DETERMINATION OF PHYSICOCHEMICAL AND ANTIOXIDANT ACTIVITIES OF SOURSOP (*Annona muricata* L.)

A THESIS

By

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Dedicated to

My

Beloved Parents

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December, 2024 The Author

ABSTRACT

Nowadays Soursop has become popular for its high antioxidant as well as anticancer property and potential medicinal benefits in Bangladesh. An experiment was carried out at Horticulture Laboratory of Agrotechnology Discipline, Khulna University, Khulna on the physicochemical characteristics and antioxidant activities of soursop (*Annona muricata* L.) leaf and fruit which were collected from five different locations of the South Western region of Bangladesh during the period from May to June, 2024. The experiment was laid out in a Completely Randomized Design (CRD) with four replications. A significant variation in respect of all the physical and chemical parameters of leaves and fruits was observed among the study areas. The highest weight (1.35mg), length (133.68cm) and breadth (6.05cm) of soursop leaf were observed in the samples collected from Khulna. The maximum weight (626g), length (15.39cm), breadth (14.43cm), weight of edible portion (474.25g). weight of non-edible portion (151.75g), percentage of edible portion (75.70%), total number of seeds per fruit (51.50) and total seed weight (12.54g) of soursop fruit were also observed in the samples collected from Khulna and the highest percentage of non-edible portion (30.38%) was found from the fruits of Kushtia. The maximum value of TSS (14.64%), TA (0.99%) and Vit. C (22.65mg/100g) was found in soursop fruit. On the other hand, the highest value of carotenoids (0.76mg/g), flavonoid (0.85g/100g), tannins (11.08mg/g), Saponins (13.93mg/g), alkaloids (35.15µg/g) and acetogenins (35.22mg/g) were found in the leaf. The highest IC_{50} (86.46µg/mL) value as well as lowest antioxidant activity was observed in soursop leaf and the lowest IC_{50} (33.36µg/mL) value as well as highest antioxidant activity was found in soursop fruit. So, the better performance in respect of physical characteristics of soursop fruits and leaves were found in the samples collected from Khulna. Soursop leaves showed better performance in respect of chemical characteristics.

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CHAPTER I

INTRODUCTION

Soursop (*Annona muricata* L.) is grown around the globe because of the latent health benefit which belongs to the Annonaceae family and mainly grown within tropical and sub-tropical regions. Other names of soursop include guanabana (Spanish), guayabano (Philippines), sirsak (Indonesia), corossol epineux (French) and graviola (Brazil) or 'Tok ata fol' in Bengali. Soursop fruit has a dark green ovoid shape which is spiny and encloses whitish, juicy, sourish and fragrant pulp (Moghadamtousi et al., 2015; Coria-Téllez et al., 2018).

Nowadays, *Annona muricata* is becoming popular all over the world because of not only for its flavor and color but also for its nutritional and medicinal values. A notable member of the Annona family, *Annona muricata* L., is praised for its high antioxidant activity. This fruit is also reported to have high amounts of flavonoids and phenols. This exotic fruit is characterized with its soft texture with a unique sweet and sour flavor. Vitamins, minerals, and fibre are abundant in soursop. The majority of the time, it is consumed fresh or processed into juice, but it is underused and not commercially exploited (Agu et al., 2017). The traditional medicinal system includes numerous plants that possess various medicinal and pharmacological uses, representing a valuable reservoir of potential new bioactive compounds. The therapeutic qualities of a number of underutilised fruits in traditional medicine for the treatment of various illnesses have drawn more attention in recent years (Singh et al., 2014). Medicinal substances can originate from the entire plant or specific parts of it, and their derivatives have played a significant role in healthcare practices. The effectiveness of both natural and active chemicals from vegetables, fruits, and other plant materials for medicinal purposes is influenced by the extraction procedures used and the levels of antioxidants present in it (Daniel, 2002). Plant-derived bioactive compounds are increasingly recognized for their diverse therapeutic effects and overall safety. According to the World Health Organisation (WHO), traditional medicine serves the primary healthcare needs of more than 80% of the world's population. They also recommended advancements in the cultivation techniques for medicinal plants (Holiman, 1989).

Soursop is indigenous to the warm climates of North and South America and has proliferated throughout the tropical and subtropical regions of Western Africa, Central and South America, and Southeast Asia (Coria-Tellez et al., 2018). The soursop fruits are 15–20 cm. The fruit's pulp contains between 55 to 170 black seeds enclosed by a green skin. The peels and seeds of the soursop fruit are inedible, and there exists a considerable amount of by-products from this fruit that remain unexplored (Aguilar Hernandez *et al.*, 2019). Nonetheless, recent years have shown an increasing interest in the utilization of fruit and vegetable by-products, owing to their potential significant amount of nutrients and bioactive compounds, including phenolics, vitamins and dietary fibre among others (Sagar et al., 2018). By-products from exotic fruits have previously been regarded as valuable natural food additives. Notably, phenolic acids, flavonoids, and tannins have been identified in the pulp, leaves and seeds of *A. muricata* L. (Nawwar et al., 2012; Marques and Farah, 2009). The anticancer properties of soursop are associated with their cytotoxic effects on cancer cells, resembling the actions of certain chemotherapy treatments. This effect is primarily linked to the presence of acetogenins, which are long-chain fatty acid metabolic derivatives consisting of 35 to 37 carbon atoms (Wu et al., 1995).

Reactive nitrogen species (RON) and Reactive oxygen species (ROS) are types of free radicals that originate from both standard metabolic activities and extrinsic sources. They play vital roles in energy production, detoxification, chemical signaling, and immune responses. However, an excess of these free radicals can cause harm to important biomolecules like proteins, lipids and DNA. They are frequently linked to oxidation processes that can contribute to various human diseases, including neurological disorders, diabetes, and specific types of cancer. When the concentration of free radicals in the body becomes too high, external antioxidants (exogenic) for example- flavonoids, vitamin A, C, and E are required (Akomolafe and Ajayi, 2015).

An antioxidant is any compound that can slow down or prevent the oxidative harm caused to lipids, proteins, and nucleic acids by reactive oxygen species (Lim et al., 2007). Consequently, antioxidants have involved significant interest in recent years, particularly in the domains of biology, food and agrochemicals. Increasing evidence indicates that the consumption of fruits and vegetables is associated with a reduced risk of chronic diseases, such as cardiovascular disorders, cancer, and cataracts (Chinnici et al., 2004). This is commonly accredited to the antioxidants found in vegetables and fruits, such as vitamins

C and vitamin E, phenolic acids, flavonoids, and carotenoids, that help to prevent damage from free radicals (Silva et al., 2004). *Annona muricata* L. is a tropical fruit known for its antioxidant capabilities. This plant is rich in annonaceous acetogenins found in its fruits, seeds, roots, twigs, and bark, that exhibit antimalarial, antiviral, antitumor, pesticidal, and antimicrobial properties, indicating numerous potential applications. The extract from ripe fruit contains three main acetogenins such as- bullatacin, bullatalicin and asimicin (Jaramillo et al., 2000).

