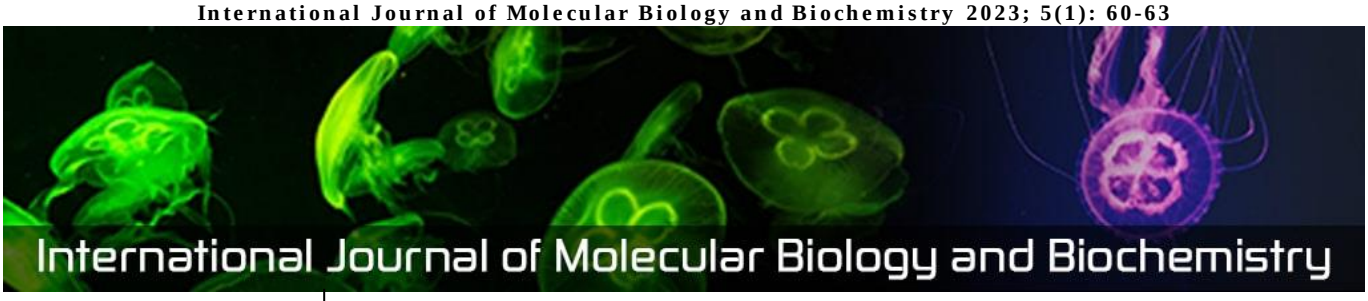

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Assessment of phytochemicals and antioxidant activity of custard apple fruit pulp (*Annona squamosa* Linn.)

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Abstract
Annona squamosa Linn. (Custard apple) has been used drastically in India traditional remedy for treatment of dysentery, cardiac problems, fainting, worm infections, constipation, hemorrhage, dysuria, fever. This study assessed the phytochemicals present in the fruit pulp of A. squamosa. The phytochemical was extracted one by one with distilled water and 96% ethanol. A wide variety of pharmacologically energetic compounds such as alkaloids, coumarins, tannins, cardiac glycosides, flavonoids, carbohydrates, phenols, and saponins were found to present in the fruit pulp of A. squamosa. However, terpenoids and phlobatannins had been absent in this plant. This study additionally assessed the contents of phenolics and flavonoids for their *in vitro* antioxidant activity. The complete polyphenol content material of ethanol extract of A. squamosa measured with the aid of Folin-Ciocalteau reagent in terms of gallic acid equal completed 238.68±4.13 mg GAE/g. The flavonoid content material of the plant pattern as quercetin equivalent executed 84,75±0.82 mg QE/g. The antioxidant undertaking of the ethanol extract of A. squamosa was correlated with complete phenolic and flavonoid content material with values IC50 of 134.761±1.83 µg/ml, 62.63±0.43 µg/ml for DPPH and ABTS scavenging activity, respectively.

Keywords: Phytochemicals, antioxidant, DPPH, ABTS, scavenging

1. Introduction
Nowadays, natural merchandise end up greater and extra popular in many topics of scientific researches due to their chemical composition, following with two most important reasons: functions as natural preferences for meals ingredients and huge influences on human fitness primarily based on their antioxidant characteristics. Moreover, scientific plant life play a critical function in the health protection and care global [1-7]. *Annona squamosa* L. (Sugar apple, custard apple, sitaphal) belongs to the Annonaceae family including about 135 genera and 2300 species. Annona. squamosa is an evergreen plant on the whole located in tropical and subtropical areas such as Bangladeshi, India, Pakistan, and Srilanka. In latest years, cultivating *Annona squamosa* L. have been receiving a excellent deal of public attentiondue to the imperative oil extracted from its flowers and fruit pulp. The previous phytochemical investigations made on the plant have proven that they possess a wide range of compounds like diterpenes (DITs), alkaloids (ALKs), and cyclopeptides (CPs) [1-2]. Numerous find out about tasks on A. squamosa have observed that it has antioxidant, antiparasitic, insecticidal, and so on. [7]. Moreover, A. squamosa peel extract has been described to have larvicidal, acaricidal, insecticidal activity, and it has also used for biosynthesis of silver nanoparticles palladium [8] Previous studies have demonstrated the function of the aqueous pulp extract of A. squamosa to ameliorate hyperthyroidism [8]. In natural, there are three foremost kinds of plant chemical substances along with alkaloids, terpenoids, and phenolic metabolites. Among these three groups, phenolic compounds play a essential function in dietary applications and appreciably researched. Discovering new and safe antioxidants from herbal sources become a amazing hobby for functions in useful foods. Antioxidants play an essential position in the human safety body against free radical disorders acting as radical scavengers. Phenolics belongs to a type of chemical compounds including simple phenols and polyphenols. Polyphenols can limit and forestall damage to the human body due to free radicals promote. Flavonoids can produce mechanisms that may additionally inhibit invasion and kill tumor cells.

