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Comparative evaluation of phenolics, flavonoids, and antioxidant activity of aqueous and methanolic extracts of *Ananas comosus* (pineapple) flesh

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Abstract. *Adefuye AO, Adegbesan BO, Ezima EN, Adefuye TA, Abisoye SB, Bello TH, Ogunbiyi EB, Onasanya AS, Oladeinde TE. 2026. Comparative evaluation of phenolics, flavonoids, and antioxidant activity of aqueous and methanolic extracts of* Ananas comosus *(pineapple) flesh. Asian J Trop Biotechnol 23 (1): c230102. https://doi.org/10.13057/biotek/c230102.* This research investigated the proximates of *Ananas comosus* Flesh (ACF), Total Phenolic Contents (TPC), Total Flavonoid Contents (TFC), and antioxidant activity of ACF extracts based on Ferric Reducing Antioxidant Potential (FRAP), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), and Nitric Oxide Scavenging Ability (NOSA). Ten fresh and ripe *A. comosus* fruits were collected, identified, authenticated, processed to a constant weight, macerated, and decanted to obtain clear filtrates of Methanolic Extract of ACF (MEACF) and Aqueous Extract of ACF (AEACF). Proximate analysis of ACF was conducted based on the standard procedure. Data analysis was conducted by two-way Analysis of Variance (ANOVA) followed by Duncan's post-hoc test; values with $p<0.05$ were considered significantly different. Fresh weight of ACF contained 81.00±1.00% moisture, 3.00±0.00% ash, 0.65±0.15% fats, 1.50±0.50% crude fiber, 1.75±0.05% protein, and 12.10±0.30% carbohydrates. AEACF and MEACF exhibited a significant ($p<0.05$) increase in TPC, TFC, FRAP, and ABTS assays by increasing concentration from 5 µg/mL to 100 µg/mL. AEACF had significantly ($p<0.05$) higher TPC than MEACF at 5, 10, and 25 µg/mL. At 50 and 100 µg/mL, TPC in MEACF was substantially ($p<0.05$) higher than in AEACF. At 5, 25, and 100 µg/mL, AEACF showed considerably ($p<0.05$) higher FRAP values than MEACF. At 10, 25, and 50 µg/mL, AEACF considerably ($p<0.05$) showed higher scavenging of ABTS radicals than MEACF. At 10 and 25 µg/mL, the NOSA of AEACF was substantially ($p<0.05$) greater than that of MEACF, whereas at 5 µg/mL, the NOSA of MEACF was substantially ($p<0.05$) greater than that of AEACF. Increased FRAP values and ABTS radical-scavenging ability indicate higher antioxidant activity, whereas increased nitrite concentration corresponds to lower scavenging activity. Correlation analysis (r=0.964-0.999) demonstrates a strong correlation between TPC, TFC, and in vitro antioxidant parameters. The findings highlight the importance of solvent selection in phytochemical extraction and show that solvent polarity significantly influences the extraction of beneficial chemicals from ACF. Pineapple (*A. comosus*) may be beneficial for health due to its potentially beneficial components.

Keywords: *Ananas comosus*, antioxidant potential, flavonoid compounds, phenolic compounds, solvent extraction

INTRODUCTION

Oxidative stress results from an imbalance between the production of Reactive Oxygen Species (ROS) and the ability of biological systems to neutralize them. Free radicals may originate from various environmental and anthropogenic sources, including pollutants, toxins, radiation, agricultural contaminants, and food preservatives (Chaudhary et al. 2023; Tumilaar et al. 2024). Excessive accumulation of these reactive species can lead to oxidative damage of biomolecules such as lipids, proteins, and nucleic acids, thereby contributing to the development of several chronic diseases. Consequently, there has been growing interest in identifying natural antioxidants that can scavenge free radicals and protect biological systems from oxidative damage.

Medicinal plants represent an important source of bioactive compounds with potential health-promoting properties. A considerable proportion of the global population relies on plant-derived products for primary healthcare due to their accessibility and therapeutic potential (Majeed 2017; Adegbesan et al. 2024; Adegbesan et al. 2024). Many of the beneficial effects of medicinal plants are attributed to secondary metabolites such as phenolic compounds, flavonoids, alkaloids, and other phytochemicals, which exhibit antioxidant, anti-inflammatory, and antimicrobial activities (Anwar et al. 2018; Ogwu et al. 2024). Fruits and vegetables, in particular, are widely recognized as important dietary sources of natural antioxidants that can mitigate oxidative stress.

