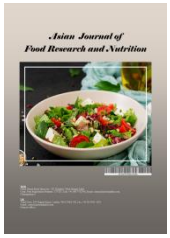

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Physicochemical, Nutritional and Antioxidant Properties of *Syzygium malaccense* Extracted with Different Solvents

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Authors' contributions

This work was carried out in collaboration among all authors. Author MO designed the study, performed the statistical analysis, wrote the protocol, and the first draft of the manuscript. Authors IYL and CDL managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Underutilized plants are nutritionally rich and contain variety of phytochemicals which need to be explored for their contribution to the improvement of human health and economic value.
Aim: To evaluate the physicochemical characteristics, nutrient, phytochemicals and antioxidant activity of *S. malaccense* grown in Nigeria.
Methodology: Fruit weight, yield, pH and nutritional composition of *S. malaccense* fruit were investigated using standard analytical methods. The samples were extracted with different solvents in order to analyze the phytochemical compounds and antioxidant activity. Different phytochemical assays were used to evaluate the polyphenolic contents of the fruit extracts. Antioxidant activity of the extracts were measured using 2,2-diphenyl- 1- picrylhydrazyl (DPPH) and total antioxidant capacity (TAC) assays.

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Results: The different part of the fruit exhibited significant differences ($p<0.05$) in their physicochemical characteristics. The flesh has a significant higher weight (104.6±19.57g) than the peel (25.85±4.838). The yield of the peel extract (11.315%) was higher than that of the flesh (6.231%) despite the higher weight of the flesh. The peel showed significant ($P<0.05$) lower value of moisture and higher contents of carbohydrates, proteins, fats, fibre, ash, dry matter and calorie than the flesh. The flesh had the highest concentration of minerals with Calcium as the predominant macro-element and Iron as the predominant trace mineral. The polyphenolic contents were higher in the peel. The phenolic, flavonoid and proanthocyanidin content of the acetone peel extract (10.917±0.01 mg GAE/g, 7.927±0.015 mg QE/g, 1.959±0.007 mg CE/g) was significantly ($P <0.05$) higher than other extracts while the aqueous flesh extract had the lowest values. All the solvent extracts showed great antioxidant activities with the acetone peel extract having the highest antioxidant activity based on DPPH and ethanol peel extract based on TAC assays with IC_{50} values of 133.339±1.87µg/ml and 31.189±6.55µg/ml, respectively. Polyphenolic contents were significantly correlated with antioxidant activity.
Conclusion: This study reveals that *S. malaccense* especially its peel contains abundant nutrients and polyphenolic compounds with high antioxidant activity that may impart health benefits when consumed.

Keywords: Myrtaceae; Syzygium malaccense; metabolite; nutrition; peel; flesh; polyphenolics, antioxidant.

1. INTRODUCTION

Since time immemorial, human civilization has used several plants as food, medicine, clothing and shelter. It has been observed that numerous plants have pharmacological effects due to the presence of metabolites. Plant-metabolites are organic compounds which can be classified into primary and secondary metabolites. Primary metabolites are organic compounds such as glucose, starch, polysaccharide, protein, lipids and nucleic acid that are beneficial for human growth and development. Plants synthesize secondary metabolites which include alkaloids, phenols, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils etc., The therapeutic efficacy of plants is because of these secondary metabolites for curing many diseases [1]. Epidemiological studies have consistently shown that regular consumption of fruits and vegetables is associated with reduced risk of developing cancer and other chronic diseases [2]. Fruits are rich sources of primary and secondary metabolites, offering numerous health benefits. Despite their abundance, many edible fruits remain underutilized and often go to waste. This is mainly due to the lack of awareness regarding their therapeutic properties and potential as alternative food sources in communities [3].

One underutilized fruit is *Syzygium malaccense* L. Merr & Perry, belonging to the *Myrtaceae* family, which includes around 3,000 species of trees and shrubs [4]. Native to Malaysia, it is known as the Malay apple and has successfully adapted to tropical and subtropical climates worldwide [5]. It was introduced to Nigeria over 55 years ago where it is found in cultivation [6]. The combination of tree, flowers and fruit has been praised as the most beautiful of the genus *Syzygium* [7]. The fruit, the main product of this tree, is pear-shaped and dark red, although some varieties have white with streaks of red or pink [8]. Some fruits are seedless while the flesh is white, crisp or spongy, and has a mild, sweetish flavor. [7].

The morphological and climatic adaptations of the Malay apple adaptations in different geographical regions affect its physical and chemical characteristics [9]. Research studies have characterized *S. malaccense*, exploring its antioxidant activity and bioactive compounds. Some studies assessed the nutrients, phytochemicals, and antioxidant capacity of different plant tissues in different geographical origins of Brazil. Results showed that the fruit peel had high nutrient content, phytochemicals, and antioxidant activity [9-11]. In Malaysia, the fruit exhibited appreciable nutrients and high antioxidant activity [12]. In Nigeria, studies revealed that the fruits contained significant mineral content, moisture, ash, fiber, and vitamin C [13,14]. In Indonesian Borneo, the fruits provided income for local communities, highlighting their economic potential [15].

Considering the necessity to utilize natural resources, it is important to investigate the nutritional and antioxidant properties of *S. malaccense* fruit, particularly in Africa where

scientific data is lacking. Therefore, the objective of this study was to evaluate the physical and nutritional properties of *S. malaccense* cultivated under Nigerian climate conditions, and assess the effect of different solvents (water, ethanol, and acetone) on its polyphenol content and antioxidant activity.