The present learn about used to be carried out to evaluate phytochemical screening, whole polyphenol, flavonoids content, and antioxidant recreation on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-and-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) of the extract of A. squamosa.

2. Materials and Methods

2.1 Sample collection and preparation

Annona squamosa L. seeds were accrued from Chapai Nawabganj district, Rajshahi city, Bangladeshi in July 2022. First, the seeds have been removed. They then have been washed with water and stored on absorbent paper towels at room temperature to dry to moisture contents of 10% and flooring to powder.

2.2 Qualiative Phytochemical Analysis

About 25g dried powder of the fruit pulp used to be extracted with one-of-a-kind solvents of growing polarities: ethanol 96% and water. Plant extracts hexane, ethyl acetate, methanol, and methanol water have been subjected to chemical checks for the presence of sterols, triterpenoids, carotenoids, tropolone, quinones, alkaloids, and flavonoids [9-12].

2.3 Quantitative Phytochemical Analysis

2.3.1 Determination of total phenolic content (TPC)

First, the 1mL extract used to be pipetted into a check tube containing 1 mL Folin-Ciocalteu reagent 10% (v/v). After 5 minutes, 1 mL Na_2CO_3 20% (w/v) used to be delivered to the sample.

Next, the combination used to be vigorously shaken and incubated for 30 minutes in the dark. Finally, the absorbance used to be spectrophotometric ally measured at 765 nm, and the results were shown in mg of gallic acid equivalents per gram of pattern (mg GAE/g) [13-15].

2.3.2 Determination of total Flavonoid content (TFC)

Based on the aluminum chloride colorimetric method, the complete flavonoid content material was once determined [13-15]. Mixing 0.5 mL the extract with 0.15 mL 5% NaNO2. After 5 minutes, mixing with 0.3 mL 10% AlCl3. Then, 1mL 1M NaOH and 2 mL distilled water was once as soon as delivered and vigorously shaken. The absorbance used to be as soon as spectrophotometric ally measured at 510 nm.

2.3.3 Determination of antioxidant capacity DPPH

The antioxidant undertaking of the character necessary oil was tested the use of 1,1-diphenyl-2- picrylhydrazyl (DPPH) assay (Analytical chemistry laboratory - University Nguyen Tat Thanh). 600 µL DPPH (OD 517 nm = 0.0403 ± 0.013) into 500 µL solution sample. The sample answer with pre-concentration and the blended the stable at room temperature in the dark within 37 min. The optical dimension of the combination with the aid of UV/VIS - 1800 Shimadzu Spectrometer at 517 nm. Blank sample, but 500 µL solution replaced EtOH 99.7%. Standard sample: Vitamin C (0.1g ÷ 0.01) was dissolved EtOH 99.7% into volume flask 100mL, in the dark (C = 100 µL/mL). The percent DPPH scavenging effect used to be calculated by the usage of the following equation [16-18].

DPPH scavenging effect (%)/% Inhibition=A0-A1/A0 × 100

Where A0= The absorbance of control and A1= The absorbance of sample.

ABTS

Based on Thaipong and Kamonwannasit, ABTS scavenging pastime used to be used [14-16]. First, adding 10 mL of 2.6 mM K2S2O8 in 10 mL of 7.4 mM ABTS answer in 15 hours. Next, making ready the working solutions through placing 1ml of stock answer into 60 mL of methanol to take the absorbance fee of 1.1 ± 0.02 at 734 nm. Then, 0.5 mL of pattern was brought with 1.5 mL of the working answer for 30 minutes RT. Using UV-VIS spectrophotometer measured the combination at 734 nm. The share of ABTS decolorization of the sample was once determined in accordance to the equation:

% Decolorization = [1-(ABS sample /ABS control] x100

3. Results

3.1 Qualitative phytochemical analysis and percentage yields

Table 1 indicates the give up end result of phytochemical parts of A. squamosa fruit pulp in water and 96% ethanol. Evaluation of chemical factors of A. squamosa leaves printed the presence of alkaloids, coumarin, tannin, cardiac glycosides, flavonoids, carbohydrates, phenols. The previous examine about demonstrates the phyto chemistry of *Annona squamosa* fruit pulp including alkaloids, flavonoids, phenols, saponins, glycosides in water, methanol, chloroform, and petroleum ether extracts [19]. Moreover, the previous analyze about tested the phytochemical studies of the unique extracts solution, along with water, methanol, chloroform, petroleum ether, and hexane. The methanol and water extracts of seed and fruit pulp had more high exceptional outcomes for alkaloids, oils, tannins, phenols, and flavonoids [20]. The thin layer chromatography scanning of the *Annona squamosa* performed by using skill of Jayshree *et al*, and the literature survey verified that chief phyto constituent of this plant is anonaine and some biological compounds (Linalool, Borneol, Eugenol, Farnesol, and Geraniol) [21].