Ananas comosus (pineapple) has attracted significant scientific interest due to its phytochemical composition and antioxidant potential. Pineapple is a tropical fruit widely consumed worldwide and valued for its nutritional composition, which includes vitamins, phenolic compounds, flavonoids, and other antioxidant constituents (Aparecida et al. 2016; Roda et al. 2017; Zhang et al. 2020). These phytochemicals contribute to the fruit's antioxidant capacity and potential health benefits. Several studies have reported that pineapple contains appreciable levels of phenolic compounds and flavonoids that act as natural antioxidants by scavenging free radicals and reducing oxidative stress (Sah et al. 2016; Chalchisa and Dereje 2021; Zakaria et al. 2021; Shivamathi et al. 2022; Singh et al. 2022). However, while these studies provide valuable insights into pineapple, relatively limited attention has been given to the phytochemical composition and antioxidant capacity of the edible flesh, which represents the most widely consumed portion of the fruit. Furthermore, previous investigations mostly employed single-solvent extraction systems, making it difficult to directly compare the efficiency of different solvents in extracting antioxidant compounds from pineapple flesh.

The recovery of phytochemicals from plant materials largely depends on the extraction method and the solvent system used. Solvent polarity plays a crucial role in determining extraction efficiency, as different classes of phytochemicals vary in their solubility. However, comparative studies evaluating the effectiveness of aqueous versus methanolic solvents specifically for the extraction of bioactive compounds from pineapple flesh remain scarce, particularly for locally cultivated cultivars. Polar organic solvents such as methanol, ethanol, and aqueous mixtures are commonly used for extracting phenolic and flavonoid compounds because they can dissolve a wide range of polar and semi-polar metabolites (Awah and Verla 2010; Arias et al. 2022). In particular, methanol-water mixtures could enhance the recovery of phenolic compounds, possibly due to their intermediate polarity, which may facilitate the extraction of diverse antioxidant constituents. Therefore, comparative evaluation of solvent systems is essential for optimizing the extraction of phytochemicals from plant materials.

In addition, variations in cultivar type and environmental growing conditions may influence the phytochemical composition and antioxidant properties of pineapple fruits. The Smooth Cayenne cultivar is one of the most widely cultivated pineapple varieties globally and is also commonly grown in southwestern Nigeria. Despite its widespread consumption and economic importance, limited information exists regarding the influence of solvent extraction on the recovery of phytochemicals and antioxidant activity of the flesh of this cultivar. In particular, comparative data on aqueous and methanolic extracts of pineapple flesh remain limited. Therefore, the present study aimed to evaluate the proximate composition of *A. comosus* flesh and comparatively assess the total phenolic, total flavonoid content, and in vitro antioxidant activities of aqueous and methanolic extracts of pineapple flesh. The antioxidant potential of the extracts was determined using the Ferric Reducing Antioxidant Power (FRAP) assay, the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay, and the Nitric Oxide Scavenging Activity (NOSA) assay.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in this study were of pure and analytical grade. The chemicals TPTZ (2,4,6-tripyridyl-*S*-triazine), Folin-Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethyl chromane 2-carboxylic acid), ABTS, 2-thiobarbituric acid, and ascorbic acid were procured from Sigma Aldrich Chemicals Co., U.S.A. Sodium phosphate, sodium hydroxide, and potassium ferricyanide were products of Randox Laboratories Ltd. (Crumlin, UK). All additional chemicals used in this research were procured from reputable outlets.

Plant collection, authentication, and extraction

The cultivar of *A. comosus* used in this study is Smooth Cayenne, picked when fully colored and slightly soft to the touch. The samples were handled with care to avoid bruising and stored at 10-13°C to prolong shelf life before their processing. Ten (10) fresh, ripe, and viable fruits were sourced from a reputable market in Ogun State, Nigeria. These samples were identified and authenticated at the Herbarium Laboratory, Department of Forestry and Wildlife Management, College of Environmental Resources Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, with a voucher number UAHA/FWM/025/05/001. Fruits were carefully collected and rinsed with clean tap water, then rinsed with distilled water.

Processing of *Ananas comosus* (aqueous extraction and methanolic extraction)

All fruit samples were peeled and chopped into small pieces. Afterward, they were dehydrated (subjected to controlled drying to reduce moisture and stabilize the sample before extraction) in a Bosch food dehydrator (BS-6608) at 60°C until constant weight. Afterward, the samples were blended (pulverized) to a constant coarse product, which was stored in an airtight container. For maceration, a solid-to-solvent ratio of 0.4:1 (w/v) was used following Azwanida (2015). Briefly, dried and pulverized ACF was mixed separately with distilled water or 80% methanol, and the mixtures were allowed to macerate at 25°C for 72 h with intermittent agitation every 4 h. The extraction vessels were protected from light to minimize the potential degradation of light-sensitive phytochemicals such as phenolic compounds and flavonoids. The macerated product was filtered through a muslin cloth (0.1 mm) and a wool-laddered funnel to collect a clean filtrate, which was used for further procedures. The methanol and distilled water from the filtrate were removed using a rotary evaporator (RotoVap 110) at 40°C and the resulting slurry or semi-solid product was collected into a beaker and dried to constant mass. The concentrated Methanolic

Extract of *A. comosus* Flesh (MEACF) and Aqueous Extract of *A. comosus* Flesh (AEACF) samples were stored in separate, dry, airtight containers until further use.