Although this plant has many medicinal applications relatively little is explored about its antioxidant effects. Again, the work on the phytochemical and antioxidant activities of soursop have not yet been studied properly in Bangladesh. So, the current investigation was carried out to assess the physical properties, phytochemical screening and antioxidant activity of soursop leaf and fruit extract.

Therefore, to fulfill the research lacks and gaps the objectives of the study are as follows:

- To determine the physical properties of the soursop leaves and fruits.
- To analyze the chemical properties and antioxidant activities of the soursop leaves and fruits.

CHAPTER II

REVIEW OF LITERATURE

The utilization of natural components as remedies for various diseases is on the rise. Plants serve as a source of natural compounds extensively utilized in medicinal applications. The phytochemicals in plants are accountable for their therapeutic effects against many illnesses, and research can be conducted to isolate the bioactive substances in plants and assess their pharmacological efficacy against diseases (Moghadamtousi et al., 2015).

Annona muricata L., commonly known as soursop, belongs to the Annonaceae family, which includes over 130 genera and 2,300 species. The plant is rich in various compounds that exhibit pharmacological properties. It is prevalent in tropical and subtropical regions, including Southeast Asia, South America, and the African rainforests. This plant yields edible fruit throughout the year and is extensively utilized in traditional medicine for ailments such as skin disorders, respiratory issues, fever, bacterial infections, diabetes, hypertension, and cancer (Moghadamtousi et al., 2015). Different parts of *Annona muricata* serve various medicinal purposes. The seeds fight against parasite infections; the fruit aids in the treatment of arthritis, neurological diseases, and diarrhea; whereas the leaves are utilized for managing cystitis, headaches, sleeplessness, and cancer (Wélé et al., 2004). The primary active constituents of soursop include acetogenins, alkaloids, and flavonoids (Coria-Téllez et al., 2018). A study of the chemicals present in *Annona muricata* leaf extract identified secondary metabolites including flavonoids, terpenoids, saponins, coumarins, lactones, anthraquinones, glycosides, tannins, and phytosterols (Gavamukulya et al., 2014).

Physical characteristics of soursop

The soursop tree has a slender, bushy appearance with low branches, growing to heights of 25 to 30 feet (7.5-9 meters) and diameters ranging from 15 to 83 cm. Its limbs curve upwards, and its leaves have a strong fragrance; they are usually evergreen and arranged alternately, featuring a smooth and glossy texture, with a dark green color on the upper side and a lighter hue underneath. The leaves are oblong, elliptic, or narrow obovate, tapering to points at both ends. This tree is native to the tropical regions of Central and South America, Western Africa, and Southeast Asia, flourishing at elevations below 1200 meters

- The residue was ground with 20 ml of 80% acetone, centrifuged, and the supernatant was transferred to the same flask.
- The procedure was repeated until the residue became colorless. The pestle and mortar were then thoroughly washed with 80% acetone, and the clear wash was collected in the flask.
- The volume was adjusted to 100 ml using 80% acetone.
- The absorbance was measured at 510nm and 480nm compared to the blank solution.

Calculations

$$\text{mg carotenoid / g tissue} = 7.6\,(A.480) - 1.49\,(A.510) \times \frac{V}{1000 \times 10}$$

Where,

A= Absorbance of specific wave length

V= Final volume of carotenoid in 80% acetone

3.4.2.6 Determination of antioxidant activity

The DPPH test protocol is described below, followed the methodology by Shimamura et al. (2014).

Reagents

- Ethanol
- 0.004% DPPH in ethanol
- Ascorbic acid (standard)

Procedure

- Initially, 21 test tubes were utilized. They were thoroughly cleansed and rinsed with ethanol.
- Out of 21 test tubes, 9 were designated for extract solutions of varying concentrations (2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/ml), while another 9 were allocated for preparing aliquots of ascorbic acid at the same concentrations, with one additional tube reserved for the blank solution preparation.
- The sample extract and ascorbic acid were measured and diluted in ethanol to prepare the stock solution.

- Various concentrations of the plant extract and the standard were generated from the stock solution using the dilution procedure.
- DPPH was measured and diluted in ethanol to prepare a 0.004% (w/v) solution.
- 2ml of each concentration of plant extract and standard were placed in a test tube, followed by the addition of 6ml of DPPH solution.
- The test tubes were placed in a dark environment for 30 min at room temperature.
- DPPH solution was added to the blank test tube containing only ethanol as the blank.
- After 30 minutes, the absorbance of each test tube was quantified using a UV spectrophotometer at 517 nm.

Calculation of DPPH

DPPH Radical Scavenging Activity (RSA) (%) = $((Ac - As) / Ac) \times 100$

Where,

A_c = absorbance of the control sample at 517nm

A_s = absorbance of the test samples at 517nm

- IC_{50} was determined through the graph % inhibition vs. concentration and verified by the Software- GraphPad Prism 10.4.0.

3.4.2.7 Determination of alkaloids

Preparation of reagents

Bromocresol green (BCG) solution: 69.8 mg of bromocresol green (BCG) was heated with 3 ml of 2N NaOH and 5 ml of distilled water until completely dissolved. The resulting solution was then diluted to 1000 ml of distilled water.

Phosphate buffer solution: In order to make the phosphate buffer solution, 0.2M citric acid (42.02gm citric acid in 1L distilled water) was added to 2M sodium phosphate (71.6gm Na_2HPO_4 in 1L distilled water) to lower the pH to 4.7.

Atropine standard solution: 1 mg of pure atropine was dissolved in 10 mL of distilled water to prepare it.

Separation of alkaloid

A portion of the extract residue was dissolved in 2N HCl and then filtered. 1ml of this solution was transferred to a separatory funnel and rinsed with 10 ml of chloroform three times. The pH was adjusted to neutral by adding 0.1N NaOH. Subsequently, 5 ml of BCG solution and 5 ml of phosphate buffer were included into this solution. The mixture was agitated, and the mixture was extracted using 1, 2, 3, and 4 ml of chloroform with vigorous agitation. The extract was then collected in a 10 ml volumetric flask and diluted with the chloroform.

Preparation of standard curve

Precisely measured aliquots (20, 40, 60, 80, and 100 µg) of atropine standard solution were transferred to various separatory funnels. 5ml of bromocresol green solution and 5ml of phosphate buffer (pH 4.7) were combined and agitated with 1, 2, 3, and 4 ml of chloroform extract. The extracts were subsequently collected in a 10 ml volumetric flask and diluted with chloroform to achieve the desired concentration.

The absorbance of the compound in the chloroform was estimated at 470nm wave length in spectrophotometer against the blank solution (Ajanal et al., 2012).