Table 1: Phytochemical constituents of A. squamosa fruit pulp in different solvent extracts

No	Compound	Fruit pulp	
		Water	Ethanol
01	Alkaloids	++	++
02	Saponins	++	+
03	Coumarins	+	+
04	Flavonoids	++	++
05	Carbohydrates	+	+
06	Cardiac Glycosides	+	+
07	Phlobatannins	-	-
08	Terpenoids	-	-
09	Phenols	++	++
10	Tanins	++	+

++ = strong positive test, + = weak positive test, = Negative test

3.2 Quantitative Phytochemical Analysis Total polyphenol and flavonoid content

Phenolic compounds extracted from plant life have been receiving a gorgeous deal of public interest all thru latest years. In facts, phenolic compound penalties on weight loss software fitness interaction in the human body. Polyphenols are the dominant plant compounds with antioxidant activity.

Phenolic compounds belong to antioxidants, which acts as free radical terminators. The free radical scavenging challenge often correlates with the complete phenolic content material fabric fabric in vegetation [18]. Total polyphenol content material was once as soon as performed as mg gallic acid equivalents per gram of dried pattern (mg QE/g). Total polyphenol content material was 238.68±4.13 mgGAE/g extract (figure 1). The complete flavonoid content material fabric was once as quickly as carried out as mg quercetin equivalents per gram of dried pattern (mg QE/g). Flavonoid contents had been 84,75±0.82 mg QE/g dry weight ethanol extract in A. squamosa fruit pulp (figure 2). The outcomes drastically advocate that the phenolics are fundamental elements of this plant, and some of the pharmacological penalties can additionally desire to be attributed to the presence of this precious component. The literature survey confirmed that a quantity of extracts of seed, leaf, fruit pulp, root and other components of *Annona squamosa* have flavonoids and phenols [19]. We have these days mentioned that the whole flavonoids of *Annona squamosa* have been estimated for unique extracts the use of quercetin as standard, amongst which water extract of leaf verified the excessive stage of flavonoids of about 9.26 mg/g, accompanied via capability of water and methanol extract of seed [20]. Moreover, in the entire phenolic content cloth cloth estimation with the aid of folin assay, amongst the a variety of extracts of leaf validated high phenolic content cloth of about 12.9846 mg/g of extract, accompanied with the aid of way of the usage of the water and methanol extract of seed [20].

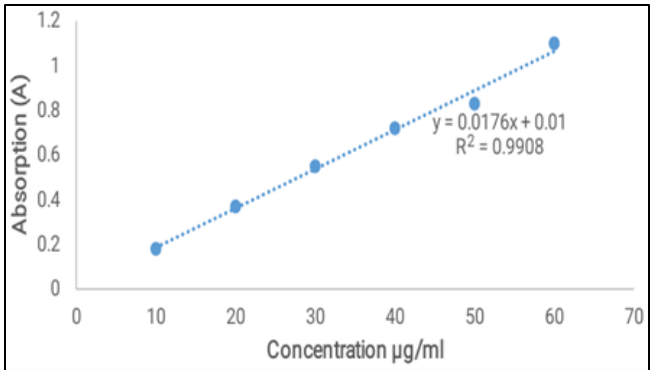

Fig 1: Standard gallic acid solution (µg/ml)\

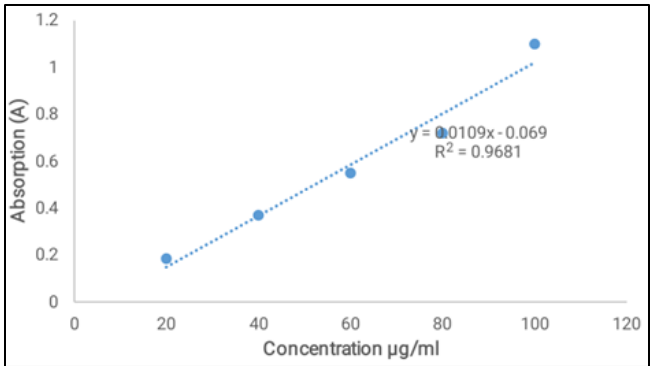

Fig 2: Standard quercetin solution (µg/ml)

DPPH

Three are distinct methods for estimating the antioxidant recreation of each synthetic compounds and natural. The DPPH assay used to be a rapid and low priced method, which commonly used for comparison of the anti-oxidative possible of specific herbal stocks. The DPPH scavenging assay is broadly applied to investigate the free radical scavenging of plant extracts thanks to its sensitive, simple, fast Antioxidants can cast off the radical via hydrogen donation, which outcomes in a reduce of DPPH absorbance at 515 nm. The IC50 price was the attention of the sample which inhibited proportion reaches 50%. Therefore, IC50 values are negatively correlated to the antioxidant activity, the decrease IC50 price capacity the absolute best antioxidant undertaking of the tested sample. Table two indicates the DPPH radical scavenging recreation of ethanolic extract of A. squamosa. Ethanol extract (IC50 performed 134.761±1.83 µg/ml) showed amazing antioxidant activity. This pastime might be due to the presence of phenolic compounds. The IC50 price of widespread ascorbic acid was once 2.60±0.03 µg/ml. The previous find out about confirmed the antioxidant analysis done for one-of-a-kind extracts by FRAP assay, amongst which water extract of seed confirmed the excessive degree of antioxidant of about
14.18 mg/g, accompanied via water and methanol extract of leaf. The amount of antioxidant current in 1 mg of extract used to be represented [20].

