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Comparative evaluation of the medicinal activities of methanolic extract of seeds, fruit pulps and fresh juice of *Syzygium cumini in vitro*

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PEER REVIEW

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Comments

This is an innovative approach to get the people know about the health benefits of berry fruits along with its pulps and seeds. Here, the authors tried to understand about the pharmacological comparisons among the berry juice, pulps and seeds. The authors clarified that berry seeds and pulps showed more potent health benefits than those of juice.
Details on Page 307

ABSTRACT

Objective: To establish the health benefits of *Syzgium cumin* to discover functional components present in the seeds, fruit pulps and fresh juice of this fruit grown in Bangladesh.
Methods: Thin layer chromatography and ultra-violet spectroscopy were used to detect the presence of various types of compound in seeds and juice. Antioxidant effects were measured by DPPH scavenging assay and total reducing assay. Receptor binding activities was performed by hemagglutination inhibition assay. Anti-inflammatory assay and hydrogen peroxide induced hemolysis assay was also investigated. Disc diffusion assay was performed to show the antibacterial effect using Gram positive, Gram negative strains of bacteria and fungi.
Results: Methanolic extract of the seeds showed stronger antioxidant, hydrogen peroxide induced hemolysis activities, hemagglutination inhibition activities and membrane stabilization activities than those of fresh juice. However, fresh juice showed stronger antibacterial and antifungal activities than those of methanolic seed extract. The seed contains higher amount of polyphenols and flavanoids than those of fruit juice.
Conclusions: Therefore, fruit juice, fruit pulp and seed of *Syzygium cumini* contain medicinal active components in different ratios.

KEYWORDS
Syzygium cumini, Antioxidant activity, Hemolysis activity, Flavanoids, Antimicrobial activity, Bangladesh

1. Introduction

Syzygium cumini (*S. cumini*) belongs to family Myrtaceae, which records the occurrence of taxonomically informative molecules, namely malic acid, oxalic acid, gallic acid, betulic acid, tannins, flavonoids and essential oil. The edible pulp of plant forms 75% of the whole fruit. Various mineral and vitamins were reported like Ca, Mg, P, Fe, Na, K, Cu, S, Cl, vitamin C, vitamin A, riboflavin, nicotinic acid, choline and folic acid. Glucose and fructose are the principle source of sweeteners in ripe fruit with no trace of sucrose[1]. Flower of plant contains oleanolic acid and other three triterpenoids also reported in the flowers are acetyl oleanolic acid (0.3%), *Eugenia* triterpenoid A (0.5%) and *Eugenia* triterpenoid B (0.3%). Flowers also contain ellagic acid (0.01%)[1]. Plant seeds are rich in protein and calcium. The seeds contain tannins (19%), ellagic acid, gallic acid (1%-2%)[2]. Leaves contain an essential oil with pleasant odour. The oil contains terpenes, 1-limonene and dipentene (20%), sesquiterpenes of cadalane type (40%), and sesquiterpenes of azulene type (10% or less), 4-(2,2-Dimethyl-6-methylenecyl) butanol, eicosane, heptacosane,

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1-chlorooctadecane, nonacosane, octacosane, triacontane, tetratetracontane, octadecane[3], jambosine, gallic acid, ellagic acid, corilagin, 3,6-hexahydroxy diphenoylglucose, 1-galloylglucose, 3-galloylglucose, quercetin, β-sitoterol and 4,6-hexahydroxydiphenoylglucose[4]. The protective efficacy of *S. cumini* seed extract against peroxidative damage contributing to skin carcinogenesis in swiss albino mice has been established[5]. The oral antihyperglycemic effect of the water and ethanolic extracts of the fruit pulp of another species of this plant *Eugenia jambolana* has also been shown[6].

Here we examined the presence of several types of functionally active components in the fruit pulp, fruit juice and seeds of *S. cumini*. We have shown the antioxidant, antibacterial, hemagglutination inhibition activity and hydrogen peroxide induced hemolysis inhibition activities of the methanolic extract of seeds, edible fruit pulps and fruit juice of *S. cumini* collected from Bangladesh.

2. Materials and methods

2.1. Plant collection and identification

The fresh fruits were collected from the surrounding of Dhaka, Bangladesh during June, 2010 and identified by the taxonomist of the Bangladesh National Herbarium, Mirpur, and Dhaka as *S. cumini*. A voucher specimen of the plant has been deposited (Accession No.: DACB 37900) in the herbarium for further reference.

2.2. Extraction of the plant material

Sun-dried and powdered seed material (750 g×2) was extracted with methanol by cold extraction process. The extracts were concentrated with a rotary evaporator (IKA, Germany) at low temperature (40-50 °C) and reduced pressure. The extracts (5 g) were stored at 4 °C until used. Fresh juice was collected by extrusion of the fruit and then the fruit pulp was soaked in methanol and extracted through cold extraction process. Fresh fruits juice was stored at -20 °C for further use.

2.4. TLC analysis

The extracts were analyzed by performing thin layer chromatography (TLC) to determine the composition of each extract. TLC was done under polar basic solvent consisted of ethyl acetate, ethanol and water (8.0 : 1.2 : 0.8). After completion of TLC, the plates were exposed to UV light for compound detection and identification. For charring the plates were exposed to 10% sulphuric acid solution, dried and then heated to 80-90 °C for charring purpose. This helped in the fixation of spot and allowed it to be prominently visible. For detection of flavanoids, the plates were dipped into 0.04% DPPH solution and dried while keeping in a dark place. For detection of polyphenols the plates were washed with Folin-Ciocalteu reagent and dried.