Table 3.1: Absorbance of atropine (standard) at different concentration

Conc. (µg/ml)	Absorbance
00	0
20	0.009
40	0.018
60	0.025
80	0.038
100	0.045

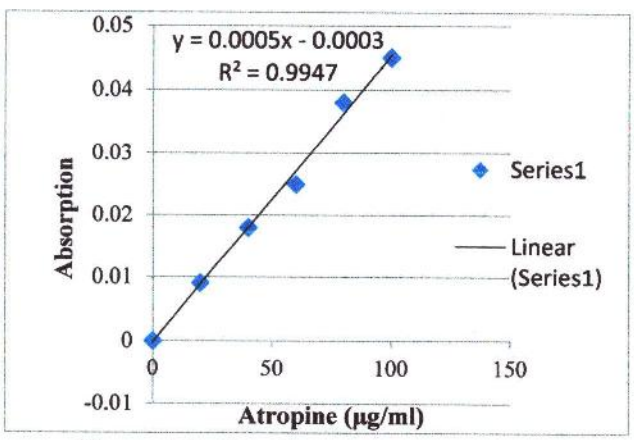

Figure 3.1: Atropine accuracy curve

3.4.2.8 Determination of tannin

Reagents required:

a) Folin-Denis reagent: 750ml water, 20g phosphomolybdic acid, 50ml of 85% phosphoric acid (H_3PO_4), and 100 g sodium tungstate ($Na_2WO_4.2H_2O$) were dissolved. Then the mixture was refluxed for 2hrs, cooled to 25°C and diluted to 1000 ml by water.

b) Saturated sodium carbonate solution: 100 ml of water was combined with 35 g of anhydrous sodium carbonate, then dissolved at 70-80°C, and allowed to cool overnight. The clear liquid was decanted before use.

c) Standard tannic acid solution: 100 mg of tannic acid was dissolved in 1 L of water. A new solution was prepared for each analysis.

Procedure

1 g of the sample was boiled with 400 ml of water for 30 minutes. Subsequently, it was transferred to a 500 ml volumetric flask and diluted to the calibration mark. Thoroughly agitated and filtered.

Preparation of standard curve

Preparation of tannic acid standard solution

100 mg of tannic acid was solubilized in 1 liter of water. A new solution was produced for each determination (1 ml = 0.1 mg of tannic acid). Then transferred the aliquots ranging from 0 to 14 ml of the standard tannic acid solution into 100ml volumetric flasks containing 75 ml of water. Added 5 ml of Folin-Denis reagent and 10 ml of Na_2CO_3 solution into each volumetric flask, then diluted to a final volume of 100 ml with water. The filtrate was utilized in an aliquot that contained not more than 0.1 mg of tannic acid. Followed the normal procedure and used the standard curve to determine the mg of tannic acid. After thoroughly mixing the samples, the color was assessed at 760 nm 30 minutes later against an experimental blank that had been corrected for absorbency (Nair et al., 2015).

Table 3.2: Absorbance of tannic acid (standard) at different concentration

Tannic acid (in ml)	Folin - denis reagent (in ml)	Sodium Bicarbonate (in ml)	Abs (760nm)
0	5	10	0.0
2	5	10	0. 051
4	5	10	0.105
6	5	10	0.158
8	5	10	0.213
10	5	10	0.261
12	5	10	0.317
14	5	10	0.373

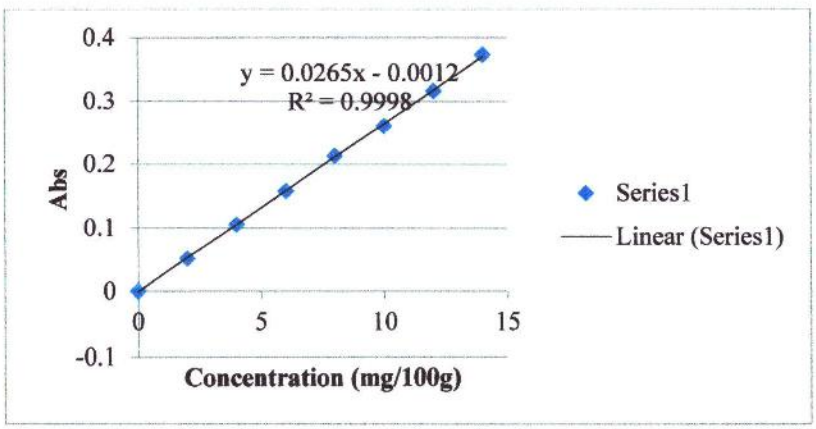

Figure 3.2: Standard curve between absorbance and concentration

3.4.2.9 Determination of saponin

Reagents

PET=Polyethylene terephthalate ethanol and chromagen reagent were used and it was ready from the standard curve prepared by using diosgenin.

Procedure

Assessment of total saponin content was performed by the method of Makkar et al. (2007).

- 1g of sample and 10ml PET ether was taken in a beaker.
- Then another 10ml of PET ether was added into the beaker.
- The solution was filtered, and the filtrate was evaporated to dryness.
- Then 6ml of ethanol was dissolved in the residue.
- After that, 2ml of the solution was taken in a test tube.
- 2ml of chromagen solution was added into the test tube.
- Then it was left for 30 min and absorbance at 544nm was taken.
- The data were quantified as diosgenin equivalents (mg DE/g extract) based on a standard curve.

Table 3.3: Absorbance of diosgenin (standard) at different concentration

Conc. (mg/ml)	Absorbance
5	0.035
10	0.072
15	0.108
20	0.144
25	0.181
30	0.219
35	0.257
40	0.288

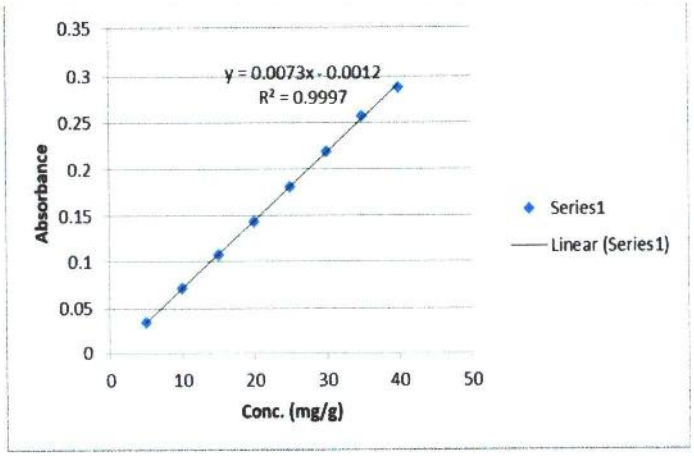

Figure 3.3: Standard curve between absorbance and concentration

3.4.2.10 Determination of acetogenins

Reagents

Kdde's reagent was prepared as solutions of 2% (2g) DNBA (3,5-dinitrobenzoic acid) in 100ml of ethanol and 5% (5g) of KOH in 100ml of ethanol.

