



Research article



Comprehensive analysis of phytochemical profiling, cytotoxic and antioxidant potentials, and identification of bioactive constituents in methanolic extracts of *Sonneratia apetala* fruit

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ABSTRACT

In the delta region of Bangladesh, *Sonneratia apetala*, also known as Keora and mangrove apple, is widely recognized for its dual role as a source of both food and medicine. Seasonal *S. apetala* fruits were gathered from Hatiya, Noakhali, in October 2021. The samples were segregated into pericarp and seeds, then fractionated into methanol segments. The anti-proliferative activities of these samples against lung A549 cells were evaluated using the Trypan blue exclusion method. Additionally, High-Performance Liquid Chromatography (HPLC) was employed to quantify phenolic compounds, while standard protocols facilitated the identification of specific phytochemical constituents. Chemical profiling via Gas Chromatography-Mass Spectrometry (GC-MS) and the isolation and detection of bioactive compounds through column chromatography and Nuclear Magnetic Resonance (NMR) analysis were undertaken. The methanol fractions of the seeds and pericarp were found to contain carbohydrates, tannins, flavonoids, steroids, alkaloids, glycosides, and terpenoids, with the absence of saponins and anthraquinones. Notably, the anti-proliferative effect demonstrated statistical significance at a concentration of 300 µg/mL for both extracts. Furthermore, HPLC analysis identified and quantified six polyphenols: catechin hydrate, (–)-epicatechin, rutin hydrate, *trans*-ferulic acid, *trans*-cinnamic acid, myricetin, and kaempferol, with the following concentrations: 46.65 and 12.72; 349.29 and 140.39; 5.26 and 33.06; 10.35

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and 29.28; ND and 11.93; and 10.03 and 7.90 mg/100 g in the methanol fraction of the seed and pericarp, respectively. GC-MS analysis of *S. apetala* fruit revealed five notable compounds with significant peak areas (%): 2-methyltetracosane, tetratetracontane, heptacosane, 1-chloro-2-hexyl-1-octanol, and phenol, 3,5-bis(1,1-dimethylethyl), exhibiting peak areas of 43.96, 35.8, and 15.95, respectively. Meanwhile, the notable peak in *S. apetala* seeds was 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester, with a peak area (%) of 100. These compounds are known for their anticancer and antioxidant properties. Therefore, *S. apetala*, particularly its seeds and fruits, shows promising potential for development into dietary supplements and functional foods.

1. Introduction

Sonneratia apetala (Buch.-Ham.) belongs to the Lythraceae family [1]. *S. apetala* is a rapidly expanding and pioneering tree species in ecological succession in the Sundarbans, along the coast of Bangladesh, India, Australia, Malaysia, and other countries [2,3]. The fruits of *S. apetala* are widely consumed by local coastal communities [2,4]. The ripened fruit of *S. apetala* tastes sour and contains a greenish-yellow pulp [5]. It produces berry-like fruits that are 1.5–2 cm in diameter, globose, and have a long, leathery calyx. Each fruit contains many seeds, sometimes up to 100, which are yellowish, compactly arranged into 6 to 8 locules, and are primarily U- or V-shaped [6]. The fruit of *S. apetala* has cytotoxic, analgesic, antidiarrheal, and antihelmintic properties, in addition to antioxidant, antibacterial, and antifungal characteristics [2,7]. Leaf and bark extracts of *S. apetala* have been found to possess antioxidant, antibacterial, antidiabetic, and anticancer properties [8]. It can be used to treat asthma, ulcers, fever, sprains, swellings, hemorrhages, bleeding, and piles.

The absence of phytonutrients in the human diet contributes to various disorders. Polyphenols are among the most consumed phytochemicals in the diet, with more than 5000 polyphenols identified to date [9]. The seed extract of *Sonneratia apetala* demonstrates strong antioxidant properties, attributed to its high reducing power, DPPH scavenging ability, NO free radicals scavenging ability, metal chelating ability, and overall antioxidant capacity [2,9]. Among the different extracts, the methanolic extract exhibits the strongest antioxidant capacity [9]. The bark and leaf extract also possesses significant antioxidant properties [10–12]. The ethanol extract of the bark is particularly rich in phenols and ascorbic acid [8]. The pericarp extract displays a noticeable antioxidant capacity, though not as potent as that of the seeds [2]. The methanolic extract of the seeds shows inhibitory effects on both gram-positive and gram-negative bacteria [2]. This supports the traditional use of *S. apetala* fruit juice by coastal communities as a tonic to treat ailments, such as diarrhea [2]. Moreover, the acetone extract of the bark and leaves also exhibits significant antibacterial properties [8], whereas the pericarp extract does not show significant antibacterial effects [2]. The JAK/STAT signaling pathway is suppressed, and renal uric acid transporters are regulated in mice with hyperuricemia treated with an extract of *S. apetala* leaves and branches [13,14]. Despite the fact that over 7000 plant species are grown or harvested for food production globally, apart from the wild (IPGRI, 2001–05) [15], the fruit has not received substantial scientific attention.