2. MATERIALS AND METHODS

2.1 Collection and Plant Identification

Fresh ripe *S. malaccense* fruits were harvested from Isefun in Lagos State, Nigeria ($6^0$34'33.8''N and $3^0$12'08.5E; $6^0$34'40.8''N and $3^0$12'01.3''E) in January, 2020. The plant materials were taxonomically identified and authenticated at the Department of Botany, University of Lagos, Lagos where a voucher specimen (LUH 7646) was deposited.

2.2 Physicochemical Characterization

20 fruits were weighed using a digital balance. The parts were manually separated using a stainless steel knife, yielding three fractions: peel, flesh and seed. Their weights were recorded. The percentage yield of the fractions were determined as the ratio of their respective weight and fruit weight, multiplied by 100. The pH of the peel (P; parts of the apple removed by the stainless steel knife), flesh (F; edible portion of the apple without the peel) and peel + flesh (PF; edible portion of the apple with the amount of flesh and peel maintained in the same proportions as in the whole apple fruit) were measured using a digital pH meter.

2.3 Nutritional Analysis

2.3.1 Proximate composition analysis

Proximate composition of fresh *S. malaccense* P, F, PF samples were reported in percentage and determined using standard analytical methods [16,17]. The percentage moisture content was determined by drying 5g of the samples at 105^0C until a consistent mass was recorded. The ash composition was determined by incinerating 5g of the samples at 550^oC for 6 h in a muffle furnace and the weight of the residue remaining after ashing was calculated as percentage ash content. The Soxhlet technique was used to extract crude fat from about 5g of each dried sample using petroleum ether. The Micro-Kjeldahl method (N x 6.25) was used to calculate the crude protein content of 5.0 g of the dried samples with a catalyst and the standard AOAC [16] method was used to assess crude fiber. The total carbohydrate content of the different samples was determined by the difference method according to the following formula:

$$\text{Carbohydrate (\%)} = 100 - (\text{\% moisture} + \text{\% ash} + \text{\% crude fat} + \text{\% crude protein} + \text{\% crude fiber}) \quad (1)$$

Dry matter or total solid of the different samples was calculated by subtracting the percentage of moisture from hundred [17]. The total energy value of the samples in kcal/100 g was obtained using the following formula [18]:

$$\text{Calorific value} = 4 \times (\text{\% protein}) + 9 \times (\text{\% fat}) + 4 \times (\text{\% carbohydrate}) \quad (2)$$

2.3.2 Mineral content analysis

Standard analytical methods according to AOAC [16] was also used for the determination of the level of mineral contents of *S. malaccense* P, F, PF by acid digestion using nitric acid and perchloric acid mixture (HNO_3: $HClO_4$, 5:1 v/v). The total amounts of minerals: Sodium, Magnesium, Calcium, Potassium, Iron, Copper, Manganese, Zinc and Lead in the digested samples were determined using atomic absorption spectrophotometer (AAS-Buck 205, USA). The values obtained were converted to mg/100 g of fresh sample.

2.4 Bioactive Compounds and Antioxidant Properties

2.4.1 Preparation of Plant Extracts

Extraction was done as described by He and Liu [19] with little modification. Fresh *S. malaccense* fruits were weighed and washed with water, separated manually into parts (peel, flesh and seed). The peel and flesh were dried separately to constant weight at room temperature and the seed was discarded. The dried peel and flesh were pulverized with the aid of Binatone electrical miller (BL-1500PRO). The ground samples were then stored in air tight bags at room temperature until use. 250g of the dried peel and flesh were soaked separately and homogenized with different solvents (water, 70% ethanol and 70% acetone; 1:2, w/v). The homogenates were filtered under vacuum with a Buchner funnel and Whatman No. 1 filter paper.

The ethanol and acetone extracts were concentrated to dryness under reduced pressure using a rotary evaporator while the aqueous filtrate was concentrated using a freeze dryer. The extracts were stored at -20^{o}C until use.

2.4.2 Phytochemical screening

Qualitative analysis was carried out to ascertain the presence of different phytochemicals such as saponin, alkaloid, tannin, flavonoid, terpenoid, sterols, phenol and condensed tannins (proanthocyanidin) in *S. malaccense* extracts [19].

2.4.3 Determination of Total Phenolic Content (TPC)

TPC was carried out using Folin-Ciocalteu colorimetric method. Briefly, 0.5 mL of each solvent extract (1 mg/mL), standard gallic acid (0.02 mg/mL to 0.1 mg/mL, and the solvent of dissolution (control) was pipetted in different test tubes. 2.5 mL Folin–Ciocalteu's reagent (10% [v/v]) was added and mixed together. The solution was left to stand at room temperature for about 5 minutes. Then, 2 mL of anhydrous 7.5% Na_2CO_3 was added to the solution and the mixtures were left to stand at room temperature for 30 minutes. The control solution was used as a blank. After incubation, absorbance was read at 765 nm using an ultraviolet- visible 3000 PC spectrophotometer. The experiment was done in triplicate. Total phenol content was extrapolated and expressed as milligram of gallic acid equivalent per gram of dry extract (mg GAE/g) [21].

2.4.4 Determination of Total Flavonoid Content (TFC)

TFC was determined using aluminum chloride colorimetric assay. Briefly, 0.5 mL of each solvent extract (1 mg/mL), different concentrations (0.02–1 mg/mL) of quercetin standard and the solvent of dissolution (control) were placed in different test tubes. Thereafter, 3 mL of methanol was added to each test tube after which, 0.1mL aluminum chloride – $AlCl_3$ (10%), 0.1 mL of 1M potassium acetate and 2.8 mL distilled water were added sequentially and mixed together. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. Total flavonoid content was extrapolated and expressed as milligram of quercetin equivalent per gram of dry extract (mg QE/g) [22].

