PHYTOCHEMICALS, ANTIOXIDANT AND ANTISTRESS PROPERTIES OF PULP, LEAVES AND SEEDS OF *PAKIA BAGLOBOSA* AND *MORINGA OLIFERA*

[1]Lawal, W.S, [2]Apata D.F, and [3]Oluwaniyi O.O[c]

[a,b]Kwara State University, Malete Department of Animal Production
[c]University of Ilorin, Ilorin Department of Industrial Chemistry.
Corresponding email: awsl2004@gmail.com

Abstract
Phytochemical (Quantitative and qualitative), Antioxidant (DPPH and FRAP), proximate composition, Vitamin and Minerals of leaves, seeds and pulp coded as (PBS as P. baglobosa seed, PBP as P. baglobosa pulp, PBPS as P. baglobosa pulp & seed, MOS as M. olifera seed, MOL as M. olifera leaves and MOLS as M. olifera leaves & seed) of both Pakia Baglobosa and Moringa Olifera was investigated. These herbs and their parts were sourced from Kwara State University, Malete. The plant parts were dried in the shade after which they were grinded so as to increase the surface area before extraction. The phytochemical screening and the antioxidant analysis were carried out using standard method. The Phytochemical analysis parameters showed their presence in an appreciable quantity while the antioxidant activities showed that all plant parts of both herbal plants are well above 50% at all dilution factors meaning that the plant parts are good sources of antioxidant because their $IC_{50.}$ The plant parts like PBS, PBP, PBPS and MOS had higher antioxidant activities and are significantly different ($P<0.05$) when compared with other plant parts and Vitamin C. The proximate composition, minerals and Vitamin content showed that they can improve the nutritional content when included in feed. It shows that PBP has the highest value ($P<0.05$) of vitamin A, B and C (31.07mg/kg, 37.07mg/kg and 20.89mg/kg respectively) which makes it significantly difference when compared to other plant part.

Keywords: Phytochemicals, Antioxidant, Dilution factor, Surface Area and Herbal Plant

Introduction

Parkia biglobosa (Jacq.) is a valuable multipurpose tree species found in the savannahs, providing food, medicines and fodder for local inhabitants (Teklehaimanot, 2014). The tree is normally spared from cutting, when the land is cleared for agriculture (Thiombiano et al., 2013). One of the aspects for which *P. biglobosa* is appreciated is the high nutritional content of its fruits (pods). The edible parts of the pods (pulp and seeds) are used as ingredients in different foods and are subjects of study aimed at improving the nutritional quality of derived products. The seeds of *P. biglobosa* have received most attention in study on nutrition and on modalities of consumption, as they are used to produce a highly appreciated and widely consumed alkaline fermented condiment, known as 'dawadawa', according to the local terminology (Ouoba et al., 2024). Besides of seeds, the pulp (a yellow powder) is largely consumed, usually raw, by collectors (such as shepherds and passers-by), or used for the preparation of drinks, cakes and/or paste (raw or fried), after mixing with water (Ouédraogo, 2015). The pulp can also be stored for use during the lean period (Thiombiano et al., 2014). So far, several of the studies have been carried out to characterize the chemical composition of the pulp. *Moringa Oleifera* is universally referred to as the miracle plant or the tree of life. The *Moringa* plant derives this name based on its uses, particularly with regard to medicine and nutrition.

It is a plant native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2015). *M. Oleifera* is the most widely cultivated among the 13 species of the *Moringaceae* family and it is exceptionally nutritious with a variety of uses. Almost all the parts of this miracle tree have been found to be very useful. Leaves are used as forage, tree trunk for making gums, flower nectar in honey and powdered seeds for water purification Fuglie (2015). *M. Oleifera* leaf has been used as an alternative food source to combat malnutrition,

especially among children and infants (Anwar et al., 2017). *M. Oleifera* leaves are reported to contain substantial amounts of vitamin A, C and E (Hekmat et al., 2015). The leaves of *M. Oleifera* have also been found to contain appreciable amounts of total phenols, proteins, calcium, potassium, magnesium, iron, manganese and copper (Hekmat et al., 2015). *M. Oleifera* leaves are also good sources of phytonutrients such as carotenoids, tocopherols and ascorbic acid (Saini et al., 2014b). These nutrients are known to scavenge free radicals when combined with a balanced diet and may have immunosuppressive effects (DanMalam et al., 2011). Besides the leaves, the flowers and fruits of *M. Oleifera* have also been found to contain appreciable amounts of carotenoids (Saini et al., 2014e).