Determination of moisture content

The moisture content of ACF was measured following Association of Official Analytical Chemists (AOAC 2005), by the oven method "via hot-air". Two grams of ACF (fresh weight) were introduced into a clean, clear crucible that had been weighed and then dried for three hours at 103°C in a hot-air oven. Afterward, the samples were cooled within a desiccator. Then, they weigh. The weight lost by the ACF sample was recorded.

Determination of ash content

Two grams of ACF were placed in a crucible and heated in a muffle furnace at 550°C for three hours, or till it turned whitish or grey (AOAC 2005). Afterward, the items were cooled within a desiccator. Then, the weight of the crucible and sample was taken and recorded.

Determination of fat content

The analysis of crude fat content was done according to AOAC (2005). Exactly 2 g of ACF was weighed into an empty round-bottom flask (for Soxhlet extraction), and the samples were wrapped in a porous material. 500 mL of petroleum ether was added to the round-bottom flask, and heating was maintained for 4 hours. After 4 hours, the power supply was turned off, and the system was allowed to cool. The samples were removed from the thimble, and the solvents were evaporated (or collected), leaving a layer of fat on the round-bottom flask.

Determination of total protein content

The Bradford (1976) method was adopted with minor modifications to determine total protein in ACF. About 50 mL of 95% ethanol was used to dissolve 100 mg of Coomassie Brilliant Blue G-250. Then, 100 milliliters of 85% (w/v) phosphoric acid was added to make 1 L of the mixture. After escaping Whatman-filter paper "No. 1," the dye was refrigerated in dark bottles. Five milliliters of Bradford reagent were added to one milliliter of ACF, and the mixture was mixed thoroughly. Absorbance was measured in a UV-VIS spectrometer at 595 nm. Five milliliters of reagent and one milliliter of distilled water were combined to create the blank. To determine the protein concentration, the value was compared to a standard curve generated with bovine serum albumin. The extraction buffer contained 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, and protease inhibitor. Sample preparation involved homogenization, centrifugation, and filtration.

% protein was derived from spectrophotometric concentration using the equation:

$$\% \text{ protein} = \frac{\textit{Protein Concentration x Extraction Volume}}{\textit{Sample Weight}}\ x\ 100$$

Determination of crude fibre

The AOAC (2005) method was followed to measure crude fiber in ACF. Approximately 5 g of ACF and 100 mL of TCA digesting reagent were added to a 500 mL Erlenmeyer flask. The mixture was boiled and refluxed for precisely 40 minutes, starting at the beginning of the boiling process. After being taken out of the heater and made to cool down slightly, filtration was allowed through a 15.0 cm Whatman paper "number 4." After cleaning with hot water and stirring with a spatula, the sediment was transferred into a porcelain dish. The collected product was dried overnight at 105 °C. The weight was recorded as W1 after drying and being dropped in a desiccator. After 6 hours of burning at 500°C in a muffle furnace and cooling, a new weight was taken and recorded as W2.

Determination of carbohydrates

After other contents of ACF had been determined, the percentage composition of carbohydrate was determined using the formula below:

% carbohydrate = 100% - (% Ash + % Protein + % Fibre + % Moisture + % Fat)

Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu assay, as described by Singleton et al. (1999), was used to measure TPC in MEACF and AEACF spectrophotometrically. The standard curve was established using gallic acid. The TPC in MEACF and AEACF was presented in mg gallic acid equivalents per gram dry weight (mg GAE/g DW). Every TPC evaluation in MEACF and AEACF was carried out in triplicate.

The calculation formula for converting absorbance to

$$\text{mg GAE/gdw} = \frac{\text{C x V x DF}}{ms}$$

Where:

C: Concentration of GAE from the calibration curve (in mg/mL)

V: Total volume of extract used (in mL)

DF: Dilution Factor

m_s: Mass of the dry sample (in g)

Determination of Total Flavonoid Content (TFC)

TFC of MEACF and AEACF was measured by the $AlCl_3$ method (Ordonez et al. 2006). Briefly, 20 µL of 2% $AlCl_3 . 6H_2O$ solution was mixed with an equal volume (20 µL) of MEACF or AEACF and shaken. Double-dilution with distilled water was done to increase the final volume to 10 mL. Following a 10-minute incubation, the absorbance of the mixture was evaluated at 440 nm. The TFC was expressed as mg Quercetin equivalents/g DW (mg QE/g DW). All the determinations were performed in triplicate.