Table 2: DPPH radical scavenging activity of ethabolic extract of A. squamosa and ascorbic acid

Concentration (µg/ml)	DPPH % A. squamosa	Concentration (µg/ml)	DPPH% Ascorbic acid
25	13.27±0.33	1	18.71±0.37
50	18.69±0.31	2	36.89±0.45
75	26.33±0.39	3	54.28±0.63
100	34.67±0.43	4	74.08±0.69
150	55.69±0.67	5	95.74±0.75

ABTS

Proton radical scavenging is an critical characteristic of antioxidants. ABTS acts as a protonated radical, which has a characteristic maximum at 734 nm. ABTS performs a vital role in identifying the antioxidant capacity of hydrogen-donating antioxidants. The ABTS scavenging capacity of A. squamosa with fee IC50 of 62.63±0.43 µg/ml, and ascorbic acid with an IC50 value of 2.60±0.03 µg/ml (Fig 3 and 4). The previous literature survey showed *in vitro* antioxidant research of A. squamosa fruit pulp in quenching ABTS with an IC50 of 38.96 µg/ml [21].

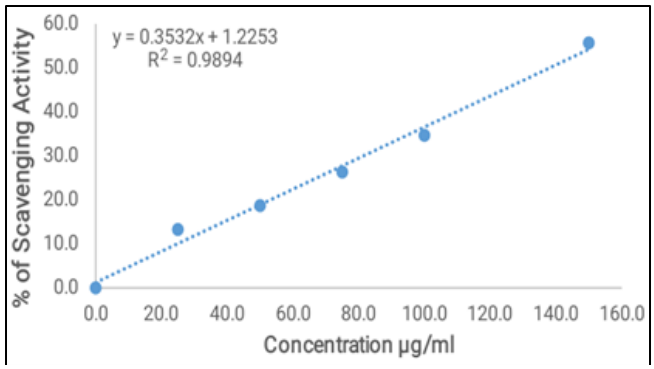

Fig 3: ABTS scavenging activity of ethanolic extract of A. squamosa

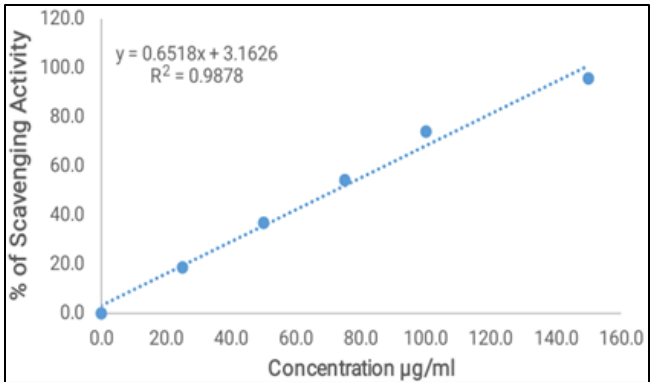

Fig 4: ABTS scavenging activity of ethanolic extract of ascorbic acid

4. Conclusion

Annona squamosa Linn (custard apple) is a treasured medicinal plant that has been used for a prolonged time in Bangladesh. Preceding lookup have confirmed that *Annona squamosa* Linn has the equal pharmacological outcomes as a large succulent fragrant perennial herb. *Annona squamosa* Linn used to be as quickly as subjected to phytochemical appear up resulted in the isolation of pretty a few flavonoids. Moreover the plant exhibited antioxidant anti-inflammatory cytotoxic and antimicrobial activities. This take a look at about is to confirm the anti-oxidant practicable and to consider whole polyphenol flavonoid contents in *Annona squamosa* Linn fruit pulp. The phenolic content cloth cloth used to be as soon as as quickly as found 238.68±4.13 mg GAE/g extract. Flavonoid content fabric used to be as soon as as soon as 84,75±0.82 mg QE/g dry weight ethanol extract in *Annona squamosa* fruit pulp. The antioxidant undertaking of the ethanol extract of a squamosa used to be correlated with total phenolic and flavonoid content material with values IC50 of 134.761±1.83 µg/ml, 62.63±0.43 µg/ml for DPPH and ABTS scavenging endeavor respectively.

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