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PROXIMATE, PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF AVOCADO PEAR SEED (Persea Americana)

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ABTRACT

Avocado pear (Persea Americana) seed is considered as one of the non- edible part of the fruit and as such is discarded as a waste. This present study is investigating dietary and therapeutic potential of avocado pear seed .Phytochemical and proximate analysis reveals the presence of nutrients, tannin ,oxalate phytic and alkaloids. Antimicrobial assessment using disk- diffusion method indicates that avocado seed extract has antibacterial activity against salmonela A and B and Salmonela Aaureus though at a lower concentration.

KEY WORD: Persea Americana, antibacterial activity, Phytochemical, anti-nutrients, Disk diffusion.

INTRODUCTION

Plants parts (seeds, leaves and barks)generally contains bioactive agents which makes such plants parts useful with nutritive and antimicrobial properties that can help fight infection and diseases .The avocado pear belongs to Lamacea family of tropical and mediteranean trees and shrubs , it is a popular food and is used for treating skin eruptions.(2)It is a source of carbohydrate, proteins, fiber, essential micronutrients for human consumptions such as polyphenols ,fats ,oils vitamins (vit.C,E ,K ,B1,B2,B6 B9)and minerals (P,Na,Mg,K,Fe and Zn) .

The avocado seed is a by-product which is normally discarded as a waste, causing increased ecological nuisance such as insects and rodents infestation. The present study is carried out to evaluate the phytochemical content and antimicrobial activity of ethanol extract of avocado seed.

MATERIAL AND METHOD

Avocado pear fruits were purchased in Nasarawa market, the fruits were identified as Avocado pear (Persea Americana) in the biochemistry department of school of applied science ,Federal Polytechnic, Nasarawa.

Preparation of sample: The fruits was deseeded by removing the flesh cover, the seed were washed, crushed into smaller size and sundried for 72 hours, milled and stored in air tight containers. Avocado pear seed flour was extracted by cold maceration method using ethanol.

Preparation of Extract: 100g powdered sample of APSF was measured into a volumetric flask and soaked for 72 hours in 90 % ethanol with intermittent shaking .The extract was filtered with what mann filter paper and concentrated on water bath at 60^{o}C.

Qualitative phytochemical screening:

Alkaloid determination: Alkaloid presence was determined using Meyers and Wagners reagent, 1ml of extract was reacted with dil. Hydrochloric acid and filtered .The filtered extract was reacted with Meyer and Wagner test reagent .The presence of alkaloid was indicated by formation of yellowish brown precipitate.

Phytic acid determination: 4g of the grounded sample was soaked in 100ml of 2% HCl for 5 hours and filtered .5ml of 0.3 ammonium thiocyanate solution was added to 25ml of the filtrate .The mixture was reacted with Iron (iii) chloride solution until a brownish yellow colour that persisted for 5minutes was obtained

Oxalate determination:1g of the sample was weighed into 100ml conical flask to which 75ml ,3M H_2SO_4 was added and stirred for I hour with a magnetic stirrer .This was filtered ,and25ml of the filterate was titrated against 0.05M $KMnO_4$ solution until a faint pink colour that persisted for at least 30 seconds was formed.

Tanin Content determination : 0.5g of the sample was weighed into a 250ml conical flask to which 50ml of distilled water was added and stirred for 1hour magnetic stirrer .This was filtered and 5ml of the filterate was pipetted into a 50ml flask and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N (HCl) and 0.008M potassium ferrocyanide.

PROXIMATE DETERMINATION

Determination of Moisture Content

Empty crucible was washed and dried in oven at 100^oC for 30 minutes, the weight was measured .5g of the sample was weighed into the crucible and dried in the oven at 105^oC for 3hours and reweighed until the weight was constant, it was transferred into a dessicator to cool and the weight of the sample was taken and calculated.

Determination of Ash

The ash content was determined using the ignition method .Exactly 2.0g of the oven dried sample used in moisture determination was weighed and placed in the pre –heated ,cooled and weighed crucible and then reweighed. The crucible was placed in a cold muffle furnace and the temperature was allowed to rise to 504^oC for 3 hours until the ash was obtained .The crucible was removed from the furnace, allowed to cool in a desiccator and reweighed. The percentage ash content was calculated.

Determination of Crude Fat

Soxhlet extractor method was used . 20g of the sample was weighed in an ether extracting thimble and placed on the soxhlet extractor connected to a round bottom flask with 200ml petroleum ether and reflux for 6 hours .The solvent was evaporated on a water bath at 60^oC .The flask was cooled and re-weighed ,and the percentage crude lipid was calculated.

Determination of Crude Protein

Determination of crude protein was carried out using kjelhdal method .2g of the sample was weighed into a kjelhdal flask and 5g of anhydrous sodium sulphate was added ,25ml of concentrated H_2SO_4 was added with few boiling chips. The mixture was heated in the fume cupboard for 2hours until a clear solution obtained .The solution was cooled and transferred into 250ml flask and made up to the mark with distilled water. About 100ml conical flask containing 5ml of 4% boric acid and 2 drops of methyl red indicator was placed under the condenser ,5ml of the digest was pipette into the apparatus through the funnel on the distillation unit .The digest was washed down with distilled water followed by addition of 40% NaOH .It was allowed to distil until the volume of the distillate reached 100ml,the solution was titrated with 0.1 mol /dm^3HCl until the first permanent pink colour appeared .The blank was

titrated the same way and the time value for the sample was obtained.

