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ANTIMICROBIAL, NUTRITIONAL AND PHYTOCHEMICAL PROPERTIES OF *Perinari excelsa* SEEDS

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ABSTRACT

Seeds of *Perinari excelsa* were investigated for its antimicrobial, nutritional and phytochemical properties. Results of the study show that the aqueous and ethanolic extracts of the seed were active against both gram negative and gram positive organisms used. Important bioactive constituents present in the extracts were alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, phenolic compounds and tannins. Yield extracts of the powdered seeds, was 8.45% for water and 6.32% for ethanol indicating that water was the better of the two solvents used. Results of the antibacterial activity of the extracts reveal that the ethanolic extracts at different concentrations were more active against the test organisms namely *Staphylococcus aureus*, *Klebsiella pneumonia, Escherichia coli* and *Salmonella typhi* than the aqueous extracts. The MIC values for the aqueous extract ranged between 13.0 and 14.0 mg/ml while that of ethanolic extract was between 12.5 and 14.0 mg/ml. The MBC values for aqueous extract ranged between 14.0 and 15.5 mg/ml while that of ethanolic extract ranged between 13.0 and 15.0 mg/ml. Nutritionally, result from the study justifies the use of the seed both as spice and food component by locals. The seed was particularly found to be very rich in iron. Therefore there is a need for further studies on the plant seed in order to isolate, identify and characterize the active components to maximize the potential of this useful seed.

KEY WORDS

Antimicrobial, Nutritional, Phytochemical, *Perinarl excelsa,* Spices

INTRODUCTION

Spices are generally defined as vegetable substances of indigenous or exotic origin which are aromatic or has a hot piquant taste, used to enhance the flavor of foods or to add to foods the stimulating ingredients contained in them[1]. In most cultures of the world, spices and herbs have been used for centuries as part of their daily food to enhance flavor and aroma. Although most spices are added to food recipes primarily to function as seasoning rather than for its nutritional benefits, their nutritional and phytochemical potential has not been overlooked. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific evidences abound of the antimicrobial properties of most spices, herbs and their components[, 2, 3, 4.] Basically when used for medicinal purpose to enhance well being, their value can be observed from the phytochemical components they possess. These phytochemicals, which have been observed to be present in small quantities as secondary metabolites include among others, alkaloids, steroids, tannins, flavonoids, and phenolic compounds[5]. Also as preservative, essential oils extracted from spices and herbs have been generally recognized as containing active antimicrobial compounds such as allicin, allyl isothocyanate, eugenol, carvacrol and thymol that can inhibit the growth of both gram positive and gram negative bacteria[2,] as well as prevent mold growth in addition to adding flavor and aroma to baked products[6,7]. Foods containing these phytochemicals not only can provide our diet with certain antioxidant vitamins like vitamin C, vitamin E and provitamin A, but also can provide a complex mixture of other natural substances with antioxidant capacity[8]. Extracts from spices have also been shown to possess very good antioxidant properties beneficial in the prevention of some off-flavor development, in snack foods and meat products[9].

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures. With many of the indigenous plants being used as spices and food, knowledge of their chemical constituent becomes very important, not only for their nutritive value, but also for discovering new sources of economic materials and drugs for the treatment of recalcitrant infectious agents. Additionally, knowledge of the chemical constituents of these plants used as spices and herbs helps in the discovery of the true relevance of folkloric medicines[10]. The plant *Perinari excelsa* Sab., which is in the family of the *Chrysobalanaceae*, is an evergreen tree found growing up abundantly in the humid rain forest and less in the Guinean forest. They are widely distributed in tropical Africa and occur gregariously at elevations between 3,000 and 6,000 ft. The tree is known to grow up to a height of 150 to 170 ft, bole cylindrical, mostly straight, and usually clear up to 60 to 90ft, buttressed to a height of 10 ft and trunk with diameter- 3 to 5 ft.[11,12]. It is variously known as Grey plum or rough skin plum (English), Mubura (Tanzania), Kpar(Liberia),and in Senegal, Mampata [13.] In Nigeria, amongst the Itsekiris, it is called Igbafilo, Nmimi (Ibos), Imako (Urhobos), and Esagho (Edo).

Figure 1: ***Perinari excelsa*** **seed**

The bark of the tree, pounded or macerated is traditionally applied as treatment to fresh wounds especially in circumcision, while the bark-decoction is taken to relieve stomach ache. The roasted bark is also added to palm wine, a traditional Nigerian beverage to improve its flavor. It is also taken as treatment for anemia and by women during pregnancy as a tonic. The most predominant however, is the use of the seed of *Perinari excelsa* (fig. 1) as spice and condiment in most traditional food preparations like pepper soup and other soup delicacies especially in the Niger- delta and Mid-western areas of Nigeria.

