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Proximate and biological activity studies of Syzygium samarangense

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ABSTRACT

Red and green varieties of Syzygium samarangense, commonly known as Jamrul, are very popular as seasonal fruits to all ages of people in Bangladesh. Objective of the work was chemical and biological activity studies of S. samarangense red and green varieties grown in Bangladesh. Air-dried fruits of S. samarangense were successively extracted with n-hexane, dichloromethane (DCM) and methanol. The moisture and ash content of the raw fruits were determined by thermal heating at 105 and 700°C, respectively. Different biologically important functional groups were identified by FT-IR spectroscopy. The n-hexane, DCM and methanolic extracts were tested for cytotoxicity against HeLa and Vero cell lines. Total phenolic content, total flavonoid content and total antioxidant capacity in the three extracts of both varieties were determined. The moisture content and ash content were 92% and 90%, and 0.12% and 0.16% in red variety and green variety, respectively. UV-Vis spectrum revealed the presence of long chain conjugation or polycyclic aromatic chromophores in n-hexane, DCM and methanolic extracts of S. samarangense. The DCM extract of both varieties had highest total phenolic content, flavonoid content and antioxidant capacity compared to n-hexane and methanol extracts. The study concludes that the fruit of S. samarangense (red and green variety) grown in Bangladesh are rich source of phytochemicals which possess antioxidant activity and safe to health for their non-cytotoxic properties. The green variety contains more phenolic content and antioxidant capacity than red variety.

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1. Introduction

Syzygium samarangense (Blume) Merr. and L.M. Perry, a member of Myrtaceae family is commonly known as wax apple and it is called Jamrul in Bangladesh. This extra-tropical tree mainly grows across Cambodia, India, Laos, Malaya, Thailand and Vietnam (Morton, 1987). The trees are generally 5-15 m long and 25-30 cm thick and produces pear-shaped fruit, which is 3.5-4 cm long and 4.5-5.4 cm wide with 4 fleshy calvx and 0-2 seeds (Morton, 1987). The fruits are edible and even can be eaten raw. Different parts of S. samarangense have been reported to have potential medicinal values. Several cytotoxic and antioxidant compounds were isolated from the methanolic extract of the pulp and seeds of S. earlier (Resurreccion-Magno et al., 2005). S. samarangense samarangense fruit can be helpful for type II diabetes patients as aqueous extract of it increases glucose uptake in TNF-α-treated FL83B cells and also increases glycogen storage (Shen et al., 2013). Another study reported that hexane extract of this plant leaves have smooth muscle relaxant activity because it can barricade calcium influx. Thus, it can show antidiarrheal activity (Ghavur et al., 2006). Moreover, the ethanolic extract of S. samarangense leaves possess antibacterial activity (Choironi and Fareza, 2018), the methanolic extract of Syzygium samarangense bark have CNS depressant activity, analgesic activity and moderate antiinflammatory properties as well (Mollika et al., 2013). Both red and green varieties of S. samarangense are seasonal fruits and are e-ISSN 2686-1623/© 2021 Institut Teknologi Bandung. All rights reserved.

naturally available in summer in Bangladesh. They are popular to all kind of people from young to old. But many do not know about the medicinal values as there is not much study. Therefore, the present study was designed for the investigation of proximate and biological activities of two varieties of *S. samarangense*.

2. Materials and methods

2.1. Collection of fruit materials

The fresh *S. samarangense* fruits were collected from the local market in Dhaka city. The fresh fruits were washed with water and the excess water was wiped out, then chopped into small pieces of nearly same diameter and finally the chopped fruits were dried in the open air.

2.2. Chemicals and reagents

All solvents, reagents used during the research work were obtained from E. Merck (Germany). Rotary vacuum evaporator (Heidolph, Germany) was used to remove organic solvents from the extracts. For freeze drying, a freeze-drier (LABCONCO, USA) was used.

2.3. Extraction

Dry fruit samples (red variety: 130.0 g and green variety: 120.0 g) were extracted with n-hexane by maceration method for three days. Then, the content of the conical flask was filtered and the

filtrate was collected and evaporated at reduced pressure with previous collection to get hexane extract (red variety: 0.90 g and green variety: 0.80 g). Similar way, the residue was successively extracted with dichloromethane (DCM) and methanol, and DCM (red variety: 1.1 g and green variety: 1.0 g) and methanol (red variety: 25.0 g and green variety: 14.2 g) extracts were obtained.