The purpose of the study on *Sonneratia apetala* fruit extracts is to investigate and understand the phytochemical composition, antioxidant, and antiproliferative activities of the fruit extracts. By examining the methanol extracts of the pericarp and seeds, the research aims to identify bioactive compounds such as polyphenols, flavonoids, and other phytochemicals that contribute to these activities. Additionally, it aims to contribute to the development of functional foods, dietary supplements, and pharmaceuticals that harness the therapeutic properties of these natural extracts. Through detailed chemical profiling and bioactivity assays, the research endeavors to add valuable knowledge to the field of natural products and their applications in enhancing human health and well-being.

2. Materials and methods

2.1. Chemical reagents for phytochemical constituent screening and polyphenol analysis

To analyze the phytochemicals in the methanolic extracts of *S. apetala* seed and pericarp, various chemicals and reagents were employed, including ethyl acetate, sulfuric acid, olive oil, phenol, dimethyl sulfoxide (DMSO), acetic acid, perchloric acid, nitric acid, hydrochloric acid, Hager's reagent, Mayer's reagent, Wagner's reagent, sodium chloride, acetic anhydride, alcoholic solution of α -naphthol, lead acetate, and ammonia solution. All solvents were of analytical grade and procured from commercial suppliers, such as Sigma Aldrich, E. Merck, and BDH in England (Germany). Additionally, the following chemicals were sourced from Sigma-Aldrich (St. Louis, MO, USA): Gallic Acid (GA), 3,4-Dihydroxybenzoic Acid (DHBA), Caffeic Acid (CA), Vanillic Acid (VA), Syringic Acid (SA), p-Coumaric Acid (PCA), Rosmarinic Acid (RA), Quercetin (QU), Catechol (CT), Catechin Hydrate (CH), (–)-Epicatechin (ET), and Rutin Hydrate (RH).

2.2. Collection, identification, and authentication of samples

In October 2021, 1.0 kg of *S. apetala* was collected from Hatiya, Noakhali, Bangladesh. Following collection, the pericarps and seeds were dried. Once dried, the samples were ground into powder and stored in airtight conditions. A taxonomist at the Bangladesh National Herbarium in Dhaka identified the plant, and a voucher specimen was preserved (DACB No. 66955).

2.3. Extraction and processing

Utilizing advanced methods and protocols [9], the air-dried and powdered pericarp and seed parts were macerated for 72 h at room temperature in 2.5 L of methanol, with intermittent shaking and stirring. The mixture was then filtered through a clean cotton plug and Whatman No. 1 filter paper (125 mm), before being concentrated under reduced pressure and temperature (below 40 °C) using a rotary evaporator to yield the crude extracts. This same process was applied to macerate the air-dried and powdered seed material (250 g) in 2.5 L of methanol for 72 h at room temperature. Following this, the methanol extract was filtered and the solvent was removed under low pressure using a rotary evaporator. Subsequently, the extracts were accurately weighed and stored in a refrigerator.

2.4. Phytochemical screening

To identify the constituents of phytochemicals, specific chemical tests were conducted using standard techniques as reported in the literature [16–18] to confirm their exclusive presence in the methanol extracts (refer to Table 1).

2.5. GC–MS analysis

GC-MS analysis was conducted using a GC-MS-QP 2010 Ultra instrument equipped with an AB Innowax column (30 m × 0.25 mm i. d., 0.25 µm film thickness). The decision to inject a 0.5 µL sample for GC-MS analysis likely considered factors such as sensitivity, concentration, analyte stability, column capacity, and instrument specifications. Initially, the oven temperature was set to 120 °C for 1 min, followed by an increase to 270 °C over 25 min. Helium was used as the carrier gas at a flow rate of 1.15 mL/min. The split ratio was set at 200, while the sample injector and mass transfer line temperatures were maintained at 200 °C and 250 °C, respectively, throughout the experiment. The ionization energy for the mass spectroscopy study was set at 70 eV. Mass spectra were recorded for 40.75 min, spanning a range of 50–650 *m/z*. The retention indices of the components were compared with those in the Wiley and NIST Libraries, as well as literature values [19].

