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Evaluation of the Antioxidant Potential of Soursop (*Annona muricata* L.) Fruit at Different Maturity Stages

H.J.K.S.S. Wijerama[1], M.K.A. Shanika[1], N.E. Wedamulla[1*] and W.A.J.P. Wijesinghe[1]

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ABSTRACT

Purpose: *Different maturity stages and extraction methods of soursop fruit could affect the antioxidant activity of the fruit. The aim of the present study was to evaluate the antioxidant activity and phenolic content of soursop at four different maturity stages namely, Immature Stage (IM), Partially Mature Stage (PM), Fully Mature Stage (FM), and Well Ripened Stage (WR) using four extraction methods.*

Research Method: *Sonication and maceration using a stir plate were used as extraction techniques, while absolute ethanol and 70% v/v ethanol were used as extraction solvents. The Ferric Reducing Antioxidant Power (FRAP) and DPPH radical scavenging activity were used to determine the antioxidant efficacy, while the Folin-Ceocalteu assay was used to determine the total phenolic content (TPC).*

Findings: *From the analyses, the TPC, FRAP assay, and DPPH activity significantly ($p<0.05$) changed with the maturity stages. The highest TPC (165.67±2.86 mg GAE/g) was observed in the 70% ethanol extract of the WR stage with the sonication extraction method. The highest ($p<0.05$) FRAP value (393.91±4.23 µmol Fe^{+2}/L) was observed in 70% ethanol extracts of FM with sonication extraction. The highest DPPH radical scavenging activity (IC_{50} 60.13±0.18 ppm) was recorded in 70% ethanol extract of the WR stage with sonication extraction. TPC and antioxidant activity increased ($p<0.05$) as maturity progressed.*

Research Limitations: *Time was limited to evaluate the IC_{50} values of the IM, PM, and FM stages.*

Originality/ Value: *Soursop at the WR stage, extracted using 70% ethanol with sonication extraction, comprised a high phenolic content and thus, exhibited strong antioxidant activity. Therefore, the findings explore its potential as a health-promoting antioxidant source.*

Keywords: *Antioxidant capacity, Extraction methods, Maturity stages, Soursop, Phenol content*

INTRODUCTION

Soursop is a tropical fruit tree genus that belongs to the family Annonaceae and genus Annona, with roughly 119 species. In Sri Lanka, seven species and one hybrid are cultivated for commercial purposes or home consumption. The fruit and other parts of the soursop tree are underutilized (Orak *et al*., 2019). Many tropical and exotic fruits have become the focus of researchers' attention in recent years owing to the possible health benefits associated with these fruits (Monarres-Cuevas *et al*., 2022; Indiarto *et al*., 2020).

Soursop fruit is classified as a climacteric fruit based on the high rate of respiration during the ripening stage. Usually, they are harvested and ripened at the postharvest stage. Climacteric fruits can be harvested at the physiological maturity stage and induced to ripen after harvest without affecting their quality (Sanusi *et al*.,

[1*]*Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University of Sri Lanka, Passara Road, Badulla, Sri Lanka.*
nishala.erandi@gmail.com
https://orcid.org/0000-0002-9131-8748

2018). Further, it is categorized as a multiple climacteric fruit, because fruits are at different maturities and ripen at different intervals.

The primary molecules involved in antioxidant action are phenolic compounds. When it comes to extraction methods, solvent extractions are frequently used to extract phenolic compounds from plant sources. Those same traditional methods can be applied to *soursop* (Orak *et al*., 2019). Furthermore, the solvent's polarity is a significant element in the extraction process. Some inventions suggest that the type of solvent used influences the bioactivity of *A. muricata* extracts (George *et al*., 2015).

Different types of bioactive compounds are modified as the fruit matures (Gyesi *et al*., 2019). Furthermore, various phytochemicals are generated due to physicochemical changes in the soursop fruit. Moreover, the chemical and physiological features of fruit at optimal maturity have a significant impact on the bioactivities and quality of the fruit. The total flavonoid and total phenolic content of the fruit increased as it matured (Shen, 2015). However, in long-term storage of fruits, antioxidant compounds degrade due to the storage conditions (Tran *et al*., 2020).

In this regard, the current invention was revealed as a result of a thorough examination of the maturational evolution of phenolic compounds as well as the antioxidant activity of A. muricata spp. On the other hand, an influx of studies has proven that the extraction techniques significantly affect the phenolic content and antioxidant activity of different extracts as assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and ferrous metal ion chelating capacity. Therefore, scientific analysis of the bioactive properties of soursop fruit in such detail is more important. Taken together, the goal of this study was to evaluate the total phenolic contents and antioxidant activities of soursop fruit at different maturity stages.