In many parts of the world including Africa, the use of *M. Oleifera and P. baglobosa* as food fortificant is on the increase. For instance, both fresh and dried *Moringa* leaves are included in meals in African countries such as Ghana, Nigeria, Ethiopia, East Africa and Malawi (Agbogidi and Ilondu, 2012). Studies has also shown that *M. Oleifera* has medicinal qualities because of its antioxidant properties. The aim of this project however is to investigate the phytochemicals, antioxidant and nutritional qualities of extracts made from the African *P. baglobosa and M. olifera* plant's seeds, leaves and pulp using DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP ((ferric reducing antioxidant power), recent research interest is geared towards the full utilization of potential medicinal and nutritious plant-based products which is in line with the Sustainable Development Goal (SDG) (Fernandez 2020). These plant-based products are expected to be accessible, cheaper, and available all year round. It is expected that the results obtained will serve as baseline data for the promotion of seed, leaves and pulp as antioxidant, medicinal substance and as food, to help reduce heat stress, malnutrition and diseases in highly affected communities.

Objectives of the Study

i. Determine both the quantitative and qualitative phytochemicals
ii Analyse the antioxidant properties using DPPH (1, 1-diphenyl-2-picrylhydrazyl) and confirm this by FRAP (Ferric reducing antioxidant power)

Methodology

Pulp, seeds and leaves of *Pakia baglobosa* and *Moringa Olifera* were obtained from surrounding of Kwara State University, Malate with 8.7063° N, 4.4622° E. The dried pulp was manually scraped from the seeds and kept in an air-tight container. The seeds and leaves were washed and sun-dried for 10 h separately and then milled into fine powder. The bioactive compounds of the fruit, pulp and leaves of both P. baglobosa and M. olifera were extracted with ethanol to obtain the extracts of the two herbs. One milliliter of aqueous extract of the sample was suspended in 1 ml of distilled water and drops of diluted hydrochloric acid (2M). To the resulting mixture 1 mL Dragendorff's reagent was added. An orange-red precipitate shows the presence of alkaloids Raal et. al., (2020). To 5 g of the sample, 5 ml of methanol was added and filtered. To a portion of the filtrate, 2 mL of sodium hydroxide solution was added. Three drops of diluted hydrochloric acid were added to the resultant mixture. The appearance of a yellow solution when the sodium hydroxide solution was added which became colourless when dilute hydrochloric acid was added, this confirmed that flavonoids are present Alqethami et.al., (2021). Two grams of powdered plant material were suspended in 5 mL distilled water. The mixture was vigorously shaken for 1 min. Persistent copious lather for more than 5 min shows the presence of saponins Fahey (2015). To 1 g of powdered plant material, 2 mL of distilled water was added after which the mixture was filtered. Ferric chloride (10 %) solution was then added to the filtrate dropwise. A dark green color inferred a positive result for the presence of phenols DanMalam et al., 2011. Five grams of the samples was suspended in chloroform (2 mL) after which 3 ml of concentrated sulfuric acid was added to the resulting solution. The formation of a reddish-brown colored interface showed a positive inference of terpenoids Jimoh and Olatidoye, 2019. To 5 g of the sample, 10 ml of distilled water, was added heated, and filtered. One milliliter of FeCl f iltrate. A blueish-green precipitate shows the presence of tannins Hekmat et al., 2015