Procedure

The acetogenin content was quantified by analyzing the colorimetric reaction with Kedde's reagent, which produces a pink color upon reaction. 50ml of the acetogenic extracts were combined with 2 ml of Kedde's reagent, and the absorbance was recorded at 505 nm using

a spectrophotometer. A standard annonacin curve was calculated, and the data were presented in milligram equivalents of annonacin per gram of dry weight (mg/g DW). (Aguilar-Hernández et al., 2020b).

Table 3.4: Absorbance of annonacin

(standard) at different concentration

Conc. (mg/ml)	Absorbance
0.5	0.017
1	0.031
2	0.064
4	0.131
8	0.259
16	0.518
32	1.038
64	2.079

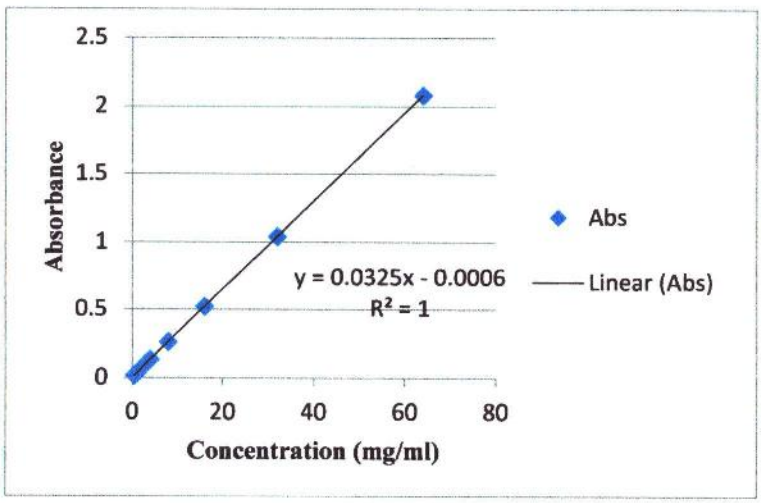

Figure 3.4: Standard curve between absorbance and concentration

3.5 Statistical analysis

The data were subjected to a two-way Analysis of Variance (ANOVA) using statistical software Statistix-10 and means were compared through the Dancan's Multiple Range Test (DMRT) (Gomez and Gomez, 1989).

CHAPTER IV
RESULT AND DISCUSSION

The results attained from the study on physicochemical characteristics and antioxidant activities of Soursop (*Annona muricata* L.) leaves and fruits of five different locations have been presented and discussed in this chapter. The data have been presented in the tables and figures for discussion, comprehension and understanding. Analysis of variance of all the parameters have been given in Appendices- I, II and III.

4.1 Physical characteristics of soursop leaves and fruits

Better performance was found in the samples of soursop leaves and fruits collected from Khulna in respect of physical characteristics which might be due to the good management practices adapted by the farmers of Khulna region.

4.1.1 Physical characteristics of soursop leaves

The data on the physical characteristics of soursop leaves collected from five different locations have been presented in Table 4.1. Physical characteristics of soursop leaves have been described based on quantitative traits in this study.

4.1.1.1 Weight of individual leaf

There were significant variations among the five locations in respect of weight of fresh soursop leaves (Appendix I). The maximum weight of soursop leaf (1.35g) was found in the samples of Khulna and the minimum weight (1.02g) was recorded from the leaf of Kushtia (Table 4.1). Average weight of leaf was recorded 1.16g and the range was 1.02-1.35g.

4.1.1.2 Length of leaf

There were significant variations found in respect of length of the soursop leaves among the five locations (Appendix I). The samples of Khulna gave the maximum length (13.68cm) and the minimum length (10.69cm) was found from the leaf collected from Kushtia (Table 4.1).

Table 4.1: Physical characteristics of soursop leaves in different locations

Locations	Leaf weight (g)	Leaf length (cm)	Leaf breadth (cm)	Leaf color	Leaf shape
Khulna	1.35a	13.675a	6.05a	Green	Oval
Jashore	1.226b	12.965b	5.388bc	Green	Oblong
Bagerhat	1.123c	12.453c	5.485b	Light green	Oblong
Satkhira	1.078c	11.725d	5.238c	Dark green	Oval
Kushtia	1.021d	10.698e	5.016d	Green	Oval
Range	1.021-1.35	10.698-13.675	5.01-6.05		
Average	1.159	12.303	5.435		
Level of significance	**	**	**		
C.V. (%)	2.66	1.30	2.31		

Note: Data are the means of four replications. In a column ** = significant at $p \leq 0.01$, CV= Coefficient of variation. Non-significant differences are indicated by similar alphabets in a column.

Average length of leaf was recorded 12.30cm and the range was 10.69-13.68cm. Similar results were also reported by Wagner and Lorence (2014). They observed that the range of soursop leaf length was 6.5 to 20cm.

4.1.1.3 Breadth of leaf

The breadth of the soursop leaves was significantly varied among the five locations (Appendix I). The maximum breadth (6.05cm) was found in the leaf collected from Khulna and the minimum weight (5.01cm) was recorded from Kushtia (Table 4.1). Average breadth of leaf was recorded 5.43cm and the range was 5.01-6.05cm. Wagner and Lorence (2014) reported similar finding. They observed that the soursop leaf breadth 2.5 to 6.5cm.

4.1.1.4 Color of leaf

Similar leaf colors were observed among the five locations. The colors of soursop leaves were green (Khulna, Jashore and Kushtia), light green (Bagerhat) and dark green (Satkhira) (Table 4.1). Similar result was found by Wagner and Lorence (2014).

4.1.1.4 Shape of leaf

The shapes of the leaves were varied from oblong (Jashore, Bagerhat) to oval (Khulna, Satkhira and Kushtia) (Table 4.1). Similar result was found by Wagner and Lorence (2014).

4.1.2 Physical characteristics of Soursop fruit

The data on physical characteristics of soursop fruit of five different locations have been presented in the table 4.2. Physical characteristics of soursop fruit have been described in this study.