Calculation formula for converting absorbance to mg

$$\text{QE/gdw} = \frac{\text{C x V x DF}}{ms}$$

Where:

C: Concentration of GAE from the calibration curve (in mg/mL)

V: Total volume of extract used (in mL)

DF: Dilution Factor

m_s: Mass of the dry sample (in g)

Assay for 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging

Free radical scavenging activity of MEACF and AEACF was performed using the ABTS radical cation decolorization assay (Re et al. 1999). $ABTS^{\cdot +}$ radical was produced when 7 mM ABTS reacted with 2.45 mM potassium persulfate (1:1) in water. The product was stored at 25°C in the dark for 12 to 16 hours before use. ABTS radical cation solution was prepared and diluted to an absorbance of 0.70±0.02 at 734 nm. An absorbance value was measured after 30 minutes of initially mixing 3995 uL of diluted $ABTS^{\cdot +}$ solution with 5 µL of MEACF. The same protocol was followed for AEACF. Results of ABTS scavenging activity were reported as mg Trolox equivalents/g DW. A suitable solvent blank was also prepared and tested in each assay. It was performed in triplicate.

$ABTS^{\cdot +}$ scavenging activity (mg, Trolox equivalents/g DW) = $\frac{AB - AA}{AB}$

Where:

AB: Absorbance $_{\text{ABTS radical}}$ + methanol

AA: Absorbance$_{\text{ABTS radical}}$ + MEACF/AEACF/standard (which was Trolox)

Ferric Reducing Antioxidant Power (FRAP)

Benzie and Strain (1996) was adopted to estimate the FRAP values of MEACF and AEACF spectrophotometrically. The mechanism includes the conversion of the colourless Fe^{3+}-TPTZ complex to the Fe^{3+}-tripyridyltriazine complex, which is blue colored, created at reduced pH by electron-donating antioxidants. The reaction was monitored by measuring the change in absorption capacity at 593 nm. The FRAP reagent was prepared by mixing 300 mM acetate buffer, 20 mM $FeCl_3 \cdot 6H_2O$, and 10 mL TPTZ in 40 mM HCl in a 10:1:1 ratio at 37°C. Using a 1-5 mL micropipette, a freshly prepared FRAP reagent (measuring 3995 uL) was thoroughly mixed with 5 µL of MEACF. Following the reduction of the ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex to the ferrous (Fe^{2+}) form, a deep blue colored complex was generated. After 30 minutes of incubation at 37°, absorbance was measured at 593 nm against a reagent blank (5 µL distilled water + 3995 µL FRAP reagent). The same protocol was followed for AEACF. Results of antioxidant activity via FRAP assay were expressed as mg Trolox equivalents/g DW.

Nitric oxide radical (NO^*) scavenging assay (NOSA)

Nitric oxide radical scavenging activity was determined according to the method described by Marcocci et al. (1994) with minor modifications. A reaction mixture (5 mL) containing 5 mM sodium nitroprusside (SNP) in phosphate-buffered saline (PBS, pH 7.3) was incubated at 25°C for 180 min under visible light generated by a 25 W tungsten lamp. The reaction mixtures contained either the Aqueous Extract of *A. comosus* Flesh (AEACF) or the Methanolic Extract (MEACF) at varying concentrations, while a control contained SNP without extract. At 30 min intervals, 1.0 mL aliquots of the incubation mixture were withdrawn and mixed with an equal volume of Griess reagent consisting of 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid. The absorbance of the resulting chromophore was measured at 546 nm using a spectrophotometer. Nitrite concentration was determined from a standard calibration curve prepared with sodium nitrite solutions, and nitric oxide scavenging activity was expressed as percentage inhibition of nitrite formation relative to the control. Lower nitrite concentrations corresponded to higher nitric oxide radical scavenging activity. All assays were performed in triplicate, and the results were expressed as mean ± standard deviation. Data presented in Table 5 were obtained from measurements taken between 120 and 180 min, corresponding to the period of stable color development in the Griess reaction.

Nitric Oxide Scavenging Ability (%) = $\frac{\text{Absorbance of control reaction} - \text{Absorbance of test reaction}}{\text{Absorbance of the control reaction}} x\ 100$

Association between components of extracts and markers of in vitro antioxidant assay

The correlation between phytochemical content (TPC, TFC) and in vitro antioxidant activity (FRAP, ABTS, and NOSA) was assessed using Pearson correlation analysis. Correlation coefficients were interpreted cautiously due to the limited number of concentration points.