2.5. Chemical analysis of the extract

UV spectroscopy of the extract was performed within 200 nm to 400 nm using a Lambda UV spectrometer (Shimadzu, Japan)[7]. Caffeine (Sigma, USA) and Epigallacto catechin gallate (EGCG) (ITOEN, Japan) was used as references.

2.6. Determination of total phenolic content

The total phenolic content of seed extracts, fresh juice and pulp extract were determined using Folin-Ciocalteu method using gallic acid as standard[8]. The extracts were oxidized with 10% Folin-Ciocalteu reagent (Merck, Germany), and were neutralized with 700 mmol/L sodium carbonate solution. The absorbance of the resulting blue color was measured at 765 nm after 60 min. The total phenolic contents were determined using a standard curve prepared with tannic acid. The estimation of the phenolic compounds was carried out in triplicate. The results were mean±SD and expressed as mg of tannic acid equivalent/g of extract.

2.7. Total flavonoid assay

The total flavonoid compounds in each seed extracts, fresh juice and pulp extract were determined as previously described by Jothy *et al*. 2011[9]. An aliquot (1.5 mL) of methanolic extract was added to 6 mL of deionized water and then 0.45 mL 5% (w/v) $NaNO_2$ and incubated for 6 min. About 0.45 mL 10% (w/v) $AlCl_3$ and 6 mL 4% (w/v) NaOH was added and the total volume was made up to 15 mL with distilled water. The absorbance was measured at 510 nm by using visible spectrophotometer. The results were expressed as mg rutin equivalents/g. The experiments were performed in three times.

2.9. DPPH radical scavenging activity

The free-radical scavenging activity of the seed extracts, fresh juice and pulp extract were measured by decrease in the absorbance of methanolic solution of DPPH[10]. The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts. Different concentrations of the seed extract (2, 4, 6, 8 and 10 μg/mL, in methanol for seed extract and 2, 4, 6, 8 and 10 μL/mL, in water for fresh juice) were added at an equal volume (10 mL) to methanol solution of DPPH (400 μg/mL). Different concentrations of L-ascorbic acid (2-10 mg/mL) were used as the standard antioxidant. After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the following equation: DPPH

antiradical scavenging capacity (%)=(Absorbance of sample-Absorbance of blank)×100/Absorbance of blank. Methanol plus different concentration of plant extract solution was used as a blank, while DPPH solution plus methanol was used as a control. IC_{50} values denoted the concentration of the sample required to scavenge 50% of DPPH radicals.

2.10. Total reducing assay

The reducing power of the samples was measured using the potassium ferricyanide [$K_3Fe_3(CN)_6$] reduction method. Various amount of extracts (0-200 mg) and L-ascorbic acid (0-1 000 mg) were taken in different test tubes as previously described by Oyaizu *et al*., 1986[12]. Then 2.5 mL of distilled water and 2.5 mL of $K_3Fe_3(CN)_6$ solution were added in all test tubes and mixed well. After incubation at 50 °C for 20 min, 2.5 mL of trichloro acetic acid (10% w/v) was added in all test tubes and centrifuged at 3000 r/min for 10 min. Afterwards, upper layer of solution (5 mL) was mixed with 5 mL distilled water. Then 1 mL of $FeCl_3$ was added each test tube. Then from each test tube we collect 1 mL of solution and mixed it with 9 mL of distilled water. Then the solution was incubated at 35 °C for 10 min. The formation of Perls Prussian color was measured at 700 nm in a spectrometer. Increased absorbance of the reaction mixture indicates increasing reducing power. L-ascorbic acid was used as a standard. The analysis was performed in twice.

2.11. Antimicrobial assay

In order to screening the antimicrobial assay, five different bacterial strains of Gram negative, three different strains of Gram positive bacteria and three different strains of fungi were used to carry out this assay. The Gram negative bacteria are *Pseudomonas aureus, Shigella boydii, Vibrio parahaemolyticus*, , *Salmonella typhi* (*S. typhi*), *Escherichia coli* (*E. coli*). The Gram positive bacteria are *Sarcina lutea, Staphylococcus aureus, Bacillus subtilis*. The fungi are *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae*. Nutrient agar was used as the culture media. Stocks of these bacterial solutions were revived in nutrient agar by incubating at 37 °C for 24 h. A single disk diffusion method was used to assess the presence of antimicrobial activities of the methanolic extract of night jasmine leaves. Whatman's filter paper was punched, and 6 mm disks were collected in a beaker. The beaker was covered with foil paper and autoclaved. About 20 μL of different concentration extracts (0-10 mg/mL) were loaded per disk. The revived test organisms were plated onto nutrient agar plates. The disks were then placed equidistant on all plates for all extracts. Standard disc (Himedia, India) of azithromycin (30 μg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. After incubation at 37 °C for 24 h, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

2.12. Hemagglutination inhibition assay

Hemagglutination activity of seed extracts, fresh juice and pulp extract were tested against human erythrocyte blood groups ABO as previously described by Saha *et al*. 2001[13]. Stock solution of the test samples was prepared at concentration of 5 mg/mL and each solution was serially diluted. Fresh blood was collected from healthy persons, centrifuged and the erythrocytes were separated. About 4% erythrocyte suspension was prepared in phosphate buffer (pH 7.4) of all blood groups. About 1 mL of the test sample dilution was taken with 1 mL of 1% erythrocyte and incubated at 4 °C. After incubation, the results were noted. Smooth button formation in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of hemagglutination was determined from the extent of deposition.