MATERIALS AND METHODS

Chemicals and Other Reagents

All the chemical reagents were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), β carotene, sodium acetate trihydrate, 2,4,6-tripyridyl-s-triazine, HCl, iron (III) chloride anhydrous, ferrous sulphate, tenfold diluted Follin Ciocalteu reagent, Acetone, anhydrous gallic acid, absolute ethanol, 70% ethanol, methanol, distilled water, and sodium carbonate anhydrous were used for the chemical analysis.

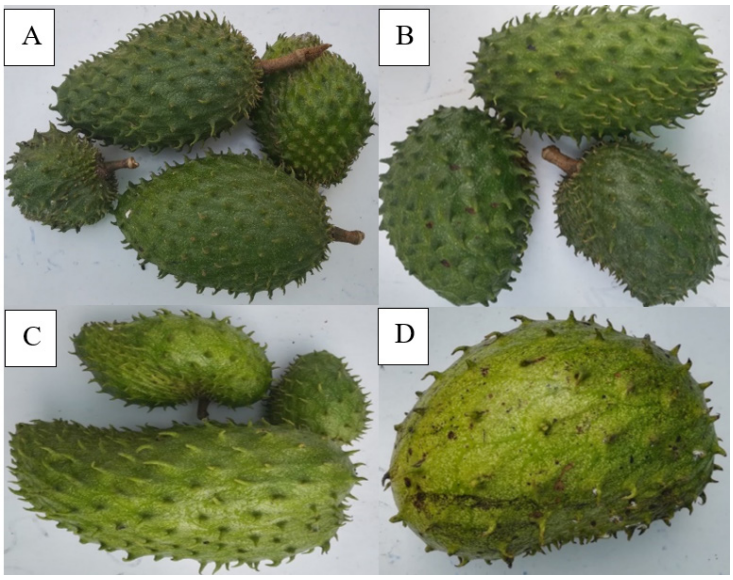

Figure 01: ***Soursop*** **fruits collected at four different maturity stages (A-Immature Stage (IM), B-Partially Mature Stage (PM), C-Fully Mature Stage (FM) and D- Well Ripened Stage (WR)**

Fruit Sample Selection

Twenty five plants were randomly selected to obtain *soursop* fruits (*Annona muricata* L.) from a commercially cultivated farm in Badulla, Sri Lanka. A minimum of three pollinated flowers were selected from each plant, and labels were placed accordingly. The fruits were harvested at four different stages as; Immature Stage (IM), Partially Mature Stage (PM), Fully Mature Stage (FM), and Well Ripened Stage (WR) corresponding to 5, 7, 8, and 9 weeks after anthesis, respectively. Fruits were harvested on August 1, 2019, and laboratory analysis was conducted from August 1, 2019 to November 1, 2019. The harvested fruits were cleaned using 100 ppm chlorinated water. Then, the pulp (without seeds) was prepared separately for each maturity stage. Finally, fruit pulps were stored at 5°C until they were analyzed.

Chemical Analysis

Well-ripen fruits were used to produce different value-added food products in the processing industry. For that, chemical analysis was conducted on the WR stage fruits to get an idea about the physicochemical properties of the fruits. All analytical procedures were conducted for fruit pulp according to the Association of Official Analytical Chemists (AOAC) method (AOAC, 2016). The total solid percentage of fruit pulp was determined by oven drying at 105°C until the steady weight was achieved. The crude fat content of the fruit pulp was evaluated according to the soxhlet extraction. The moisture content was analyzed using the oven drying method. The ash content was determined by igniting for 5 hours at 550°C. The titratable acidity (%) of fruit pulp was determined according to the alkali titration method (titration with standardized NaOH) using phenolphthalein as an indicator. The total soluble solids were analyzed by using a laboratory refractometer (HI96816, USA).

Extraction of Antioxidants

First, fresh fruits (without seeds) were blended at low pressure to obtain a uniform pulp mixture. Then, sonication and stir plate extraction were used as extraction techniques, while absolute ethanol and 70% v/v ethanol were used as extraction solvents. In the sonication method, fruit pulp and solvent (1:10 v/v) extracted at 40°C for 30 minutes. In the stir plate method, fruit pulp and solvent (1:10 v/v) were mixed and placed on a laboratory heating magnetic stirrer plate (T.ARE, USA). Three extraction cycles were performed at 30°C. The rotary evaporation method (20 rpm at 40°C) was used to evaporate the solvent. Extraction was performed on fruit pulps obtained from all the selected maturity stages. Finally, the extracted crude samples were kept at 5°C until they were analyzed.