2.4.5 Determination of Condensed tannin (Proanthocyanidin) content

An aliquot (0.5mL) of each solvent extract (1 mg/mL), standard solution (0.02 to 1 mg/mL) and the solvent of dissolution (control) was pipetted in different test tubes and mixed with 3 mL of 4% (w/v) vanillin- methanol and 1.5mL of conc. HCl. The well-mixed solution was incubated at ambient temperature in the dark for 20 minutes. The absorbance against blank was read at 500 nm. Condensed tannin content was extrapolated and expressed as milligram of catechin equivalents per gram of dry extract (mg CE/g) [23].

2.4.6 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

A solution of 0.1 mM DPPH in methanol was prepared. 1 mL of the DPPH solution was mixed with 1 mL (62.5 µg/mL to 1000 µg/mL) of the solvent extract/standard drug (ascorbic acid). A control containing DPPH solution and methanol only was also prepared. The reaction mixture was thoroughly vortexed and left to stand in the dark at room temperature for 30 minutes. Absorbance of the mixture was measured at 517 nm using a spectrophotometer blanked with methanol. The DPPH scavenging ability was calculated using the equation below [24].

[(Absorbance of control – Absorbance of sample) / Absorbance of control] × 100 (3)

2.4.7 Determination of Total Antioxidant Capacity (TAC)

The TAC of the solvent extracts was determined by phosphomolybdenum method. Briefly, 0.3 mL of the solvent extracts and standard drugs (31.75 µg/mL to 500 µg/ mL) was taken in test tubes and dissolved in 3 mL of reagent solution (0.6M sulphuric acid, 4mM ammonium molybdate and 28mM sodium phosphate). The test tubes were covered and incubated at 95°C in a water bath for 95 minutes. The mixture was allowed to cool to room temperature and the absorbance was measured at 695nm against a blank using a spectrophotometer. A mixture containing distilled water instead of the samples served as control. Ascorbic acid was used as standard drug. The

percentage inhibition was calculated using the equation below [25]

[(Absorbance of sample– Absorbance of control)/ Absorbance of sample] X 100 (4)

The IC_{50} (Half Inhibitory Concentration) value in the DPPH free radical scavenging and phosphomolybdenum method was calculated.

2.5 Statistical Analysis

All of the analyses were carried out in triplicate and results expressed as mean ± S.D (Standard Deviation). The data were analyzed using GraphPad Prism 9 software, Incorporated, USA. One-way Analysis (ANOVA) was used to compare differences among the groups. The results were considered significant at the value of $p< 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics of *S. malaccense* Fruit

The result of the physico-chemical characteristics of *S. malaccense* is represented in Table 1. There are significant differences ($p<0.05$) in the physicochemical characteristics between the different part (peel, flesh and seed) of the fruit. The average weight of the fruit, peel, flesh and seed were 143.70g, 25.85g, 104.6g and 13.25g which is high when compared to 35.57g - 75.86, 9.97g - 11.52g, 38.80g - 40.93g, 12.09g - 11.11g obtained from different geographical locations in Brazil [9,10,26]. The percentage yield of the peel, flesh and seed were 17.989%, 72.792%, and 9.222%. Despite the greater weight of the flesh, the yield of extract from the fresh peel is higher than from the flesh. Furthermore, the weight of the fresh peel (517.04g) compared to the flesh (2092.23g) is in a ratio of 1:4, while the extract yield from the fresh fruit's weight is reduced to a ratio of 1:2 (2.04:4.54). This suggests that the peel contains a higher concentration of bioactive compounds. The pH value of *S. malaccense* peel is significantly lower than that of the flesh as observed by Nunes et al. [9] who reported 3.54-3.60 (P), 3.75-3.82 (F), although these values are lower than the those in this study. Due to the fact that the physico-chemical characteristics of fruits are influenced by genetic and environmental factors [27] as well as production practices [28], the difference between this result and other studies' findings is expected.

3.2 Proximate Composition of *S. malaccense*

The composition of *S. malaccense*, expressed as % (g/100 g, wet basis), and its energetic value (Kcal/100 g, wet basis) are presented in Table 2. The peel exhibited lower moisture content and higher levels of carbohydrates, proteins, fats, fiber, ash, dry matter, and calories compared to the flesh. Moisture content is influenced by humidity, temperature, and harvest time. Significant differences in moisture content were observed among the peel, flesh, and peel+flesh. The flesh had significantly higher moisture content than the peel and peel+flesh. The moisture content of the peel (84.56±0.6746) was lower than the reported value of approximately 90% by Nunes et al. [9]. The moisture content of the peel+flesh (86.94±1.131) was lower than the reported value of 90.07±0.06 by Ogundare [13] and higher than 83.28±0.16 reported by Lim and Rabeta [12]. The high moisture content in the flesh contributes to its reduced extract yield in section 3.1. Additionally, high moisture content is typical of mature fresh fruits and plays a role in enzyme function and metabolic processes [29]. However, it also makes fruit susceptible to microbial attack during storage, posing a significant challenge in food preservation [30]. The carbohydrate content of the flesh was significantly lower ($p < 0.05$) than the peel and peel+flesh. The peel and flesh have lower carbohydrate content compared to the reported values of approximately 7% (peel) and 5.05-6.48% (flesh) by Nunes et al. [9]. The PF (5.77±1.08) has lower carbohydrate content than the reported values of 6.74-12.68 [12,13]. Fruits with very low carbohydrate content are considered poor sources of energy or low-calorie fruits, such as Blueberries [31]. Therefore, *S. malaccense* (L.) fruits are a good source of pharmaceutical agents that may be suitable for managing hyperglycemic conditions like obesity and diabetes mellitus [13]. There are no significant differences in the protein, fat, fiber, and ash content of *S. malaccense* in all fruit fractions. The protein, fat, fiber, and ash content in the fruit fractions are higher than previously reported values [9, 12, 13]. The protein content in *S. malaccense* is low as seen in other fruits such as breadfruit, cactus pear, sweetsop and bacuri [32]. Fruit proteins, if present, have low biological value and cannot replace meals. They should be consumed with other food sources that provide the required quantities and qualities of proteins [33]. The fat content in the fruit fractions are very low which is common for fruits like eggfruit,