2.13. Anti-hemolytic assay

Inhibition of H_2O_2 induced red blood cell (RBC) hemolysis of methanolic extract was examined by the *in vitro* method described previously by Tavazzi *et al*. 2001[14]. The erythrocytes from human blood were separated by centrifugation and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant was colorless. The erythrocytes were then diluted with saline or phosphate buffer to give a 4% suspension. Varying amounts of sample (0-1 mg/mL) with saline or buffer were added to 2 mL of the suspension of erythrocytes and the volume was made up to 3.5 mL with saline or buffer. This mixture was pre incubated for 120 min and then 0.5 mL H_2O_2 solutions of appropriate concentration in saline or buffer were added. The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring 90% hemolysis of blood cells after 120 min incubation. Incubation was concluded after these time intervals by centrifugation during 5 min at × 1 000 g and the extent of hemolysis was determined by measurement of the absorbance at 540 nm corresponding to hemoglobin liberation. Antihemolytic activity was expressed as the inhibition percentage and was calculated using the following formula:

Antihemolytic activity (%)=($Control_{540\ nm}$-$Sample_{540\ nm}$)×100/$Control_{540\ nm}$

Where, $Sample_{540\ nm}$ was the absorbance of the sample and $Control_{540\ nm}$ was the absorbance of the control.

2.14. In vitro anti-inflammatory test (Erythrocytes membrane stabilization method)

In vitro anti-inflammatory test has been performed as previously described with slight modifications[15]. Alsever solution was prepared by 2% (w/v) dextrose solution, 0.8%

(w/v) sodium citrate, 0.05% (w/v) citric acid, and 0.42% (w/v) sodium chloride dissolved in distilled water, then the solution was sterilized. Blood was collected from cubital vein of healthy volunteers (HRBC). The collected blood was mixed with equal volume of sterilized alsever solution. The blood was centrifuged at 3 000 r/min for 5 min and packed cells were washed with isosaline and a suspension of 10 % (v/v) isosaline was made. Various concentrations of seed extracts, fresh juice and pulp extract were prepared in 1 mL phosphate buffer (pH 6.8), 2 mL hyposaline and 0.5 mL HRBC suspension. Diclofenac sodium was used as standard drug. The assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3 000 r/min for 5 min. The hemoglobin content in the supernant solution was estimated using UV analysis at 560 nm. The percentage hemolysis was calculated by assuming hemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization produced was calculated by using the following equation:

$$\% \text{ Membrane Stablization}=100-\left\{\left(\frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}}\right)\times 100\right\}$$

3. Results

3.1. Phytochemical screening

Polar basic solvent consisted of ethyl acetate, ethanol, and water (8.0 : 1.2 : 0.8). The separation performed by the polar basic solvent is shown here in Figure 1. TLC plates were seen under UV light and found some compounds were separated at the bottom of the plates. Charring with H_2SO_4 in high temperature the separated compounds transformed into black color indicates the presence of organic compounds in the samples. Staining the plate with Folin–Ciocalteu reagent the color of the separated compounds changed into bluish color indicates the presence of polyphenols in the samples. Staining the plate with DPPH solution the color of the separated compounds changed into yellow color indicates the presence of flavanoids in the samples. The separation in polar solvent system has been shifted from the bottom to the top the stationary TLC plates.

UV spectroscopic analysis of the methanolic extract of seeds and fresh juice showed in comparison with caffeine and EGCG as references (Figure 2 and Figure 3). Such an absorbance values in UV spectroscopic analysis indicates the presence caffeine or its derivatives and EGCG like compounds may presesent in the samples. 273 nm was found to be the λ_{max} for caffeine. The absorbance wavelengths for aqueous extract of seeds of *S. cumini* were 243 nm and 318 nm, where 243 nm was the absorption maximum. The absorbance wavelengths for the fruit juice of *S. cumini* were 249 nm, 217 nm and 364 nm, where 271 nm was the absorption maximum. The curve for the fruit juice of *S. cumini* strongly resembles the curve for caffeine and the λ_{max} for caffeine is very close to the absorption maximum of the fruit juice. This strongly indicates that caffeine might be present in the fruit juice of *S. cumini*. From the scanning curve the λ_{max} for EGCG was found to be 273 nm. The curve for the methanolic extract of seeds of *S. cumini* displayed absorbance at 230 nm, 236 nm and 305 nm, where 236 nm was the absorption maximum. The curve for the fruit juice of *S. cumini* displayed absorbance at 253 nm and 271 nm, where 271 nm was the absorption maximum.

