Journal of Nutritional Health & Food Engineering

Research Article

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Proximate, functional, anti nutrient and antimicrobial properties of avocado pear (*Persea americana*) seeds

Abstract

The proximate, functional, antinutrient and antimicrobial properties of avocado pear (*Persea americana)* seeds were studied using standard methods. The proximate contents (%) of avocado peat seed flour, ASF, in decreasing order were moisture (13.09±0.14) followed by ash (3.82±0.00), fibre (2.87±0.00), protein (2.64±0.01) and fat (0.33±0.00). The energy value, carbohydrate and dry matter content respectively was 334.01±1.05%, 80.12±0.15% and 86.91±0.14% while the water absorption capacity and oil absorption capacity respectively was 2.70±0.00% and 1.80±0.00%. The anti-nutrients (mg/100g) in decreasing order were saponins (8.10±0.01) followed by alkaloids (2.14±0.00), flavonoids (1.81±0.01), tannins (1.14±0.01), cyanogenic glycosides (1.02±0.00) and phenols (0.29±0.01). The avocado pear seed extract (ASE) elicited antibacterial activity (mm) against *Proteus mirabilis* (23±0.14), *Staphylococcus aureus* (16±0.04) and *Pseudomonas aeruginosa* (15±0.11) though lower than the corresponding activity by the standard, Ciprofloxacin. ASE had higher activity (18±0.31mm) against the fungus, *Aspergilus niger* compared with the standard antifungal, Ketoconazole (8±0.22mm) while it had a comparable activity as the standard against *Candida albicans* but no activity against *Penicillum notatum* in contrast to the standard drug (6±0.24mm). Thus, ASF had appreciable nutrient and antinutrient contents with apparently moderate storage value while, out of the tested pathogens, ASE had a broad spectrum antibacterial activity, a selective antifungal activity and an overriding activity against *Aspergilus niger.* These while highlighting possible diet and drug potentials of ASF and ASE, respectively, provided basis for ethno-medicinal applications of avocado pear seeds, hence warranting further studies.

Keywords: pathogens, anti-nutrients, antimicrobial, *Aspergilus niger*, *Proteus mirabilis*

Volume 8 Issue 1 - 2018

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Received: October 22, 2017 | **Published:** February 15, 2018

Abbreviations: ASF, avocado pear seed flour; ASE, ethanolic extract of avocado pear seed flour; OAC, oil absorption capacity; WAC, water absorption capacity; AOAC, association of analytical chemists; MHA, mueller hinton agar; ANOVA, analysis of variance; SD, standard deviation

Introduction

Generally, plant parts (seeds, leaves, bark, fruits and stems) contain bioactive agents. These confer such plant parts with nutritive and antimicrobial properties which could contribute to the management of diseases.[1–6] However, proper assessment on the possible dietary and therapeutic potentials of such plant parts are required for informed use in animals, including human.

Avocado plant (*Persea americana*), a plant belonging to the family of *Lauraceae* and genus, *persea* bears fruit known as avocado pear or alligator pear that contains the avocado pear seed. Reported uses of avocado pear seed include use in the management of hypertension, diabetes, cancer and inflammation.[7–9] The fruit is known as *ube oyibo* (loosely translated to 'foreign pear') in Ojoto and neighboring Igbo speaking communities south east Nigeria.[10] Different parts of avocado pear were used in traditional medications for various purposes including as an antimicrobial.[11,12] That notwithstanding, the avocado pear seeds are essentially discarded as agro-food wastes hence underutilized. Exploring the possible dietary and therapeutic potentials of especially underutilized agro-food wastes will in addition reduce the possible environmental waste burden.[13–15] Thus, this study was warranted and aimed at assessing the proximate, functional, anti-nutrients and antimicrobial properties of avocado pear seed to provide basis for its possible dietary use and justification for its ethno-medicinal use. The objectives set to achieving the study aim as stated were by determining the proximate, functional, antinutrient and antimicrobial properties of avocado pear (*Persea americana)* seeds using standard methods as in the study design.