lychee and banana [32]. The fiber content is comparable to other dark-colored fruits like blueberries and strawberries, making them a good source of fiber [9]. Fiber is essential for digestion, increasing food bolus and reducing the risk of constipation. It can also help manage hypercholesterolemia and related nutritional disorders [13]. The ash content value compared favorably with most fruits like orange, watermelon, bush mango but higher than pawpaw, banana, apple, guava, soursop and pineapple [29]. The percentage ash of the sample provides insights into the inorganic content, which can be used to determine mineral concentrations. High ash content indicates higher mineral elements that can enhance metabolic processes and promote growth [34]. The wide range in dry matter and moisture in *S. malaccense* is similar to other fruits, leading to rapid deterioration if not processed soon after harvesting [29]. Carbohydrate content is the main calorie contributor in tropical fruits such as strawberries, pineapple, pawpaw, while lipid and protein contents have a minimal impact on total energy value [9,12]. Therefore, *S. malaccense* can be included in calorie-restricted diets.

3.3 Mineral Composition of *S. malaccense*

Table 3 shows the mineral composition of *S. malaccense*. The flesh has a higher mineral concentration than the peel, with Calcium as the predominant macro-element and iron as the predominant trace mineral. Minerals are crucial for proper bodily functioning, and reference values for intake are periodically reviewed based on new findings [35]. Recommended dietary allowances (RDAs) and adequate intakes (AIs) determine nutrient levels that meet the needs of healthy individuals. Based on these parameters, the average daily mineral requirements for adults (men and women, 19 to 70 years old) are as follows: Ca, 1000 mg; Mg, 320 to 420 mg; Na, 1500 mg; K, 4700 mg; Fe, 8 to 18 mg; Zn, 8 to 11 mg; Cu, 0.9 mg/day; and Mn, 1.8 to 2.3 mg/day [36]. *S. malaccense* fruit is rich in minerals such as Calcium, Magnesium, Sodium, Potassium, Iron, Copper, Manganese, and Zinc, with the highest quantity found in the flesh. This finding aligns with Enidiok and Attah [14]. The mineral content in *S. malaccense* follows this order: Mg > K > Ca > Na > Fe > Cu > Zn > Mg (P), Ca > K > Mg > Na > Fe > Cu > Zn > Mg > Pb (F and PF).

Table 1. Physicochemical characteristics of *S. malaccense* fruit

Parameters	Fruit (Peel+Flesh)	Peel	Flesh	Seed
Weight of 20 apples (g)	2894.24	517.04	2092.23	265.06
Mean weight of 20 apples (g)	143.70±24.91[a]	25.85±4.84[b]	104.60±19.57[c]	13.25±3.991[b]
Yield of fruit parts (%)	-	17.99	72.79	9.22
Weight of aqueous extract (g)	-	58.50	130.36	-
Yield of extract from fresh part weight (%)	-	11.32	6.23	-
Yield of extract from fresh fruit weight (%)	-	2.04	4.54	-
pH	3.95±0.04[a,b]	3.80±0.06[a]	4.00±0.00[b]	-

Values with superscript in a row are significantly (P<0.05) different

Table 2. Proximate composition of *S. malaccense* peel, flesh and peel+flesh

Parameters	Peel	Flesh	Peel+Flesh
Moisture	84.56±0.67[a]	89.97±1.08[b]	86.94±1.13[c]
CHO	6.43± 1.22[a]	3.04±1.18[b]	5.77±1.08[a]
Protein	2.14±0.19	1.41±0.01	1.61±0.07
Fat	1.15±0.07	0.83±0.04	0.93±0.09
Fibre	3.33±0.00	2.46±0.22	2.330±0.00
Ash	2.40±0.28	2.30±0.14	2.44±0.05
Dry matter (g)	15.44±0.67[a]	10.03±1.08[b]	13.06±1.13[b]
Calorific value (kcal)	44.59±3.48[a]	25.25±4.40[b]	37.81±5.18[a]

Results are expressed as mean ± Standard deviation of three determinations. Values with superscript in a row are significantly (P<0.05) different

Evidence suggests the importance of considering sodium and potassium consumption for cardiovascular health outcomes. According to the World Health Organization, the ideal Na:K ratio is 1.0 and a lower sodium to potassium (Na:K) ratio is associated with better blood pressure and cardiovascular health [37,38]. *S. malaccense* has a lower Na:K ratio, promoting better cardiovascular health. Trace amounts of lead were detected only in *S. malaccense* flesh, which is lower than in *S. cumini* flesh [39]. High concentrations of lead which can be ingested by various means like contaminated food, use of lead contaminated plants and from the environment can cause toxic effects [40]. The permissible limit of lead for herbal material according to WHO is 1mg/100g while the daily recommended consumption for 70kg adult is 0.250 mg/day [41]. *S. malaccense* flesh has a lead concentration below the daily recommended consumption, therefore it is a natural source of minerals, providing over 50% of the minimum RDA for Cu and Fe, and 2-20% for other minerals.