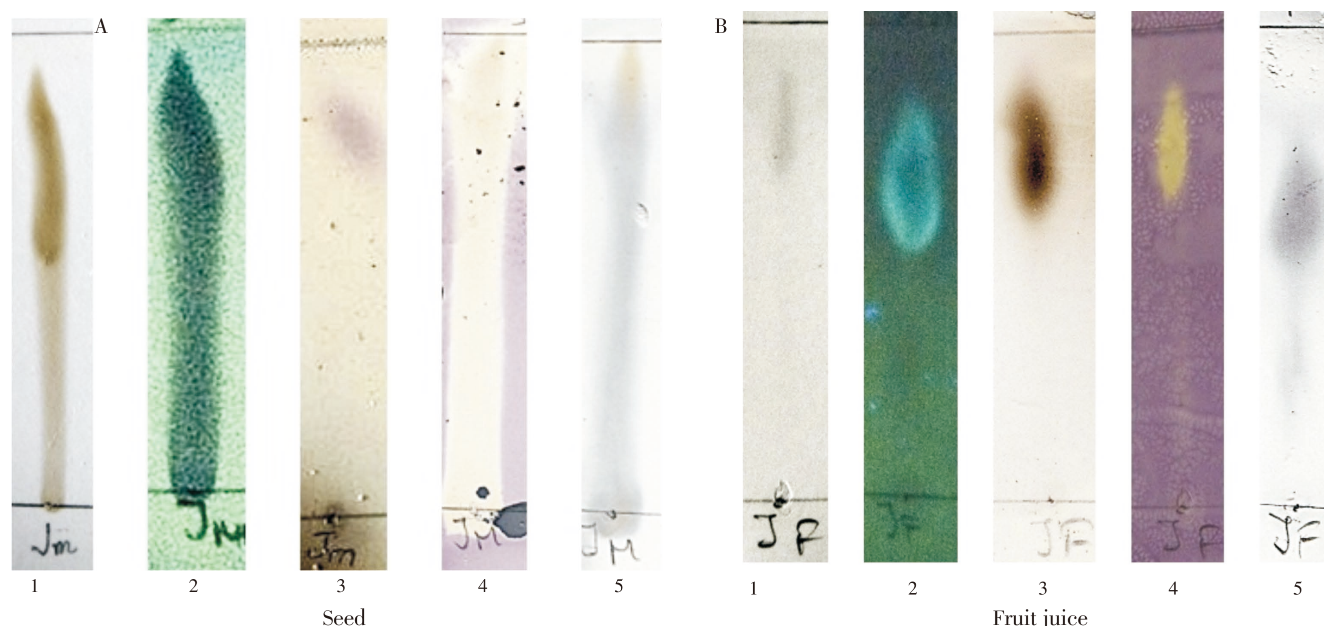

Figure 1. Separation of methanolic seed extract (A) and fresh juice (B) of *S. cumini* in TLC system using ethyl acetate, ethanol and water (8.0 : 1.2 : 0.8) solvent system.

Key: 1=Normal view; 2=UV view; 3=Sulphuric acid charring view; 4=DPPH staining view; 5=Folin–Ciocalteu reagent staining view.

Just like the pervious graph of caffeine, the λ_{max} of EGCG and the absorption maximum for the fruit extract are very close together. This may indicate the presence of EGCG or similar compounds such as flavonoids and catechins in the fruit juice. In order to confirm the presence caffeiene and EGCG in seeds and juice, we performed TLC analysis (Figure 2B and 3B) using them as reference standards. We found the retardation factor (R_f) of EGCG was found to be 0.82 and the R_f of caffeine was 0.64 and we found similar spots present in seeds and fruit juice. We further analysis the amount of caffeiene present in aqueous seed extract, fresh juice and fruit pulps using UV–spectroscopy. We establish a standard curve by using different concentrations of pure caffeiene using Beer–Lambert`s law in a concentration dependent manner. The equation we found was: Y=55.35X+0.007; where, R^2=0.999. We found that caffeine content in seed powder, fruit juice and fruit pulp is 24.1 mg/g, 7.86 mg/mL and 23.04 mg/g respectively. Therefore, *S. cumini* contains polyphenols and flavanoids like molecules in a greater extent.

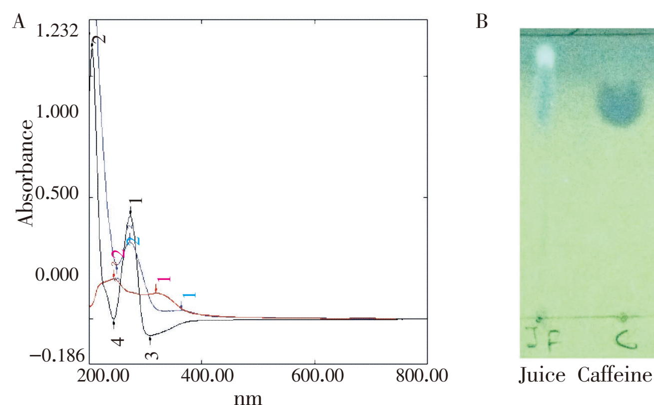

Figure 2. A. UV–scanning of aqueous seed extract, fresh juice and caffeine. B. TLC separation of fresh juice in comparison with caffeine and visualization under UV lamp.
Key: Black line: Caffeiene; Red line: Aqueous seed extract; Blue line: Fresh juice.