2.4. Moisture and ash contents

The washed fruits were chopped finely and the exact amount was taken into pre-dried porcelain crucibles. Both red and green varieties of fruit samples were separately taken into three crucibles. The crucibles, containing samples were heated at 105 °C in a muffle furnace (CARBOLITE – GSM 11/8). After heating, the residue was cooled in a desiccator and the weight of the residue was measured in an analytical balance and the percentage of moisture was calculated for each crucible and the mean value was determined for both red and green varieties of *S. samarangense* separately using equation (1). The residues of the moisture content in the same porcelain crucibles were further heated for another 4 h at 700 °C in a muffle furnace. After heating, the residue was cooled and weighted for the determination of ash content. The ash contents were calculated using equation (2) (Park, 1996).

%Moisture =
$$\frac{\text{Loss of weight}}{\text{Weight of the sample}} \times 100 \dots \dots (1)$$

%Ash = $\frac{\text{Weight of the residue}}{\text{Weight of the sample}} \times 100 \dots \dots (2)$

2.5. FT-IR experiment of different crude extracts

Different extracts were thoroughly mixed with powdered potassium bromide separately and made into pellets under high pressure. The prepared KBr pellets were inserted into the FT-IR spectrophotometer (Shimadzu FT-IR 4800S) and experiments were recorded.

2.6. UV-Vis experiment of different crude extracts

The dried n-hexane, DCM and methanolic extracts were dissolved in respective solvents. The values of λ_{max} and corresponding absorbances were measured by a double beam UV-Vis spectrophotometer (SHIMADZU UV – 1800).

2.7. Cytotoxicity

Three extracts of both red and green varieties were subjected to cytotoxicity assay against HeLa (a human cervical carcinoma cell line) and Vero (a kidney epithelial cells extracted from an African green monkey) cell lines. The extracts were dissolved in 2.5% DMSO (extract concentration: 2.5 mg/ml) separately. Both cell lines were cultured in DMEM (Dulbecco's Modified Eagles' Medium) which contains 0.2% gentamycin, 1% penicillin-streptomycin (1:1) and 10% FBS (Fetal Bovine Serum). HeLa cells ($4 \times 104 / 200 \mu$ l) and Vero cells ($4 \times 104 / 200 \mu$ l) were seeded onto 48-well plate. The incubation was done at 37°C and 5% CO2. To each well 25 μ l of filtered sample was added on the following day. After 48 h of incubation the cytotoxicity was investigated under an inverted light microscope (Nesa et al., 2021; Patel et al., 2009; Saroar et al., 2020).

2.8. Total phenolic content

Total phenolic content was determined by Folin-Ciocalteau method (Alhakmani et al., 2013). 0.5 ml (1 mg/ml) methanolic solution of n-hexane, DCM, and methanolic extracts were taken into separate test tubes. 5 ml Folin-Ciocalteu (1:10 v/v distilled water) was added to all of those solution followed by 4 ml Na₂CO₃ solution.

Solutions were vortexed and incubated for 30 min. After 30 min of incubation the absorbance at 765 nm was measured against a blank with a double beam UV-Vis spectrophotometer. Standard solutions of gallic acid of concentration 6.25, 12.5, 25, 50, 100, 200 and 400 μ g/ml were prepared by the same process using gallic acid instead of extracts. A calibration curve (y = 0.0062x + 0.0825, R² = 0.9989) was drawn from the plot of the absorbance (at 765 nm) versus concentration. Then the total phenolic content was determined from the calibration curve and expressed as mg GAE/g of dry extract.