A DPPH solution (0.2 mM) was prepared (0.0078864 g of DPPH in 100 ml methanol). In a microtiter plate, 150 µl of 0.2 mM DPPH was added and followed by a 50 µl of standard (ascorbic acid; ranging 40 µg/ml to 200 µg/ml) or extract (BO/DBEE/BFWE; ranging 200 µg/ml to 1000 µg/ml). The mixture was left to stand in the dark at 27 ◦C for 30 min. From the resulting solution absorbances were taken against a blank at 517 nm with a spectrostar nano, spectrophotometer. The scavenging activity of the extracts against DPPH radical was calculated as scavenging activity (%) =(DPPHabs – Extractabs/DPPHabs) ×100 Jongrungruangchok et al., 2010. 1ml of water and 80µl of test bee product sample was pipetted in to a standard 4ml plastic cuvette. 600µl of the incubated FRAP reagent was added to the cuvette, which was briefly inverted to mix the solutions. A reagent blank was also prepared as described above but 80µl of water was added instead of a test sample. Change in absorbance at 593nm (a result of reduction of the Fe3+-TPTZ complex to the blue Fe2+ TPTZ complex at low pH) was recorded at exactly four minutes using a spectrophotometer. Each test sample dilution was tested in triplicate to allow a mean absorbance to be calculated. A number of dilutions of each bee product were tested allowing dose response curves to be produced.

The nutritional composition such as moisture, ash, protein, fat, fiber, and carbohydrate content of the seeds and fruit pulp were determined using techniques certified by the Association of Official Agricultural Chemists (AOAC) with slight modifications Nkukwana et al., (2014a). Each analysis was done in triplicates. A weighed sample (5g) was placed in a preheated oven at 105 ◦ C and dried to a contant weight to measure the percentage moisture. The micro-Kjeldahl technique was used to determine the crude protein concentration Nkukwana et al., (2014b) Percentage fatin the seeds and fruit pulp were determined by extracting from a 5 g sample in petroleum ether through the Soxhlet extraction method. After digesting a measured amount of the fat-free residue produced from the crude fat determination, the crude fiber was evaluated gravimetrically. With 1.25 % sodium hydroxide and 1.25 % sulfuric acid, the digestion was carried out under reflux. The sample was burned for 10 h at 600 ◦ C in a muffle furnace to ascertain its ash content. Carbohydrate percentages of the various samples were estimated as follows: Carbohydrate (%) = 100 - % yield [moisture + fat + ash + protein + fiber] The Total Energy values of the plant materials were obtained by calculations according to the Atwater factor as shown below: Energy value (kcal) = 4(% crude protein) + 9(% crude fat) + 4(% carbohydrate), Nkukwana et al., (2014c). All data were subjected to analysis of variance (ANOVA) (Steel and Torrie, 2012), and Duncan's Multiple Range Test was used to separate the means (Duncan 2000)

Result and Discussion

The plant under investigation are African P. baglobosa and M.olifera, the parts to be considered are PBS (Pakia baglobosa seed), PBP (Pakia baglobosa pulp), PBPS (Pakia baglobosa pulp and seed), MOS (Moringa olifera seed), MOL (Moringa olifera leaves) and MOLS (Moringa olifera leaves and seed). Table 1 show qualitative phytochemical screening of *P. baglobosa and M. olifera* herbal plants, all parameters considered are present in all the sample except flavonoids, saponins and Tannins are present only in PBS, PBPS, MOS and MOLS.

Table 1: PHYTOCHEMICAL SCREENING OF *P. Baglobosa and M. Olifera* HERBAL PLANTS

samples Parameters	PBS	PBP	PBPS	MOS	MOL	MOLS
Alkaloids (Wagner's test)	+	++	+	++	++	+
Flavonoids (Alkaline test)	-	+	-	-	++	++
Saponins (Foam test)	+	++	-	-	++	++
Phenols (Ferric chloride test)	+	-	+	++	+	+
Terpenoids	+	+	++	++	++	+
Tannins	-	++	-	-	++	-

PBS(P. baglobosa seed), PBP(P. baglobosa pulp), PBPS(P. baglobosa pulp & seed), MOS(M. olifera seed) MOL(M. olifera leaves) and MOLS (M. olifera leaves & seed) Key: (+) means present (-) means absent (++) means abundantly present.

