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Antioxidant potentials and anti-inflammatory properties of methanol extracts of ripe and unripe peels of *Ananas comosus* (L.) Merr.

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Abstract. Studies suggest that extracts from plant materials could play protective roles against various disorders associated with the interplay between oxidative response and inflammatory disorders. The aim of this study was to investigate the potential antioxidant and anti-inflammatory properties, and phytochemical analyses of methanol extract of ripe and unripe peels of *Ananas comosus* (L.) Merr. The antioxidant properties were investigated through the analyses of ferric ion reducing antioxidant capacities, ascorbic acid equivalent antioxidant capacities, and nitric oxide scavenging capacities of the extracts. The anti-inflammatory potentials of the extracts were assessed through albumin denaturation inhibition and proteinase inhibition assays. Further investigation was carried out on the phytochemical composition of the extracts. There was no significant difference in the antioxidant potentials of the extracts assessed through the reduction of ferric ion. However, the ascorbic acid equivalent capacities and nitric oxide scavenging potential revealed that the antioxidant potentials of the extract of the unripe peel of *A. comosus* were significantly higher ($p < 0.05$) than the antioxidant potentials of the extract of ripe peel. Albumin denaturation inhibitory potential was significantly higher ($p < 0.05$), but there was no significant difference in the protease inhibitory potentials of both extracts. The estimated amounts of total flavonoids present in the extract of ripe peel of *A. comosus* were significantly higher ($p < 0.05$) than the estimates in the extract of unripe peels. This study gave a comprehensive insight into the antioxidant properties, anti-inflammatory properties and phytochemical compositions of the methanol extracts of the peels of ripe and unripe *A. comosus* which could be exploited as an alternative and complementary medicine in the treatment of different ailments associated with inflammatory disorders.

***Keywords*:** *Ananas comosus* (L.) Merr.; ripening; peel; total phenolics; antioxidant; anti-inflammation.

1. Introduction

Processing of food materials such as fruits and vegetables and the lack of suitable handling procedures could generate high levels of plant-derived food wastes that could cause a nuisance to the environment as solid waste. The negative environmental effect might lead to emission of detrimental greenhouse gases, microbial decomposition and leachate production [1-3]. Recently, plant-derived food wastes have been explored as possible sources of valuable nutrients and natural products in food, pharmaceutical and biotechnological development besides being used as animal feeds or manures. This attempt is in accordance with the advocate by the *Food and Agriculture Organization (FAO*) suggesting wastes of fruits and vegetables as probable sources of health-promoting ingredients [4]. The by-products of the plant-derived food wastes can be used in the productions of dietary fibres, dietary antioxidants, flavour compounds and functional ingredients and to fortify some foods [5, 6].

As progress towards utilisation of plant-derived food wastes, various food peels had been assessed for the possible presence of health-promoting compounds [7]. The plant-derived food products could be suggested to be beneficial for health promotion vis-à-vis alleviation of disorders that are associated with dysfunctional redox status and inflammatory response [8]. Pineapple (*Ananas comosus* (L.) Merr.) is a common fruit crop that is commonly consumed as fresh fruits and processed juices due to its savoury properties. The peel is usually dumped into landfills after consuming the edible part where it could result in solid-waste management problems. The present investigation was carried out to evaluate the comparative antioxidant and anti-inflammatory activities of ripe and unripe peels of *A. comosus*.

2. Experimental

2.1. Materials and reagents

Ripe and unripe pineapple fruits (*A. comosus*) were obtained from local suppliers in Ajebo, Ogun State, Nigeria and authenticated at the Department of Biological Sciences, McPherson University, Seriki Sotayo, Nigeria. The entire chemicals used were of analytical grade. Trypsin was purchased from Molychem (India), 1, 1- diphenyl-2-picrylhydrazyl was obtained from E-Labscience (China), Folin-Ciocalteu's phenol reagent was a product of Loba Chemie (India).

2.2. Preparation of plant samples

The peels of fruits were removed and dried at room temperature of 30±1 ºC. 50 g of each dried sample were mixed with 200 ml MeOH and the mixture was filtered

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after 24 h. The filtrates were used for the subsequent analyses.