Determination of Crude Fibre

2g of the sample was weighed into 500ml beaker and 1.25% H_2SO_4 was added and heated on the heating mantle for 30 minutes ,it was filtered and the filtrate was transferred into a beaker and dissolved with 1.25% NaOH and heated for another 30 minutes .The filterate was rinsed with 25ml of ethanol ,and transferred into the oven and dried for 1hour,the residue was weighed and the crude fibre content was calculated.

MICROBIAL ANALYSIS

Stocks culture of staphylococcus aureus and salmonella typhi were obtained from the microbiology laboratory department of the Federal polytechnic Nasarawa.

Media preparation:

All used media were prepared with instructions indicated on them by the manufacturer .The prepared media were properly mixed and sterilized in the autoclave for 15 minutes at 121^{O} C

Antimicrobial Assay :Disk diffusion method : Antimicrobial activity test was carried out with disk diffusion method. Muller Hutton agar and the petri dishes were sterilized in the autoclave at 121^{O} C for 15 minutes . 10ml of the agar medium was poured into the sterile petri dishes in a laminar flow to obtain plates of a uniform depth of 5mm and allow to solidify .A loopful of the test microorganism staphylococcuss aureus and salmonella thphy were inoculated and streaked unto the surface of the well labelled solidified nutrient agar plates

Varying concentration 25,50 75 and 100ml of the ethanol extracts of Americana persea seed mg /ml of the ethanol extract were impregnated using sterile forceps on the antimicrobial discs surface on the inoculated pates .The plates were incubated for 24 hours at 37^{O}C .The diameter for zone of inhibition concentration was measured in millimetre .The minimum inhibition concentration is seen as the lowest concentration of antimicrobial agent at which the micro organism fails to show visible growth on the agar plates. The positive control for the bacterial is commercial chloramphenicol and the negative control for the bacterial is the ethanol extract.

RESULT AND DISCUSSION

The proximate profile of the ASF showed lower moisture content compared to others which indicates a comparatively higher storability of the avocado pear seed flour ,the protein value (3.4%) is more compared to the value reported (0.16%) reported by Vinha et al ,while the ash content is higher (10.9%) compared with the value range (2.48-3.35%)for watermelon seed and round orange carica papaya seed, but oil/fat content is low compared to that reported for the avocado pear seed .In particular ,the difference in oil content compared to that obtained by Paramsewaran & Murthi may be due to location related variation .The crude fibre content (15%) of the avocado pear seed is higher compared with the value range (2.02-3.10) reported for water melon and mango ,therefore avocado pear seed could serve as a good dietary fibre source and perhaps fibre health benefit since fibre 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 of plague .The carbonhydrates content of ASF was higher than that (25.47%) reported by Akpabio for almond seed, this could be a pointer that avocado pear seed flour could serve as a high carbonhydrate source.

Qualitative phytochemical screening of Avocado pear seed (Americana persea)

The result for qualitative phytochemical tests are shown in the table 2.The table showed that all tested phytochemicals were present in the ethanol extract of Americana persea .The tannin content of avocado seed flour (ASF) was quite lower compared with that in Mangifera indica seed kernel ,the alkaloid ,oxalate and phytic acids content of avocado seed flour was present than that reported for watermelon seed,thus it is speculated that avocado seed flour could have antimicrobial activity .

As shown on Table 1, the proximate contents (%) of avocado pear seed flour, ASF, were in the order: Crude fibre (15) > Ash (10.9) > Crude fat (7.5) > protein (3.4) > moisture . The carbohydrate content was (76.2).

Table .1: Proximate composition of Avocado peer seeds

Compositions	**Concentration (%)**
Moisture content	**2**
Ash Content	**10.9**
Crude protein	**3.4**
Crude Fat	**7.5**
Crude Fibre	**15**
Carbohydrate	**76.2**

The anti-nutrients as shown on Table 2 were in the order: Tanin (present), Oxalate (present), phytic (present), Alkaloids (present).

Table .2: Antinutrients constituents of ethanolic extract of Avocado Peer seeds

Antinutrients	**Colour**	**Indication**
Tanin	Brownish green precipitate	+
Oxalate	Pale pink	+

Phytic	Brownish yellow	+
Alkaloids	Yellowish brown precipitate	+

Key + : present - : absent

The presence of alkaloids in the extract is responsible for its antibacterial properties ,the presence of tannin in Americana persea seed can be further studied for anti diarrheal and haemostatic and anti hemorrhoidal activity.

Antimicrobial Activity

Ethanol extract of Americana persea showed the highest zone of inhibition to salmonella A,salmonella B,at 100mg/ml while the minimum inhibition zone was 50 mlg/ml has no antibacterial activity against staphylococcuss aureus A and B

As depicted on Table 3, the avocado pear seed extract (ASE) elicited antibacterial activity (mm) against *salmonella (A)* (14.84; 14.63; 13.22), *salmonella (B)* (14.54; 10.46; 4.60) which was higher than that against *S. aureus* (0.00). The activity of ASE against these pathogens was however lower than the corresponding activity elicited by the standard drug, chloramphenicol.

Table .3:

Species	**Diameter of Zone inhibition (mm) concentration**			**Control (Chloramphenicol)**
	100mg/ml	**75mg/ml**	**50mg/ml**	
Staphylococcus aureus (A)	0.00	0.00	0.00	30.06
Staphylococcus aureus (B)	0.00	0.00	0.00	31.32
Salmonella (A)	14.81	14.63	13.22	36.30
Salmonella (B)	14.54	10.46	4.60	28.99

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