African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 15 No.3; pp. 1-6 (2023)

Original Research Article

IN-VITRO ANTI-INFLAMMATORY EVALUATION OF METHANOL AND BUTANOL EXTRACTS OF AVOACADO PEAR (*PERSEA AMERICANA* MILL) FRUIT PEEL

NDIDIAMAKA HANNAH OKORIE[1,*], SUNDAY CHIDOZIE UMERIE[2], EMEKA LEONIE CHISOM[2], IBEABUCHI JUDE ALI[1]

1. Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.
2. Department of Biochemistry, Faculty of Natural and Applied sciences. Paul University, Awka, Anambra State, Nigeria

ABSTRACT

Inflammatory diseases such as arthritis, rheumatoid or non-rheumatoid joint inflammations are dominant among people all around the world. Plant-derived anti-inflammatory drugs are widely used against these disease conditions. This study investigated the in-vitro anti-inflammatory activity of the aqueous methanol and butanol extracts of *Persea americana* fruit peel. Three different concentrations of test samples: 10, 20 and 40 mg/ml from the concentrated methanol extract (ME) and butanol extracts (BE) were evaluated in vitro by albumin denaturation, heat induced hemolysis, anti-proteinase action, anti-lipooxygenase spectrophotometric assay methods using aspirin as a reference drug. Results showed that at sample concentrations of 10, 20 and 40 mg/ml, the inhibition of albumin denaturation by the ME and BE were in the range 26.826-51.471 % and 40.329- 54.797 % respectively. The inhibition of heat-induced haemolysis by ME at the same concentrations was in the range 30.252-44.727% and 34.52-49.897% for BE. Anti-proteinase inhibitory activity for ME was within the range of 17.073-52.1 20% while 31 .781-37.01 4% was for BE. In all four assays, the BE elicited a relatively higher inhibitory activity than the ME. These suggest that the fruit extract possesses anti-inflammatory properties which may be attributed to the occurrence of bioactive compounds in the fruit peel.

ARTICLE INFO

Received 28 May, 2023
Revised 29 September, 2023
Accepted 02 October, 2023

KEYWORDS

Persea americana,
In-vitro,
Anti-inflammatory,
Spectrophotometry,
Methanol extract,
Butanol extract

INTRODUCTION

Nature has provided mankind with plethora of herbs for prevention and treatment of different ailments. Since time immemorial, plants were used by different tribes to treat several diseases due to their availability and effectiveness coupled with low toxicity index [1]. Hence, medicinal plants have gained much interest in the discovery and development of novel drug molecules which points to presence of phytoconstituents [1]. According to W.H.O reports, nearly 80 % of the World's population depends primarily on medicinal plants for their basic health care needs[2]. Medicinal plants contain reservoirs of potential bioactive metabolites that are the major sources of

***Corresponding author:** hannah.okorie@esut.edu.ng; +234-8037777736*

https://doi.org/10.59493/ajopred/2023.3.1 *ISSN: 0794-800X (print); 1596-2431 (online)*

drugs, which intensifies the interest of global pharmaceutical industries in the search for substances obtained from plant sources, particularly as great majority of species have not been studied chemically or biologically [3] for specific ailment.

Inflammation is a protective response purposely to get rid of organisms that cause injury and the consequence of the injury; as such without inflammation, infection will continue developing, wounds will not heal and the injured organ will continue suppurating. In the process to inactive or eliminate the invading stimuli or organisms and set the stage for tissue repair, orthodox drugs are usually administered. Most of the orthodox drugs used in treating inflammatory condition, non-steroidal anti-inflammatory drugs (NSAID), have several adverse effects, especially gastric irritation, leading to the formation of gastric ulcers [4] and bleeding or perforation of gastro duodenal tract [5]. Therefore, the search for natural product based anti-inflammatory agent has increased greatly over the years.