Determination of Total Phenolic Content (TPC)

The total phenolic content was determined according to the method described by Silva and Sirasa (2018), with slight modifications. Tenfold diluted Folinceocalteu reagent (1.0 mL) was added to a 200 µL extract and solution series of gallic acid and kept for 8 minutes. Then $Na_2CO_{3(aq)}$ (800µL of 7.5% w/v) was added and kept for 1 hour. The absorbance at 765 nm was measured against a reagent blank. A standard curve was prepared using gallic acid. The phenolic contents of fruit extracts were expressed in mg galic acid equivalents, (GAE), per gram of fresh fruit.

Determination of Ferric Reducing Antioxidant Power Assay (FRAP)

The working FRAP solution was prepared according to the method described by Silva and Sirasa (2018), with some modifications. Acetate buffer (150 mM, pH 3.6) was prepared by dissolving 1.55 g of sodium acetate trihydrate and 16 mL of glacial in 1000 mL of distilled water. Then 5 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in

40 mM HCl and 10 mM $FeCl_3.6H_2O$ in distilled water were also prepared. Three solutions were mixed at a 10:1:1 v/v ratio to prepare the working FRAPS solution. An aliquot (100 µL) of fruit extract was mixed with 3.0 mL of the prepared FRAP solution. The tubes were mixed well and allowed to stand for 5 minutes at 25°C. The absorbance of each extract was measured against a blank at 594 nm wavelength at 37°C. The results were expressed as µM of Fe^{2+}/g dry weight using a Fe^{2+} standard curve.

DPPH radical scavenging activity % = $\frac{As}{Asd}$ X FRAP value of standard (1000 µM)

A_s- Change in absorbance of the sample, A_{sd} - Change in absorbance of standard

Determination of DPPH Radical Scavenging Activity Assay (DPPH)

The DPPH radical scavenging activity of extracts was determined as described by Silva and Sirasa (2018), with slight modifications. Thus, 4×10^{-3} M DPPH 100 mL was prepared by adding methanol. Dilution series (50, 100, 150, 200 ppm etc.) were prepared accordingly. In this microplate, a sample was incubated in the dark for 20 minutes at room temperature (25°C). Then the absorbance value of the sample was measured using a spectrophotometer (MultiskanGo.100.40, China) at 517 nm against a blank assay. The amount of sample needed to decrease the initial DPPH concentration by 50% v/v, IC_{50} (Inhibition Concentration), was calculated graphically.

DPPH radical scavenging activity % = $\frac{Ac-As}{Ac}$ X 100

A_c-Absorbance of the control, A_s-absorbance of the test sample

Statistical Analysis

The data was expressed as the mean of three independent experiments. Statistical comparisons of the results were performed by one-way ANOVA using Minitab 16. Significant differences ($p<0.05$) among the maturity stages were analyzed by Duncan's triplicate range test.

RESULTS AND DISCUSSION

Variation in Proximate Composition

According to the proximate analysis of the well ripe (WR) stage fruit, it contains a considerable amount of moisture (80.52±1.34% w/w), crude fat content (3.28±0.03% w/w), crude protein content (2.98±0.14% w/w), Brix value (11.5±0.01 degrees Brix), titratable acidity (1.02±0.67% w/w), pH (3.7±0.83), and low amount of ash content (78±0.05% w/w). Those results were in accordance with previous studies that analyzed the composition, nutritional value, and medicinal values of *soursop* (*Annona muricata*) fruit (Agu and Okolie, 2017; Tiencheu *et al*., 2021).

