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IN-VITRO ANTIFUNGAL ACTIVITY OF LEAF, STEM BARK AND ROOT EXTRACTS OF *Irvingia gabonensis* AGAINST FUNGI ASSOCIATED WITH ITS SEED DETERIORATION

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ABSTRACT

Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) Baill seeds are often contaminated in storage by postharvest spoilage fungi. This study investigated the nutritional contents of the seeds, phytochemical contents and antioxidant activity of different parts of the plant as well as their antifungal effects against fungi isolated from the deteriorated seeds. Proximate, mineral and phytochemical analyses were carried out using standard laboratory techniques. The antioxidant activity was against DPPH radicals. Ethanol extracts (100 and 200 mg/ml) of the various parts were screened against the isolates using pour plate method in Potato Dextrose Agar (PDA) plates. The plates were examined for growth daily for 10 days. Data were statistically analysed. The healthy seeds contained higher protein (13.73%), fat (58.73%) and fibre (2.50%), whereas, higher content of moisture (10.17%) and carbohydrate (15.47%) were observed in the spoilt seeds. The leaf had highest contents of alkaloids (1228.3 mg/100 g), flavonoids (1345.9 mg/100 g), tannins (1345.9 mg/100 g), saponins (578.3 mg/100 g) and carotenoids (2348.3 μg/100 g). The antioxidant activity of the leaf (50.30%) was the highest. Fungi isolated from the deteriorated seeds were *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Curvilaria lunata*. The leaf was most effective against *A. niger, A. flavus* and *C. lunata* whereas the stem bark extract was most effective against *A. fumigatus*. The F-values were significant for model, treatment, concentration and number of days for the leaf and root. This study confirmed the antifungal activity of extracts of various parts of the plant against isolates implicated in the deterioration of its seeds. The extracts could be used as preservative to enhance the shelf-life and nutritional quality of the seeds.

Keywords: *Irvingia gabonensis*, Spoilage of seeds, Antifungal activity, Free radical scavengers, Preservatives.

INTRODUCTION

Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) Baill belongs to the family Irvingiaceae, it is commonly known as Wild mango, African mango, Bush mango, Dika or Ogbono, it is a species of African trees and grows at altitudes from 200-500 m (660-1640 ft). *I. gabonensis* is an economic and nutritional plant (Ejiofor, 1994). The seeds are used as fodders for cattle cake in Ghana, fruits also as food for pigs (Ayuk *et al*., 1999). The wood is used locally for heavy construction work and for making ships' decks, paving blocks and planking. Young trees are used for making poles and stakes, while branches are made into walking sticks or thatched roof supports. Dead branches are used as firewood (Tchoundjeu and Atangana, 2007). It contains tannins in both bark and roots therefore useful in dye making. In Nigeria, the kernels are used as a condiment and are highly valued for their food thickening properties (Ndjouenekeu *et al*., 1996) in preparing "Ogbono" or draw soup. The kernels of *I. gabonensis* are widely marketed in Cameroon (Ndoye *et al*., 1997) and form an important diet, providing carbohydrate, oil, and protein, to enhance health and nutrition.

Ethnomedicinal treatments utilise the bark, kernels, or roots for a variety of ailments (Lowe *et al*., 2000). *I. gabonensis* is used in the treatment of hernia, diarrhoea and obesity. It is valuable as antimicrobial, purgative and aphrodisiac. A major lapse in the sales and consumption of *I. gabonensis* kernels is the susceptibility to postharvest spoilage fungi with their health risk. Several studies have shown that *I. gabonensis* kernels displayed on shelves for sale in Nigeria markets are often contaminated with spoilage fungi (Iyayi *et al*., 2010; Frimpong *et al*., 2019). Furthermore, studies carried out by (Adebayo-Tayo *et al*., 2006) showed that fungal-contaminated *I. gabonensis* kernels are potentially harmful to those who consume them, as the fungal-contaminated kernels possess aflatoxin. Aflatoxins are carcinogenic substance produced by the fungi *Aspergillus flavus* and *A. parasiticus* (Wuand and Khlangwiset, 2010). Consumption of high levels of aflatoxin in food has been reported to have caused illnesses among several hundreds of Kenyan in 2004, and leaving

125 people dead (Lewis *et al*., 2005).