2.3. Assessment of in vitro antioxidant potentials

2.3.1. Ferric reducing antioxidant potential assay. The estimation of the reducing potential of the extract was carried out as described by Oyaizu [9]. Filtrate (0.25 ml) was added into a test tube containing 0.5 ml of 0.5% (w/v) potassium ferricyanide, $K_3[Fe(CN)_6]$, and 2.5 ml of 0.1M phosphate buffer (pH 6.6) and allowed to stand for 30 min at room temperature of 30 °C. Exactly 0.1 ml of 5% trichloroacetic acid, Cl_3CCOOH, was added to the mixture and centrifuged at 600×*g* for 5 min. The supernatant (2.0 ml) was transferred into a separate test tube containing 0.2 ml of 0.1% (w/v) ferric chloride, $FeCl_3$, and absorbance was measured at 700 nm after 15 min. The reducing potential was computed from the standard curve ($y = 0.0108x + 0.0552$) obtained from varying concentrations of ascorbic acid, $C_6H_8O_6$, and expressed in µg/g of dry weight of the sample.

2.3.2 Ascorbic acid equivalent antioxidant capacities. The ascorbic acid equivalent antioxidant capacity of each extract was estimated using DPPH (1,1-diphenyl-2 picrylhydrazile) radical (DPPH•) discoloration assay. The discoloration assay was carried out as described by Oso and Ogidi [10]. Exactly 0.5 ml of each extract was added into a test tube containing 2.0 ml 0.1 mM DPPH• freshly prepared in methanol. The absorbance was read at 517 nm after 20 min of incubation in the dark at the room temperature of 30 °C. The antioxidant activity of each sample was calculated from the standard curve ($y = -0.004x + 0.209$) obtained from varying concentrations of ascorbic acid and expressed in µg/g of dry weight.

2.3.3. Nitric oxide scavenging potential. Nitric oxide scavenging potential of each extract was estimated in accordance to the method of Garrat [11]. Precisely 2.0 ml of 1% sodium nitroprusside, $Na_2[Fe(CN)_5NO]$, prepared in 0.1 M phosphate buffer (pH 7.4) was added into a test tube containing 0.1 ml of the reconstituted extract and the mixture was incubated for 60 min at room temperature of 30 °C. Afterwards, 0.5 ml of the incubated mixture was pipetted into another test tube containing 1.0 ml sulfanilic acid, reagent prepared by adding 33% sulfanilic acid, $C_6H_7NO_3S$, to 20% glacial acetic acid, CH_3COOH, and incubated at room temperature for 5 min. Successively, 1.0 ml of 0.1 % naphthylethylenediamine dihydrochloride, $C_{12}H_{16}Cl_2N_2$, was pipetted into the reaction mixture and allowed to develop for 30 min at room temperature of 30 °C. The absorbance was measured at 540 nm.

The percentage inhibition was calculated using:

$$\% \text{ inhibition } = \frac{Ao - A1}{Ao} \times 100 \qquad (1)$$

where A_0 = absorbance of the control and A_1= absorbance of extract. The inhibition concentration at 50% (IC_{50}) was calculated and expressed in mg/ml.

2.4. Determination of anti-inflammatory properties

2.4.1. Inhibition of albumin denaturation. Determination of albumin denaturation inhibitory potential of the extract was carried out as described by Williams et al. [12]. The reaction mixture consisted of the filtrates (1.0 ml) in separate test tubes containing 200 µl of 1% aqueous solution of bovine albumin solution and 2.0 ml of 0.05 M phosphate buffer (pH 6.5). The mixtures were incubated at 37 °C for 10 min and then heated to 51°C for 20 min. The turbidity was measured at 660 nm after cooling the test tube. The percentage inhibition of albumin denaturation was calculated using Eq. 1.

2.4.2. Proteinase inhibitory action

The test was performed according to Kunitz [13] with slight modifications. The reaction mixture contained 0.06 mg trypsin, 1.0 mL of 0.1M phosphate buffer (pH 7.4) and 1.0 ml of the filtrate. The mixture was incubated at 37 °C for 5 min. Subsequently, 1.0 ml of 0.8% (w/v) casein was added. The mixture was allowed to incubate for an additional 20 min at 37 °C. Finally, 0.1 ml of 5% Cl_3CCOOH was pipetted into the reaction solution and centrifuged at 600×*g*. The absorbance of the supernatant was measured at 280 nm.

The percentage inhibition of proteinase inhibitory activity was calculated from Eq. 1.