Because of the increasing reported cases of multiple drug resistance in medically important strains of bacteria and fungi [13,14] and the fact that many published reports have shown that plants and spices are one of the bedrocks for modern medicine to attain new principle, there is a need to further carry out research in this very interesting area. As the plant *Perinari excelsa* is easily available and widely used in the Nigerian diet, this study was carried out to investigate the nutritional, phytochemical and antimicrobial properties of the aqueous and ethanolic extracts of the seeds of the plant. The result is expected to provide further information regarding the plant and add to the body of knowledge on its nutritional and medical value.

MATERIALS AND METHODS

Source of *Perinari excelsa* seeds

Dried seeds of *Perinari excelsa* were purchased from different vendors in the "Yanga" sub-section of Oba market, Benin City, and the scientific name authenticated by Dr J. E. Ehiagbonare of the Department of Biological Sciences, Igbinedion University, Okada.

Preparation of Sample

The seeds were sun dried in the open for five days, after which they were shelled and milled into powder with a dry sterilized Panasonic blender model MX-J120P. The powdered seeds were then sieved through a 2.0 mm filter and subsequently stored in an air tight sterile container until it was used.

Seed extract preparation

The seed extract was obtained by using the method previously described[15]. 100g of the powdered sample was soaked in 400ml of solvent in a sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminum foil and shaken vigorously. The mixture was left to stand for 24 hours in a shaking water bath maintained at 40^{o}C. The mixture was then filtered using a clean muslin cloth and Whatman No. 1 filter paper. Thereafter the filtrate was evaporated to dryness by means of a rotary evaporator attached to a vacuum pump. The percentage yield of each of the crude

extract was determined for each solvent and estimated as dry weight (extract) / dry sample weight x 100. The extracts were stored in refrigerator until needed for further analysis.

Microorganisms

The species of microorganisms used in the investigation were *Staphylococcus aureus*, *Klebsiella pneumonia, Escherichia coli* and *Salmonella typhi.* The organisms were clinical isolates obtained from the microbiology laboratory of the Lahor Research laboratory and Diagnostic center, Benin City. The cultures of bacteria were maintained on nutrient agar slants at 4^{o}C, re-identified by biochemical tests[16, 17] and sub-cultured on nutrient broth for 24h prior to testing.

Phytochemical screening of seed extract

Phytochemical screening of the seed extract was carried out using the methods of[4,18] as highlighted below.

Alkaloids

About 0.5g of the extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff's reagent were used to treat 1ml of the filtrate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids.

Saponins

0.5g of the extract was dissolved in 5ml distilled water in a test tube and shaken vigorously. Frothing which persisted on warming was observed. The frothing was mixed with 3 drops of olive oil and shaken again after which it was observed for the formation of an emulsion which was indicative of a positive result.

Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish-green or a blue-black coloration which indicated the presence of tannins.

Anthraquinones

About 0.5 g of the extract was boiled with 10 ml of sulphuric acid (H2SO4) and filtered using Whatman filter paper No.1.The filtrate was shaken with 5 ml of chloroform. The chloroform layer was transferred into another test tube and 1 ml of 10% dilute ammonia was added. The resulting solution observed for color changes was indicative of the presence of anthraquinones.

Flavonoids

10% of dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow coloration that disappears on standing indicated the presence of flavonoids.

Cardiac glycosides (Keller-Killiani test)

0.5 g of extract was diluted to 5 ml in water and 2 ml of glacial acetic acid containing one drop of ferric chloride solution was then added. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout the layer.

Phenolics

Total phenolic compounds were determined using the Tannic Acid Equivalent (TAE) assay method of relative astringency of the seed extract as a direct measurement of total soluble tannin.

Antibacterial Activity

Antibacterial activity of the seed extracts were tested using the agar-well diffusion test[19]. About 0.2 ml of a 24 hours broth culture containing 1 X 10^{6} cells/ml of organism was ascetically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Mueller-Hinton agar plates. Three wells of about 6.0 mm diameter were ascetically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between

peripheral wells and the edge of the Petri dish. Fixed volumes (0.1 ml) of the extract were then introduced into the wells in the plates. A control well was put in the center with 0.01 ml of the extracting solvent. The plates were allowed on the bench for 40 minutes for pre-diffusion of the extract to occur and then incubated at 37^{o}C for 24 hours. The resulting zones of inhibition were measured using a ruler calibrated in millimeters. The average of the three readings was taken to be the zone of inhibition of the bacterial isolate in question at that particular concentration. The result is presented as mean plus standard deviation of three determinations.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacteria Concentration (MBC)

The MIC was determined using the tube dilution method. Standardized suspensions of the test organism was inoculated into a series of sterile tubes of nutrient broth containing different concentrations of leaf extracts and incubated at 37^{o}C for 24hours. The MICs were read as the least concentration that inhibited the growth of the test organisms [19].