Reducing postharvest losses is very important; ensuring that sufficient food, both in quantity and in quality is available to every inhabitant in our planet. Preservatives may be added to prevent the growth of fungi and bacteria which could be antimicrobial preservatives. Traditional methods of preservation usually aim to exclude air, moisture and microorganisms or to provide environments in which organisms that might cause spoilage cannot survive (Abdulmumeen and Sururah, 2012). Effects of food preservatives such as sodium benzoate, sodium nitrite and sulphur dioxide on human health can vary with age and health status, the side effects include cancer, heart damage, asthma, behavioural changes, headache, acidity, increase in blood pressure, dermatological reactions and allergic reactions (Gomaa *et al*., 2013; Sarac and Sari, 2019).

The demand on this plant-based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, non-narcotic, easily biodegradable, possess minimum environmental hazards, have no adverse side-effects and are easily available at affordable prices (Kannan *et a*l., 2009; Thomas-Charles and Fennell, 2019). Higher plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity known as secondary metabolites (Wang *et a*l., 2019). Most phytochemicals have antioxidant activity and protect the cells against damage and reduce the risk of developing certain types of cancer.

In view of postharvest spoilage of the seeds and reported side effects of synthetic preservatives, this work was designed to study the nutritional contents of healthy and spoilt seeds, analyse the leaf, stem bark and root for phytochemical composition and antioxidant activity, as well as screen the extracts of the various parts against fungal isolates that are associated with the deterioration of *I. gabonensis* seeds. This was with a view to presenting the extracts of its various parts as preservatives for the seeds, with the hope that the extracts might improve the shelf-life of the seeds.

MATERIALS AND METHODS

Preparation of Medicinal Plant Materials

The healthy seeds of *Irvingia gabonensis* were purchased from the market in Ibadan, Oyo state, Nigeria. The stem barks and roots were collected from the premises of the University of Ibadan, Nigeria. The samples were identified and authenticated at the University of Ibadan Herbarium (UIH - 23067). The plant materials were then thoroughly washed and air-dried at room temperature for three weeks. They were ground into powder and the powdered materials were stored (4 ^{o}C) in bottles for further use.

Proximate Analysis of *Irvingia gabonensis* Seeds

The proximate composition of the seeds was determined using the standard methods of analysis described in the (ASEAN, 2011). The ash content was determined using a muffle furnace set at 550 ^{o}C for 4 hours. Crude fibre was determined using enzymatic-gravimetric method and % carbohydrate was calculated by subtracting the sum values of the other nutrients from 100. Crude fat was determined by Soxhlet extraction method using petroleum ether as extracting solvent. Moisture content of the samples was determined by air oven (Gallenkamp) method at 105 ^{o}C. The crude protein of the sample was determined using micro-Kjeldahl method.

Mineral Analysis of *Irvingia gabonensis* Seeds

The dry seeds were digested using 5 ml of concentrated HNO_3 and 1 ml of concentrated $HCIO_4$. The mineral analysis of the samples was carried out after wet digestion of samples. The methods described in ASEAN (2011) were used for the analysis. After digestion, potassium, sodium, calcium, magnesium, iron, copper and zinc were analysed using atomic absorption spectrophotometer (FC210/211 VGP Bausch scientific AAS). Phosphorus was determined using Vanadomolybdate (Yellow method) and percentage transmittance was read at 400 nm using Spectronic 20 (Bausch and Lomb) Colorimeter.