2.5. Estimation of phytochemical composition

2.5.1 Total phenolic content. The phenolic contents of the extracts were estimated using the Folin-Ciocalteu reagent (FCR) as described by Singleton et al. [14]. Exactly 0.1 ml of each extract was added into a test tube containing 0.5 ml of FCR (prepared in ratio 1 to 10 with distilled water). The reaction mixture was allowed to stand for 30 min after which 1.5 ml of 7.5 % Na_2CO_3 solution was added to the mixture. The absorbance was measured at 765 nm after 20 min. The phenolic content was computed from the standard curve ($y = 0.0202x - 0.0382$) obtained from varying concentrations of gallic acid and expressed in µg/g of dry weight of the sample.

2.5.2. Total flavonoid content. Total flavonoid content was estimated colorimetrically based on the method earlier described by Zhishen et al. [15]. In a 10 ml test tube containing 0.1 ml of the extract, 5.0 ml of 5% $NaNO_2$ was added. 5 min later, precisely 3.0 ml of 10 % $AlCl_3$ was added to the mixture and allowed to incubate at room temperature of 30±1 °C for 15 min after which 2.0 ml of 1.0 M NaOH solution was added and the absorbance at 510 nm was measured.

The flavonoid content was computed from the standard curve ($y = 0.002x + 0.069$) obtained from varying concentrations of quercetin and expressed in µg/g of dry weight of the sample.

2.6. Statistical analysis

The results obtained were subjected to statistical analyses using a one-way analysis of variance with the differences between means determined by Duncan's New Multiple post-hoc test using IBM SPSS Statistics 20 software. The values were expressed as the mean ± standard deviation of three determinations.

3. Results and discussion

3.1. In vitro antioxidant properties

The results of the *in vitro* antioxidant properties of the methanol extracts of dried peels of ripe and unripe *A.*

comosus, evaluated through ferric reduction antioxidant potential (FRAP), ascorbic acid equivalent antioxidant capacity (AEAC) and nitric oxide scavenging activity (NO), are presented in Table 1. There was no statistical difference ($p < 0.05$) between the reducing potentials of the extracts. The ascorbic acid equivalent antioxidant capacity of the extract of ripe peel of *A. comosus* was significantly lower than that of the extract of the unripe peel of *A. comosus* with the IC_{50} values of 359.0±10.39 mg/ml and 416.0±12.49 mg/ml, respectively. Correspondingly, the nitric oxide scavenging potential of the extract of ripe peel had a lower activity with the IC_{50} value of 339.33±61.533 mg/ml compared to the IC_{50} value of the unripe peel (253.67±8.386 mg/ml).

Table 1. Antioxidant capacity of ripe and unripe peel of *A. comosus*

Extracts	FRAP (µg/g)	AEAC (µg/g)	NO (IC_{50} mg/ml)
ERP	187.33±8.33	359.0±10.39*	339.33±61.533*
EUP	186.67±2.517	416.0±12.49	253.67±8.386

The values represent the mean ± standard deviation of three determinations. * shows the values within a column are statistically different at $p < 0.05$. FRAP = Ferric Reducing Antioxidant Potentials, AEAC = Ascorbic acid Equivalent Antioxidant Capacity, NO = Nitric Oxide scavenging potential. ERP = Extract of the ripe peels of *A. comosus*, EUP = Extract of the unripe peels of *A. comosus.*

3.2. Anti-inflammatory properties

The anti-inflammatory properties of the extracts are presented in Table 2. The ADIP of the ripe peel extract of *A. comosus* is significantly lower ($p < 0.05$) compared to the extract of the ripe peel. However, there was no statistical difference between the proteinase inhibition potentials (PIP) of the extracts.

Table 2. Albumin denaturation inhibitory potentials (ADIP) and proteinase inhibition potential (PIP) of methanol extracts of ripe and unripe peels of *A. comosus*

Extracts	ADIP (% inhibition)	PIP (% inhibition)
ERP	12.76±3.02*	11.27±2.13
EUP	16.79±2.93	9.84±3.80

The values represent the mean ± standard deviation of three determinations. * shows the values within a column are statistically different at $p < 0.05$. ERP= Extract of the ripe peels of *A. comosus*, EUP= Extract of the unripe peels of *A. comosus.*

3.3. Total phenolic and total flavonoid content

The results of the estimation of phenolic and flavonoid contents of ripe and unripe peels of methanol extract of *A. comosus* are presented in Table 3. There was no significant difference between the phenolic contents of the extracts of the ripe peel (71.33±8.08 µg/g) and the unripe peel (68.33±2.08 µg/g). However, the flavonoid contents were significantly higher in the extract of the ripe peel compared to the extract of the unripe peel.