Persea americana (Avocado pear), belonging to the family of Lauraceae is a berry from a tree containing pericarp with skin (exocarp), edible part (mesocarp) and a big seed having covering layer at the center. It has a high nutritional and phytochemical content [6]. When compared to other tropical fruit peels (banana, melon, passion fruit, papaya, pineapple and watermelon), avocado dried peel has the highest total phenolic content and the raw peel of the fruit presents the highest flavonoid content [7]. Furthermore, dried avocado peel shows the highest antioxidant activity with the comparison of the peels of other fruits [7]. Previous studies have reported that avocado peel contains more than 30 individual phenolic compounds and high polymeric compounds which can be extensively categorized into 3 groups; hydroxycinnamic acids, flavonols and flavan-3-ols. The oligomers and polymers, known as procyanidins, are one of the largest phenolic compounds in the food products which have been studied to propose prevention of cancer, inflammation and some chronic diseases [8, 9]. It was reported that the procyanidin level of avocado peel is comparable to the one determined in natural cocoa powder which have been declared to have great procyanidin amount. Dried peels of avocado were used to produce a novel functional beverage. Avocado peel activated carbon (APC) is reported to be a promising cheaper alternative to high-priced activated carbon as the adsorption capacity of APC was comparable to the commercial ones [9]. These previous reports and more on avocado pear, has recently drawn more attention on extensive investigation of the fruit. *P. americana* fruits as rich in polyphenols, anti-oxidant vitamins and has anti-inflammatory properties as well. Though this fruit has been studied for its antioxidant activities [10], studies on the evaluation of its anti-inflammatory properties have not been reported extensively. Therefore, the present study was conducted to evaluate the in-vitro anti-inflammatory activity of *P. americana* fruit.

MATERIALS AND METHODS

Materials

Methanol and Butanol, (Sigma Aldrich Germany), HCl (1 M, pH7.4), (Alpha), Chemika Mumbai), Borate buffer (2M, Ph9.0) (Krishna Chem Industry), Butylated hydroxytoluene (Seidler Chemical Co), Perchloric acid (Mercki), Bovine albumin fraction (1 50mM, pH5.2) (Bayer AG), 1 ml of 1 0% RBCs, (A&Z group Co. LTD), Aspirin (Bayer AG), Trypsin (0.06mg) (Barentzi), Linoleic acid solution (Quanghua Sci-Tech Co LTD), Vanillin reagent (A&Z group Co. LTD) Folin-Ciocalteau (Mercki), Distilled water Beaker, Volumetric flask,, Filter paper, Oven, Incubator (Duran Group GmbH),, Spectrophotometer, (Model 371 , Elico India Ltd) Water heater, Measuring cylinder, Pipette, Centrifuge, Gloves, Conical flask, Spatula (Swastik Scientific Instruments),

Collection and Preparation of Sample

The avocado pears fruit used for the study was obtained from Eke Awka market in Awka Town, Anambra State, Nigeria in 2022. The plant part with voucher's No, NAUH No 219A was authenticated by the Taxonomist in charge of herbarium unit of Department of Plant Science Nnamdi Azikiwe University Awka. The unripe fruits were kept at room temperature in moisture-free environment to ripe. The rind was removed, washed and dried under shade for two weeks. The dried material was pulverized into powder and put in an air tight container till further analysis.

Extraction

Ten grams (10 g) of the sample was weighed separately and transferred into two different 250 ml beakers. Then, 80 ml each of methanol and butanol was added to the sample and shaken vigorously for a few minutes. The samples were then allowed to stand for 24 hours for extraction. After the 24 hours the mixture was filtered and concentrated using rotary evaporator at 40 degree centigrade to obtain the methanol extract and butanol extract respectively. The extracts were then put in the refrigerator before being used for analysis.

