide complete information on the antioxidant capacity of a particular plant.

In this study, we used DPPH radical scavenging, FRAP and TAC assay to evaluate antioxidant activity of the two plants seeds. DPPH radical scavenging assay is a rapid, simple and inexpensive method to assess antioxidant capacity of extract or drug that elicits antioxidant properties (Uddin et al., 2008). The seeds protein extracts of the two plants displayed the capacity for scavenging free radicals by reducing the stable purple violet coloured DPPH radical to yellow coloured diphenylpicryl hydrazine. The results indicated the potential electron and/or hydrogen donating ability of the extracts. Based on the scavenging ability of the free radicals, *P. osun* showed the highest antioxidant activity with the least IC_{50} value (0.58 ± 0.05 mg/ml) and the scavenging activity of the extracts are concentration dependent. DPPH scavenging activity results obtained in this study is within the range of value reported by Manukumar and Madhu (2013) for red gram seed coat protein and Zhou et al. (2012) for *Gingko biloba* seeds crude protein and ammonium sulphate precipitate. But the result showed better radical scavenging activity than crude protein extract of *Lablab purpureus* seed (Saha et al., 2014). Conversely, the standard antioxidant (ascorbic acid) gave a better scavenging effect than the extracts.

The reducing power test is regularly used to evaluate the ability of an antioxidant to donate electron or hydrogen and at times serves as an indicator of antioxidant activity (Zhou et al., 2012). Many reports have revealed that there is a direct relationship between the antioxidative activity and the reducing power of bioactive compounds. For the reducing power assay, the occurrence of antioxidants in the seeds protein extract caused the reduction of the Fe3+/ferricyanide complex to the ferrous form. The values for ferric reducing antioxidant power revealed that the *P. osun* (29.57±0.25) had a significantly higher reducing power than *B. angolensis* (2.67±0.28). *P. osun* and *B. angolensis* seeds protein extract gave values that are similar to values reported in the literature (Kumaran and Karunakaran, 2006; Ani and Naidu, 2011).

The human blood types have different sugar moieties on the surface of the cell. Type A has N-acetyl- D-galactosamine, D-galactose for type B and L- fructose in type O. Blood type AB contains the sugar determinants for both A and B. Agglutination occurs when the lectin interacts with these sugar moieties (Saha et al., 2014). The ability of lectins to agglutinate cells is a recognized physiological effect that depends on their specificity and high binding affinity for a particular carbohydrate moiety

on the cell surface (Lam and Ng, 2011). The presence of lectin has been detected in large number of plant species, but very not many lectins have been isolated and purified in to pure form. The present study revealed the occurrence of lectins in *P. osun* and *B. angolensis*, which are from different plant families. The aqueous seeds protein extracts of the two plants were shown to possess agglutinating activity when tested against human ABO blood group and rabbit erythrocytes. *P. osun* was seeds extract agglutinated only rabbit blood cells and agglutination was completely inhibited by mannose/glucose and some of their derivatives. This classified the lectin present in the extract as mannose/glucose-binding lectin. *B. angolensis* seed extract nonspecifically agglutinated human red blood cell and agglutination was inhibited by galactose. The haemagglutination inhibition by simple sugars proved that there in sugar residue on red blood cells that the lectin binds. Lectin specificity differ from one lectin to another however, some lectins have common inhibitory sugars. The sugar specificity gives a primary indication of the nature of the lectin present in the extracts, which helps in the purification procedure. Mannose-binding lectin (MBL) has been shown to bind to HIV (Ji et al., 2005). Binding of MBL to HIV was reported to depend on the high mannose glycans on gp120. Some lectins with high antiviral activity, that bind mannose, have been described in the past and they include jacalin, concanavalin A, *Urtica diocia* agglutinin, *Myrianthus holstii* lectin, and *Narcissus pseudonarcissus* lectin (Charan et al., 2000). Antifungal activity of a mannose/glucose-specific lectins from *Capsicum annum* seed (Kuku et al., 2009) and *Pisum sativum* seed (Sitohy et al., 2007) has been reported. Galactose-binding lectin from *Sphenostylis stenocarpa* was shown to possess insecticidal activity against cowpea major pests (*Clavigralla tomentosicollis* and *Callosobruchus maculatus*) (Omitogun et al., 2001). Antifungal and mitogenic activities were reported for the Galactose-binding lectin purified from Red cluster pepper (*Capsicum frutescens*) (Ngai and Ng, 2007).

The needs to provide better alternatives for the control of mosquitoes, which are the most important vectors for the transmission of malaria, filariasis, and viral diseases, have been focus of many researchers. Recent development of resistance by mosquitoes to various chemicals used in their control has also prompted scientist to look towards biologically active larvicides from plant origin. It has been established that plant contain certain phytochemicals that can be used as insecticide for larvae or adult mosquitoes or as repellant for protection against mosquito bites. Yadav et al. (2015) reported that leaves of *Vernonia cinerea* and *Prosopis juliflora* extracted with solvent of medium polarity showed significant larval toxicity. The lectins from *Glycine max* and *Erythrina indica* seeds showed toxic effect on various insects (Singh et al., 2009). In the present study, aqueous seed extracts from *P. osun* and *B. angolensis* showed no toxicity towards *Culex quinquefasciatus* larvae even at 100 mg ml^{-1}. It is possible that the protein extract does not possess larvicidal potential or that a much higher concentration is needed for the extract to be effective larvicide. The toxicity of plant extract against against mosquito larvae has been shown to vary with plant species, mosquito species, geographical location and season, plant parts, extraction methodology and polarity of solvent used (Singh et al., 2009; Reegan et al., 2013; Dohutia et al., 2015; Yadav et al., 2015).

Conclusion

The present study concludes that phosphate buffer saline (pH 7.2) is capable of extracting good quantity of protein from the plant material used. The plants seeds protein extracts possessed significant antioxidant activity but no larvicidal effect was detected. Hemagglutinating activity of the seeds protein extracts revealed presence of lectins which are specific for mannose/glucose and galactose. The lectins present in the extracts may have antimicrobial, anti-inflammatory or other biological activities. However, further investigations are required to purify the lectin and establish its bioactivity. More studies are also necessary to fully characterize the protein(s) present in the seeds that is responsible for the antioxidant activity. The protein isolates may possibly have the capacity to be useful in biotechnological and pharmaceutical industries.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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How to cite this article:
Odekanyin, O. O., Akeredolu, E. O., 2016. Comparative investigation of the biological activity of the *Pterocarpus osun* and *Bosqueia angolensis* seeds protein extracts. Int. J. Curr. Res. Biosci. Plant Biol. 3(9), 16-26. doi: http://dx.doi.org/10.20546/ijcrbp.2016.309.003