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was sub-cultured onto extract free agar plates, incubated for another 24 hours at 37^{o}C. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony [19].

Nutritional and Mineral content Estimation

The recommended methods of the Association of Official Analytical Chemists [20], were used for the determination of moisture, protein, lipid, fiber, and carbohydrate contents respectively

Moisture

Ten grams of powdered seeds were dried in a thermostatically controlled ventilated oven at 105^{0}C until constant weight was obtained. The loss in weight was recorded as moisture content.[20]

Protein

Crude protein was determined by the Kjeldahl method[20]. Powdered seed (0.2g) was digested in 2ml concentrated H_2SO_4 in the presence of selenium catalyst, until a clear digest was obtained. The nitrogen content of digest was determined calorimetrically at 630nm. Protein was calculated as: Nitrogen content x 6.25.

Lipid

Two grams (2 g) of each of the dried samples were weighed into the porous timble of the Soxhlet extractor with the mouth plugged with cotton wool. The timble placed in the extraction chamber was suspended above the weighed receiving flask containing petroleum ether (b.p.40 60^{0}C) below the condenser. The flask was heated for eight hours to extract the crude lipid. The flask containing the crude lipid was disconnected from the Soxhlet, and then oven dried at 100^{0}C for 30 minutes, cooled in a dessicator and weighed. The difference in weight is expressed as percentage crude lipid content.

Fiber

The crude fiber was determined as the organic residue left after treating the sample under standard conditions with petroleum ether , then boiled in 1.25% H2SO 4 (w/v) and 1.25% NaOH (w/v) solutions. The residue after crude lipid extraction was used for the assay. Crude fiber content was expressed as percent loss in weight on ignition.

Carbohydrate

Available carbohydrate was calculated using the difference method, by subtracting total or sum of crude protein, crude lipid, crude fiber and ash from 100% dry weight sample [20].

Minerals

Mineral contents were obtained by ashing 2.0g dried and powdered seed sample in a muffled furnace at 550^{0}C. The ash was dissolved in 10ml, 20% nitric acid and filtered through acid washed Whatman No:541 filter paper into a

100ml volumetric flask. The filtrate was made up to the mark with de-ionized water and the resulting solution used for the analysis of Calcium, sodium, potassium, magnesium, zinc and Iron. The analyses were determined by atomic absorption spectrophotometry at 630nm [21]. All analysis was done in triplicate.

RESULTS

Results of the study are presented in tables 1 to 6. The results showed the presence of important phytochemical and nutritional constituents. It also showed significant antimicrobial activity against the organisms tested.

Table 1
Percentage yield of the crude extracts of *Perinari excelsa* seed

Extraction Solvent	Seed Powder (g)	Extracted Seed Powder (g)	Yield (%)
Aqueous	100	8.45	8.45
Ethanol	100	6.32	6.32

Table 2
Phytochemical Constituents of crude extracts of *Perinari excelsa* seeds

	Solvents (mg/ml)	
Constituents	**Aqueous**	**Ethanol**
Alkaloids	+	+
Anthraquinones	+	+
Cardiac glycosides	+	+
Flavonoids	+	+
Phenolic compounds	+	+
Saponins	+	+
Tannins	+	+

+ = Present - = Not Present.

Table 3
Proximate Composition of *Perinari excelsa* Seeds

Nutritional Property	Content (g/100g)
Moisture	67
Lipids	7.5
Protein	3.9
Carbohydrate	5.3
Fibre	2.6
Ash	0.7

Table 4
Mineral Content of *Perinari excelsa* Seeds and the recommended daily allowance in mg/100ml

Mineral	Content (mg/100ml)[A]	RDA[B] (% of RDA)
Iron	42.3	18(235)
Magnesium	12.9	400(3.23)
Calcium	14.7	1000(1.47)
Zinc	2.6	15(17)
Sodium	115.1	2300(5.0)
Potassium	81.6	3500(2.33)

[A] = This Study; [B] = Recommended daily allowance (RDA) [22].