3.4 Percentage Yields and Phytochemical Content of *S. malaccense*

S. malaccense extracts were obtained using water, 70% aqueous ethanol, and acetone. The resultant percentage yield after extraction was reported in Table 4. The extraction yields of the peel and flesh of S. malaccense by the solvents followed this order: AP > AF > EP > EF > ACP > ACF. Extraction is the initial step in phytochemistry research, preceding the isolation of effective constituents. Its purpose is to obtain the desired chemical constituents while minimizing unwanted ones [42]. The extraction yield varies depending on the solvents used and the sample's chemical nature [43]. Results showed that extraction yield increased with solvent polarity, and the peel had a higher yield, suggesting more bioactive compounds compared to the flesh. This study is the first to explore the acetone extracts of *Syzygium malaccense* for bioassays. Acetone, a versatile extractant, can dissolve both hydrophilic and lipophilic compounds, making it suitable for dried plant materials [44].

Table 5 shows the results of the phytochemical screening on the solvent extracts of *S. malaccense* peel and flesh. The analysis revealed the presence of saponin, alkaloid, tannin, flavonoid, terpenoid, sterols, phenol, condensed tannin, and reducing sugar. The peel has higher concentrations of alkaloids, tannins, flavonoids, phenols, and proanthocyanins compared to the flesh. A higher intensity of flavonoid and phenol

Table 3. Mineral composition of *S. malaccense* peel, flesh and peel+flesh

Minerals (mg/100g)	Peel	Flesh	Peel+Flesh
Ca	44.91±16.63[a]	156.20±38.70[b]	106.90±17.47[c]
Mg	70.05±11.92	78.60±20.63	72.21±21.15
Na	29.41± 1.95	53.33±5.19	40.73±0.26
K	63.92±3.46[a]	137.50±1.78[b]	82.17±9.04[a]
Fe	0.91±0.68	8.47±0.28	6.10±2.49
Cu	0.68±0.15	1.18±0.51	1.00±0.52
Mn	0.08±0.05	0.32±0.04	0.12±0.04
Zn	0.39±0.03	0.67±0.22	0.51±0.12
Pb	-	0.13±0.18	-
Na:K	0.46	0.39	0.50

Results are expressed as mean ± Standard deviation of three determinations. Values with superscript in a row are significantly (P<0.05) different

Table 4. Percentage yield of different extracts of *S. malaccense*

Sample	Aqueous Peel	Aqueous Flesh	Ethanol Peel	Ethanol Flesh	Acetone Peel	Acetone Flesh
Weight of dried fruits (g)	250g	250g	250g	250g	250g	250g
Weight of dry extracts (g)	166.20	158.20	158.28	151.10	95.01	91.98
Percentage yield (%w/w)	66.48	63.28	63.31	60.44	38.00	36.79

Table 5. Phytochemical Constituents of *S. malaccense* fruit extracts

Phytochemicals	Test	AP	AF	EP	EF	ACP	ACF
Saponin	Frothing	+	+	+	+	+	+
Alkaloid	Mayer's	++	+	++	+	++	+
Tannin	Ferric chloride	++	+	++	+	++	+
Flavonoid	Alkaline reagent	+++	++	+++	++	+++	++
Terpenoid	Salkowski	+	-	+	-	+	-
Sterols	Liebermann-Burchard	+	+	+	+	+	+
Phenol	Ferric chloride	+++	++	+++	++	+++	++
Proanthocyanin	HCl, ammonium solution	++	+	++	+	++	+
Reducing sugar	Fehling's Test	++	+++	++	+++	++	+++

Keys: indicates the intensity of the phytochemical; - Absent, + Present in low concentration, ++ Present in moderate concentration, +++ Present in high concentration

were detected in the peel and reducing sugar in the flesh. Terpenoid was not detected in all the flesh extracts. Phytochemicals are non-nutritive plant chemicals with protective or disease preventive properties. Medicinal value of plants lies in their phytochemical constituents which differ widely in terms of structure, biological properties and mechanisms of actions. These phytochemical constituents are known to be responsible for antioxidant, antimicrobial, anti-larvicidal, and anti-inflammatory activities [45]. Fruits are good sources of phenolics, flavonoids, and anthocyanins which are responsible for antioxidant, anti-carcinogenic and health-promoting properties [46]. The presence of these secondary metabolites in *S. malaccense* may contribute to its medicinal value.