Materials and methods

Collection, identification, preparation and extraction of plant materials

Avocado pear fruits were bought in a local market in Umuahia during the fruiting season (June, 2015). The fruits were identified as that of *Persea americana* mill (*Lauraceae*) in the Plant Science department of Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. The fruits were deseeded by removing the fleshy cover. The resultant seeds were washed with clean tap water, crushed into smaller pieces with the help of manual grater and sun-dried for three days. The sun-dried seeds were subsequently milled into powder using a laboratory miller (ED-5, U.S.A) and shared into two.

One part of the avocado pear seed flour, ASF, was stored in an air tight container until used for the determination of proximate, functional and anti-nutrient contents. The other part was extracted by cold maceration method using ethanol as the extracting solvent. The extraction method involved weighing 700g of the avocado pear seed flour into a volumetric flask, soaking the weighed ASF in 1400ml of 90% ethanol with intermittent shaking and stirring for three days and thereafter filtering with No 1 What mann filter paper. The filtrate was concentrated using water bath at 60°C and was further dried in an oven at 50°C. The resultant avocado pear seed extract (ASE) was placed in a sample bottle and stored in a refrigerator until used in determining the antimicrobial (antibacterial and antifungal) activity of selected pathogens.

Determination of studied parameters

The proximate contents (dry matter, moisture, protein, ash, crude fibre and fat) of ASF were determined according to corresponding method of Association of Analytical Chemists (AOAC, 1990). From the determined proximate contents, carbohydrate content and energy value of ASF were respectively calculated by difference thus:

Carbohydrate (%)=100-(protein+ash+fat+moisture content)%.

Energy value (%)=(% carbohydrate×4)+(% protein×4)+(% fat×9).

The functional properties (water absorption capacity, WAC and oil absorption capacity, OAC) were determined as the weight of water or oil absorbed and held by 1g of the sample.[16] Alkaloids content was determined by the method of Harbone[17] as described by Okwu & Morah[18] while saponins content was determined by the method as described earlier.[18,19] Tannins content was determined by Folin-Dennis colorimetric method as described by Pearson[20] and reported earlier.[21] Phenols content in the flour sample was determined by the method in Association of Analytical Chemists, AOAC,[22] while cyanogenic glycosides content was determined using the method described by Onwuka[23] whereas flavonoids content was determined by the method described by Okwu & Omodamiro.[24]

The determination of antimicrobial activity of avocado pear seeds flour extract (ASE) against six clinical isolates comprising three bacteria (*Pseudomonas aeruginosa, Proteus mirabilis and Staphylococcus aureus*) and three fungi (*Aspergulus niger, Canidida albican and Penicillum notatum*) obtained from Federal Medical Centre Umuahia, Abia State, Nigeria was according to the disc diffusion method.[25] In brief, discs (6mm in diameter) were made from Whatman No.1 grade filter paper with the aid of a mechanical perforator and sterilized by autoclaving at 0°C. The blank sterile discs were placed on the inoculated Mueller Hinton Agar (MHA) surface and impregnated with 20µl of stock solution (2000µg/disc). Ciprofloxacin and Ketoconazole were respectively used as antibacterial and antifungal controls. The plates were incubated at 37°C for 24hours. All tests were performed in triplicate and the antimicrobial activity was expressed as the mean diameter of inhibition zones (mm) produced by the plant extract.

Statistical analysis

Descriptive statistics and test for significant difference in mean of the generated data were carried out by analysis of variance (ANOVA) using the statistical package for social sciences for Windows version 16. The turkey post hoc test was used to identify the means that differ significantly at p<0.05. Results were expressed as mean±standard deviation, SD of duplicate for proximate, functional and anti-nutrient properties) or triplicate (for antimicrobial property) determinations.

Results and discussion

Results

As shown on Table 1, the proximate contents (%) of avocado peat seed flour, ASF, were in the order: moisture (13.09±0.14) > ash (3.82±0.00) > fibre (2.87±0.00) > protein (2.64±0.01,) > fat (0.33±0.00). The energy value, carbohydrate and dry matter content respectively were 334.01±1.05%, 80.12 ±0.15% and 86.91±0.14%. Out of the determined functional properties, the water absorption capacity (2.70±0.00%) was higher than the oil absorption capacity (1.80±0.00%).