Phytochemical Analysis of Leaf, Stem Bark and Root of *Irvingia gabonensis*

The quantitative screening of samples for alkaloids flavonoids, saponins, tannins, carotenoids, proanthocyanidins contents using

standard procedures were as follows:

Alkaloids

The method of Harborne (2005) was used for the analysis of alkaloids in samples. 200 ml of 10 % acetic acid in ethanol was added to 5.0 g of powdered sample in 250 ml beaker. The mixture was covered and allowed to stand for 4 h. The mixture was filtered and the filtrate was concentrated on a water bath to one-fourth of its original volume, concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until precipitation was completed and the solution was allowed to settle. The precipitate collected was washed with dilute NH_4OH and then filtered. The residue was dried and weighed. The alkaloid content was calculated using the formula:

% alkaloid = (Final weight of the sample / Initial weight of the extract) x 100.

The experiment was replicated three times. The result was expressed as mg/100 g.

Flavonoids

The method of Ordonez *et al*, (2006) was used for the flavonoids analysis of the samples. The sample (0.5 ml) was added to 0.5 ml of 2% $AlCl_3$ ethanol solution. The mixture was allowed to stand for 1 h at room temperature and a yellow color indicated the presence of flavonoids. The absorbance was read at 420 nm. The samples were evaluated at a final concentration of 0.1 mg/ml. The result was expressed as mg/g using the equation:

$$Y = 0.0255x, \quad R^2 = 0.9312,$$

where x is the absorbance and Y is the quercetin equivalent. The experiment was replicated three times. The result was expressed as mg/100g.

Saponins

The powdered sample (20 g) was added to 100 ml of 20% aqueous ethanol and kept on a shaker for 30 min. The sample was heated on a water bath for 4 h at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 ml on a water bath at 90 °C. The concentrate was transferred into a 250 ml separating funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 ml n-butanol was added. Then n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride (NaCl). The remaining solution was heated on a water bath. After evaporation, the sample was dried in the oven at 40 °C to a constant weight (Harborne, 2005). The saponin content was calculated and expressed as mg/100 g.

Tannins

The sample (0. 20 g) was added to 20 ml of 50% methanol. The mixture was shaken thoroughly and placed in a water bath at 80 °C for 1 h to ensure uniform mixing. The mixture was filtered into a 100 ml volumetric flask, followed by the addition of 20 ml of distilled water, 2.5 ml of Folin-Denis reagent and 10 ml of 17% aq. Na_2CO_3. The solution was thoroughly mixed. The mixture was made up to 100 ml with distilled water, mixed and allowed to stand for 20 min. The bluish-green colour developed from the reaction mixture of different concentrations (0-10 ppm). The absorbance of the tannic acid standard solutions as well as sample was read at 760 nm using the spectrophotometer (Harborne, 2005). The results were expressed as mg/g of tannic acid equivalent using the calibration curve:

$$Y = 0.0593x - 0.0485, R^2 = 0.9826,$$

where x is the absorbance and Y is the tannic acid equivalent. The experiment was replicated three times. The result was expressed as mg/100 g.

Carotenoids

The sample (1 g) was weighed into 20 ml acetone and left for 1 hour, filtered and mixed with 10 ml of water. The filtrate was poured into separating funnel and 5 ml of petroleum ether was added to the funnel allowing it to flow into it by the side of the funnel and left it for some minutes to separate, the lower layer was discarded and the absorbance was measured at 440 nm and read off a standard graph. The result was expressed as µg/100 g (ASEAN, 2011),

Proanthocyanidins

The extract (0.5 ml of 1 mg/ml) solution was mixed with 3 ml of vanillin-methanol (4% v/v) and 1.5 ml of hydrochloric acid. The mixture was left for 15 min at room temperature and the absorbance was read at 500 nm (Sun *et al*., 1998). The result was expressed as catechin equivalent (QE/g) using the calibration curve equation:

$Y = 0.5825x, R^2 = 0.9277$,

where x is the absorbance and Y is the quercetin equivalent. The experiment was replicated three times. The result was expressed as mg/100 g.

