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ASSESSMENT OF AVOCADO (*Persea americana* MILL) SEED COMPOSITION AND IMPACT OF FUNGAL PATHOGENS ON THE FRUIT ROT AND SEED NUTRITION

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SUMMARY

The growing trend of consuming raw avocado seeds, framed within sustainability and the principles of a circular economy, has prompted evaluation of avocado seeds nutritional value and potential to valorise the waste. This study evaluated the antinutrients of the avocado seed, assessed the fungi associated with avocado fruit deterioration, and the pathogenicity of the isolates. The antifungal activity of avocado seed extract, and the effects of the pathogens on the healthy and unhealthy seed proximate compositionsv were determined. The results showed the percentage occurrence of fungi on avocado fruits, including *Aspergillus niger* (28.42 %), *A. flavus* (26.50 %), *Rhizopus stolonifer* (18.30 %), *Botryodiplodia theobromae* (14.35 %), and *Fusarium oxysporum* (12.43 %). Pathogenicity tests showed that all isolates except *A. niger* caused disease symptoms on inoculated fruit, including soft rot and browning. The strongest mycelial radial growth inhibition against the pathogens wasat 25mg/mL, seed extract concentration, with inhibition values for *A. flavus*(18.27mm),*R. stolonifer*(20.93mm), *F. oxysporum* (19.63mm), and *Lasiodiplodia theobromae* (23.33mm). Mancozeb produced stronger inhibition against the pathogens, than the extract. Healthy seeds had substantially higher proximate values (g/100 g) than diseased seeds: carbohydrate 45.74 vs 11.38, crude fat 23.00 vs 5.33, protein 14.15 vs 3.45, ash 1.66 vs 0.34, and fibre 1.77 vs 0.35; moisture was higher in diseased seeds (17.34% vs 14.50%). Oxalate (5.11 mg/100 g) was the predominant antinutrient; phytate was lowest (0.31 mg/100 g). These findings demonstrate the potential of avocado seed as a sustainable and eco-friendly solution for post harvest protection and waste valorisation.

Keywords: avocado seed, Antifungal, antinutrient, proximate, fungi.

INTRODUCTION

Avocado (*Persea americana*Mill.) is one of the most widely consumed and tropical fruits, with demand growing exponentially over the last decade (1). The increase in avocado fruit consumption and industrial use has contributed to a vast amount of peels and seeds waste products, causing environmental pollution and human health problems (2;3). Global avocado production in 2022 was approximately 8.98 million metric tons, representing an increase of 1.05 million metric tons compared to 7.93 million metric tons totalin2021(4).

The annual seeds and peel production ismorethan 1.2 milliontons(5). Different parts of the avocado pear have been reported to be used in traditional medications for various purposes, including antimicrobial (6; 7). Avocado

seed has been reported to be used in ethnomedicine for the treatment of diarrhoea, dysentery, toothache, intestinal parasites,and skin treatment(8). Avocado seed extracts also have many health-related bioactive properties, such as antihypertensive, anti-hyperglycaemic, anticancer, anti-hypercholesterolemia, antioxidant, anti-inflammatory,analgesic effects, and anti-neurogenerative effects (9;10). The seed harbours bioactive agents, such asphenolics, fatty acids, and other constituents (10), that can add value to food, feed, and pharmaceutical applications. However, several by-products are generated by the avocado sector, which could be exploited by industries in beneficial ways to contribute to the zero-waste target as well as the circular economy idea proposed in the Sustainable Development Goals (SDGs), which seek the reduction of poverty, the elimination of hunger, the protection of the planet, and sustainable agriculture (11).

However, avocado seeds' antinutrient constituent and basic proximate composition of healthy and diseased avocado seed, remain incompletely characterised.

This study therefore identified the fungal pathogens responsible for the seed deterioration; assessed the antifungal activity compared the healthy and diseased seed's proximate composition; identified the antinutrient composition. By combining the seed compositional analysis, and pathogen testing, the work clarifies how intrinsic seed chemistry influences susceptibility to rot and highlights opportunities to valorise avocado seed biomass while managing postharvest losses.

MATERIALS AND METHODS

Location of the study

The study was conducted in the Departments of Plant Science and Biotechnology laboratory, and Nutrition and Dietetics, Imo State University, Owerri, situated in the humid rainforest ecosystem of Nigeria, located from longitude 3°E to 12°E and latitude 4°N to 9°N. Farmers predominantly inhabit this region, and the ecosystem is known to contribute to the growth of the Avocado pear.