4.1.2.1 Weight of individual fruit

There were significant variations among the five locations in respect of weight of the soursop fruits (Appendix II). The maximum weight of fruit (626.00g) was found in the samples collected from Khulna and the minimum weight (377.31g) was recorded from Kushtia (Table 4.2). Average weight of the fruit was recorded 489.53g and the range was

Table 4.2: Physical characteristics of soursop fruits in different locations

Locations	Fruit weight (g)	Fruit length (g)	Fruit breadth (g)	Weight of edible portion (g)	Weight of non-edible portion (g)	Percentage of edible portion (%)	Percentage of non-edible portion (%)	Total number of seeds per fruit	Weight of seeds per fruit (g)	Fruit color	Fruit shape
Khulna	626.00a	15.39a	14.43a	474.25a	151.75a	75.70a	24.30c	51.50a	12.54a	Green	Conical
Jashore	482.38c	13.57b	12.21c	349.14c	133.23b	72.38b	27.63b	41.50c	10.78c	Light green	Oval
Bagerhat	526.88b	14.15b	13.03b	390.07b	136.81b	74.03ab	25.98bc	45.00b	11.56b	Yellowish green	Oval
Satkhira	435.06d	12.37c	10.89d	302.74d	132.32b	69.56c	30.44a	37.25d	9.85d	Light Green	Oval
Kushtia	377.31e	10.45d	8.75e	262.77e	114.54c	69.62c	30.38a	31.75e	9.01e	Green	Conical
Range	377.31-626.00	10.45-15.39	8.75-14.43	262.77-474.25	114.54-151.75	69.56-75.70	24.30-30.44	31.75-51.50	9.01-12.54		
Average	489.53	13.18	11.86	355.79	133.73	72.26	27.74	41.4	10.75		
Level of significance	**	**	**	**	**	**	**	**	**		
C.V. (%)	5.77	2.99	3.87	7.07	5.29	2.06	5.36	4.81	3.69		

Note: Data are the means of four replications. In a column **= significant at $p \leq 0.01$. CV= Coefficient of variation. Non-significant differences are indicated by similar alphabets in a column.

377.31-626.00g. Pinto et al. (2005); Coria-Téllez et al. (2018) and Wagner and Lorence (2014) reported similar findings. They mentioned that the weight of soursop fruit can be 0.4 to 4kg.

4.1.2.2 Length of fruit

There were significant variations found in respect of length of the soursop fruits among the five locations (Appendix II). The maximum length of fruit (15.39cm) was found in the samples collected from Khulna and the minimum length (10.45cm) was recorded from Kushtia (Table 4.2). Average length of fruit was recorded 13.18cm and the range was 10.45-15.39cm. Similar result was revealed by Pinto et al. (2005) and Wagner and Lorence (2014). They mentioned the range of soursop fruit length was 4 to 12 inches.

4.1.2.3 Breadth of fruit

The breadth of the soursop fruits was significantly varied among the five locations (Appendix II). The maximum breadth (14.43cm) was found in the fruits of Khulna and the minimum weight (8.75cm) was recorded from Kushtia (Table 4.2). Average breadth of fruit was recorded 11.86cm and the range was 8.75-14.43cm. Similar findings was found by Pinto et al. (2005) and Wagner and Lorence (2014). They mentioned that the soursop fruit breadth was up to 6 inches.

4.1.2.4 Weight of edible portion of soursop fruit

There were significant variations among the five locations in respect of the weight of edible portion of soursop fruit (Appendix II). Maximum weight of edible portion of fruit (474.25g) was found in the samples of Khulna and the minimum weight of the edible portion (262.77g) was recorded from Kushtia (Table 4.2). The average weight of edible portion of the fruit was recorded 355.79g and the range was 262.77-474.25g. Onimawo et al. (2002) reported similar finding.

4.1.2.5 Weight of non-edible portion of soursop fruit

Significant variations were found among the five locations in respect of weight of non-edible portion of the soursop fruits (Appendix II). The maximum weight of non-edible portion of fruit (151.75g) was found in the fruits of Khulna and the minimum weight of non-edible portion (114.54g) was recorded from Kushtia (Table 4.2). Average weight of non-edible portion of the fruit was recorded 133.73g and the range was 114.54-151.75g. Onimawo et al. (2002) reported similar finding.

4.1.2.6 Percentage of edible portion of soursop fruit

There were significant variations among the five locations in respect of percentage edible portion of the soursop fruits (Appendix II). The maximum percentage of edible portion of fruit (75.70%) was found in the fruits of Khulna and the minimum percentage of edible portion (69.62%) was recorded from Kushtia which was statistically similar to Satkhira (69.56%) (Table 4.2). Average percentage of edible portion of the fruit was recorded 75.26% and the range was 69.56%-75.70%. Onimawo et al. (2002) reported similar finding and it was 67.5%.

4.1.2.7 Percentage of non-edible portion of soursop fruit

Significant variations were found among the five locations in respect of percentage of non-edible portion of the soursop fruits (Appendix II). The maximum percentage of non-edible portion of fruit (30.38%) was found in the samples of Kushtia which was statistically similar to Satkhira (30.44%) and the minimum percentage of non-edible portion (24.30%) was recorded from Khulna (Table 4.2). Average percentage of non-edible portion of the fruit was recorded 27.74% and the range was 24.30%-30.44%. Onimawo et al. (2002) reported similar finding.

4.1.2.8 Total number of seeds per fruit

Significant variations were found among the five locations in respect of total number of seeds per fruit (Appendix II). The maximum number of seeds per fruit (51.50) was found in the samples of Khulna and the minimum (31.75) was recorded from Kushtia (Table 4.2).

Average number of seeds per fruit was recorded 41.4 and the range was 31.75- 51.50. Pinto et al. (2005) and Coria-Téllez et al. (2018) reported similar findings.

4.1.2.9 Weight of seeds per fruit

There were significant variations among the five locations in respect of weight of seeds per fruit (Appendix II). The highest weight of seeds per fruit (12.54g) was found in the samples of Khulna and the lowest weight of seeds per fruit (9.01g) was recorded from Kushtia (Table 4.2). Average weight of edible portion of the fruits was recorded 10.747g and the range was 9.01-12.54g. Pinto et al. (2005) and Coria-Téllez et al. (2018) reported similar findings.

4.1.2.10 Color of fruit

Similar fruit colors were observed among the five study locations. The colors of soursop fruits were green (Khulna and Kushtia), light green (Jashore and Satkhira) and yellowish green (Bagerhat) (Table 4.2). Pinto et al. (2005) and Coria-Téllez et al. (2018) reported similar findings.

4.1.1.11 Shape of fruit

The shapes of the fruits were varied from conical (Khulna and Kushtia) to oval (Jashore, Bagerhat and Satkhira) (Table 6). Pinto et al. (2005) and Coria-Téllez et al. (2018) reported similar finding.

4.2 Chemical characteristics of soursop leaves and fruits

The data on chemical characteristics of soursop leaves and fruits in five different locations have been presented in Table 4.3. The chemical characteristics of soursop leaves and fruits have been described in this study.

4.2.1 Total soluble solids (TSS) (%)

There were significant variations found among the five locations in respect of TSS of the soursop leaves and fruits (Appendix III). The highest percentage of TSS was found in soursop fruits (14.64%) collected from Jashore and the lowest was found in the soursop leaves (3.31%) from Bagerhat which was statistically similar to Khulna (3.38%) and Kushtia (3.41%) (Table 4.3). The range of TSS in soursop fruit was 13.41% to 14.64% and in soursop leaf was 3.31% to 3.67%. Budiastra and Jannah (2022) and Terán-Erazo et al. (2022) also reported that the TSS of soursop fruit was 5.73 to 17.93%.