Total phenolic contents (TPC), total flavonoid content (TFC), and condensed tannin (CT) are shown in Fig. 1. TPC, TFC, and CT of the peel were significantly higher than the flesh ($p < 0.05$). The acetone peel extract had significantly higher TPC, TFC, and CT content (10.92±0.01 mg GAE/g, 7.93±0.02 mg QE/g, 1.96±0.01 mg CE/g) compared to other extracts ($P < 0.05$), while the aqueous flesh extract had the lowest values (6.02±0.02 mg GAE/g, 4.49±0.01 mg QE/g, 0.85±0.00 mg CE/g). The findings reveal higher polyphenolic contents in the peel compared to the flesh. Environmental stresses are known to lead to higher accumulation of phenolics in exposed fruit parts [47]. These results are consistent with previous studies reporting phenolic values in the peel (8.58 mg GAE/g dry weight) [48] and in the flesh (12.58 - 12.93) [9]. The phenolics value in *S. malaccense* is lower when compared to other fruits from the *Myrtaceae* family such as *Myrciaria dubia* (101.0), *Myrciaria vexator* (44.1), *S. curranii* (39.6), *Myrciaria cauliflora* (31.6), *Eugenia* aggregate (25.3), *S. cumini* peel and flesh (59.90 and 51.03) [9,49]. Flavonoids, like phenols, are water-soluble secondary metabolites with polyphenolic structure. They exhibit great antioxidant activities and are reported to be more potent than Vitamins C, E, and carotenoids [50]. The higher flavonoid content in the peel compared to the flesh is consistent with previous findings [49]. Condensed tannin, a group of polyphenolic bioflavonoids, is of great interest in nutrition and medicine due to its potent antioxidant capacity and potential protective effects on human health [51]. Therefore, *S. malaccense* exhibits a rich composition of polyphenolic compounds, concentrated in the peel, contributing to its medicinal value [9,10].

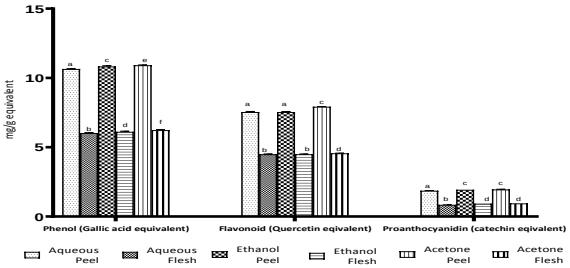

Fig. 1. Phytochemical content of the solvent extracts of *S. malaccense* fruit in standard equivalents

Results are expressed as mean ± Standard deviation of three determinations. Values with superscript are significantly (P<0.05) different

3.5 Antioxidant Activity of Solvent Extracts of *S. malaccense*

Fig. 2 and Table 6 present the DPPH radical scavenging activity of the solvent extracts of *S. malaccense*, along with the antioxidant Vitamin C

and their respective concentrations that inhibited 50% (half maximal inhibitory concentration (IC_{50})) of the radicals. This comparison highlights the effectiveness of the extracts in scavenging radicals. The order of decreasing scavenging activity based on IC_{50} values is: Vitamin C > ACP > AP > EP > ACF > EF > AF. Fruits have a wide range of antioxidants and antioxidant capacity. Fruits with higher antioxidant capacity are assumed to have more antioxidants [50]. The study found that all solvent extracts showed great DPPH scavenging activities even at low concentrations (65 µg/mL). The extracts had high DPPH radical scavenging activity, although significantly lower than ascorbic acid ($p<0.05$). However, there was a dose-dependent relationship between the concentration of the extracts and the DPPH radical scavenging activity. The peel had significantly higher scavenging activity compared to the flesh, similar to *S. cumini* [49]. Acetone extracts had significantly higher DPPH scavenging activity than other solvent extracts ($P < 0.05$), suggesting acetone as a better solvent for extracting antioxidants from *S. malaccense*.

The total antioxidant capacity of the solvent extracts and standard increased with concentration (Fig. 3). The order of decreasing TAC for the solvent extracts and standard drug is Vitamin C > EP > ACP > AP > ACF > EF > AF (Table 6). TAC is a relevant tool for studying the relationship between dietary antioxidants and oxidative stress-induced pathologies [51]. It is determined by the synergic and redox interaction among the different molecules present in food, rather than the levels of individual antioxidants [52]. The TAC of the solvent extracts was determined based on the reduction of molybdenum (VI) to molybdenum (V) and the subsequent formation of a green phosphate/molybdenum (V) complex at acidic pH. The solvent extracts showed a dose-dependent increase in TAC with increasing concentrations. At 62.5 µg/mL, the peel extracts had already reduced >50% molybdenum (VI) to molybdenum (V), with EP exhibiting the most potency (IC_{50} value of 27 µg/mL), followed by ACP (IC_{50} value of 33 µg/mL).