Collection and preparation of fresh Avocado seed

Thirty healthy unriped avocado pear (*P. Americana*Mill) fruits were randomly, purchased fromfive traders, by lottery method in Relief market in Owerri municipal Council city, Imo State, Nigeria, and securely sealed polythene bag, and brought to the laboratory.Cities have served as major outlets for fruits, including avocado pear commercialization whereas the consumption fuels the expansion in its production. Personal communications with the market traders confirm that avocado pear were sourced from the foremost production locations, round the year.

According to the methodology outlined by (12), with slight modifications, five avocado seeds were surface sterilized with sodium hypochlorite, followed by rinsing with sterilized distilled water, sliced and dried at room temperature, and then blended to powder. Then, 500g of the avocado sterilized powder was extracted using methanol (80%),(to extract both polar and nonpolar compounds) and filtered with filter paper.Then 20g of the filtrate was subsequently dissolved in 250 mL of a 20% aqueous methanol solution (v/v) to create a stock solution. This stock solution was serially diluted with sterile distilled water to

obtain concentrations, 5,10,15,20,25(mg·mL^{-1}). The sterilized specimen was stored preserved inside the refrigerator, for further use.

Isolation and identification fungi associated with avocado fruits rot disease

The fungi were isolated from fruits that were left for five days to over ripe and subsequently identified based on their morphological and microscopic features attributes (colony color and texture, septation of hyphae, spore shape and colour, and conidiophore structure(13).

Pathogenicity test

The identified fungi were inoculated on healthy avocado fruits through the use of small sterile knife to induce wound in the plant tissue, where the spore suspension is placed(14) and were examined for their ability to initiate decay. The pathogenicity of each fungus on the inoculated samples was determined by measuring the length and width of rotten parts at two-day intervals for ten days, according to the procedure of (15).

Assessment of antifungal activity of the seed extracts

The isolated fungal pathogens were cultivated on Potato Dextrose agar(Oxoid, UK), at room temperature for24 hours. Then the colonies were resuspended in test tubes containing 5 mLof Potato Dextrose Broth. Then 5g of the avocado seed extract was diluted in test tubes containing 1 mL of brothat concentrations(5, 10, 15, 20, and 25mg/ml), as outlined by (16), with slight modifications. Mancozeb served as positive control, while DMSO served as negative control. The experiments were replicated three times. The plates were incubated at a25–30°C for 24–72 hours.The antifungal activity was evaluated by determining the diameter of the inhibitory zone.

The formula for calculating inhibition percentage is expressed as follows:

$$\text{Inhibition}\,(\%) = \frac{dc - de}{dc} \times 100$$

(Where "dc" represents the diameter of the control mycelium, and "de" represents the diameter of the mycelium subjected to extracts).

Determination of the proximate composition of healthy and diseased avocado seed

Seeds with physical symptoms of disease (discoloration, distortion) were used for the analysis. Both diseased and healthy avocado seeds were used to assess the level of proximate composition, as impacted by the fungal pathogens.

Protein was measured as total nitrogen (Kjeldahl), then multiplied by a conversion factor (commonly 6.25), as described in **(**17**).**

Crude fat was measured using Soxhlet extraction, with hexane to extract fat as described in (17)**.**

Moisture was measured using gravimetric oven drying at 105 °C until constant weight, as described in (17)**.**

Ash was measured by the Direct Muffle Furnace Ash Method, according to (17)**.** The weighed sample was transferred into the crucible and placed in a muffle furnace, and then ignited at 525–550°C until the residue is white or light grey and the mass is constant.

Carbohydrate was not measured directly, but calculated by difference, as described by (17)**.**

Determination of the Avocado seedantinutrient

Phytate (Phytic Acid) was measured by colourimetric determination using ferric chloride precipitation, as described in **AOAC 986.11** (17)**.**

Tannins weremeasured**by**Vanillin–HCl spectrophotometric method for condensed tannins, as described in AOAC 952.03 (17)**.**

Oxalates were measured by the titrimetric method using calcium oxalate precipitation followed by $KMnO_4$ titration, as described in AOAC 974.24 **(**17)**.**

Saponins were measured by validated spectrophotometric assays(vanillin sulfuric acid) as described in (17)**.**

Data and statistical analysis

All the collected data were subjected to an analysis of variance (ANOVA) to determine significant differencesbetween the means, and 'Duncan Multiple Range Test's post hoc test was applied, assuming a significance level of $p < 0.05$, using the MINITAB 19 software package.

RESULTS

Percentage occurrence of the fungal isolates.