Statistical analysis

All experiments were conducted in triplicate, data were analyzed using SPSS" version 20.0. Results were presented as mean ± Standard Error of the Mean (SEM). Statistical comparisons were performed using two-way Analysis of Variance (ANOVA) "to evaluate the effects of extraction solvent type (distilled water or 80% methanol) and varying concentration levels of extracts (AEACF and MEACF)", including their interaction effects. When significant differences were detected, Duncan's Multiple Range Tests (DMRTs) were used for post-hoc comparisons. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Ananas comosus Flesh (ACF) was analyzed for its nutritional value (Table 1). The ACF (fresh weight) contained fat (0.65±0.15%) and moisture (81.00±1.00%). The ash, fiber, protein, and carbohydrate contents were 3.000±0.000%, 1.500±0.500%, 1.750±0.050%, and 12.100±0.300%, respectively. The results provide insights into the proximate analysis of pineapple flesh and the influence of solvent selection on TPC, TFC, and antioxidant activity. The difference in antioxidant activity between MEACF and AEACF suggests that MEACF and AEACF contain different antioxidant compounds. The proximate composition in this study aligns with previously reported nutritional profiles of pineapple fruits, which are characterized by high moisture content, moderate carbohydrate levels, and low lipid content (Ali et al. 2020).

The TPC and TFC of MEACF and AEACF are presented in Table 2 with the extract concentrations of 5-100 µg/mL. TPC varied considerably in MEACF and AEACF, ranging from 5.000±0.887 mg GAE/g DW to 80.615±0.577 mg GAE/g DW and from 6.641±0.128 mg GAE/g DW to 78.436±0.427 mg GAE/g DW, respectively. TPC in MEACF and AEACF increased with increasing extract concentration. The total flavonoid content also varied considerably in MEACF and AEACF, ranging from 7.122±0.190 mg QE/g DW to 87.439±1.896 mg QE/gDW and from 7.618±0.389 mg QE/g DW to 69.707±2.643 mg QE/g DW, respectively. However, TFC in MEACF and AEACF at 5 and 10 µg, respectively, was below the Limit of Detection (LOD).

Phenolic compounds are widely recognized for their antioxidant properties due to their ability to donate hydrogen atoms or electrons to neutralize free radicals. The higher phenolic content in methanolic extracts likely contributed to the stronger reducing power in FRAP assays. Similar relationships between phenolic content and antioxidant activity have been reported in numerous plant-based studies (Singleton et al. 1999; Anwar et al. 2018). Flavonoids represent another important group of plant secondary metabolites that contribute to antioxidant activity through radical scavenging and metal-chelating mechanisms. The higher flavonoid content detected in methanolic extracts may explain the enhanced antioxidant activity in some assays. These compounds are known to interact with free radicals via electron-transfer mechanisms, thereby stabilizing reactive species and preventing oxidative damage (Tumilaar et al. 2024).

The increase in TPC and TFC observed for MEACF and AEACF aligns closely with the work of Hossain and Rahman (2011), who reported that pineapple fruit contains appreciable levels of total phenolics and flavonoids. Their study confirmed a strong positive correlation between TPC and in vitro antioxidant assays (ABTS and DPPH radical scavenging activities in pineapple extracts), consistent with the high correlation coefficients observed in the present work. Similarly, Du et al. (2016) reported that phenolics and flavonoids in pineapple fruit significantly contribute to its antioxidant activity following the comparison of seventeen pineapple varieties. The study showed considerable variability in phenolic profiles and antioxidant capacity across cultivars, supporting the present study's findings, particularly the differential extraction efficiencies of methanol and distilled water. The strong correlations between phenolic content and antioxidant activity (FRAP values) reported by Du et al. (2016) mirror the high R^2 values obtained in this study, reinforcing the central role of phenolics in determining antioxidant potential. The ABTS assay showed that both MEACF and AEACF have the potential to scavenge free radicals (Table 3).

Table 1. Proximate compositions of ACF (fresh weight)

Component	Proximate Analysis of *Ananas comosus* flesh (%); n = 3
Ash	$3.000±0.000^{b}$
Moisture	$81.000±1.000^{d}$
Fat	$0.650±0.150^{a}$
Fibre	$1.500±0.500^{ab}$
Protein	$1.750±0.050^{ab}$
Carbohydrate	$12.100±0.300^{c}$

Note: The value was expressed as mean ± SEM (n=3). Different alphabets (superscripts) within the column are statistically significantly different at $p<0.05$

Table 2. Total phenolic and total flavonoid contents of MEACF and AEACF

Concentration	Total flavonoid content (mg QE/g DW)		Total phenolic content (mg GAE/g DW)	
	MEACF	AEACF	MEACF	AEACF
5 µg/mL	Below the LOD	Below the LOD	$5.000±0.887^{a}$	$6.641±0.128^{bc}$
10 µg/mL	Below the LOD	Below the LOD	$5.923±0.194^{ab}$	$8.103±0.321^{c}$
25 µg/mL	$7.122±0.190^{a}$	$7.618±0.389^{a}$	$31.436±0.370^{d}$	$40.692±0.160^{e}$
50 µg/mL	$69.959±1.810^{c}$	$43.350±1.993^{b}$	$53.256±0.921^{g}$	$51.000±0.311^{f}$
100 µg/mL	$87.439±1.896^{d}$	$69.707±2.643^{c}$	$80.615±0.577^{i}$	$78.436±0.427^{h}$