Inhibition of Albumin Denaturation

Bovine serum albumin [11] were used as protein. Denaturation of protein was induced by keeping the reaction mixture at 70°C in a water bath for 10 minutes according to Sakat *et al*. [12] with minor modifications. The reaction mixture consisted of test extracts and 1 % aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted to 7.4 with 1 M HCl. The sample extracts were incubated at 37 °C for 20 minutes and then heated for 20 minute. After cooling the samples, the turbidity was measured at 660 nm using UV Visible Spectrophotometer. Aspirin was used as a standard drug and the positive control (blank) is methanol. The experiment was performed in duplicate. Percentage inhibition was calculated using Equation 1.

$$\%\ Inhibition = \frac{Absorbance\ (blank) - Absorbance\ (sample)}{Absorbance\ (blank)} \times 100 \text{.....Eqn. 1}$$

Heat-induced Haemolysis

Heat induced haemolysis was studied by the standard method as described [13. 14]. The reaction mixture (2 ml) consisted of 1

ml test sample of different concentrations (100 - 500 µg/ml) and 1 ml of 10% red blood cells suspension, instead of test sample only, saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30 minutes and cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples and percentage inhibition calculated using Eqn. 1

Antiproteinase Action

The antiproteinase assay was performed according to the modified method [14, 15] .The reaction mixture (2 ml) contains 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The mixture was incubated at 37 ^{o}C for 5 minutes and then 1 ml of 0.8 % (w/v) casein was added. The mixture was incubated for an additional 20 minutes. Then, 2 ml of 70 % perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. Aspirin was used as a standard drug. The experiment was performed in duplicate. The percentage inhibition of proteinase inhibitory activity was calculated using Eqn. 1.

Anti-lipoxygenase Activity

The anti-lipoxygenase activity test was performed according to the standard method [14] and was studied using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and added 0.25 ml of enzyme solution (20,000 U/ml) and incubated for 5 minutes at 25 ^{o}C. After this, 1 ml of linoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. Aspirin was used as reference standard and percentage inhibition was calculated using Eqn. 1.

Statistical Analysis

Results of the study were expressed as mean ± standard deviation (Mean ± SD). Comparison of all the tested parameters among groups and statistical significance was determined by one way ANOVA and a 2 sided Dunett post hoc comparison test. The results were considered significant when $p< 0.05$

RESULTS

Inhibition of Albumin Denaturation

The percentage inhibition of albumin denaturation on the methanol and butanol extracts of *P. americana* fruit peel was compared with standard drug in Table 1. The result reveals that there was a significant reduction in the mean percentage inhibition of all the groups treated with the different concentrations of the methanol and butanol extract of the plant when compared to the standard control group that was treated with the standard drug (aspirin) at $p<0.05$ level of significance. Thus, the group that received the standard drug significantly inhibited albumin denaturation higher than the samples that received the plant extracts. Therefore, there is evidence to show that the sample treated with the 40 mg/ml of the standard drug inhibited albumin denaturation better when compared to other samples that received the different concentrations of the methanol and butanol extract of the plant.

Heat-induced Haemolysis

The percentage inhibition of heat-induced hemolysis of methanol extract and butanol extract was also compared with the standard drug as presented in Table 2. The results reveals that there was a significant reduction in the mean percentage inhibition of heat induced hemolysis of all the groups treated with the different concentrations of the methanol and butanol extract of the plant when compared to the standard control group that was treated with the standard drug (aspirin) at $p<0.05$ level of significance. Thus, the group that received the standard drug significantly inhibited lysis of haemoglobin higher than the samples that received the plant extracts.

Anti-proteinase Action

The percentage inhibition of anti-proteinase action of the methanol and butanol extracts of *P. americana* fruit peel compared with the standard drug was shown in Table 3. The result in Table 3 reveals that there was a significant reduction in the mean percentage inhibition of all the groups treated with the different concentrations of the methanol and butanol extract of the plant when compared to the standard control group that was treated with the standard drug (aspirin) at $p<0.05$ level of significance. Thus, the group that received the standard drug significantly exerted higher anti-proteinase action than the samples that received the plant extracts.