2.9. Total flavonoid content

The total flavonoid content was determined by aluminum chloride colorimetric method (Bhaigyabati et al., 2014). The stock solution was prepared by dissolving 20 mg of each extract: n-hexane, DCM and methanolic, in 20 ml methanol. To the 5 ml of the stock solution 2.5 ml of AlCl3 reagent (2% AlCl3 + 1M CH₃COONa) was added. The mixtures were incubated for 30 min and the absorbance was measured at 430 nm in a double beam UV-Vis spectrophotometer against a blank. A calibration curve (y = 0.0071x - 0.0003, R² = 0.9948) was prepared with standard quercetin solution (2, 5, 10, 20, 40, 50 and 100 μ g/ml) prepared by the same process as samples. The result was expressed in mg QE/g of dry extract.

2.10. Total antioxidant capacity

Total Antioxidant capacity assay was carried out by the formation of phosphomolybdenum complex (Prieto et al., 1999). To 0.3 ml of each extract solution (1 mg/ml in methanol) 3 ml of reagent was added. The reagent contains mixture of 0.6 M H₂SO₄, 28 mM Na₃PO₄ and 4 mM ammonium molybdate in 4:2:4 ratio. The mixtures were incubated for 90 min at 95°C. After incubation the absorbance was measured in a double beam UV-Vis spectrophotometer against a blank (containing same amount of solvent instead of extract). A calibration curve (y = 0.0074x – 0.649, R² = 0.9837) was drawn with standard ascorbic acid solution (5, 10, 20, 40, 50 and 100 μ g/ml) prepared by same process as sample. Total antioxidant capacity was expressed in mg ascorbic acid equivalent per gram of dry sample.

3. Results and discussion

3.1. Moisture and ash contents

Water in food is present both in free and bound form. It regulates the quality, stability, texture and freshness of a food. A small change in water content in foodstuff may have a significant effect on the stability of that product (Park, 1996). The water/moisture content in the experimental fruit is 92 and 90% for red and green variety, respectively. Another study of two water apple (*S. samaragense*) cultivars reported the moisture content in the range 90-92% (Rosnah et al., 2012). Ash content in a foodstuff represents the amount of minerals present there (Liu, 2019). Ash content of a food is important for its nutritional value, quality, processing and microbial stability (Sonkamble and Pandhure, 2017). The ash content of the research fruit for red and green variety is 0.12 and 0.16%, respectively

3.2. FT-IR experiment

The FT-IR spectrum of methanolic extract of both varieties includes -OH, C=O and C-O stretching peaks which indicate the presence of phenolic, alcoholic and carbonyl type compounds where aromatic ring is found between 1600 and 1400 cm⁻¹ and DCM extract also includes -OH, C=O, C-O and C-H stretching peaks which indicate the presence of phenolic, ketonic and alcoholic compounds. The n-hexane extract shows N-H, C-N and C=O

stretching peaks which infer the presence of amine and carbonyl type compounds. Spectrum of all three extract contains C=C stretching vibration which may be either conjugated with C=O or aromatic. Furthermore, the sp3 C-H stretching, -CH2- and -CH3

bending peaks suggest the presence of hydrocarbon chain (Pavia et al., 2015). Observed vibrational frequencies and corresponding groups are represented in Table 1.

	Table 1. FT-I	R absorption	frequency a	and peak	assignment
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n-hexane extract (red variety)		DCM extract			MeOH extract			
Frequency (cm ⁻¹)	Inferred group	Frequency (cm ⁻¹)		T. C	Frequency (cm ⁻¹)		T. C 1	
		Red variety	Green variety	 Inferred group 	Red variety	Green variety	Inferred group	
3435.04(st)	-OH	3430.75(st)	3433.61(st)	-OH	3379.27(st)	3373.55(st)	-OH	
2920.21(st)	sp ³ C–H	2924.50(st)	2925.93(st)	sp ³ C-H	2931.65(st)	2931.65(st)	sp ³ C–H	
2851.57(st)	sp ³ C–H	2854.43(st)	2854.43(st)	sp ³ C-H	1736.11(st)	1737.54(st)	С=О	
1706.08(st)	Č=O	1710.37(st)	1721.81(st)	Č=O	1638.87(st)	1633.15(st)	C=C	
1633.15(st)	C=C	1633.15(st)	1624.56(st)	C=C		1405.76(b)	$-CH_2-$	
1564.50(b)	N-H	1460.11(b)	1461.54(b)	$-CH_2-$		1231.29(st)	C-O	
1465.83(b)	$-CH_2-$		1382.88(b)	$-CH_3$		1078.28(st)	C-O	
1378.59(b)	$-CH_3$		1073.99(st)	C-0				
1169.80(b)	C-N		942.42(oop)	C-H				
			863.77(oop)	C-H				
			767.95(oop)	C-H				