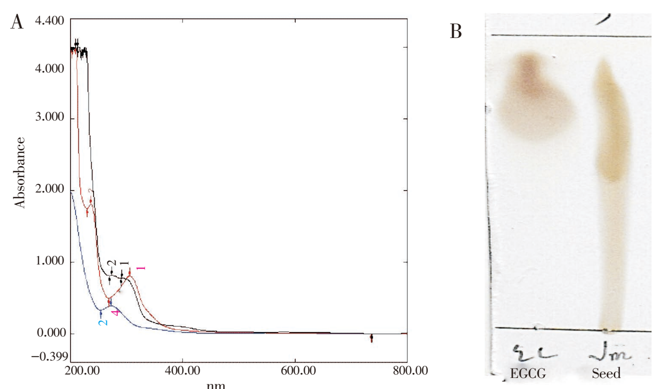

Figure 3. A. UV–scanning of methanolic seed extract, fresh juice and EGCG. B. TLC separation of methanolic seed extract in comparison with EGCG and visualization after sulphuric acid charring.
Key: Black line: EGCG; Red line: Methanolic seed extract; Blue line: Fresh juice.

3.2. Total phenolic content

Quantitative analysis of polyphenols and flavanoids were performed as described previously[8]. In case of polephenols quantifiacation, we establish a standard curve by using different concentrations of tannic acid using Beer–Lambert`s law in a concentration dependent manner. The equation we found was: Y=17.82X+0.124; where, R^2=0.978. From the standard curve, the total phenolic compounds as tannic acid equivalent present in fruit juice, fruit pulp and dry seed powder was 6.9 mg/mL, 20.7 mg/g and 21 000 mg/g respectively. In case of flavanoid quantification, a standard curve was used[5], where the equation is Y=0.007 1X+0.113 9, R^2=0.992 7. From the standard curve the amount of catechin present in the methanolic seed extract and fruit juice was 7 715 mg/g and 10 mg/mL respectively of catechin equivalent per gram of sample.

3.3. DPPH radical scavenging activity

From the analyses of Figure 4, we found the scavenging effect of methanolic seed extract and fresh juice in a concentration dependent manner. The 50% inhibitory concentration (IC_{50}) values of the methanolic seed extract and fruit juice was 2.9 μg/mL and 188.5 μL/mL respectively. Whereas, the 50% inhibitory concentration (IC_{50}) values of ascorbic acid was 1 290 μg/mL. Therefore, the seeds and juice of *S. cumini* showed stronger radical scavenging activities than that of ascorbic acid.

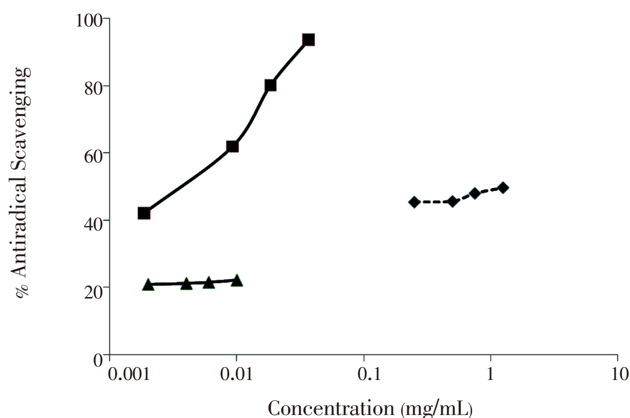

Figure 4. DPPH scavenging activity of the methanolic seed extracts and fresh juice of *S. cumini*.
Key: Dotted rhombus line: Ascorbic acid; Solid rectangular line: Methanolic seed extract; Solid triangular line: Fresh juice.

3.4. Total reducing assay

Total reducing assay of the methanolic seed extract and fresh juice in a concentration dependent manner was investigated in compared with ascorbic acid as shown in Figure 5. The seeds and juice of *S. cumini* showed weaker reducing activities than that of ascorbic acid.

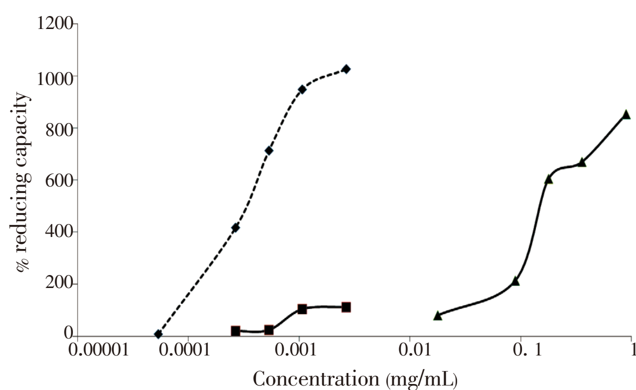

Figure 5. Total reducing activity of the methanolic seed extracts and fresh juice of *S. cumini*.

Key: Dotted rhombus line: Ascorbic acid; Solid rectangular line: Methanolic seed extract; Solid triangular line: Fresh juice.

3.5. Hydrogen peroxide induced hemolytic inhibition activity

Hemolysis caused by hydrogen peroxide was inhibited by methanolic seed extract and fruit pulps at various concentrations have been shown in Figure 6. About 300 μL of H_2O_2 was used for complete lysis of RBC.