Table 1 Proximate and some functional properties of avocado pear seeds

Parameters	Concentration (%)
Moisture Content	13.09±0.14
Dry Matter	86.91±0.14
Crude Protein	2.64±0.01
Ash	3.82±0.00
Crude Fat/Lipid	0.33±0.00
Crude Fibre	2.87±0.00
Carbohydrate	80.12±0.15
Energy Value	334.01±1.05
Water Absorption Capacity	2.70±0.00
Oil Absorption Capacity	1.80±0.00

Values are means±standard deviations of duplicate determinations

The anti-nutrients (mg/100g) as shown on Table 2 were in the order: saponins (8.10±0.01) > alkaloids (2.14±0.00) > flavonoids (1.81±0.01) > tannins (1.14±0.01) > cyanogenic glycosides (1.02±0.00) > phenols (0.29±0.01).

Table 2 Anti-nutrients composition of ethanolic extract of avocado pear seeds

Anti-nutrients	Concentration(mg/100g)
Alkaloids	2.14±0.00
Saponins	8.10±0.01
Tannins	1.14±0.01
Flavonoids	1.81 ±0.01
Cyanogenic Glycosides	1.02±0.00
Phenols	0.29±0.01

Values are means±standard deviations of duplicate determinations

As depicted on Table 3, the avocado pear seed extract (ASE) elicited antibacterial activity (mm) against *P. mirabilis* (23±0.14) which was higher than that against *S. aureus* (16±0.04) followed by that against *P. aeruginosa* (15±0.11). The activity of ASE against these pathogens was however lower than the corresponding activity elicited by the standard drug, Ciprofloxacin (Disc diffusion pictures not provided).

Table 3 Antibacterial activity of ethanolic extract of avocado pear seeds and standard antibacterial, Ciprofloxacin, against *P. aeruginosa, P. mirabilis and S. aureus*

Organisms	Diameter of zone of inhibition(mm)	
	Extract	Ciprofloxacin
Pseudomonas aeruginosa	15±0.11	41±0.02
Proteus mirabilis	23±0.14	33±0.13
Staphylococcus aureus	16±0.04	45±0.06

Values are means±standard deviations of triplicate determinations

The sample extract, ASE, had higher activity, mm, (18±0.31) against the fungus, *Aspergilus niger* when compared with the standard drug, Ketoconazole (8±0.22). And, the activity of ASE against *Candida albicans* was comparable with that of Ketoconazole (32±0.14), a standard antifungal. However, the extract elicited no activity against the pathogenic fungus, *Penicillum notatum* in contrast to the standard drug (6±0.24) (Table 4).

Table 4 Antifungal activity of ethanolic extract of avocado pear seeds and standard antifungal, Ketoconazole, against *A. niger, C. albican and P. notatum*

Organisms	Diameter of Zone of Inhibition (mm)	
	Extract	Ketoconazole
Aspergilus niger	18±0.31	8±0.22
Candida albican	32±0.14	48±0.11
Penicillum notatum	0	6±0.24

Values are means±standard deviations of triplicate determinations

Discussion

The proximate profile of the ASF (Table 1) showed higher moisture content compared to the others suggesting poor storability of the avocado pear seed flour. However, while this compared with the value (9.92 ± 0.01%) reported by Arukwe et al.,[26] the value was much lower than the range (54 - 65.62%) reported by Oluwole et al.,[27] and Vinha et al.,[28] This could indicate a comparatively higher storability (shelf life) hence apparent moderate storage value of ASF.[29] The dry matter content of ASF (86.91±0.145%) compared with that (94.88-96%) reported for watermelon rind and seed and indicated high nutrient content in the ASF.[1] The protein content (2.64±0. 01%) compared with the value (2.19±0.16%) reported by Vinha et al.,[28] while the ash content (3.82%) compared with the value range (2.48-3.35%) for watermelon seed and round orange *Carica papaya* seed[1,30] but the oil/fat content was low compared to that reported previously for avocado pear seed.[31] In particular, the difference in oil content compared to that obtained by Parameswaran & Murthi[31] may be attributed to location-related variation.[29]