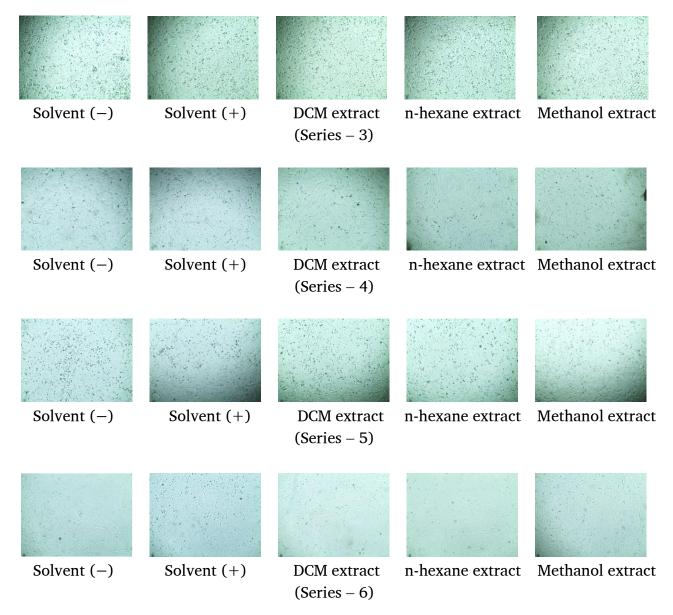


Fig 1. Cytotoxic assay of different extracts of *S. samarangense* against HeLa cell line {series -3 (red variety) and series -5 (green variety)} and Vero cell line {series -4 (red variety) and series -6 (green variety)}

3.3. UV experiments

The UV-visible spectral data of hexane, DCM and methanol extracts were observed at 250, 301, 326 and 247, 293 and 315 nm for red and green variety respectively. These indicated the presence of conjugated dienes and chromophores with $n \rightarrow \pi^*$ and $\pi \rightarrow \pi$ transition (Pavia et al., 2015).

3.4. Cytotoxicity

The n-hexane, DCM and methanolic extracts of both varieties were dissolved in 2.5% DMSO solvent and tested for cytotoxicity against HeLa and Vero cell lines. None of the samples showed cytotoxic activity (Fig. 1).

3.5. Total phenolic content

One or more hydroxyl group substituted aromatic rings are called phenolics and they are secondary metabolites produced by

plants (Dai and Mumper, 2010). Phenolics have antioxidant properties as they can absorb free radicals and also can quench reactive oxygen species (Zheng and Wang, 2001). The total phenolic contents of hexane, DCM and MeOH extracts in red and varieties were 46.32±0.2, 87.77±0.09, 49.59±0.16, green 53.93±0.02, 87.77±0.09, 57.32±0.02 and 119.58±0.02 mg GAE/g, respectively (Table 2). The total phenolic content was found greater in DCM extract of both variety than n-hexane and methanolic extracts (Table. 2, Fig. 2). The data revealed the difference of TPC between red and green variety where hexane, DCM and MeOH extracts of green variety contained more phenolic content than that of red. DCM extract of green and red showed phenolic content around 119 and 87 GAE/g of dry extract, respectively. However, the lowest amount of phenolic content was found in hexane extract of red variety with 46 GAE/g.

Table 2. Total phenolic content in different extracts of S. samarangense

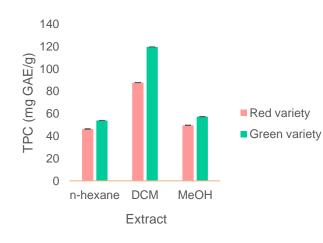
	n-hexane extract		DC	M extract	MeOH extract		
Parameter	Red variety	Green variety					
TPC (mg GAE/g) 46.32±0.24 53.93±0.02 87.77±0.09 119.58±0.02 49.59±0.16 57.32±0.02							
Note: TPC = total phenolic content, $GAE =$ gallic acid equivalent. The result (n=3) is expressed as (mean±SD),							