Table 2: Quantitative Phytochemical Screening Of *P. Baglobosa And M. Olifera* Herbal Plants (Ml/G)

Parameters	PBS	PBP	PBPS	MOS	MOL	MOLS
Alkaloids	4.45	6.15	5.70	6.10	10.25	10.50
Flavonoids	5.55	5.60	6.75	6.35	4.25	4.50
Saponins	2.75	3.05	3.10	3.40	6.10	6.20
Phenols	1.15	0.80	0.84	0.89	1.29	1.29
Terpenoids	0.63	0.59	0.65	0.68	0.99	0.87
Tannins	0.94	1.07	1.01	0.85	0.93	1.02

PBS(P. baglobosa seed), PBP(P. baglobosa pulp), PBPS(P. baglobosa pulp & seed), MOS(M. olifera seed) MOL(M. olifera leaves) and MOLS (M. olifera leaves & seed)

Table 3 shows the antioxidant activities of P. baglobosa and M.olifera parts in DPPH invitro analysis. It reveals that the higher the concentration or dilusion factor, the higher the antioxidant activities, however irrespective of the medium concentration or dilusio factor, the antioxidant activities in Vitamin C was always higher. The plant parts like PBS, PBP, PBPS and MOS had higher antioxidant activities and are significantly different ($P<0.05$) when compared with other plant parts and Vitamin C.

Table 3: EFFECT OF *P. Baglobosa And M. Olifera* PLANT PARTS IN ANTIOXIDANT ACTIVITIES IN DPPH IN-VITRO ANALYSIS

Conc.	PBS	PBP	PBPS	MOS	MOL	MOLS	VIT C	SEM
3.125	12.06±0.05^{b}	86.90±0.01^{a}	68.90±0.05^{a}	66.60 ±0.05^{b}	76.30.04^{ab}	70.50±0.03^{a}	70.20±0.01^{a}	8.15
6.25	78.70±0.05^{a}	92.00±0.09^{a}	76.20±0.04^{b}	78.90±0.0^{a}	70.10±0.02^{b}	71.70±0.04^{b}	80.20±0.03^{a}	0.96
12.5	86.13±0.05^{a}	94.70±0.04^{a}	91.10±0.03^{a}	86.40±0.02^{a}	66.80±0.02^{b}	76.70±0.03^{ab}	86.00±0.01^{a}	2.10
25.0	91.30±0.04^{a}	95.90±0.05^{a}	84.90±0.05^{ab}	93.20±0.01^{a}	64.90±0.05^{b}	82.60±0.04^{ab}	92.50±0.05^{a}	1.22
50.0	94.70±0.04^{a}	97.10±0.01^{a}	92.20±0.02^{a}	93.90±0.02^{a}	63.70±0.05^{b}	83.10±0.02^{ab}	89.50±0.02^{a}	2.72

Table 4 shows the antioxidant activities of P. baglobosa and M.olifera parts in FRAP invitro analysis, it shows the higher the concentration or dilution factor the higher the antioxidant activities though not equal to that of vitamin C which perform better than plant parts. MOS, MOL and MOLS had no significant difference ($P>0.05$) between them and vitamin C, meaning they do well like vitamin C at 3,125 dilution factor or concentration but at higher factors between 6.25 and 50 a significant difference occurred showing vitamin C performed better than plant parts ($P<0.05$).

Table 4: EFFECT OF *P. Baglobosa and M. Olifera* PLANT PARTS IN ANTIOXIDANT ACTIVITIES IN FRAP IN-VITRO ANALYSIS

Conc.	PBS	PBP	PBPS	MOS	MOL	MOLS	VIT C	SEM
3.125	16.98±0.01^{c}	17.921±1.4^{b}	17.19±1.2^{b}	21.35±0.09^{a}	21.49±2.1^{a}	19.86±1.1^{a}	20.70±0.5^{a}	2.3
6.25	17.33±4.0^{d}	19.78±0.6^{c}	19.09±0.06^{c}	21.90±1.5^{b}	21.80±0.08^{b}	19.90±0.03^{c}	23.58±0.02^{a}	1.4
12.5	18.70±0.4^{d}	20.52±0.07^{c}	20.27±0.6^{c}	22.62±0.09^{b}	22.21±0.01^{b}	20.31±0.03^{c}	26.68±1.9^{a}	1.13
25.0	18.82±0.6^{e}	20.94±0.07^{d}	20.96±0.4^{d}	23.58±0.09^{b}	22.78±0.01^{c}	20.49±0.09^{d}	29.00±1.7^{a}	1.7
50.0	20.07±0.1d	21.27±1.2c	21.54±1.5c	23.96±0.9b	23.00±0.7b	20.66±0.08d	31.98±1.5a	0.9