Note: Values of TPC and TFC are expressed as mean ± SEM (n=3). Values with different alphabets (as superscripts) are significantly different at $p<0.05$. Below the LOD: Below the Limit of Detection

Table 3. ABTS radical scavenging activity of MEACF and AEACF

Concentration	ABTS scavenging ability (mg TE/g DW)		
	MEACF	AEACF	Vitamin C
5 µg/mL	$19.516±0.358^{b}$	$10.376±0.040^{a}$	$49.856±1.729^{f}$
10 µg/mL	$21.586±3.004^{bc}$	$27.940±0.160^{d}$	$80.340±0.192^{h}$
25 µg/mL	$23.910±0.731^{c}$	$44.933±0.594^{e}$	$82.271±0.054^{h}$
50 µg/mL	$43.615±0.824^{e}$	$49.949±0.495^{f}$	$82.832±0.675^{h}$
100 µg/mL	$65.314±0.385^{g}$	$64.274±2.537^{g}$	$84.120±0.125^{h}$

Note: Values of ABTS radical scavenging ability were expressed as mean ± SEM (n=3) as mg TE/g DW. Values with different alphabets (as superscripts) are significantly different at $p<0.05$

FRAP assays are a common method for evaluating the antioxidant potential of a substance because they tend to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Findings from the FRAP assay are presented in Table 4. Findings from the FRAP assay revealed that both MEACF and AEACF possess free radical scavenging potential.

Table 5 shows the NO scavenging ability of MEACF and AEACF, represented by the nitrite concentration produced (in µM). Nitric oxide ($NO^{\cdot}$) produced from SNP degradation exhibits a potent radical character (Awah and Verla 2010). The NO radical generated alters the structure and function of several cellular components. Both MEACF and AEACF exhibited good NO· scavenging ability as their concentration decreased from 100 µg/mL to 5 µg/mL, resulting in a reduction in nitrite concentration in the assay medium. The concentration of nitrite produced in the reaction medium serves as an indirect indicator of nitric oxide radical activity. The lower nitrite concentrations correspond to stronger scavenging activity, whereas higher nitrite levels indicate reduced scavenging efficiency.

The ability of AEACF and MEACF to reduce Fe^{3+} to Fe^{2+} suggests their potential as natural antioxidants. Such an attribute may be due to their phytochemical content and apparent bioactivity. Thus, AEACF and MEACF may be rich origins of biologically active substances with strong antioxidant capabilities. The ability of MEACF and AEACF to reduce oxidants is likely due to single-electron transfer mechanisms described by Huang et al. (2021), in which antioxidants in pineapple are suggested to act as effective electron donors. Although the study by Li et al. (2014) focused primarily on pineapple peels, it demonstrated that major polyphenolics interact synergistically, which may partly explain the strong positive relationships in the current study between TPC/TFC and FRAP, ABTS, and NO scavenging assays. Thus, the antioxidant performance for ACF suggests that the edible portion of pineapple remains a meaningful source of bioactive compounds. Moreover, while many pineapple studies emphasize ABTS, DPPH, and FRAP assays, fewer explore nitric oxide scavenging.

The apparent inverse relationship observed in the nitric oxide scavenging assay may be attributed to the complex reaction mechanisms underlying nitric oxide generation and its interaction with phytochemicals in MEACF and AEACF. Unlike stable radical assays such as FRAP and ABTS, nitric oxide scavenging involves the continuous generation of NO radicals from sodium nitroprusside under light exposure, followed by their conversion to nitrite in the presence of oxygen. There are also matrix effects at higher extract concentrations, where increased phytochemical content may interfere with the reaction between nitric oxide and oxygen or affect the diazotization reaction of the Griess reagent, despite this being atypical concentration-dependent. Both MEACF and AEACF demonstrated measurable nitric oxide scavenging potential within the tested concentration range. The activity observed is likely associated with the presence of phenolic compounds and flavonoids in pineapple flesh, which are known to possess antioxidant properties through electron donation and radical stabilization mechanisms.