Antioxidant Activity of Leaf, Stem Bark and Root of *Irvingia gabonensis* using DPPH Assay

The powdered sample (500 g) was extracted in 100 ml of 80% ethanol using cold extraction method. The extract was concentrated using a rotary vacuum evaporator at 40 °C. The extract of each sample was prepared at 100 mg/ml; 0.2 ml of the extract solution was added to 2.8 ml of freshly prepared 20 mg/dm^3 of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) in methanol. The mixture was then incubated for 30 mins in the dark at room temperature. Methanol only and DPPH in methanol were used as the control. Absorbance was read at 517 nm using UV/Vis spectrophotometer (Brand-Williams *et al.*, 1995). The scavenging activity of plant extract was calculated as follows:

$$\% \text{ DPPH Inhibition} = (1-A_{sample}/A_{control}) \times 100$$

Where: $A_{control}$ = Absorbance for control; A_{sample} is Absorbance for sample

Preparation of Extracts of Leaf, Stem Bark and Root of *Irvingia gabonensis*

The powdered samples -leaf, stem bark and root, (500 g) were extracted separately in 1.5 litre of ethanol (95%) for 6 hours using Soxhlet apparatus. The extract was transferred into sample holder of the rotary vacuum evaporator, where it was concentrated to dryness at 40 °C and then air dried to constant weight. The extract was then refrigerated at 4 °C prior to use. A 2 g portion of the extract was reconstituted in 10 ml sterile distilled water to obtain a concentration of 200 mg/ml, while 1 g of the extract was reconstituted in 10 ml of sterile distilled water to give 100 mg/ml. The extracts were used for the antifungal assay.

Induction of Spoilage and Isolation of Fungi from Seeds of *Irvingia gabonensis*

Fungi intended for this study were isolated from diseased seeds of *I. gabonensis* that were collected from four different markets in Ibadan. Spoilage was induced by adding sterile distilled water in an air tight bottle. Noticeable changes were seen on the seed after 5 days to show spoilage. Different fungi were isolated from the spoilt seeds and inoculated on Potato Dextrose Agar (PDA) which was prepared, autoclaved at 121 °C for 15 minutes. It was allowed to cool slightly before the addition of lactic acid so as to prevent bacterial contamination. Then, the agar was poured aseptically into the Petri dish beside a Bunsen flame. Plate was incubated at 25 °C for 5-7 days and observed daily for fungal growth. To obtain a pure culture, each fungal culture was repeatedly sub cultured into fresh PDA plates until each plate contained one type of fungal isolate. The resulting pure cultures were identified at the Pathology Laboratory of the University of Ibadan, Nigeria.

Antifungal Assay of Various Parts of *Irvingia gabonensis*

The antifungal activity of the plant extracts was examined on the isolated fungi at concentrations of 100 mg/ml and 200 mg/ml. 1 ml of each extract concentration was first pipetted into a sterile Petri dish, after which 15 ml of Potato Dextrose Agar (PDA) was poured into the Petri dish. The Petri plates were agitated and allowed to cool and gel. Each test fungal isolate was inoculated onto the surface of the medium using an inoculating needle and incubated at room temperature (28 °C). Each treatment was replicated 3 times. The plates were observed and the mycelia growth was taken daily for 10 days of incubation using a meter rule for each concentration. Each of the organisms was also cultured on PDA plate alone as control experiment.

Statistical Analysis

The data obtained were analysed using SAS version 9.3 and means were separated using Duncan Multiple Range Test (DMRT) at $p \leqslant 0.05$.