4.2.2 Titratable acidity (TA) (%)

Significant variations were found among the five different locations in respect of titratable acidity of the soursop leaves and fruits (Appendix III). The highest percentage of TA was found in soursop fruit (0.99%) collected from Khulna and the lowest value was found in the soursop leaf (0.31%) of Kushtia which was statistically similar to the leaves collected from Jashore (0.34%) (Table 4.3). The range of TA in soursop fruit was 0.73% to 0.99% and in soursop leaf was 0.31% to 0.50%. Terán-Erazo et al. (2022) also reported that the TA of soursop fruit was 0.9-1.4%.

4.2.3 Vitamin C (mg/100g)

It was observed that the differences in vitamin C value of soursop leaves and fruits were significant among the five locations (Appendix III). The highest value of Vit. C was found in the fruit (22.65mg/100g) collected from Bagerhat and the lowest value was estimated in the leaf (18.95mg/100g) of Jashore (Table 4.3). The range of Vit. C content in soursop leaf was 18.95mg/100g to 20.19mg/100g and in soursop fruit was 20.63mg/100g to 22.65mg/100g. Similar result was reported by Badrie and Schauss (2009) and Afzaal et al.

Table 4.3: Proximate chemical composition of soursop leaves and fruits in different locations

Plant parts/ Locations	Total soluble solid (%)	Titratable acidity (%)	Vitamin C (mg/100g)	Carotenoids (mg/g)	Flavonoids (g/100g)	Tannins (mg/100g)	Saponins (mg/g)	Alkaloids (µg/g)	Acetogenins (mg/g)
Khulna									
Leaf	3.38g	0.47f	19.46g	0.69c	0.69d	10.86b	12.36b	28.85b	31.29d
Fruit	13.49d	0.99a	21.69b	0.42e	0.54e	6.78g	5.88d	17.85d	16.49fg
Jashore									
Leaf	3.54f	0.34h	18.95h	0.76a	0.74c	11.08a	11.06c	33.85a	33.53b
Fruit	14.64a	0.81c	20.85c	0.36h	0.36h	7.08f	4.72f	15.35fg	19.56e
Bagerhat									
Leaf	3.31g	0.50e	20.16e	0.72b	0.85a	10.59d	11.09c	35.15a	35.22a
Fruit	13.41d	0.73d	22.65a	0.46e	0.46g	6.06i	6.06d	17.30de	18.90e
Satkhira									
Leaf	3.67e	0.39g	19.96f	0.68c	0.72cd	10.37e	11.50c	28.20bc	32.79c
Fruit	14.26b	0.87b	21.64b	0.39g	0.344h	7.05f	5.26ef	14.30g	16.16g
Kushtia									
Leaf	3.41g	0.31h	19.61g	0.63d	0.83b	10.74c	13.93a	27.40c	34.81a
Fruit	13.71c	0.76d	20.63d	0.35h	0.49f	6.40h	5.85de	16.25ef	16.85f
Level of significance	**	**	**	**	**	**	**	**	**
C.V. (%)	0.86	3.70	0.62	2.72	2.46	0.55	4.66	8.67	1.77

Note: Data are the means of four replications. In a column **= significant at $p \leq 0.01$. CV= Coefficient of variation. Non-significant differences are indicated by similar alphabets in a column.

(2022) that the range of Vit. C in soursop fruit was 20.90 to 29.60mg per 100g. Abara et al. (2017) reported that the Vit. C of soursop leaf was 11.50 to 20.0mg per 100g.

4.2.4 Carotenoids (mg/g)

The carotenoid content of soursop leaves and fruits showed significant differences among the five locations (Appendix III). The highest carotenoid content was found in soursop leaf (0.76mg/g) collected from Jashore and the lowest content was observed in soursop fruit (0.35mg/g) of Kushtia which was statistically similar to the fruits collected from Jashore (0.36mg/g) (Table 4.3). The range of carotenoid content in soursop fruit was found 0.35mg/g to 0.46mg/g and in soursop leaf was 0.64mg/g to 0.76mg/g. Similar result was reported by Santos et al. (2023) that the soursop fruit contained 6.79μg g^{-1} of carotenoid content. Usunomena and Okolie (2015) reported that the beta-carotene of soursop leaf was 6.60mg/100g.

4.2.5 Flavonoids (g/100g)

It was observed that the differences in flavonoid value of soursop leaves and fruits were significant among the five different locations (Appendix III). The highest value of flavonoid was found in the leaf (0.85g/100g) collected from Bagerhat and the lowest value was estimated in the fruit (0.34g/100g) from Satkhira which was statistically similar to the fruits of Jashore (0.36g/100g) (Table 4.3). The range of flavonoid content in soursop leaf was 0.69g/100g to 0.85g/100g and in soursop fruit was 0.34g/100g to 0.54g/100g. Makuasa et al. (2020) also reported that the flavonoid content in soursop leaf was 0.41 to 0.55g per 100g. Guevara et al. (2019) reported that the flavonoid content of soursop fruit was 215mg/100g.

4.2.6 Tannins (mg/100g)

Significant variations were found among the five locations in respect of tannin content of the soursop leaves and fruits (Appendix III). The highest tannin content was found in soursop leaf (11.08mg/100g) collected from Jashore and the lowest value was found in the soursop fruit (6.06mg/100g) collected from Bagerhat (Table 4.3). The range of tannin

content in soursop fruit was 6.06mg/100g to 7.08mg/100g and in soursop leaf was 10.37mg/100g to 11.08mg/100g. Similar result was revealed by Akinseye et al. (2016) and Badrie and Schauss (2009).

4.2.7 Saponins (mg/g)

The saponin content in soursop leaf and fruit showed significant variations among the five locations (Appendix III). The highest value was estimated in soursop leaf (13.93mg/g) collected from Kushtia and the lowest value was found in the fruit (4.72mg/g) of Jashore (Table 4.3). The range of saponin content was found in soursop leaf 11.055mg/g to 13.93mg/g and in fruit was 4.72 mg/g to 6.06 mg/g. Similar result was revealed by Akinseye et al. (2016) (saponin content in leaf- 12.07mg/g) and Mutakin et al. (2022) (saponin content in fruit 4.63mg/g).

4.2.8 Alkaloids (µg/g)

It was observed that the differences in the alkaloid value of soursop leaves and fruits were significant among the five different locations (Appendix III). The highest value of alkaloid was found in the leaf (35.15µg/g) collected from Bagerhat which was statistically similar to the leaf of Jashore (33.85µg/g) and the lowest value was estimated in the fruit (14.30µg/g) of Satkhira (Table 4.3). The range of alkaloid content in soursop leaf was 27.40µg/g to 35.15µg/g and in soursop fruit was 14.30µg/g to 17.85µg/g. Similar result was reported by Setyaningrum et al. (2022) and Aguilar-Hernández et al. (2020a).