Anti-lipooxygenase Activity

The percentage inhibition of anti-lipooxygenase of the methanol extract and butanol extract were compared with the standard drug as presented in Table 4. The result reveals that that there was a significant reduction in the mean percentage inhibition of all the groups treated with the different concentrations of the methanol extract and butanol extract of the plant when compared to the standard control group that was treated with the standard drug (aspirin) at $p<0.05$ level of significance. Thus, the group that received the standard drug significantly exerted higher anti-lipoxygenase action than the samples that received the plant extracts.

DISCUSSION

The comparison of percentage inhibition of albumin denaturation on the methanol and butanol extracts of *P. americana* fruit peel with standard drug showed that the extracts were able to inhibit albumin denaturation in a concentration-dependent manner. The ranges are 26.83-51.47% for methanol extract and 40.33-54.80% for butanol extract at the concentrations 10.00-40.00 mg/ml for each of the extract. The inhibition rate of egg albumin denaturation for both the methanol

extract and butanol extract increased gradually with concentration. At the concentration of 40.00 mg/ml, the

Table 1: Effect of methanol and butanol extract on albumin denaturation

Sample	Concentration (mg/ml)	% Inhibition
Methanol extract	10.00	26.83 ± 1.09*
	20.00	42.16 ± 0.53*
	40.00	51.47 ± 0.72*
Butanol extract	10.00	40.33 ± 2.85*
	20.00	42.16 ± 0.01*
	40.00	54.80 ± 1.63*
Aspirin (Standard)	40.00	72.87 ± 0.93

*Values are expressed as mean ± standard deviation of duplicate determination. Significant difference is set at *p<0.05 when compared with standard control group one-way, ANOVA followed by 2 sided Dunnett post hoc comparison test.*

Table 2: Effect of methanol and butanol extract on heat induced hemolysis

Sample	Concentration (mg/ml)	% Inhibition
Methanol extract	10.00	30.26±0.93*
	20.00	40.66±0.58*
	40.00	44.73±0.07*
Butanol extract	10.00	34.53±0.27*
	20.00	44.66±0.56*
	40.00	49.90±0.44*
Aspirin (Standard)	40.00	56.65±0.66

*Values are expressed as mean ± standard deviation of duplicate determination. Significant difference is set at *p<0.05 when compared with standard control group one-way, ANOVA followed by 2 sided Dunet poc hoc comparison test.*

inhibitory effect of butanol was 75.19% while that of methanol extract was 70.62% as efficient as the standard drug (aspirin), which is in tandem with previous works done [16]. Significantly ($p<0.05$) higher inhibition was observed in the butanol extract than the methanol extract at the concentration of 40 mg/ml as shown in Table 1. This could be probably due to its much phenolic compounds and antioxidant capacity [17]. Denaturation of protein causes the production of auto antigens in conditions like rheumatic arthritis, cancer and diabetes which are conditions of inflammation. Hence, by inhibition of protein denaturation, inflammatory activity can be inhibited [17].

The anti-inflammatory activity of the extract showed a good protective effect of RBCs against heat solution induced haemolysis. It is a confirmation of a previous study on avocado peel extract used to inhibit the release of the pro-inflammatory TNF-α and the inflammation mediator nitric oxide [18, 19].

Neutrophils are known to be a rich wellspring of serine proteinase and are delimitated at lysosomes. It was recently revealed that leukocytes proteinase assumes a significant role in the improvement of tissue harm during inflammatory responses and a huge degree of defense was given by

Table 3: Effect of methanol and butanol extract on anti-proteinase action

Sample	Concentration (mg/ml)	% Inhibition
Methanol extract	10.00	17.08±1.14*
	20.00	23.99±1.36*
	40.00	52.13±0.36*
Butanol extract	10.00	31.78±1.46*
	20.00	39.39±1.27*
	40.00	37.02±1.28*
Aspirin (Standard)	40.00	71.18±0.64

*Values are expressed as mean ± standard deviation of duplicate determination. Significant difference is set at *p<0.05 when compared with standard control group one-way, ANOVA followed by 2 sided Dunet poc hoc comparison test.*