Pearson correlation analysis revealed strong positive relationships between TPC, TFC, and FRAP in both AEACF and MEACF (Table 6). In MEACF, FRAP showed a very strong positive correlation with TPC ($r=0.998$, $p<0.001$, $n=5$). Similarly, in AEACF, the relationship between FRAP and TPC was also extremely strong ($r=0.999$, $p<0.001$, $n=5$). A comparable trend was observed for flavonoids. FRAP correlated strongly with TFC in MEACF ($r=0.998$, $p=0.042$) and AEACF ($r=0.999$, $p=0.028$, $n=3$). Strong positive correlations were also observed between FRAP and ABTS radical scavenging activity in both extracts. FRAP and ABTS of AEACF exhibited a very strong correlation ($r=0.996$, $p=0.0001$, $n=5$), while a similarly strong relationship was observed in MEACF ($r=0.995$, $p=0.0002$, $n=5$). Correlations between TPC and NOSA were significantly positive in both extracts. In AEACF, NOSA correlated strongly with TPC ($r=0.975$, $p=0.005$), while a similar trend was observed in MEACF ($r=0.964$, $p=0.008$). In MEACF, NOSA showed an extremely strong correlation with TFC ($r=0.999$, $p=0.028$), indicating that flavonoids may significantly contribute to nitric oxide scavenging activity. However, the relationship was not statistically significant in AEACF ($r=0.987$, $p=0.105$). Strong positive correlations were also observed among the antioxidant assays, with FRAP correlating strongly with NOSA in AEACF ($r=0.992$, $p=0.001$) and MEACF ($r=0.978$, $p=0.004$). Similarly, ABTS showed strong correlations with NOSA in AEACF ($r=0.991$, $p=0.001$) and MEACF ($r=0.971$, $p=0.006$).

Table 4. FRAP scavenging activity of MEACF and AEACF

Concentration	Reducing potential as FRAP values (mg TE/gdw)		
	MEACF	AEACF	Vitamin C
5 µg/mL	0.193±0.222[a]	1.318±0.569[b]	14.005±0.441[d]
10 µg/mL	3.950±0.077[c]	3.497±0.325[c]	36.355±1.741[g]
25 µg/mL	12.955±0.303[d]	15.180±0.364[e]	51.783±0.399[i]
50 µg/mL	22.667±0.242[f]	22.101±0.297[f]	53.384±0.626[ij]
100 µg/mL	38.616±0.122[g]	42.542±0.545[h]	58.844±1.227[j]

Note: Absorbance values were extrapolated in terms of Trolox per g of dry weight based on a standard curve. Values from the FRAP assay were expressed as mean ± SEM (n = 3) as mg TE/gdw. Values with different alphabets (as superscripts) are significantly different at $p<0.05$

Table 5. Nitric Oxide radical scavenging (NOSA) activity of MEACF and AEACF

Concentration	Nitrite concentration (µM)		
	MEACF	AEACF	Vitamin C
5 µg/mL	20.044±0.251[a]	22.198±0.276[b]	28.660±0.459[d]
10 µg/mL	30.613±0.239[e]	25.758±1.741[c]	29.701±0.263[d]
25 µg/mL	31.763±0.321[ef]	27.747±0.159[d]	33.279±0.066[g]
50 µg/mL	32.749±0.333[fg]	33.954±0.684[g]	36.948±0.256[h]
100 µg/mL	37.240±0.114[h]	36.729±0.097[h]	44.998±0.102[i]

Note: Nitric oxide radical (NO^*) scavenging ability was expressed as percentage inhibition of nitrite formation. Nitric oxide radical (NO^*) scavenging ability was expressed as mean ± SEM (n = 3). Values with different alphabets (as superscripts) are considered to have statistical significance at $p < 0.05$. Tendency of MEACF and AEACF to scavenge NO^* radical is presented as an aggregate of nitrite generated following the degradation of 5 mM sodium nitroprusside (SNP) at 25°C.

Table 6. Correlation between TPC and TFC vs in vitro antioxidant parameters (FRAP, ABTS, NOSA) for MEACF and AEACF

Comparisons	Sample	Pearson r value (approx.)	p-value (approx.)	Significant (at p<0.05)
FRAP vs TPC (n=5)	AEACF	0.999	< 0.001	Yes
FRAP vs TPC (n=5)	MEACF	0.998	< 0.001	Yes
FRAP vs TFC (n=3)	AEACF	0.999	0.028	Yes
FRAP vs TFC (n=3)	MEACF	0.998	0.042	Yes
ABTS vs TPC (n=5)	AEACF	0.976	0.004	Yes
ABTS vs TPC (n=5)	MEACF	0.987	0.002	Yes
ABTS vs TFC (n=3)	AEACF	0.992	0.083	No
ABTS vs TFC (n=3)	MEACF	0.999	0.036	Yes
NOSA vs TPC (n=5)	AEACF	0.975	0.005	Yes
NOSA vs TPC (n=5)	MEACF	0.964	0.008	Yes
NOSA vs TFC (n=3)	AEACF	0.987	0.105	No
NOSA vs TFC (n=3)	MEACF	0.999	0.028	Yes
FRAP vs ABTS (n=5)	AEACF	0.996	0.0001	Yes
FRAP vs ABTS (n=5)	MEACF	0.995	0.0002	Yes
FRAP vs NOSA (n=5)	AEACF	0.992	0.001	Yes
FRAP vs NOSA (n=5)	MEACF	0.978	0.004	Yes
ABTS vs NOSA (n=5)	AEACF	0.991	0.001	Yes
ABTS vs NOSA (n=5)	MEACF	0.971	0.006	Yes