RESULTS AND DISCUSSION

The nutritional profiles of healthy and spoilt seeds of *I. gabonensis* are shown in Table 1. The healthy seeds had higher protein (13.73%), fat (58.73%) and crude fibre (2.50%) than the spoilt seeds. The spoilt seeds had higher moisture content (10.17%), ash (3.67%) and carbohydrates (15.47%). In this study, the healthy seeds of *I. gabonensis* were more nutritious than the spoilt seeds, they were richer in protein, fat, and crude

fibre compared to the spoilt seeds. On the other hand, the spoilt seeds had higher moisture, ash and carbohydrate. The proximate components of *I. gabonensis* are comparable to the reports of previous authors (Sahoré *et al*., 2012; Aremu *et al*., 2014; Mgbemena *et al*., 2019). Healthy *Irvingia* seeds can be considered a good source of protein which is used by the body to build and repair tissues. Crude fat is needed for energy and crude fibre for reducing blood pressure and inflammation. As moisture content in plants enhances deterioration, the high moisture content of the seeds could be responsible for spoilage. Consumption of healthy seeds could be a good source of protein, fat and crude fibre for healthy growth (Sahoré *et al*., 2012).

The mineral components of the healthy and spoilt seeds of *I. gabonensis* are presented in Table 2. The healthy seeds contained iron (10.50 mg/100 g), zinc (0.53 mg/100 g), copper (0.30 mg/100 g) and potassium (36.67 mg/100 g), whereas the spoilt seeds had higher sodium (851.67 mg/100 g), calcium (266.67 mg/100 g), phosphate (199.67 mg/100 g) and magnesium (165.00 mg/100 g). The healthy seeds were richer in potassium, zinc, copper, and iron than the spoilt seeds. This is in line with the report of Ndamitso *et al.* (2012) on the mineral content of *I. gabonensis*, which showed that it was higher in copper and zinc. The consumption of the healthy seeds could be valuable in the management of anaemia, immune system building, promotion of healthy growth in childhood, healing wounds, diarrhoea and decrease risk of age related chronic diseases, helps maintaining healthy bones, prevents cardiovascular disease, reduction of blood pressure and protection against stroke (potassium). Overall, minerals are needed by humans to perform functions necessary for life (Ndamitso *et al*., 2012).

Table 3 shows the phytochemical contents of various parts of *I. gabonensis*. The phytochemical components of leaf, stem bark and root varied significantly. The alkaloids, flavonoids, saponins, tannins and carotenoids were highest in the leaf, also alkaloids, flavonoids; proanthocyanidins were higher in the stem bark than the root. However, the root had higher contents of saponins, tannins and carotenoids than the stem. The variation in proanthocyanidins content was in the order: stem bark > root > leaf, whereas, anthraquinones content was in the order: stem bark > leaf > root. The plant parts contained alkaloids, anthraquinones, carotenoids, flavonoids, proanthocyanidins, saponins and tannins in varied concentrations. Leaf was the richest in all the phytochemicals tested in this study. It contained alkaloids (1228.3 mg/100 g), flavonoids (1345.9 mg/100 g), saponins (578.3 mg/100 g), tannins (773.3 mg/100 g) and carotenoids (2348.3 µg/100 g), meaning that the leaf could be used for the treatment of an array of diseases. As examples, it could be used in the treatment of blood related diseases (carotenoids and flavonoids), malaria (alkaloids), infections (alkaloids and tannins) and cancer (saponins). The stem bark could be used for laxative effect with its high content of anthraquinones. This is in line with the findings of Fadare and Ajaiyeoba (2008) and Etebu (2012) that reported the presence of these phytochemicals in the *I. gabonensis* seeds. Phytochemicals have been reported to be responsible for plant bioactivity (Adedapo *et al.,* 2009; Khan *et al.,* 2019).