Table 5
Antimicrobial activity of seed extract at different concentrations

Isolates	Ciprofloxacin (control) 10mg/ml	Zones of Inhibition in mm at different concentration (mg/ml)							
		30mg/ml		20mg/ml		10mg/ml		5mg/ml	
		Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
S. aureus	24.50	12.42 ±0.16	22.38 ± 0.48	9.44 ± 0.18	16.63 ± 0.10	7.33 ± 0.42	13.65 ± 0.13	4.10 ± 0.13	8.60 ± 0.15
E.coli	23.00	9.60 ± 0.25	15.28 ± 0.45	7.25 ± 0.48	11.45 ± 0.64	4.38 ± 0.19	6.30 ± 0.36	1.43 ± 0.31	2.28 ± 0.18
K. pneumonia	25.00	11.43 ± 0.08	16.77 ± 0.31	7.43 ± 0.40	12.37 ± 0.25	3.83 ± 0.25	5.77 ± 0.45	2.17 ± 0.40	3.47 ± 0.25
S. typhi	24.50	10.50 ± 0.20	15.55 ± 0.13	8.07 ± 0.25	11.93 ± 0.42	4.20 ± 0.20	6.50 ± 0.50	2.40 ± 0.26	3.40 ± 0.20

Table 6
Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of the Crude Extract in (mg/ml).

Isolates	**Solvents**			
	Aqueous		**Ethanol**	
	MIC	**MBC**	**MIC**	**MBC**
S. aureus	3.0	4.0	2.5	3.0
E.coli	4.0	5.5	4.0	4.0
K. pneumonia	4.0	4.0	4.0	5.0
S. typhi	3.5	4.0	3.0	5.0

DISCUSSION

Percentage yield of the aqueous and ethanolic extracts of the powdered seeds of *Perinari excelsa* shown in table 1, is for water 8.45% and ethanol 6.32%.Though this yield is low when compared to result of extract yield from plant sources of other authors[23,24,19], water however seem to be the best of the two solvents used in this study in terms of extract yield. This is consistent with the results of [15 and 19] who also found water to be the best of all the solvents used for their analysis.

Basically, extraction of bioactive components from medicinal plant is known to permit the demonstration of their physiological activity as well as facilitate pharmacological studies on the plant leading to synthesis and discovery of pure and potent compounds that have low toxicity when used as drugs. Result of the phytochemical screening in this study (table 2) shows the presence of some bioactive components in the seed extract. It contains alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenolic compounds, saponins and tannins. These compounds have been shown severally to be active against potentially significant pathogens including those that are responsible for enteric infections [24, 25, 15].

Apart from their potential antibacterial activity, compounds present in this study such as alkaloids are known antimalarial agents, analgesics and can act as stimulants. Glycoside moieties such as saponins, anthraquinones, cardiac glycosides and flavonoids can inhibit tumor growth, act as an antiparasitic agent, and can be used as an antidepressant[26]. Tannins are polymeric phenolic compounds which are capable of tanning leather or precipitating gelatin from solution (astringency). They are widely used in traditional medicines in treating wounds and to arrest bleeding [27]. Tannins are also known to possess general antimicrobial and active antioxidant property[28, 29]. Report also indicates that tannins have now found use in the manufacture of plastics, paints and water softening agents[30]. Though quantification of individual components was not done in this study to determine their level of presence, it was however noted that result of the analysis showed abundant presence of flavonoids and phenolic compounds.

Result of the nutritional analysis of the seeds of *Perinari excelsa* shown in table 3 indicate that the dominant constituent was moisture which accounted for 67% of their weight. Lipid content was 7.5%, protein 3.9%, carbohydrate 5.3%, while Fiber and ash were 2.6% and 0.7% respectively. Nutritionally, plant foods that provide less than 12% of its calorific value from protein are normally not considered good source of protein. Therefore Seeds of *Perinari excelsa with* 3.5% protein cannot be an important source of protein in the diet. However it should be noted that the seed is used mostly as spice and condiment. They are generally noted for their low protein content which is mostly present as enzymes, rather than as a storage pool, as seen in grains and some nuts [31]. The carbohydrate values too followed the same pattern as that of the protein and as such the plant seeds may not be an important source of carbohydrate for the body. Although the value recorded for lipid (7.5%) may be considered low, it is significant from the standpoint of its nutritional importance[32]. Oils derived from the cotyledons of such seeds have significantly high saponification value, low iodine and acid values which makes them very suitable as a good source of essential oils for the body. Such oils are equally very useful in soap making. Essential oils from the seeds could also be used for their medicinal and antioxidant values (further work on this is presently being undertaken by the authors of this study).

The metabolic functions of minerals for life are well documented in literature[33,34].