PBS(P. baglobosa seed), PBP(P. baglobosa pulp), PBPS(P. baglobosa pulp & seed), MOS(M. olifera seed) MOL(M. olifera leaves) and MOLS (M. olifera leaves & seed) a,b and c mean on same row with difference superscript are significantly different (P<0.05)

Table 5 shows the Vitamin content of all the plant parts, it shows that PBP has the highest value (P<0.05) of vitamin A, B and C (31.07mg/kg, 37.07mg/kg and 20.89mg/kg respectively) significantly difference when compared to other plant part (P<0.05) while MOLS has the least value of Vitamin A (1.08mg/kg), PBS has least value of Vitamin Band C with (0.58mg/kg and 7.28mg/kg) respectively.

Table 5: VITAMIN CONTENT OF PULP, LEAVES AND SEEDS OF *PAKIA BAGLOBOSA* AND *MORINGA OLIFERA* PLANTS (mg/kg)

Vitamin Samples	A	B	C	SEM
PBS	5.265^{c}	0.582^{e}	7.285^{e}	0.1
PBP	31.075^{a}	37.771^{a}	20.890^{a}	1.0
PBPS	20.185^{b}	26.180^{b}	17.388^{c}	0.98
MOS	3.770^{d}	12.429^{c}	4.885^{f}	0.3
MOL	4.252^{c}	0.781^{e}	18.158^{b}	1.33
MOLS	1.087^{e}	10.476^{d}	8.940^{d}	0.7

PBS(P. baglobosa seed), PBP(P. baglobosa pulp), PBPS(P. baglobosa pulp & seed), MOS(M. olifera seed) MOL(M. olifera leaves) and MOLS (M. olifera leaves & seed)

Table 6 shows the proximate composition of the plant parts, it reveals that MOS has the highest value of energy 405.74 kcla/kg while PBS has the least energy content 390.79kcal/kg. MOL has the highest protein content 15.50% and the least protein content from PBP 12.05%. The fibre content of all the plant parts are moderate enough and can be tolerated by Broiler chicken. MOS has the highest value of fat content 7.06% while the least value was obtained from PBS 4.05%.

Table 6: PROXIMATE COMPOSITION OF PULP, LEAVES AND SEEDS OF *PAKIA BAGLOBOSA* AND *MORINGA OLIFERA*

Sample	Moisture(%)	Ash(%)	Crude fibre(%)	Crude protein(%)	Crude fat(%)	Carbohydrate(%)	Energy(kg/kcal)
PBS	5.15	2.00	0.22	12.05	4.05	76.54	390.79
PBP	4.95	2.65	0.29	13.45	6.85	71.82	402.71
PBPS	6.40	2.70	0.26	12.35	7.05	71.25	397.83
MOS	5.15	2.75	0.17	14.25	7.60	70.09	405.74
MOL	5.85	3.05	0.21	15.50	5.50	69.90	391.08
MOLS	4.65	2.25	0.16	14.25	6.25	72.45	403.03