The total polyphenol content and the antioxidant activity of the leaf, stem bark and root are shown in Table 4. There was correlation between polyphenol contents and antioxidant activity, the higher the polyphenol content, the higher the antioxidant activity. Overall, polyphenol contents and antioxidant activity were in the order: leaf > root > stem bark. The various parts of *I. gabonensis* showed varied antioxidant activity against DPPH radicals; the leaf (50.30%) had the highest antioxidant activity, followed by the root (42.37%) and the stem bark (36.17%) had the least. There was correlation between the antioxidant activity and total polyphenol contents of various parts i.e. the higher the polyphenol content the higher the antioxidant activity. However, the total polyphenol and antioxidant content of *I. gabonensis* seeds as reported by Boakye *et al.* (2015) was given as 20.95 mgGAE/100 g and 78% respectively. As there is scarcity of information on the antioxidant activity and total polyphenol contents of the stem bark, root and leaf of *I. gabonensis*, this study provides additional information on antioxidant activity of the plant parts. Polyphenol has protective effects against cardiovascular diseases

and could serve as anticancer, antidiabetics and antiaging (Fraga *et al*., 2019). Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and altitude sickness (Baillie *et al*., 2009). Antioxidants are additives capable of delaying or preventing rancidity of food due to oxidation and therefore lengthen the shelf life of products (Kebede and Admassu, 2019). The antioxidant activity of various plant parts could be responsible for the observed antifungal activity against isolated organism from the deteriorated seeds.

The antifungal activity of the stem bark extract of *I. gabonensis* against *A. niger, A. flavus, A. fumigatus* and *C. lunata* was highly significant (P>0.0001) for the model, treatments and number of days of incubation. However, the concentrations used were not significant (P>0.151) (Table 5). Table 6 shows the antifungal effect of the root extracts against *A. niger, A. flavus, A. fumigatus* and *C. lunata* and was significant (P>0.0001). The effect of *I. gabonensis* leaf extract on *A. niger, A. flavus, A. fumigatus* and *C. lunata* (Table 7), the F-values for models, treatments, and days of incubation (P>0.0001) and concentration used (P>0.0014) were all highly significant.

The cumulative antifungal effect of all extracts against all organisms is presented in Table 8. The leaf extract was most effective on *A. niger, A. flavus* and *C. lunata* whereas the stem bark was most effective against *A. fumigatus*. All extracts showed significant (p≤0.05) activity compared to the control experiments. The various parts of *I. gabonensis* displayed varied antifungal activity. Antifungal activity of the leaf and stem bark was significant (P>0.0001) for the model, treatment, number of days and concentration whereas, the root showed antifungal effect that was significant (P>0.0001) for the model, treatment and number of days. Cumulatively, the leaf extract was most effective on *A. niger, A. flavus and C. lunata* whereas the stem bark extract was most effective on *A. fumigatus*. Overall, *A. fumigatus* was most susceptible to all extracts followed by *A. flavus, A. niger and C. lunata* was the least susceptible. The susceptibility of the organisms was in the order of *A. fumigatus* > *A. Flavus* > *A. niger* > *C. lunata*.

The effect of the treatment on daily growth of fungi is presented in Table 9, although there was no significant difference in the antifungal activity of all extracts. However, the stem bark extract was the most effective. In Table 10, 100 mg/ml extract concentration was more effective than 200 mg/ml against organisms. The incubation days (10 days) reflected increased growth along increased number of days; however the extract showed significant antifungal activity on the 10^{th} day compared to the control experiment. Of the two concentrations used in this work, 100 mg/ml displayed higher antifungal activity than 200 mg/ml.

Table 1: Proximate Contents of Healthy and Spoilt Seeds of *Irvingia gabonensis*

Parameters (%)	Seeds	
	Healthy	Spoilt
Moisture	8.7 ±0.1	10.17±0.15
Protein	13.73±0.15	11.63±0.47
Ether Extract (Fat)	58.73±0.15	57.07±0.06
Ash	3.17±0.15	3.67±0.15
Crude Fibre	2.50±0.10	2.00±0.10
Carbohydrates	13.17±0.40	15.47±0.35

Legend: Mean ± Standard Deviation, n=3

Table 2: Mineral Components of Healthy and Spoilt *Irvingia gabonensis* Seeds.