Iron is required in mammalian nutrition to prevent anemia, and is part of hemoglobin and myoglobulin molecules involved in oxygen transport to and within cells. Zinc forms metallo-proteins and enzymes complexes which cannot be dissociated without loss of activity, calcium is an important constituent of body fluids and bone formation in conjunction with phosphorus. Magnesium is an activator in enzyme systems which maintain electrical potential in nerves, while sodium and potassium influences osmotic pressure and contributes to normal pH equilibrium.

Result of the mineral analysis of the seeds as shown in table 4; indicate that iron constitute 42.3, magnesium 12.9, calcium 14.7; zinc 2.6, sodium 115.1 and potassium 81.6 all in mg/100ml. RDA values are added for comparison. The seed appear to be very rich in iron even surpassing the RDA values by more than 200%. This justifies its use by locals to treat anemic people and used extensively to prepare pepper soup for women immediately after delivery. The contributions of the other minerals studied to their total dietary requirement when compared to the RDA values appears to be very small. Thus seeds of *Perinari excelsa* seem to be a minor dietary source of these minerals. It is important to note however, that the nutritional value of spices depends not only on the concentration of nutrients in the produce, but also on the amount consumed in the diet.

Since spices are usually eaten in combination with other dietary components, some of which may be better sources of the minerals under consideration, this spice seed could be of value in supplementing the minerals available from these other sources. The fact that the seeds equally have medicinal properties also makes them an important addition to diet.

Generally, antimicrobials provide the main basis for the therapy of microbial infections, and their effectiveness depends largely on the ability of such antimicrobial compound to stop or inhibit the growth of any microorganism in the body system they infect. However the high genetic variability of microorganisms enables them to rapidly evade the action of antimicrobials by developing resistance. Result of the antibacterial activity of the seed extract of *Perinari excelsa* against four clinical isolates namely *S. aureus, E.coli, K. pneumonia, and S. typhi* at different concentration is presented in table 5. The result indicates that the seed extract is effective, though at varying degrees, to all the test organisms. This result is in agreement with that of [35] who reported that susceptibility of bacteria to plant extracts on the basis of zones of inhibition varies according to strains and species.

Both aqueous and ethanolic extracts were most effective against *S. aureus*, a gram positive bacterium than the other three bacterial isolates which are all gram negatives. This result is also in agreement with that of [36,] who gave the likely reason for the difference in sensitivity between gram positive and gram negatives to antibacterial agents to the morphological differences between these organisms. Gram negatives have an outer phospholipid membrane carrying the structural lipopolysaccharide components which makes the cell wall partially impermeable. Gram positive bacteria are thus more susceptible; having only an outer peptidoglycan layer which is not an effective permeability barrier[37].

Interestingly it is observed that at various concentrations, the ethanolic extract was more active against the test organisms than the aqueous extracts. Some researchers have reported alcohol based extracts to be more effective than water extracts[38,26, 39], while others have also reported contrary result[40,15]. Traditionally, plant parts are soaked in water and alcohol based solvents for days before they are administered. The result from this study therefore, lends credence to the folkloric use of these solvents in traditional medicine as extracts from both solvents were highly

effective against all the test organisms. The fact too that the extracts showed a broad spectrum of activity is significant in the drive to developing therapeutic substances against multidrug resistant organisms.

Minimum inhibitory concentration (MIC) and Minimum bacteria concentration (MBC) values from the analysis is reported in table 6. MIC for aqueous extract ranged between 13.0 and 14.0 mg/ml while the ethanolic extract ranged between 12.5 and 14.0 mg/ml. The MBC obtained from the study was between 14.0 and 15.5mg/ml for aqueous extract and that for the ethanolic extract between 13.0 and 15.0mg/ml. These figures when compared to that normally obtained for conventional antibiotics can be said to be reasonable enough to warrant its possible pharmacological significance.[15], indicated that such observed differences could be due to the fact that synthetic antibiotics are in a pure form, whereas crude plant extracts contains some impure substances that may be inert and do not have any antibacterial activity. MBC values can either be the same or higher than the MIC values. In this study, the MBC values were either the same or slightly higher than the MIC values. MIC and MBC values are .

predictive indices of the efficacy of antimicrobial agents [41].

CONCLUSION

Conclusively, this study has provided some preliminary evidence of positive antimicrobial activity of the seed extract of *Perinari excelsa* against important infectious agents. Significant number of useful bioactive components was found to be present also in the extract. Nutritionally, result from this study justifies the long and continuous use of the seed as both a spice and food component by locals. Therefore further studies on the plant seed in order to isolate, identify and characterize the active components to maximize the potential of this useful seed are necessary today.

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