Table 3. Phenolic and flavonoid contents of ripe and unripe peels of methanol extracts of *A. comosus*

Extracts	Phenolic (µg/g)	Flavonoid (µg/g)
ERP	71.33±8.08	24.0±0.03*
EUP	68.33±2.08	22.33±2.31

The values represent the mean ± standard deviation of three determinations. * shows the values within a column are statistically different at $p < 0.05$. ERP = Extract of the ripe peels of *A. comosus*, EUP = Extract of the unripe peels of *A. comosus.*

The capacities of plant-based natural products to act as antioxidants and their role in the prevention of diseases that are associated with inflammatory disorders have been employed in the development of substitutes in place of synthetic agents with biological properties. The results of the study indicated that extracts of the peels of *A. comosus* exhibited corresponding antioxidant and anti-inflammatory properties which could be attributed to the characteristic phytochemical constituents. The antioxidant capacities assessed through DPPH radical and NO were higher in the extract of the unripe peel. The observed differences in the antioxidant capacities between the ripe and unripe peel could be related to structural and functional natures of the bioactive compounds in the peels. Several studies had reported that the antioxidant capacity of plant materials is ripening-dependent [16-18].

Similarly, the extracts exhibited anti-inflammatory activities in both ADIP and PIP assays. The anti-inflammatory potentials of the extracts assessed through ADIP correlated with AEAC and NO antioxidant activities. The results of the present study were consistent with a number of reports regarding the strong relationship between antioxidant property and anti-inflammatory potential of plant materials [19, 20]. Consequently, peel of *A. comosus* could be used as a source of antioxidants and anti-inflammatory agents and thus may be effective against disorders that are related to oxidative stress and inflammation.

Furthermore, the study provides evidence for the presence of biologically active compounds such as phenolics and flavonoids. These are compounds containing one or more aromatic rings and are essential dietary factors naturally found in plant-based foods. The phenolics comprise one of the main groups of secondary metabolites in plant materials with essential health-promoting properties. Studies had shown that concentrations of these phytochemicals could be influenced by several factors which include post-harvest handling, processing techniques, and extraction procedures [21]. Ripening appears not to influence the phenolic contents of the peel; however, there were higher values of flavonoid contents in the ripe peel than the unripe peel. The flavonoid contents correlated inversely with the observed antioxidant and the anti-inflammatory potentials of the extracts which aggress with the observation of Liu et al. [22]. Generally, the flavonoids become less-reactive radical and more stable when oxidized by radicals [23]. The negative correlation could be attributable to the changes in the phytochemical profile in the peel of *A. comosus*. Examples of phytochemicals that had been reported to increase with ripening include anthocyanin, naringenin, lycopene, rutin and luteolin [24, 25]. Moreover, ripening could modulate the synergistic effects among the different phytochemical groups in the peels and subsequently contribute to the observed biological activities. In addition, flavonoids had been demonstrated to have undefined reactivity against DPPH radical

whereas they could be related to the reduction of pathological disorders [26].

Moreover, the study indicate the peels could contain a wide range of secondary metabolites with favourable biological properties which could be similar with edible parts of the fruits which had been reported to be a good source of phytochemicals and micronutrients with therapeutic properties in the food industry, pharmaceuticals and cosmetic products [27, 28]. Thus, the peels of ripe and unripe of *A. comosus* could also be used in the manufacture of nutraceuticals such as polyphenols with antioxidant and anti-inflammatory function. Additionally, the peels could improve waste management, reduce environmental pollution, and generate value-added food products.

4. Conclusion

This study reveals the probable exploitation of the peel of *A. comosus* in alternative and complementary medicine most especially for ailments that associated with inflammatory disorders. It also showed that the overall compositions and biological properties of the peel of *A. comosus* could be affected by ripening process. *In vivo* studies of the extracts could be considered in future studies in order to establish the toxicological effects of the extracts.

Conflict of interest

The authors declare that there is no conflict of interest as regards the content of this article

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