Note: Comparisons between parameters are considered statistically significant at $p<0.05$

However, despite the very high correlation coefficients observed across several comparisons, these relationships should be interpreted with caution, given the relatively small sample sizes used in the correlation analyses. In some cases, the number of observations was limited (e.g., n = 3 for certain flavonoid measurements), which can artificially inflate correlation coefficients and reduce the reliability of statistical significance testing. Small datasets may therefore produce extremely high correlation values that do not necessarily reflect true biological relationships across larger sample populations. Furthermore, the lack of statistical significance observed in the relationship between NOSA and TFC in the aqueous extract may also be attributed to the limited number of detectable flavonoid values in that dataset. This limitation underscores the need for larger datasets and additional experimental replicates to more robustly evaluate the relationships between phytochemical composition and antioxidant activity.

The solvent for extraction plays a critical role in determining the yield and composition of the phytochemicals recovered from plant materials. In this study, methanol-water extraction yielded a slightly higher extraction yield and greater recovery of phenolic and flavonoid compounds compared with aqueous extraction. The finding is consistent with a previous study by Arias et al. (2022), which indicated that mixed solvent systems often provide improved extraction efficiency because they combine the solubilizing properties of both organic and aqueous phases. Methanol-water (80:20) is considered to be more polar than absolute methanol and may be more effective at extracting lipophilic antioxidant compounds, while water extracts more polar antioxidants. This observation underscores the importance of solvent choice when extracting bioactive compounds. The superior antioxidant performance of MEACF at higher concentrations reinforces the well-established principle that methanol efficiently extracts semi-polar and moderately non-polar phenolics. This finding agrees with observations from Li et al. (2014) and Du et al. (2016), who demonstrated that the extraction solvent significantly influences phenolic yield and antioxidant activity. The differential performance of AEACF and MEACF across assays suggests that each solvent preferentially extracts distinct phenolic subclasses.

This study suggests that *A. comosus* flesh represents a potential natural source of antioxidants, with its efficacy largely influenced by extraction conditions, particularly solvent polarity. Also, the findings of this study support the

optimization of pineapple flesh as both a dietary antioxidant source and a candidate for nutraceutical development. The strong positive correlations between TPC, TFC, and antioxidant assays (FRAP, ABTS, and NOSA) highlight a synergistic interaction among these bioactive compounds. However, solvent selection is critical for maximizing phytochemical recovery and antioxidant potential. Likewise, while pineapple consumption offers health benefits, factors such as bromelain activity, natural sugar content, and acidity may limit excessive intake, emphasizing the importance of moderation.

Limitations

Variations in fruit maturity, storage conditions, and moisture content may influence phytochemical concentrations and contribute to assay variability. In addition, measurements approaching the Limit of Detection (LOD) may introduce analytical uncertainty, particularly at low extract concentrations. The antioxidant assays employed (ABTS, FRAP, and nitric oxide scavenging) evaluate chemical reactivity under controlled in vitro conditions and therefore do not necessarily reflect biological efficacy in vivo. Consequently, further cellular and in vivo studies are required to determine the bioavailability, stability, safety, and physiological relevance of the antioxidant activity observed for the methanolic and aqueous extracts of *A. comosus* flesh. The study evaluated only two extraction solvents (water and methanol), whereas other food-grade solvents, such as ethanol or mixed solvent systems, may yield different extraction efficiencies.

Additionally, chromatographic characterization of individual phytochemicals was not performed. Advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) or Liquid Chromatography-Mass Spectrometry (LC-MS) would enable more precise identification and quantification of specific phenolic and flavonoid compounds responsible for the antioxidant activity. The correlation analysis was conducted using a relatively small number of extraction replicates and concentration points, which may limit statistical robustness. Future studies should incorporate larger sample sizes, additional experimental replicates, and complementary antioxidant assays to improve the reliability and biological interpretation of the results.

In conclusion, the findings of this research provide vital information on the nutritional composition, phenolic content, flavonoid content, and antioxidant properties of *A. comosus* flesh, which may be beneficial for health. MEACF and AEACF showed strong in vitro antioxidant activity. contained measurable phenolics/flavonoids, and significant NO-scavenging activity. TPC and TFC correlated strongly with FRAP, NOSA, and ABTS, which contribute to its antioxidant potential. ACF is suggested to have the potential to manage both non-oxidative and oxidative damage associated with reactive oxygen and nitrogen species.

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