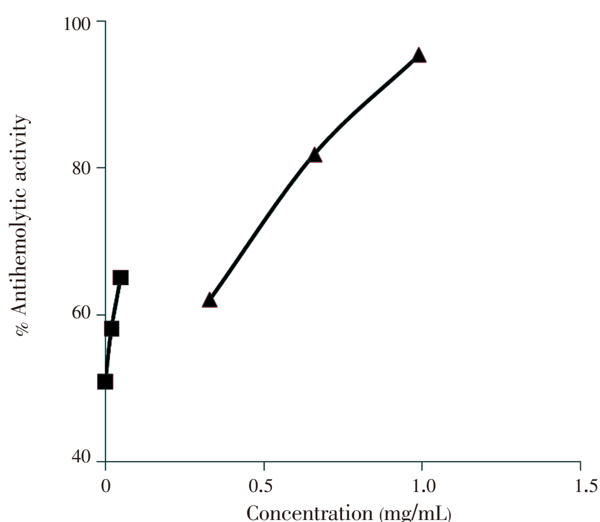

Figure 6. Hemolytic inhibition activity of the methanolic seed extracts and fruit pulps of *S. cumini*.

Key: Solid rectangular line: Methanolic seed extract; Solid triangular line: Methanolic fruit pulps extract.

3.6. In vitro anti–inflammatory test (Erythrocytes membrane stabilization method)

The methanolic seed extract and the fruit juice of *S. cumini* were subjected to the *in vitro* anti–inflammatory test using diclofenac as a standard as shown in Figure 7. As the concentration of the extracts goes up, their membrane stabilizing capacity also goes up. This can be implied that the extracts have anti–inflammatory properties because they have antioxidant molecules such as flavonoids and polyphenols that prevent oxidative stress.

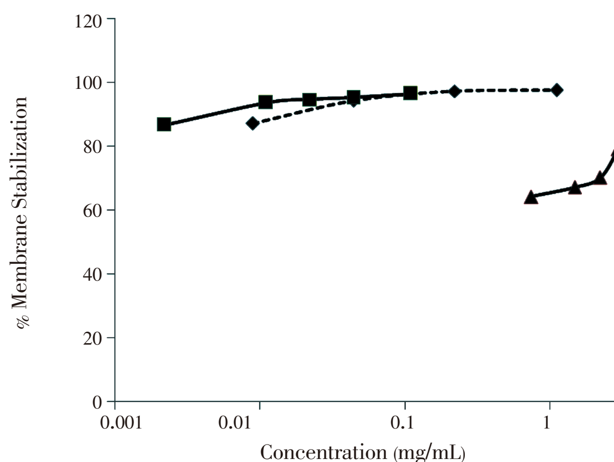

Figure 7. *In vitro* anti–inflammatory activity of the methanolic seed extracts and juice of *S. cumini*.

Key: Dotted rhombus line: Diclofenac sodium; Solid rectangular line: Methanolic seed extract; Solid triangular line: Fresh juice.

3.7. Antimicrobial assay

Different types of Gram positive, Gram negative bacteria and fungi were subjected in this test. The positive control used was erythromycin (15 μg/disc) and the negative control used was pure isotonic saline solution. The zones of inhibition for the microbes were measured in millimeters using a transparent ruler after 24 h of incubation. The results are shown in Table 1.

Table 1

Antimicrobial sensitivities of methanolic seed extract and methanolic pulp extract in disc diffusion assay.

Microorganisms	Negative control	*S. cumini*		Erythromycin
		Seed methanolic	Fruit pulp	
	Zone of inhibition in mm			
Fungi				
Candida albicans	0	10	11	30
Aspergillus niger	0	6	11	26
Saccharomyces cerevisiae	0	0	13	28
Gram Positive Bacteria				
Sarcina lutea	0	9	10	25
Staphylococcus aureus	0	10	7	32
Bacillus subtilis	0	15	0	20
Gram negative bacteria				
Pseudomonas aureus	0	8	10	29
Shigella boydii	0	0	9	18
Vibrio parahaemolyticus	0	0	10	26
S. typhi	0	14	13	36
E. coli	0	10	11	34

In order to investigate the minimum inhibitory concentration (MIC) of the methanolic seed extract and the fruit pulp of *S. cumini* required on *E. coli* and *S. typhi* infection, a dose dependent disc diffusion assay was performed as shown in Figure 8. MIC was calculated at that concentration where no inhibition was seen. MIC of the methanolic extract of the seed of *S. cumini* for *E. coli* and *S. typhi* is 0.08 mg/mL and the MIC for the fruit pulp of *S. cumini* is 2.67 mg/mL.