The most frequently isolated fungus was *A.niger*(28.43%),followed by *A.flavus* (26.50%), *Rhizopus stolonifer,* (18.30),*Lasidiploidiatheobromae*(14.35), whereas *Fusarium oxysporium* was the least frequent (12.43%)(Table 1)

Table 1: Percentage Frequency of occurrence of fungal isolates from avocado pear

Fungal isolates	Frequency
Aspergillus niger	28.42
Aspergillus flavus	26.50
Rhizopus stolonifer	18.30
Fusarium oxysporium	12.43
Lasidioploidiatheobromae	14.35

Pathogenicity test of the identified Avocado fruit fungal pathogens.

Pathogenicity assays showed that, with the exception of *Aspergillus niger*, *Aspergillus flavus, Rhizopus stolonifer, Fusarium oxysporium,* and *Lasidioploidia theobromae* induced typical symptoms on avocado fruit, primarily soft rot and browning.

Inhibitory effect of avocado seed extracts on the mycelial growth of the Avocado fruit. fungal pathogens

Ahighly significant difference was observed in the mycelial growth inhibition of the isolated fungal pathogens in response to different concentrations of avocado seed extract. The results indicated that mancozeb had highest inhibitory effect on the growth of *Aspergillus flavus* (22.80mm), *R. stolonifer* (24.70mm), *Fusarium oxysporium* (25.40mm),and *Lasidioploidia theobromae I* (18.57mm) (Table 2).

Table 2.Effects of different concentrations of Avocado seed extracts' inhibition(mm)against the fungal pathogens

Avocado seed concs (mg/mL)	*A. flavus*	*R. stolonifer*	*F. oxysporium*	*L. theobromae*
5	6.11(0.27)f	8.530(0.26)f	7.11(0.37)^{f}	7.53(053)^{bc}
10	6.99(0.083)e	14.103(0.34)^{e}	8.626(0.08)^{e}	11.09(0.32)^{bc}
15	12.53(0.21)d	16.867(0.31)^{d}	14.267(0.493)^{d}	14.53(0.21)^{ab}
20	15.733(0.25)c	18.167(0.66)^{c}	16.20(0.27)^{c}	17.73(0.25)^{ab}
25	18.267(0.25)b	20.93(0.32)^{b}	19.63(0.15)^{b}	23.33(0.153)^{a}
Mancozeb	22.800(0.20)a	24.700(0.60)^{a}	25.40(0.53)^{a}	18.57(0.12)^{ab}
DMSO	0.2833(0.12)g	0.283(0.042)^{g}	0.203(0.04)^{g}	0.2300(0.04)^{c}

Numbers in brackets are ± standard deviation .Means that do not share a letter are significantly different.(Turkey's HSD test ($p < 0.05$).

Effects of fungal pathogens on avocado seed proximate composition

The study revealed a clear and consistent significant variations in healthy and unhealthy avocado seeds,except for moisture content, which was higher in unhealthy avocado seeds.(Table 4). Healthy seeds contain higher carbohydrate (45.74g/100 g) than unhealthy fruits (11.38). Crude fat is markedly higher in healthy seed (23.00g/100 g) than in unhealthy seed (5.33). Protein content in healthy seed (14.15 g/100 g) is substantially greater than in unhealthy (3.45). Unhealthy seed shows a modestly higher moisture content (17.34), than healthy seed (14.50).Ash content in healthy seed (1.66 g/100 g) is greater than in unhealthy seed (0.34). Healthy seeds show higher fibre content (1.77g/100 g) than unhealthy seeds (0.35) (Table 3).

Table 3: Proximate analysis of healthy and unhealthy avocado pear seed.

	Proximate composition(g/100g)				
Carbohydrate	Ash	Moisture	Crude fat	Protein	Fibre
Healthy	1.66(±0.13)a	14.50(±0.40a	23.00(±0.6)a	14.15(±0.10)a	1.77(±0.2)a
Unhealty	0.34(± 0.61	**17.34(**±0.23**b**	5.33(±0.31)b	**3.45(**±0.33**)b**	0.35(±0.4)b

Numbers in brackets are ± standard deviation.Means that do not share a letter are significantly different.(Turkey's HSD test ($p < 0.05$).