Parameters (mg/100g)	Seeds	
	Healthy	Spoilt
Iron (Fe)	10.50±0.10	10.20±0.10
Zinc (Zn)	0.53±0.06	0.33±0.06
Copper (Cu)	0.30±0.10	0.23±0.06
Sodium (Na)	835.00±5.00	851.67±7.64
Potassium (K)	36.67±2.89	31.67±2.89
Calcium (Ca)	253.33±7.64	266.67±10.41
Phosphate ($PO_{4)}$	188.33±7.64	191.67±2.89
Magnesium (Mg)	160.00±5.00	165.00±10.00

Legend: Mean ± Standard Deviation, n=3

Table 3: Phytochemical Components of Various Parts of *Irvingia gabonensis*

Phytochemicals	Plant Parts		
	Leaf	Stem bark	Root
Alkaloids (mg/100 g)	1228.3±12.6^{c}	955.0±15.0^{a}	251.7±18.9^{b}
Flavonoids (mg/100 g)	1345.9±22.9^{c}	358.3±7.6^{a}	218.3±7.6^{b}
Saponins (mg/100 g)	578.3±12.58^{b}	245.0±18.03^{a}	258.3±29.30^{a}
Tannins (mg/100 g)	773.3±24.66^{c}	433.3±10.41^{a}	545.0±13.23^{b}
Anthraquinones (mg/100 g)	46.7±2.89^{a}	26.7±2.89^{b}	43.3±2.89^{a}
Carotenoids (µg/100 g)	2348.3±12.6^{c}	226.7±16.1^{a}	276.7±10.4^{b}
Proanthocyanidins (QE/g)	0.8±0.29^{c}	2.7±0.29^{a}	1.7±0.29^{b}

Legend: Means with the same alphabets within a row are not significantly different at p≤0.05
Mean ± Standard Deviation, n=3

Table 4: Total Polyphenol and Antioxidant Activity of *Irvingia gabonensis*

Plant parts	Total polyphenols (mgGAE/g)	% inhibition of $DPPH^{+}$
Stem bark	23.53±0.21^{b}	36.17±0.15^{b}
Leaf	45.17±0.15^{c}	50.30±0.17^{c}
Root	36.87±0.15^{a}	42.37±0.21^{a}

Legend: Means with the same alphabets within a column are not significantly different at p⩽0.05.
Mean ± Standard Deviation, n=3

Table 5: ANOVA Table for the Effect of *Irvingia gabonensis* Stem Bark Extract on the Isolated Fungi

Source	**DF**	**SS**	**MS**	**F value**	**Pr> F**
Model	19	4875.82	256.62	66.97	0.0001**
Treatments	7	2766.91	395.27	103.15	0.0001**
Days	9	2083.88	231.54	60.42	0.0001**
Concentrations	1	22.79	22.79	5.95	0.151
Error	460	1762.70			
Total	479	6638.52			

Legend: **= highly significant
Abbreviations: DF = Degree of Freedom, SS: Sum of Squares, MS: Mean Square.

Table 6: ANOVA Table for the Effect of *Irvingia gabonensis* Root Extract on the Isolated Fungi.

Source	**DF**	**SS**	**MS**	**F value**	**Pr>F**
Model	19	4950.66	260.56	76.02	0.0001**
Treatments	7	2506.89	358.13	104.48	0.0001**
Days	9	2303.15	255.91	74.66	0.0001**
Concentrations	1	95.05	95.05	27.73	0.0001**
Error	460	1576.74	3.43		
Total	479	6527.39			

Legend: **=highly significant
Abbreviations: DF = Degree of Freedom, SS: Sum of Squares, MS: Mean Square.