Total Phenolic Content

Phenolic compounds are very important fruit constituents because they exhibit antioxidant activity by inactivating free radicals or preventing the decomposition of hydroperoxides into free radicals. The presence of hydroxide groups in the phenolic compounds affects the antioxidant activity of *soursop* fruit (Akomolafe *et al*., 2015). The TPC determined by two extraction methods with two solvents of *soursop* fruit pulp is shown in Figure 02. According to the results, TPC ranged widely from 65.28±3.24 to 165.67±2.86 mg GAE/g FW. The highest ($p<0.05$) TPC (165.67±2.86 mg GAE/g) was recorded in the absolute 70% ethanol extract of the WR stage with the sonication extraction method. The lowest ($p<0.05$) TPC (65.28±3.24 GAE mg/g) was recorded in ethanol (v/v) extract of the UR stage with the stir plate method. Therefore, a significant difference ($p<0.05$) was observed in TPC with the maturity stage of *soursop* fruit. The

TPC of Sri Lankan *A. muricata* was in accordance with those results (Padmini *et al*., 2014). The two extraction methods with two solvents obtained different results in one maturity stage of fruit pulp. Therefore, the extraction method of compounds was highly affected by the TPC of the same stage fruits. In addition, food processing methods are able to improve the extractability of antioxidant compounds by breaking down cell walls (Orak *et al*., 2019). Therefore, some of the different sample processing methods, storage conditions, and extraction techniques affected the variation of the final antioxidant activity of the same fruit (Orak *et al*., 2019; Renard *et al*., 2018). In addition, Babbar *et al*, (2011), reported that the total phenolic content of plant extracts could be influenced by geographical origin, cultivar, harvest, and storage time, as well as drying and extraction techniques. In the present study, the sonication extraction method obtained a higher yield of phenolic content when compared with the stir plate method.

Ferric Reducing Power Assay (FRAP)

FRAP variations in *soursop* fruits at the four different maturity stages are shown in Figure 03. According to the results, the FRAP of *soursop* pulp at different maturity stages changed with fruit maturity. The highest ($p<0.05$) FRAP (393.91±4.23 µmol Fe^{+2}/L) value was recorded in 70% v/v ethanol extracts of FM stage with sonication extraction method. It is important to note that the entire antioxidant capacity of the samples was derived by measuring their capacity to reduce Fe^{+3} to Fe^{+2} using the FRAP test. Accordingly, the lowest ($p<0.05$) FRAP (236.24±1.35 µmol Fe^{+2}/L) value was recorded in the absolute ethanol extract of the WR stage with the sonication method. According to the results, FRAP values ranged widely from 236.24±1.35 to 393.91±4.23 µmol Fe^{+2}/L extract. Orak *et al*, (2019), investigated that successive extraction of *soursop* leaves, fruit pulp, seeds, and peels with hexane, chloromethane, ethyl acetate, and methanol extracts with different antioxidant activities resulted in different yields of antioxidant activity in the same part of the *soursop* fruit. In addition, FRAP values of the same fruit in different ripening stages changed due to the metabolic activity of the ripening stages and the climacteric period and the action of ascorbic acid, which presents a high concentration day after harvest (Anaya Esparza and Montalvo-Gonzalez, 2020). Nevertheless, the antioxidant activity (FRAP) can also depend on the diverse types of substances with antioxidant activity in the fruit and the different action sites of these substances (Nuguyen *et al*., 2020).

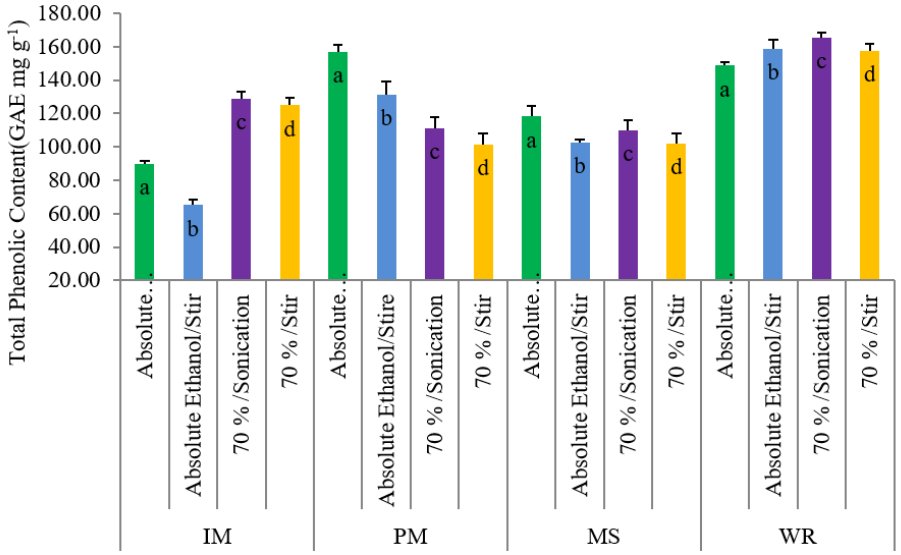

Figure 02: **Total Phenolic Content of *Soursop* Fruit at Different Maturity Stages under different solvent extraction (Immature Stage (IM), Partially Mature Stage (PM), Fully Mature Stage (FM), and Well Ripened Stage (WR). Bars accompanied by different [a,d] superscripts represent means that differed significantly by ANOVA test at P<0.05.**