Table 3. Total flavonoid content in different extracts of S. samarangense

Parameter	n-hexane extract		DCM extract		MeOH extract		
Red variety Green variety Red variety Green variety Green variety Green variety Green							
TFC (mg QE/g) 54.92±0.08 39.24±0.01 131.31±0.14 129.78±0.01 63.75±0.08 96.27±0.01							
Note: TFC = total flavonoid content, QE = quercetin equivalent. The result is expressed as (mean±SD)							

Table 4. 'Total antioxidant capacity' of different extracts of S. samarangense

Parameter	n-hexane extract		DCM extract		MeOH extract	
Parameter	Red variety	Green variety				
TAC (mg AAE/g) 21.79±0.20 52.91±0.20 67.55±0.14 136.65±0.16 32.46±0.08 116.02						
Note: TAC = total antioxidant capacity, AAE = ascorbic acid equivalent. The result is expressed as (mean±SD)						



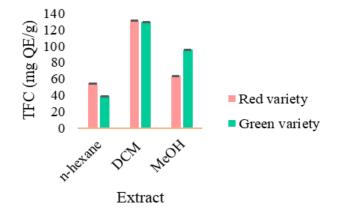


Fig 2. TPC of S. samarangense

Fig 3. TFC of S. samarangense

3.6. Total flavonoid content

Flavonoids are one kind of plant secondary metabolite and they posse's polyphenolic structure. They are important for their anti-inflammatory, anti-mutagenic anti-oxidant. and anticarcinogenic properties (Panche et al., 2016). The total flavonoid content was found greater in DCM extract of both variety than nhexane and methanolic extracts (Table 3, Fig. 3). Flavonoids are one kind of plant secondary metabolite and posse's polyphenolic structure. They are important for their anti-oxidant, antiinflammatory, anti-mutagenic and anti-carcinogenic properties (Panche et al., 2016). Kuo et al. (2004) reported the isolation of sixteen flavonoids from the acetone extract of the leaves of S. samarangense and these compounds were evaluated for immunopharmacological activity in a dose dependent manner. Red variety of both hexane and DCM extracts contained higher amount of flavonoid content with 54 and 131 QE/g, respectively than their green variety. The green variety of MeOH extract contained more flavonoid content than red variety.

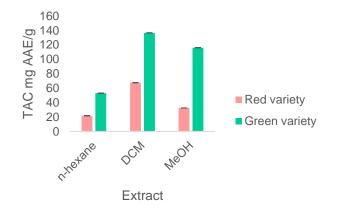


Fig 4. Total antioxidant capacity (TAC) of S. samarangense

3.7. Total antioxidant capacity

Antioxidants provide cells guard against oxidative damage (Kasangana et al., 2015). The total antioxidant capacity assay by phosphomolybdenum method using ascorbic acid as a standard showed greater value for DCM extract of both varieties than other two extracts respectively and the extracts of green variety showed more total antioxidant capacity than red variety (Table 4, Fig. 4). Natural phenolic and flavonoids are important antioxidant compounds which have the ability to deactivate free radicals by donating protons or transferring electrons. Many studies have found that these compounds play a vital role against the action of free radicals with potent antioxidant activity are related to reduce the risk of cancer, cardiovascular diseases, diabetes and neurodegenerative diseases (Aryal et al., 2019; Adebooye et al., 2008). The data shows that the green variety of extracts contained more phenolic content and antioxidant capacity than red variety. Thus, the green variety may be a good source of antioxidant compounds.

4. Conclusion

The FT-IR studies of different extracts have shown the presence of different functional groups which infer the presence of wide range of organic compounds in all those extracts. The UV-Visible spectrum showed the presence of highly conjugated and polycyclic aromatic system. The DCM extract of the fruit of both varieties has shown higher phenolic, flavonoid content and antioxidant capacity than hexane and methanol extracts. Green variety showed greater antioxidant capacity, containing higher number of phenolic compounds. Further studies can be carried out for the isolation of the specific compounds which are significant for their different biological activity.

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Conflict of interest

The authors declare that there is no conflict of interest.

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