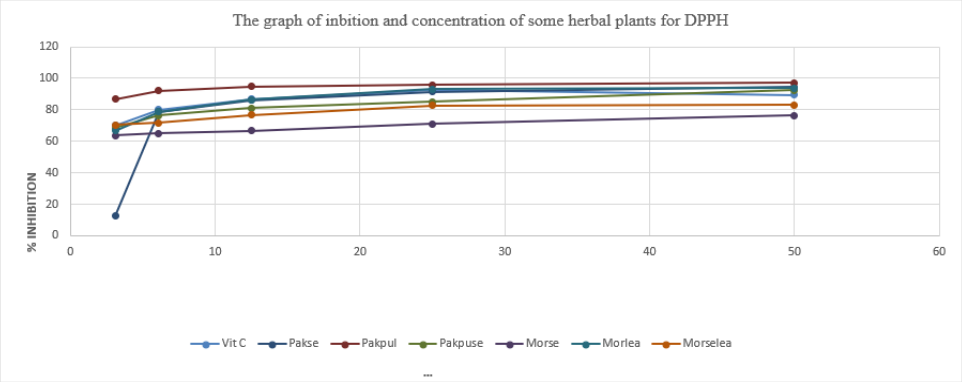
PBS(P. baglobosa seed), PBP(P. baglobosa pulp), PBPS(P. baglobosa pulp & seed), MOS(M. olifera seed), MOL(M. olifera leaves) and MOLS (M. olifera leaves & seed)

Table 7 show the mineral composition of all plant parts ant it reveals that all part has appreciable amount of minerals that are concern with antioxidant and antistresss that work synergistically with vitamin to give a very good result. The analysis of plant parts revealed that all the seeds parts contain compounds including Tannins, Glycosides, Steroids, Flavonoids, and Saponins. Alkaloids were found only in the seeds and not in the fruit pulp. The chemical compounds obtained from plants are very important due to their potential pharmacological activities in the body He et al., 2010. Secondary metabolites from plants comprise many related compounds that have different physiological properties. The nature of these compounds is dependent on the type of plant as well as the part of the plant from which the compound is obtained Ogunsina et al., 2010. Anti-oxidant properties of the plant was evaluated by quantifying the presence flavonoids, tannins, and phenols. The two plants with their parts shows high antioxidant activities with DPPH and they were well above 50% at all dilution factors, the invitro activities shows that the plant parts can be good source of antioxidant and so can be used for manufacturing antistress drugs. The plant parts are well above IC_{50} value for antioxidant activities and also contribute to nutritional value of the feed because they are good source of protein and Energy as shown in the proximate analysis in Table 6.

Table 7: MINERAL CONTENT OF PULP, LEAVES AND SEEDS OF *PAKIA BAGLOBOSA* AND *MORINGA OLIFERA* PLANTS (ppm)

Samples	Ca	Zn	Cu	Fe	K	Mg	Na	Cr	Mn	Ph
PBS	8.118	0.490	0.349	9.429	8.257	6.921	2.706	1.906	1.548	2.554
PBP	11.058	0.603	0.394	5.689	9.420	7.066	2.562	0.377	2.582	2.393
PBPS	4.843	0.444	0.245	2.404	7.995	6.886	2.603	0.102	1.219	1.765
MOS	6.053	0.424	0.108	3.311	8.659	6.839	2.582	0.105	0.318	1.940
MOL	3.295	0.413	0.433	4.501	9.640	7.114	2.713	0.114	1.130	2.343
MOLS	9.448	0.374	0.085	3.858	9.752	6.801	2.694	0.058	0.388	2.375

PBS(P. baglobosa seed), PBP(P. baglobosa pulp), PBPS(P. baglobosa pulp & seed), MOS(M. olifera seed), MOL(M. olifera leaves) and MOLS (M. olifera leaves & seed).



Conclusion

Both *Pakia baglobosa* and *Moringa olifera* are good source of antioxidant, antistress, contain some useful phytochemicals, vitamins, minerals and also rich in protein and energy. They are therefore useful when used in feed and can also be uses as antioxidant in poultry birds and this make the herbs to cure a number of diseases in farm animals. The use of herbal plants in farm animals is advised because it is very effective, readily available all year round because they are locally sourced so they are very cheap, no side effect, rich in antioxidant, antistress, contains some vitamins and minerals, no residual effect and no resistance to drug. The two plants are good source of antioxidant based on the qualities they contains and so could be used in manufacturing drugs for both human and animals. The herbs could be used raw for both animals and human to cure some diseases. The plants/herbs is therefore recommended for use for both human and animals.

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