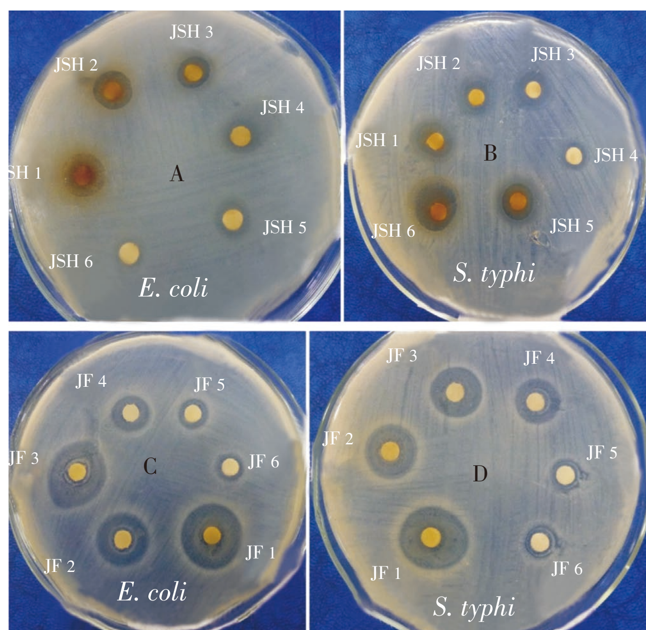

Figure 8. MIC determination of methanolic seed extract and methanolic pulp extract on *E. coli* and *S. typhi* infection.
A: methanolic seed extract on *E. coli*; B: methanolic seed extract on *S. typhi*; C: fresh juice required on *E. coli*; D: fresh juice required on *S. typhi*.

3.8. Hemagglutination inhibition assay

Various concentrations of methanolic seed extract (0-10 mg/mL) and methanolic pulp extract (0-333 mg/mL) were taken to investigate hemagglutination inhibition activity on different types of human blood groups. Hemagglutination inhibition activities of the methanolic extracts of the seeds and fresh fruit extract were tested against four different types of human blood groups and the results are as shown in Figure 9. The MIC required for hemagglutination is listed as shown in Table 2.

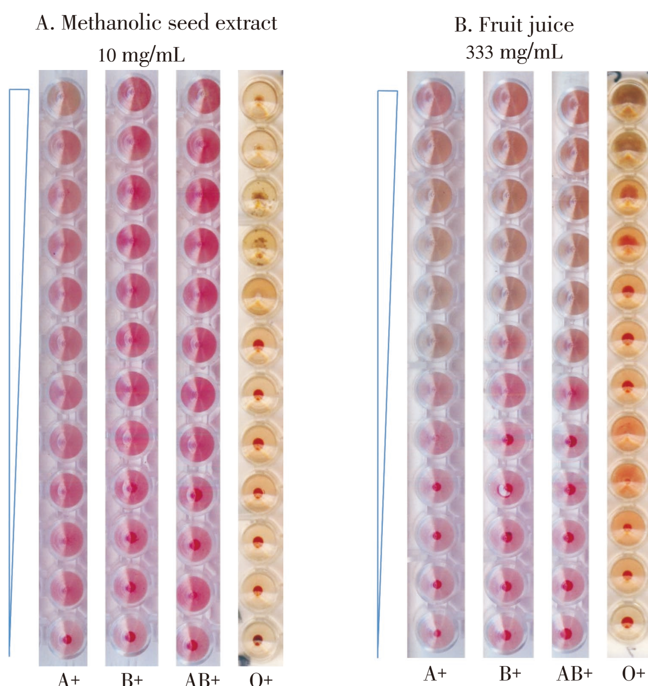

Figure 9. Hemagglutination inhibitory concentration determination of methanolic seed extract and methanolic pulp extract.

Table 2
Minimum hemagglutination inhibitory concentration of methanolic seed extract and methanolic pulp extract in disc diffuision assay.

	Concentration (mg/mL)	
Blood Group	Methanolic seed extract	Methanolic pulp extract
A+	0	2.6
B+	0.039	5.1
AB+	0.039	5.1
O+	0.3125	41.6

4. Discussion

4.1. Phytochemical screening

TLC is used to separate mixtures of compounds. TLC provided a qualitative idea about the components that are present in the crude mixture of the methanolic extract of seeds, fruit pulp and fresh juice. The developed plates were tested with DPPH and turned in yellow color ensures that the extracts posses any antioxidant activities. Folin-Ciocalteu reagent was used to test the developed TLC plates and found bluish color indicates the presence of phenolic and polyphenolic antioxidants present in the extract. We found the relative separation of the components can be studied by calculating the R_f of the extracts in comparison with caffeine and EGCG. Such a similarity in R_f values indicates the presence of caffeine or EGCG and its simple derivatives may present in *S. cumini*. Therefore, we performed UV scanning of the solution of methanolic seed extract and fresh juice to investigate their wavelength maximum in comparison with pure caffeine and EGCG. We found some of the peaks are closely match with the wavelength maximum of caffeine and EGCG.

4.2. Total phenolic and flavanoids content

The Folin-Ciocalteu reagent is used for the colorimetric *in vitro* assay of phenolic and polyphenolic antioxidants. Flavonoids are all ketone containing compounds and are secondary plant metabolites. They not only have various physiological functions in plants but also they are potent antioxidants. Measuring the content of flavonoids in plant samples can give us a measure of their antioxidant potential. In order to investigate the total phenolic content in the methanolic extract of the seed, fruit pulps and fresh juice we performed total phenolic and flavanoid content. We found that both the seed and pulp extract and juice contained a siginificant amount of phenols and flavanoinds. Therefore, *S. cumini* has a potential to show antioxidant activities.

4.3. DPPH radical scavenging and total reducing power activities

DPPH is a very good free radical scavenger for other radicals, therefore reduction of DPPH upon addition gives a very good measure of antioxidant activity. On the other hand, total reducing power activity test is a good test to estimate the potential antioxidant activity of a sample. Substances that have reducing potential such as antioxidants react with $K_3Fe_3(CN)_6$ (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form a ferric ferrous complex that is prussian blue in color and that has is absorption maximum at 700 nm. We found that methanolic extract of the seed and fresh juice showed DPPH scavenging and total reducing activities.