The crude fiber content (2.87%) of the avocado pear seed flour compared with the value range (2.02 - 3.10%) reported for water melon, mango and avocado pear seeds.[1,26,32,33] Thus, the avocado pear seed could serve as a good dietary fibre source and perhaps fibre health benefits. Fiber improves food bulk, appetite satisfaction and motility through the digestive system and by improving the absorption and re-absorption of cholesterol and bile acids respectively could lower cholesterol level and prevent the formation plaque.[34–36] The carbohydrate content of ASF was higher than that (25.47%) reported by Akpabio[37] for almond seed. This observation, in concert with energy value of ASF (334.01±1.05%), could be a pointer that avocado pear seed flour could serve as a high carbohydrate source with high energy value. The water absorption capacity (2.70%) and the oil absorption capacity (1.80%) of ASF in this study were comparable with the values (3.01%) and (1.39%) respectively reported by Egbuonu et al.,[38] for bitter yam, suggesting that avocado pear seed flour could have flavour retention and mouth feeling properties.[1]

The tannin (mg/100g) content of ASF (1.14) was quite lower compared with that (29.21mg/100g) in *Mangifera indica* seed kernel.[39] Also, the cyanogenic glycosides (mg/100 g) content (1.02±0.00) was lower than the value (21.6) reported by Akpabio et al.,[37] while the phenol (mg/100 g) content (0.29±0.01) was lower than the value (6.14±1.28) reported by Arukwe et al.,[26] probably indicated low derivation potential of these antinutrients from ASF. Saponin (mg/100g) recorded the highest value (8.10±0.01) among all the determined antinutrients which was higher than the value (1.55±0.05) reported for watermelon seed by Oseni & Okoye.[40] This could be highlighting apparent higher derivation of saponins than the other anti-nutrients from ASF. The alkaloids (mg/100g) content (2.14±0.00) of ASF was higher than the value range (0.72-1.23%) reported for avocado pear and watermelon seeds.[26,41] but compared with the value (1.64±0.10%) for glycine max reported by Okwu & Orji.[42] Alkaloids are plant metabolites with bactericidal activity (Rimando & Perkins-veazie,[43] implying that avocado pear seed flour could have anti-bacterial potentials. Furthermore, the flavonoids (mg/100g) content compared with the value (1.90±0.07) reported earlier[26] and reportedly exhibited antibacterial activity.[24] Thus we speculated that avocado pear seed flour could have antimicrobial activity.

In apparent support of our speculation, the avocado pear seed extract (ASE) elicited antibacterial activity (mm) against *P. mirabilis* (23±0.14) which was higher than that against *S. aureus* (16±0.04) followed by that against *P. aeruginosa* (15±0.11) though respectively lower than the corresponding activity elicited by the standard antibacterial, Ciprofloxacin. The present result underscored the prospect of ASE as a broad spectrum antifungal following purification warranting further studies. Further to this, ASE elicited higher activity (18±0.31mm) against the fungus, *Aspergilus niger* on comparison with the standard drug, Ketoconazole (8±0.22mm) indicating apparent overriding potency of ASE over the standard against *A. niger* and perhaps *A. niger*-related diseases. However, while ASE showed activity against *Candida albicans* that was comparable with that of the standard antifungal, Ketoconazole (32±0.14) it had no activity against the pathogenic fungus, *Penicillum notatum* in contrast to the standard drug (6±0.24) suggesting that ASE could not serve as a potential broad spectrum antifungal. The spectra of antimicrobial activity shown by ASE could be attributed to the anti-nutrients, notably saponins, alkaloids and flavonoids contained in ASF as reported in this study, and were in consonance with earlier reported antimicrobial activity of avocado pear seeds.[12,44,45]

Conclusion

Thus, ASF had appreciable nutrient and antinutrient contents with apparently moderate storage value while, out of the tested pathogens, ASE had a broad spectrum antibacterial activity, a selective antifungal activity and an overriding activity against *Aspergilus niger*. These while highlighting possible diet and drug potentials of ASF and ASE, respectively, provided basis for ethno-medicinal applications of avocado pear seeds hence warranting further studies to harness the possibility of applications in food industries and in medicine.

Acknowledgments

None.

Conflict of interest

Author declares that there is no conflict of interest.

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