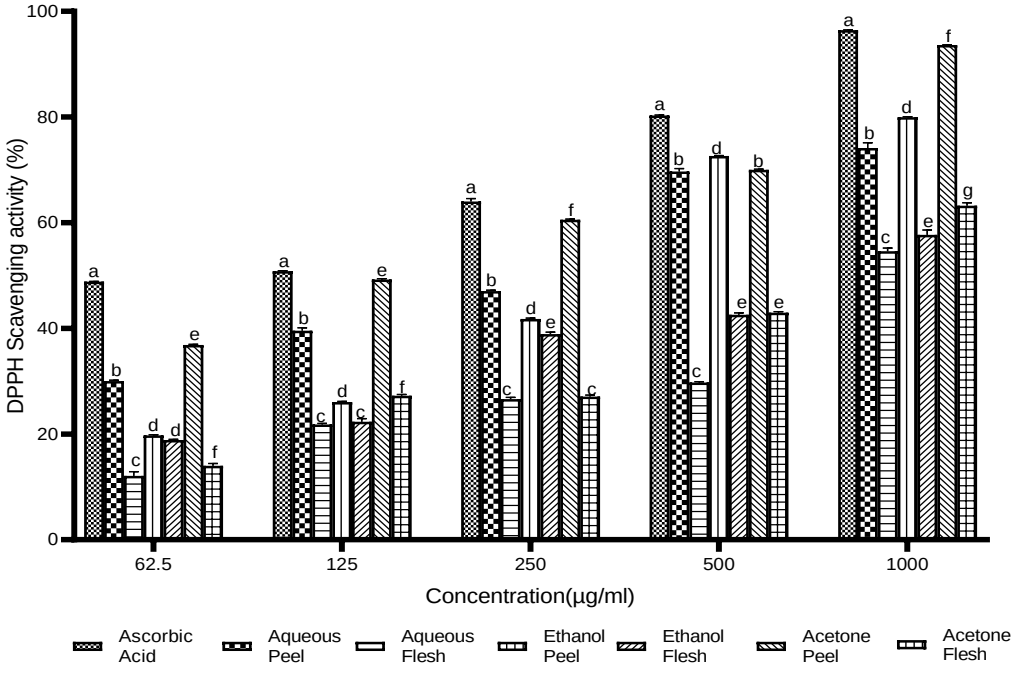

Fig. 2. DPPH radical scavenging activity of the solvent extracts of *S. malaccense* fruit as a function of concentration

Results are expressed as mean ± SD of three determinations. Values with superscript are significantly (P<0.05) different.

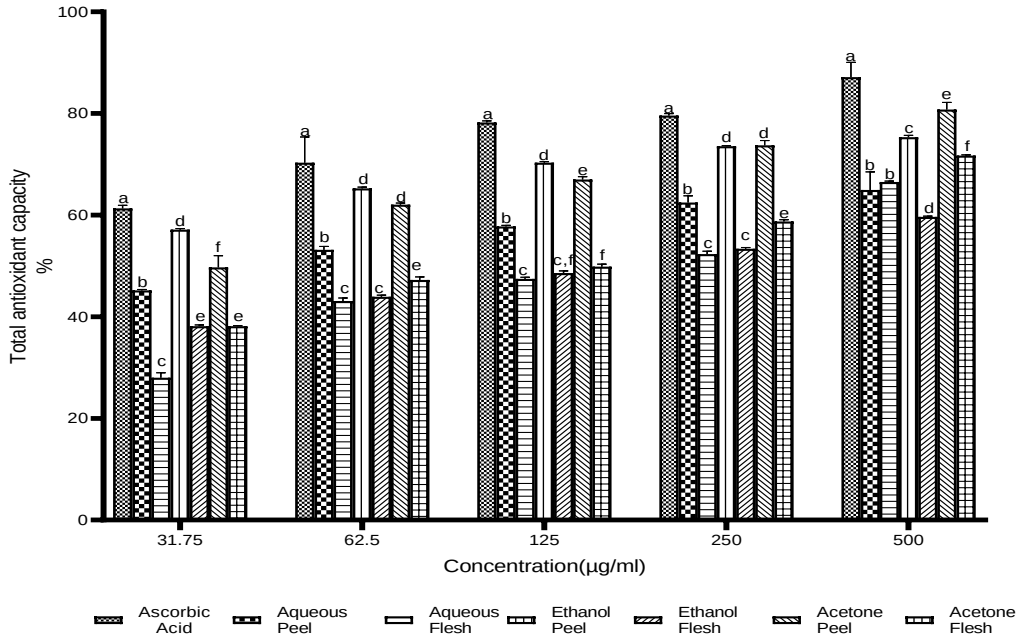

Fig. 3. Total antioxidant capacity of the solvent extracts of *S. malaccense* fruit as a function of concentration

Results are expressed as mean ± SD of three determinations. Values with superscript are significantly (P<0.05) different.

Table 6. Antioxidant IC_{50} Values (µg/mL ± SD) of *S. malaccense* Extracts and Standard

Treatment	DPPH	Phosphomolybdenum
Aqueous Peel	282.30±2.14	50.15±1.32
Aqueous Flesh	907.23±10.41	190.58±10.14
Ethanol Peel	316.67±1.22	27.34±0.09
Ethanol Flesh	745.70±13.29	161.18±9.30
Acetone Peel	133.34 ± 1.87	33.25±3.02
Acetone Flesh	673.65±4.61	125.70±7.74
Vitamin C	100.65±1.27	25.48±0.25

The Pearson product moment correlation coefficients between the polyphenolic contents and the IC_{50} of the antioxidant activities analyzed are shown in Fig. 4a- e. Based on DPPH and TAC assays, the polyphenolic contents had a high negative correlation of R2 = 0.9178 and 0.9214 (for phenols), R2 =0. 9254 and 0.9202 (flavonoids), R2 = 0.9152 and 0.9186 (Proanthocyanidin) at p< 0.005. The higher polyphenolic contents in *S. malaccense* led to lower IC_{50} antioxidant activity, suggesting synergistic effects between the polyphenolic compounds present. Previous studies have shown a positive correlation between antioxidant activity and polyphenolics in plants, particularly fruits [9, 53-54]. The present study identified positive correlations between polyphenolics (phenol, flavonoid, and proanthocyanidin) and antioxidant activities, highlighting the significant role of polyphenolics in the antioxidant activity of *S. malaccense*. Antioxidants are well-known for their role in preventing cancer and other oxidative stress-related diseases [55]. This study demonstrates the potential of *S. malaccense*, especially its peel, as an excellent antioxidant.