Table 4: Effect of methanol and butanol extract on anti-lipoxygenase activity

Sample	Concentration (mg/ml)	% Inhibition
Methanol extract	10.00	20.03±1.53*
	20.00	36.60±1.72*
	40.00	49.30±0.28*
Butanol extract	10.00	29.93±0.04*
	20.00	43.11±1.30*
	40.00	45.58±2.44*
Aspirin (Standard)	40.00	80.05±0.45

*Values are expressed as mean ± standard deviation of duplicate determination. Significant difference is set at *p<0.05 when compared with standard control group one-way, ANOVA followed by 2 sided Dunet poc hoc comparison test.*

proteinase inhibitors [20, 21]. Proteinases of leukocytes assume a critical job in the advancement of tissue harm during incendiary procedures. As indicated by previous work [21], a critical degree of protection was given by proteinase inhibitors. *P. americana* displayed significant anti-proteinase action at various concentrations The methanol extract demonstrated maximum inhibition of 52.13 % at 40 mg/ml when compared with the standard drug which demonstrated the greatest inhibition (71 .18 %) at same concentration. The findings of this study demonstrate that *P. americana* extract displayed significant anti-proteinase action at different concentrations when contrasted with the standard drug.

Lipoxygenases are the key catalysts in the biosynthesis of leukotrienes which assume a pivotal role in diverse inflammatory illnesses such as joint pain, asthma, malignancy and unfavorably susceptible ailments [22]. It was observed from the result that the methanol extract exhibited more anti-lipooxygenase activity than the butanol extract. Past

investigations have also demonstrated that a few herbs have high lipooxygenase inhibitory action, for example *Leptadenia pyrothecnica* [22] ·These outcomes propose that *P. americana* has a conceivably high antiinflammatory potential, which may be identified with the polyphenolic constituents of the extracts. The extracts of *P. americana* fruit peel are a potential source of natural antioxidants and could serve as an effective free radical inhibitor [23]. Hence, *P. americana* might be a good plant-based pharmaceutical product for several diseases caused by free radicals.

CONCLUSIONS

In conclusion, the present results show that extracts of *P. americana* may have anti-inflammatory effects at varying levels. It is also very possible that these anti-inflammatory activities may be due to the occurrence of bioactive compounds like the phenolic compounds and flavonoids. This finding supports the use of the peel of *P. americana* in the traditional medicine for the treatment of inflammation.

ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Faculty of Pharmaceutical Sciences ESUT for their technical support during the research.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest

AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

REFERENCES

1. Ali IJ, Ugwu OC, Onugwu SO, Mbah T, Okorie NH Eze R. Evaluation of in vivo anti- inflammatory effect of Newbouldia laevis (p. Beauv) seem root, Bignoniaceae, World Journal of Pharmacy and Pharmaceutical Sciences, 11 (8), 2022: 32-42.
2. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Valuation of in vitro anti-inflammatory activity of coffee against the denaturation of the protein, Asian Pacific Journal of Tropical Biomedical, 2 (1), 2012: 78–84.
3. Shah B, Seth A, Maheshwari K. A Review on Medicinal Plants as a Source of Antiinammatory Agents. Research Journal of Medicinal Plant, 5(1), 2011: 101 –115.
4. Okorie NH, Mbah CJ, Ugodi GW, Magbo CC. Investigation into the Gastroprotective Properties of Alstoniaboonei (De Wild) Leaves and Stem Bark. Tropical Journal of Natural Products Research, 6(3), 2022: 403-407.
5. Okorie NH, Ezealisiji KM, Obieze CJ. Evaluation of Anti-Ulcer activity of crude methanol extract of aerial parts of Costus afer in ethanol- induced and Indomethacin-induced ulcer models. Journal of Pharmacy and Allied Sciences, 19(3), 2022: 3722-3731.
6. Yahia EM, Woolf AB. Avocado (Persea americana Mill.). In: Postharvest Biology and Technology of Tropical and Subtropical Fruits. Wood head Publishing Limited, Cambridge. 2020.
7. Kosińska A, Karamać M, Estrella I, Hernández T, Bartolomé B, Dykes GA. Phenolic compound profiles and antioxidant capacity of Persea americana Mill. Peels and seeds of two varieties. Journal of Agricultural and Food Chemistry, 60 (18), 2012: 4613-4619.
8. Ahangarpour A, Sayahi M, Sayahi M. The antidiabetic and antioxidant properties of some phenolic phytochemicals: A review study. Diabetes & Metabolic Syndrome: Clinical Research and Reviews, 13 (1), 2019: 854-857.
9. Rodríguez-Carpena JG, Morcuende D, Estévez M. Avocado by-products as inhibitors of colour deterioration and lipid and protein oxidation in raw porcine patties subjected to chilled storage. Meat Science, 89(2), 2011: 166-173.
10. Mark R, Lyu X, Lee JL, Parra-Saldívar R, Chen WN. Sustainable production of natural phenolics for functional food applications. Journal of Functional Foods, 57(2), 2019: 233- 254.
11. Osman NI, Sidik NJ, Awal A, Adam NA, Rezali NI. *In vitro* xanthine oxidase and albumin denaturation inhibition assay of *Barringtonia racemosa* L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. Journal of Intercultural Ethnopharmacology, 5(4), 2016: 343-349.
12. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activityof methanol extract of Oxalis corniculata Linn. International Journal of Pharmacy and Pharmaceutical. Sciences, 2(2), 2010: 146–155.
13. Oyedepo FAJ. Anti-protease and membrane stabilizing activities of extracts of Fagra zanthoxiloides, Olax subscorpioides and Tetrapleura tetraptera. International. Journal of Pharmacy, 33(1), 1995: 65–69.
14. Mensor LL,, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research, 15(2), 2001: 127-30.
15. Tremocoldi, MA, Rosalen PL, Franchin M, Massarioli AP, Denny C, Daiuto ÉR, JAR, Melo PS, de Alencar SM. Exploration of avocado by-products as natural sources of bioactive compounds. PLoS One, 13(2), 2018:192-577.
16. Calderón-Oliver M, Escalona-Buendía HB, Medina-Campos ON, Pedraza-Chaverri J, Pedroza-Islas R, Ponce-Alquicira E. Optimization of the antioxidant and

antimicrobial response of the combined effect of nisin and avocado byproducts. LWT-Food Science and Technology, 65(2), 2016: 46-52.
17. Palma C, Lloret L, Puen A, Tobar M, Contreras E. Production of carbonaceous material from avocado peel for its application as alternative adsorbent for dyes removal. Chinese Journal of Chemical Engineering, 24(4), 2016: 521-528.
18. Leelaprakash G, Mohan SD. In-vitro anti-inflammatory activity of methanol extract of Enicostem maaxillare. International Journal of Drug Development & Research, 3(4), 2011: 189-196.
19. Das SN, Chatterjee S. Long-term toxicity study of ART-400. Indian Indigeneous. Medicine, 16(1), 1995: 117–123.
20. Rackova L Oblozinsky M, Kastalova D, Kettmann V, Bezakova L. Free radical scavenging activity and lipoxygenase inhibition of Mahonia aquafolium extract and isoquinoline alkaloids. Journal of Inflammation, 4(1), 2007:1-7.
21. Rotta EM, Morais DR, de Biondo PBF, Santos VJ, dos Matsushita M, Visentainer JV. Use of avocado peel (Persea americana) in tea formulation: a functional product containing phenolic compounds with antioxidant activity. Acta Scientiarum-Technology, 38(1), 2016: 23-29.
22. Akinwunmi KF, Oyedapo OO. In vitro anti-inflammatory evaluation of African nutmeg (Monodoramyristica) seeds. European Journal of Medicinal Plants, 8(1), 2015:167-174.
23. Azab AN, Nassar A, Azab AN. Anti-Inammatory Activity of Natural Products. Molecules, 21(1), 2016: 321-332.