4.4. Hydrogen peroxide induced hemolytic inhibition activity

Both the crude methanolic extract of seeds and fresh juice showed hydrogen peroxide scavenging activity, reducing activity and hydrogen peroxide induced hemolytic inhibition activity.

4.5 In vitro anti-inflammatory assay

The percentage membrane stabilization of RBCs is considered to correlate to the anti-inflammatory activity. The RBC membrane is similar to lysozomal membrane components. Hypotonicity brings about oxidative stress in the cell and promotes cell lysis. Prevention of the destruction of the RBC membrane means that the extracts may also stabilize the lysozomal membrane. In comparison with diclofenac sodium, fruit extracts and seed extracts of *S. cumini* showed a considerable anti-inflammatory activity.

4.6. Hemagglutination and hydrogen peroxide induced hemolysis inhibition assay

Many viruses attach to molecules present on the surface of RBCs. Hemagglutination inhibition assay was performed to investigate the receptor binding affinity of the compounds present in the crude methanolic seed extract and fresh juice on human erythrocytes or othere receptors may present in viruses. We found that both of the crude methanolic seed extract and fresh juice have the binding affinity to the receptors of erythrocytes and prevent agglutination. The results showed a possible benefits of *S. cumini* extract as an antiviral therapeutics.

Biomembranes may be most susceptible to free radical attacks due to its content of polyunsaturated fatty acids. The lipid oxidation by the presence of hydrogen peroxide leads to the formation of free radicals that leads to the hemolysis of the erythrocyte and expulsion of its hemoglobin content. The content of expelled hemoglobin is monitored spectroscopically. This test tests the antioxidant levels in a sample by its potential to prevent hemolysis. Both fruit extracts and seed extracts of *S. cumini* showed a considerable anti-hemolytic activity may be due to the presence of high amount of polyphenols and flavanoids present in the extracts.

4.7. Antimicrobial assay

The disk diffusion method is a means of measuring the effect of an antimicrobial agent against bacteria grown in culture. Crude methanolic seed extract and pulp extract of *S. cumini* showed broad spectrum antibacterial and antifungal activities. The extracts also showed the dose-response relationship of antibacterial activities on *E. coli* and *S. typhi* strains of bacteria. Therefore, *S. cumini* may be considered as a useful source for discovering a safe and novel antibacterial compound.

Therefore, our present study on the methanolic seed extract, fruits pulp and fresh juice of *S. cumini* showed the potentiality of it as an antioxidant, receptor binding activity with RBCs, hydrogen peroxide induced hemolysis activity, *in vitro* anti-inflammatory activities, antifungal activities and antibacterial activity. *S. cumini* is a popular seasonal fruit in many tropical countries. Therefore, *S. cumini* may be considered as a medicinal fruit for future health benefits against many infectious diseases and life style related chronic disease. Further research is required to isolate functional molecules present in the fruit and seeds of *S. cumini*.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

S. cumini is a berry type fruit plant widely found in Bangladesh and Indian sub-continents. It has shown several medicinal values of its leaves, barks, flowers, seeds and fruits. Its different parts contain different types of polyphenols, flavanoids and other plants constituents. This paper examined the presence of several types of

functionally active components in the fruit pulp, fruit juice and seeds of *S. cumini*.

Research frontiers

This study has shown the antioxidant, antibacterial, hemagglutination inhibition activity and hydrogen peroxide induced hemolysis inhibition activities of the methanolic extract of seeds, edible fruit pulps and fruit juice of *S. cumini* collected from Bangladesh. It found the presence of caffeine and EGCG in its seed extract. Seed extract also showed antibacterial activities. The hypothesis of its antibacterial activities is that some components bind with the bacterial cell surface receptors and inhibit bacterial growth.

Related reports

The authors tried to find out the functional molecules present in the fruits and its seeds responsible for health benefits. Therefore, they performed several experiments using various methods. It found that they showed antioxidant and total reducing power activities. Expectedly they inhibited hydrogen peroxide induced hemolytic activities due to their potential as antioxidants. They inhibited bacterial growth and showed hemagglutination inhibition activities. Such results also reflect the possibility of binding affinity of their components on bacterial cells.

Innovations and breakthroughs

This paper tried to discover the health benefits of local berry fruit in Bangladesh. The unused parts of the fruit are its seed and sometimes pulps. *S. cumini* seed and pulp are very rich in catechins, caffeine and other medicinal components. They also showed stronger antioxidant, total reducing power, anti-inflammatory, hemagglutination inhibitory and antibacterial activities than those of fresh juice. Therefore, we may get more medicinal activities from the fruit by taking its seeds and pulps.

Applications

Black berry (*S. cumini*) is a seasonal fruit and generally found in summer. There are some infectious diseases widely spread during summer. The results of this project suggested that by taking fruit pulps and seeds with its berry juice people may get rid of many infectious diseases during this season. They may also get free from free radicals by taking the fruits more.

Peer review

This is an innovative approach to get the people know about the health benefits of berry fruits along with its pulps and seeds. Here, the authors tried to understand about the pharmacological comparisons among the berry juice, pulps and seeds. The authors clarified that berry seeds and pulps showed more potent health benefits than those of juice.

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