Antinutrient composition of Avocado seeds

The antinutrient composition of avocado seeds revealed significant variations among the concentrations of the antinutrient.The following antinutrients were observed in avocado seed, Saponins (0.49mg/100g), Phytates (0.31mg/100g), Oxalates (5.11mg/100g), and Tannins (1.04mg/100g) (Table 5). Oxalates exhibited highest in concentration among others, whilePhytateshas the lowest concentration of antinutrients (Table 5). Overall antinutrient burden in the avocado seed is modest in absolute terms (6.95 mg/100 g) (Table 5).

Table 4:Antinutrient composition of avocado seeds

Anti-nutrients	(mg/100g)
Saponins	0.49(±0.01)
Phytates	0.31(±0.10)
Oxalates	5.11(±0.08)
Tannins	1.04(±0.03)

Numbers in brackets are ± standard deviation.

DISCUSSIONS

The result on the percentage occurrence of the fungal isolates was consistentwith the report by(18;19**)**, who observed the high percentage occurrence of *Aspergillus niger*, *Rhizopus*spp, *Candida* spp, *Lasidioploidia theobromae*, in avocado fruit. Similar studies have implicated *Aspergillus*.The presence of these fungi may be attributed to the high deterioration of avocado fruits at harvest resulting from improper handling and wounds created during transportation and storage.

The different sensitivities of the fungal pathogens to the avocado seed extract could be a consequence of the composition of the fungal cell wall that occurs during the stages of development and across different fungal species (20,21).The suppressive effect of avocado seed extract on mycelial growth can be attributed to the presence of antifungal secondary metabolites. These findings are in agreement with previous reports on the antifungal properties of avocado seed extract against some phytopathogens, including *Fusarium oxysporum* (22).Recent studies have demonstrated that avocado seed extracts possess antifungal activity against several plant-pathogenic fungi, including *Fusarium oxysporum* and *Colletotrichum gloeosporioides*.(23), and *Rhizoctonia solani* and *Fusarium* spp(24), and *A.niger*.(25).

The decrease in proximate composition of the unhealthy avocado seed collaborates with the reports by (26), who reported that fungal pathogens cause measurable declines in total soluble solids, dry matter percentage, and crude fatover the storage period, and (27,28),who reported that fungal pathogens secrete cell wall degrading enzymesthat break down structural carbohydrates, reducing total soluble solids and dry matter,enabling the fungus to access nutrients and causing measurable declines in proximate constituents. Similarly, (29) reported that fungal pathogens secrete a broad arsenal of extracellular proteases that hydrolyse host macromolecules in the apoplast into peptides and free amino acids, which support fungal growth and sporulation. In addition, (30,31), reported an increased moisture content in diseased tissue and ascribed it to cell integrity breakdown and water redistribution, causing softer and wetter plant tissue. These reductions in the proximate composition of diseased avocado seeds undermine their nutritional value, seed viability, and suitability for oil extraction or animal feed production. Therefore, addressing these impacts requires targeted mitigation strategies in postharvest handling and processing.

The antinutrient composition in avocado seed was low (6.95 mg/100 g), so for most people it does not pose a major health risk, although processing is still advisable (32). Avocado seeds contain phytate, tannins, oxalates, saponins, and cyanogenic glycosides (10,33),which can reduce nutrient absorption, protein digestibility, and mineral bioavailability. Saponins, at low doses show anticancer and immune-supporting effects and can lower bloodpressure (34), but at high doses saponins have poor membrane permeability, low oral bioavailability, and can damage red blood cells and lower cholesterol. (35). High oxalate intake is linked to impaired calcium absorption, kidney injury, and gastrointestinal symptoms, while low dietary oxalate is safe for most people but may raise kidney-stone risk in susceptible individuals (36,37). Low phytate intake can be antioxidant and anticancer and may help prevent kidney stones, but high phytate levels inhibit mineral absorption and disrupt mineral

homeostasis(38,39). Low tannins intake can improve appetite and cardiovascular markers, whereas high intake may harm the liver and metabolism (40).Simple processing, for example, grating, cooking, dewatering, roasting, fermentation, or drying can reduce antinutrient levels to the recommended safe limit set by the (41,42).

CONCLUSION

Avocado seed extract showed clear antifungal activity against postharvest pathogens, including*A. flavus, R. stolonifer, F. oxysporum, and B. theobromae*. Fungal infection reduced seed nutritional quality, carbohydrates, fats, and proteins declined while moisture increased. Antinutrient levels were low.These results revealed avocado seeds as promising, eco-friendly option for postharvest disease control.Further research should be carried out in field trialsto validate these findings, optimization of the extraction and formulation should be carried out to achieve effective doses, assess safety and toxicity, and clarify its mode of action against both fungal and bacterial pathogens.

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