4.2.9 Acetogenins (mg/g)

Significant variation was found among the five locations in respect of acetogenin content of the soursop leaves and fruits (Appendix III). The highest amount of acetogenin content was found in soursop leaf (35.22mg/g) collected from Bagerhat which was statistically similar to the leaf of Kushtia (34.81mg/g) and the lowest value was found in the fruit (16.16mg/g) collected from Satkhira (Table 4.3). The range of acetogenin content in soursop fruit was 16.16mg/g to 19.56mg/g and in soursop leaf was 31.29mg/g to 35.22mg/g. Similar result was reported by Aguilar-Hernández et al. (2020b).

4.2.10 Antioxidant activities of soursop leaf and fruit

Table 4.4: Absorbance of blank solution

Blank	Abs1	Abs2	Abs3	Avg. abs
Solution	0.981	0.979	0.975	0.97833

Table 4.5: %RSA and IC_{50} value of ascorbic acid (standard) at different concentration

Conc. (µg/mL)	Abs1	Abs2	Abs3	Avg. abs	%RSA	IC_{50} value
2	0.859	0.863	0.855	0.859	12.198	
4	0.733	0.737	0.729	0.733	25.077	
8	0.611	0.617	0.621	0.616	37.0017	
16	0.488	0.481	0.478	0.482	50.698	17.21
32	0.367	0.373	0.364	0.368	62.385	µg/mL
64	0.242	0.247	0.237	0.242	75.264	
128	0.122	0.117	0.125	0.121	87.598	
256	0.063	0.069	0.068	0.0667	93.186	
512	0.043	0.041	0.042	0.042	95.7069	

Table 4.6: %RSA and IC_{50} value of soursop leaf at Khulna and Jashore

Khulna				Jashore			
Conc. (µg/mL)	Avg. abs	%RSA	IC_{50} value	Conc. (µg/mL)	Avg. abs	%RSA	IC_{50} value
2	0.824	15.775		2	0.817	16.49	
4	0.745	23.85		4	0.736	24.77	
8	0.683	30.187		8	0.678	30.698	
16	0.571	41.635	35.38	16	0.566	42.147	33.36
32	0.511	47.768	µg/mL	32	0.505	48.381	µg/mL
64	0.388	60.340		64	0.378	61.363	
128	0.302	69.131		128	0.311	68.211	
256	0.288	70.562		256	0.276	71.789	
512	0.199	79.659		512	0.189	80.681	

Table 4.7: %RSA and IC_{50} value of soursop leaf at Bagerhat and Satkhira

Bagerhat				Satkhira			
Conc. (µg/mL)	**Avg. abs**	**%RSA**	**IC_{50} value**	**Conc. (µg/mL)**	**Avg. abs**	**%RSA**	**IC_{50} value**
2	0.821	16.082		2	0.819	16.286	
4	0.781	20.170		4	0.793	18.944	
8	0.695	28.960		8	0.702	28.245	
16	0.577	41.022	43.44	16	0.592	39.489	46.70
32	0.531	45.724	µg/mL	32	0.544	44.395	µg/mL
64	0.426	56.457		64	0.445	54.514	
128	0.373	61.874		128	0.361	63.101	
256	0.287	70.665		256	0.299	69.438	
512	0.211	78.433		512	0.214	78.126	

Table 4.8: %RSA and IC_{50} value of soursop leaf at Kushtia

Kushtia			
Conc. (µg/mL)	**Avg. abs**	**%RSA**	**IC_{50} value**
2	0.811	17.104	
4	0.771	21.193	
8	0.693	29.165	
16	0.585	40.204	
32	0.533	45.519	41.9 µg/mL
64	0.414	57.683	
128	0.357	63.509	
256	0.293	70.051	
512	0.207	78.841	

IC_{50} value denotes the concentration standard of inhibition. Low IC_{50} value indicates the highest antioxidant activity whereas the higher the IC_{50} value the lower the antioxidant activity.

Here, we can see that the IC_{50} value significantly varied among the five study locations in respect of leaf analyses. The highest IC_{50} value (46.70µg/mL) (Table 4.6) of soursop leaf

was recorded in the samples collected from Satkhira and the lowest value (33.36µg/mL) (Table 4.7) from Jashore. So, the leaves collected from Jashore showed the highest antioxidant activity and lowest antioxidant content was observed in the leaf of Satkhira.

Table 4.9: %RSA and IC_{50} value of soursop fruit at Khulna and Jashore

Khulna				Jashore			
Conc. (µg/mL)	**Avg. abs**	**%RSA**	**IC_{50} value**	**Conc. (µg/mL)**	**Avg. abs**	**%RSA**	**IC_{50} value**
2	0.826	15.570	68.71 µg/mL	2	0.829	15.264	75.05 µg/mL
4	0.751	23.237		4	0.783	19.966	
8	0.719	26.507		8	0.709	27.529	
16	0.662	32.334		16	0.654	33.151	
32	0.563	42.453		32	0.589	39.795	
64	0.546	44.191		64	0.552	43.577	
128	0.437	55.332		128	0.449	54.105	
256	0.316	67.700		256	0.325	66.780	
512	0.246	74.855		512	0.255	73.935	

Table 4.10: %RSA and IC_{50} value of Soursop fruit at Bagerhat and Satkhira

Bagerhat				Satkhira			
Conc. (µg/mL)	**Avg. abs**	**%RSA**	**IC_{50} value**	**Conc. (µg/mL)**	**Avg. abs**	**%RSA**	**IC_{50} value**
2	0.845	13.628	76.85 µg/mL	2	0.866	11.482	86.46 µg/mL
4	0.759	22.419		4	0.785	19.762	
8	0.722	26.201		8	0.725	25.894	
16	0.643	34.276		16	0.615	37.138	
32	0.574	41.329		32	0.598	38.876	
64	0.561	42.657		64	0.543	44.497	
128	0.451	53.901		128	0.451	53.901	
256	0.329	66.371		256	0.383	60.852	
512	0.274	71.993		512	0.292	70.153	

Table 4.11: %RSA and IC_{50} value of soursop fruit at Kushtia

Kushtia			
Conc. (µg/mL)	**Avg. abs**	**%RSA**	**IC_{50} value**
2	0.857	12.402	81.34 µg/mL
4	0.773	20.988	
8	0.712	27.223	
16	0.611	37.547	
32	0.584	40.306	
64	0.551	43.679	
128	0.443	54.719	
256	0.377	61.465	
512	0.285	70.869	

In soursop fruit, the highest IC_{50} value (86.46µg/mL) (Table 4.10) was found in the samples collected from Satkhira and the lowest value was observed from Khulna (68.71µg/mL) (Table 4.9). That means soursop fruit of Khulna showed the highest antioxidant activity and the lowest antioxidant activity was found in the fruits of Satkhira.