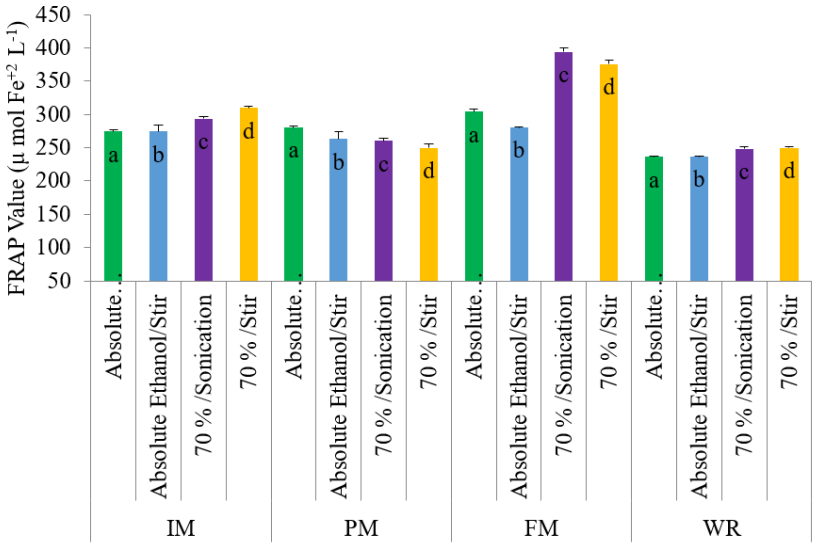

Figure 03: **FRAP values of *Soursop* fruit at different maturity stages under different solvent extractions (Immature Stage (IM), Partially Mature Stage (PM), Fully Mature Stage (FM), and Well Ripened Stage (WR) Bars accompanied by different $^{(a,d)}$ superscript values represent means that differed significantly by ANOVA test at P<0.05.**

DPPH Radical Scavenging Activity

The radical scavenging activity was expressed as Inhibition Concentration (IC_{50}) values. IC_{50} (ppm) value was determined only for the WR stage fruits due to the time shortage for the present study. The highest DPPH radical scavenging activity (IC_{50}-60.13±0.18 ppm) was identified in the 70% ethanol extract of WR stage with sonication extraction method while the lowest amount of DPPH radical scavenging activity (IC_{50}-57.13±0.16 ppm) was identified in absolute ethanol extract of the WR stage with the sonication extraction method. Further, in the stir plate extraction method, DPPH radical scavenging activity was higher in 70% ethanol (56.32±0.16 ppm) and lower (55.12±0.19 ppm) in absolute ethanol extract. Padmini *et al*, (2014) also reported the antioxidant properties (IC_{50} 724.98±3.0 ppm) of ethanolic fruit pulp extract in the mechanical stirrer plate method. These results imply that antioxidant compounds such as ascorbic acid, β-carotene, α-carotene, and different xanthophylls have been detected in *soursop* and may have contributed to the antioxidant activity of the fruit extract. In general, the most abundant antioxidants in fruits are phenols, vitamins, flavonoids, and carotenoids (Anaya Esparza and Montalvo-Gonzalez, 2020). In addition, Acetogenins are long-chain fatty acids common in plants belonging to the *Annonaceae* family (Sanusi *et al*., 2018). Notably, the total phenolic content, FRAP values, and IC_{50} values of the *soursop* fruit pulp in the present study were in accordance with the findings reported by Orak *et al*. (2019).

CONCLUSION

The present study employed different extraction techniques and extraction solvents to determine the phenolic content and antioxidant efficacy of *soursop* fruits at different maturity stages. Results of the study revealed that the maturity stage of the *soursop* fruit significantly affects the total phenolic content and the antioxidant activity. Moreover, extraction technique and extraction solvent also greatly alter the total phenolic content and the antioxidant activity of *soursop* fruit. The current study has identified sonication extraction and 70% ethanol as an excellent extraction technique and extraction solvent respectively, to extract bioactive compounds from *soursop* fruit under the experimental conditions used in the current study.

Conflict of Interest

The authors declare that there is no conflict of interest.

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