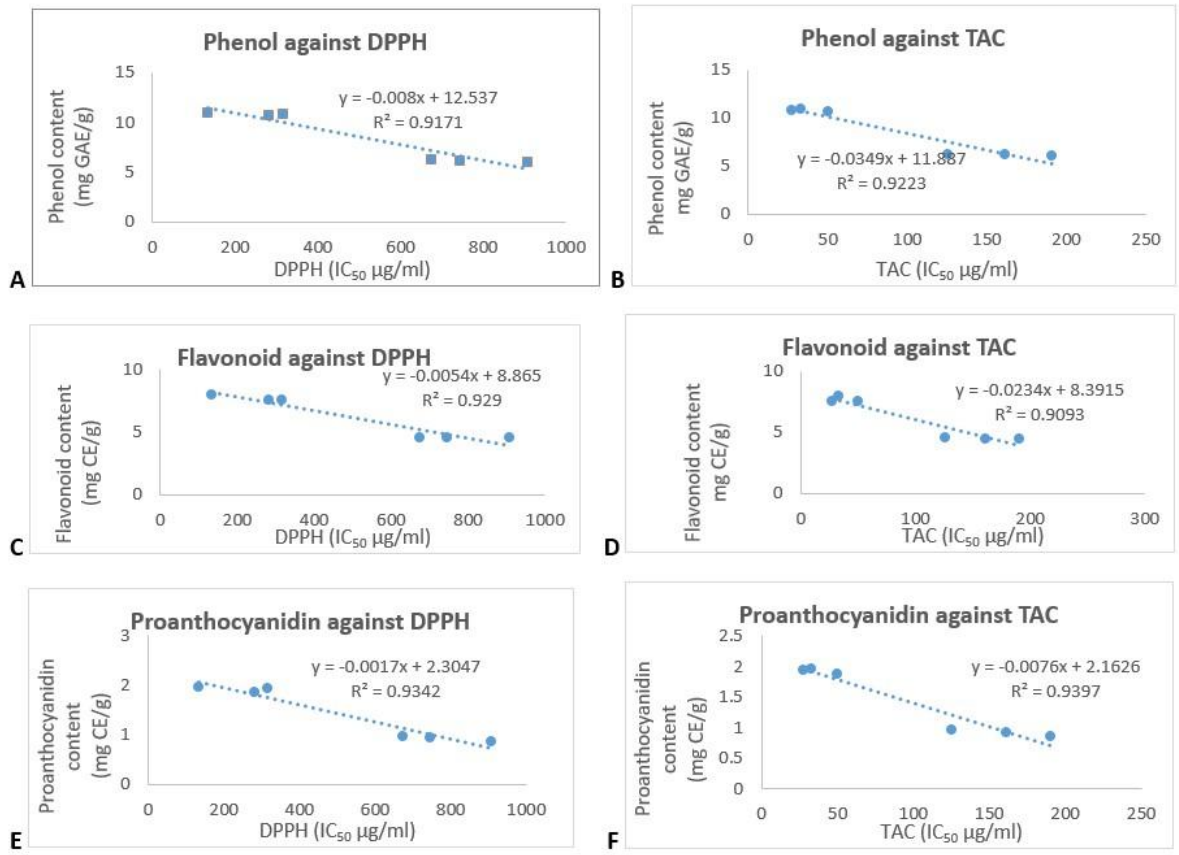

Fig. 4. Correlation of the polyphenolic compounds against the IC_{50} of the antioxidant activity: Phenol against DPPH and TAC respectively (A-B), Flavonoid against DPPH and TAC respectively (C-D) and Proanthocyanidin against TAC (E-F)

3.6. Effect of Solvent System

The choice of solvent is crucial in the extraction process. The polyphenolic content obtained in this study confirms that different solvents have varying abilities to extract phytochemicals from the sample. Acetone extract showed significantly higher polyphenolic contents ($P < 0.05$) compared to other solvent extracts, making it the optimal choice for extracting polyphenolics from *S. malaccense*. However, water (aqueous), being the cheapest and most readily available solvent, had the lowest quantity of polyphenolics. This could be due to the presence of nonphenol compounds like carbohydrates and terpenes in water extracts. Complex formation of certain phenolic compounds soluble in ethanol and acetone could also contribute to the lower level of polyphenols in aqueous solvent. These phenolic compounds may have more phenol groups or higher molecular weights compared to those in the water extract [56]. Studies suggest that samples extracted with acetone exhibit higher antioxidant capacity when measured using DPPH [12, 57, 58]. The results of this study indicate that the chemical characteristics and polarities of the solvent affect the polyphenolic content and antioxidant values. The high polyphenolic content and antioxidant activity observed in acetone extracts may be attributed to the presence of more lipophilic compounds, such as lipophilic phenols, which can be extracted by less polar solvents like acetone. This aligns with Batista et al. [10], who observed the highest value in the lipophilic antioxidant capacity of *S. malaccense* peel and suggested the potential of other nonpolar compounds in the fruit for scavenging peroxyl radicals. These results revealed the potency of the *S. malaccense* as an excellent antioxidant.

4. CONCLUSION

The study revealed that *S. malaccense* contains significant amounts of primary and secondary metabolites, which can meet nutritional and antioxidant needs. The extract yield, polyphenol recovery, and antioxidant activity varied based

on the fruit part and solvent system. Higher yields were observed in the peel, indicating higher bioactive compounds presence, resulting in increased polyphenolic content and antioxidant activity. Water was the preferred solvent for yield, while acetone was preferred for polyphenol content and antioxidant activity. A strong correlation was found between polyphenol content and antioxidant capacity. The peel of *S. malaccense* has potential as a functional fruit for disease prevention and health promotion. Expanding the cultivation and utilization of *S. malaccense* is recommended to enhance the fruit's economic value.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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