So, we can reveal that the soursop leaf showed the lowest IC_{50} value as well as highest antioxidant activity which was observed in the samples of Jashore (33.36µg/mL) (Table 4.7). On the other hand, the soursop fruit showed the highest IC_{50} value as well as lowest antioxidant activity which was observed in the fruits collected from Satkhira (86.46µg/mL) (Table 4.10). Similar results were found by Orak et al. (2019), Qorina et al. (2019) and Widyastuti et al. (2017).

CHAPTER V

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

A study on the physical characteristics and antioxidant activities of soursop (*Annona muricata* L.) leaf and fruit collected from five different locations were carried out during the period from May to June, 2024. The study locations were selected from the south western region of Bangladesh. The collected soursop leaves and fruits were brought to the Horticulture Laboratory of Agrotechnology discipline of Khulna University, Khulna and kept in ambient temperature to determine the physical characteristics and antioxidant activity.

The study was laid out in a completely randomized design (CRD) with four replications. Five study areas were selected for the collection of the experimental materials for this investigation. Data were recorded and statistically analysed to study the variabilities among these selected study areas.

The highest weight (1.35mg), length (133.68cm) and breadth (6.05cm) of soursop leaf were observed from study area-Khulna and the lowest value of weight (1.02mg), length (10.69cm) and breadth (5.02cm) were found in the leaf collected from Kushtia. The average leaf weight, length and breadth were found 1.16mg, 12.30cm and 5.44cm respectively.

The maximum weight (626g), length (15.39cm), breadth (14.43cm), weight of edible portion (474.25g), weight of non-edible portion (151.75g), percentage of edible portion (75.7%), total number of seeds per fruit (51.5) and total seed weight (12.54g) of soursop fruit were also observed in the samples of Khulna and the highest percentage of non-edible portion (30.38%) was found in the samples of Kushtia. The minimum value of weight (377.31g), length (10.45g), breadth (8.75cm), weight of edible portion (262.77g), weight of non-edible portion (114.54g), total number of seeds per fruit (31.75) and total seed weight (9.01g) were found in the fruits collected from Kushtia. Again, the lowest percentage of edible portion (69.56%) in the sample of Satkhira and lowest percentage of non-edible portion (24.3%) from the sample of Khulna were found. The average fruit weight- 489.53g, length- 13.18cm, breadth- 11.86cm, weight of edible portion- 55.79g,

weight of non-edible portion- 13.73g, percentage of edible portion- 72.26%, percentage of non-edible portion- 27.74%, total number of seeds per fruit- 41.4 and total seed weight- 10.75g.

The maximum value of TSS (14.64%) was found in the soursop fruit collected from Jashore and the minimum TSS (3.31%) was found in the soursop leaf of Bagerhat.

The highest TA (0.99%) was found in the fruit of Khulna and lowest TA (0.31%) was found in the leaf of Kushtia.

Maximum value of Vit. C (22.65mg/100g) was found in the fruit, the maximum flavonoid (0.85g/100g) and alkaloids (35.15μg/g) were observed in the leaf collected from Bagerhat. And the minimum value of Vit. C (18.95mg/100g) was found in the leaf of Jashore, minimum flavonoid (0.34g/100g) and alkaloids (14.30mg/μg) were observed in the fruit of Satkhira.

Highest value of carotenoids (0.76mg/g) and tannins (11.08mg/g) were found in the leaf collected from Jashore. On the other hand, the lowest value of carotenoids (0.35mg/g) from Kushtia and tannins (6.06mg/g) from Bagerhat were observed in the fruit.

Maximum Saponin (13.93mg/g) was found in the samples of Kushtia and acetogenin (35.22mg/g) content from Bagerhat were found in the leaf. And the minimum content of saponins (4.72mg/g) from Jashore and acetogenins (16.16mg/g) from Satkhira were observed in the fruit.

Soursop leaf showed the lowest IC50 value as well as highest antioxidant activity which was observed from the samples of Jashore (33.36μg/mL). On the other hand, the soursop fruit showed the highest IC_{50} value as well as lowest antioxidant activity which was observed from the fruits of Satkhira (86.46μg/mL).

5.2 Conclusion

1. It could be concluded from the results of the present study that the physical properties of both soursop leaf and fruit were higher in the samples collected from Khulna and the lowest performance was showed by the samples of Kushtia.
2. By considering the chemical characteristics, superiority was showed by the soursop leaf in respect of carotenoids, flavonoids, tannins, saponins, alkaloids, acetogenins and

antioxidant activity than the soursop fruit. And the soursop fruit showed superiority in respect of TA, TSS and Vit C.

5.3 Recommendation

As soursop contains a high nutritive value, therefore further experiment is recommended to find out more nutritional as well as chemical properties for the betterment of human health.

CHAPTER VI

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CHAPTER VII

APPENDICES

Appendix I

Analysis of variance of the data in respect of physical characteristic of soursop leaves at different locations

Source of Variation	Degrees of Freedom	Mean square		
		Leaf weight (mg)	Leaf length (cm)	Leaf breadth (cm)
Study Area	4	0.06799**	5.25469**	0.59734**
Error	15	0.00095	0.02558	0.01571
Total	19	–	–	–

** Significant at 1% level of probability.

Appendix II

Analysis of variance of the data in respect of physical characteristic of soursop fruits at different locations

Source of Variation	Degrees of Freedom	Mean Squares								
		Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Weight of edible portion (g)	Weight of non-edible portion (g)	Percentage of edible portion (%)	Percentage of non-edible portion (%)	Total no. of seeds per fruit	Weight of seeds per fruit
Study Area	4	35629.4**	14.0792**	18.663**	26717.7**	704.793**	29.2219**	29.2219**	225.325**	7.68074**
Error	15	796.9	0.1558	0.2103	632.2	50.038	2.2081	2.2081	3.967	0.15719
Total	19	–	–	–	–	–	–	–	–	–

** Significant at 1% level of probability.

Appendix III

Analysis of variance of the data in respect of chemical characteristic of soursop leaves & fruits at different locations

Source of Variation	Degrees of Freedom	Mean Squares								
		Total soluble solid (%)	Titratable acidity (%)	Vitamin C (mg/100g)	Carotenoids (mg/100g)	Flavonoids (g\100g)	Tannins (mg/100g)	Saponins (mg/100g)	Alkaloids (mg/100g)	Acetogenins (mg/100g)
Study Area	4	0.85**	0.04306**	2.8011**	0.00986**	0.02867**	0.630**	4.696**	33.03**	14.50**
Plant part	1	1090.56**	1.79734**	34.7450**	0.91113**	1.07158**	164.254**	413.775**	2096.70**	2539.86**
Interac-tion	27	0.35**	0.02737**	0.6363**	0.00648**	0.01791**	0.424**	2.363**	25.53**	4.93**
Error	27	0.01	0.00052	0.0162	0.00022	0.00022	0.002	0.167	0.96	0.20
Total	39	–	–	–	–	–	–	–	–	–

** Significant at 1% level of probability.