Table 7: ANOVA Table for the Effect of *Irvingia gabonensis* Leaf Extract on the Isolated Fungi

Source	**DF**	**SS**	**MS**	**F value**	**Pr>F**
Model	19	4914.03	258.63	71.96	0.0001**
Treatments	7	3061.70	437.39	121.69	0.0001**
Days	9	1779.83	197.76	55.02	0.0001**
Concentrations	1	37.02	37.02	10.30	0.0014**
Error	460	1653.35	3.60		
Total	479	6567.39			

Legend: **= highly significant
Abbreviations: DF = Degree of Freedom, SS: Sum of Squares, MS: Mean Square.

Table 8: Diametric Growth of the Isolated Fungi on PDA Impregnated with *Irvingia gabonensis* Extracts

	Means of fungal growth (cm)		
Treatments (Fungi)	Leaf	Stem bark	Root
A. niger	2.19[e]	3.68[e]	3.56[d]
A. fumigates	1.08[f]	0.55[f]	0.91[f]
A. flavus	1.10[f]	1.19[f]	1.65[e]
C. lunata	4.08[d]	4.47[d]	4.94[c]
Control 1 (*A. niger* only)	8.24[a]	7.85[a]	7.89[a]
Control 2 (*A. fumigatus* only)	6.67[b]	6.75[b]	6.69[b]
Control3 (*A. flavus* only)	6.23[b]	5.93[c]	6.16[b]
Control 4 (*C. lunata* only)	5.35[c]	5.30[c]	5.37[c]
$LSD_{0.05}$	0.68	0.70	0.66
R^2	0.75	0.73	0.76

Legend: Each value is a mean of three replicates. Means with different letters across the column are significantly different from each other

Table 9: Daily Growth of the Isolated Fungi on PDA impregnated with Extracts of Certain Parts of *Irvingia gabonensis*

	Means of fungal growth (cm)		
Incubation days	Leaf	Stem Bark	Root
10	6.80[a]	6.68[a]	7.10[a]
9	6.60[a]	6.53[a]	6.88[a]
8	5.58[b]	6.38[a]	6.48[ab]
7	5.37[b]	6.08[ab]	6.28[cb]
6	5.26[bc]	5.46[cb]	5.61[cd]
5	4.60[dc]	4.74[c]	4.89[ed]
4	4.04[d]	3.96[d]	4.37[e]
3	3.19[e]	2.76[e]	2.84[f]
2	1.41[f]	1.30[f]	1.27[g]
1	0.81[f]	0.77[f]	0.75[g]
Control	9.00[g]	9.00[g]	9.00[h]
$LSD_{0.05}$	0.76	0.79	0.74
R^2	0.75	0.73	0.76

Legend: Means with different letters across the column are significantly different from each other. $p \leq 0.05$

Table 10: Inhibitory Effects of Two Different Concentrations of Extracts from Parts of *Irvingia gabonensis* on the Isolated Fungi

	Means of fungal growth (cm)		
Concentrations	Leaf	Stem Bark	Root
200 mg/ml	4.64[a]	4.68[a]	5.09[a]
100 mg/ml	4.09[b]	4.25[b]	4.20[b]
$LSD_{0.05}$	0.34	0.35	0.33
R^2	0.75	0.73	0.76

Legend: Means with different letters are significantly different from each other

CONCLUSION

The healthy seeds were more nutritious than the spoilt seeds. The proximate and mineral composition of the healthy seeds could be responsible for the medicinal uses of the seeds in cancer, obesity, hypertension, diabetes and diarrhoea. Extracts of the leaf, stem bark and root showed significant antifungal activity against fungi associated with seed deterioration. The observed antifungal activity of the plant could be attributed to the antioxidant activity and phytochemical contents especially in the leaf. Based on this study, *I. gabonensis* leaf, stem bark and root extracts could be said to have a potential use as preservative for its seed. This could be a better alternative to synthetic fungicides that have been reported to be toxic and carcinogenic.

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