2.6. Analysis of antiproliferative activity using ATCC cell lines

The antiproliferative activity of the samples was evaluated in lung A549 cells using the Trypan blue exclusion method. Lung A549 cells (passage 92) were obtained from ATCC. The cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % (v/v) fetal bovine serum and 1 % (v/v) antibiotic solution (10,000 U/mL penicillin and 10,000 µg/mL streptomycin, HyClone Laboratories, USA). Cultures were maintained in a CO₂ incubator at 37 °C with 5 % CO₂. Cell passaging involved aspirating the old media, washing off the media residue with phosphate-buffered saline (PBS, pH 7.4), and trypsinizing with

Table 1

Several standard phytochemical tests were conducted to identify the plant metabolites in the pericarp and seed extracts of *S. apetala*.

P.chemical group	Tests	Observations
Saponins	i) Frothing: 1/2 mg of pericarp extract and seed extract were taken in the test tube and shaken vigorously with distilled water (5 mg). ii) Emulsification: Approximately 2 mg of each crude extract was added to a few drops of olive oil and shaken well.	i) Frothing was not formed. ii) No emulsified layer was formed.
Flavonoids	i) Alkaline reagent test: Aqueous sol. of each extract was made, mixed with 5 mL of a dilute sol. of ammonia, and added with conc. H ₂ SO ₄ . ii) Lead acetate test: 1–3 mL solution of 10 % lead acetate was added to small amounts of each extract.	i) A buff-colored precipitate has been found. ii) A yellow color has appeared.
Alkaloids	i) Mayer's test: 1 mL of each extract, one/two drops of Mayer's reagent (Mixture of HgCl ₂ and KI with DI) was given by side tube. ii) Hager's test: Hager's reagent was added to a small amount of each filtrate extract.	i) White or creamy precipitate. ii) Yellowish precipitate.
Carbohydrates	i) Molisch's test: To 2 mL of each crude filtrate extract, a few drops of Molisch's reagent (solution of α-naphthol and 10 % ethanol) were added by the side of the test tube.	i) A reddish-violet ring was formed between two layers.
Anthraquinones	i) Borntrager's test: 5 mg of each crude extract was added to 5 mL of suitable organic reagent in a test tube and shaken well. Then, 10 % ammonia solution was added in equal volumes.	i) No red/pink/violet color was observed.
Steroids	Liebermann-Burchard's test: 1 mL of acetic acid and 1 mL of chloroform mixed with 0.0 °C. Then, the combined solution was mixed with each crude extract and conc. H ₂ SO ₄ was added.	i) A greenish color was observed.
Terpenoids	i) Salkowski's test: 2 mL of each crude extract was mixed carefully with 1 mL CHCl ₃ and 1 mL conc. H ₂ SO ₄ .	i) A ring of reddish brown color was observed.
Tannins	i) Lead acetate test: 5 mL of each crude extract was mixed with a solution of 1 % lead acetate (a few drops) in the test tube.	i) Yellowish/reddish precipitate.
Glycosides	i) Keller-Killiani test: Glacial acetic acid, 5 % FeCl ₃ and conc. H ₂ SO ₄ was added to each of the crude extracts. ii) Concentrated H ₂ SO ₄ test: 5 mL of each crude extract was added to glacial acetic acid (2 mL) few drops of 5 % FeCl ₃ and conc. H ₂ SO ₄ .	i) A reddish-brown color was observed at the junction of two layers. ii) Appearance of the brown ring.
Coumarins	i) 10 mg of crude extract was added to 5 mL of MeOH, and a few drops of KOH (alcoholic) were added.	i) Yellow color in the upper layer.

0.25 % trypsin-0.5 mM EDTA solution (HyClone Laboratories, USA). Prior to treatment, cells were seeded at approximately 1×10^4 cells/mL in T25 flasks using trypsin-EDTA solution in the aforementioned growth media. After 24 h, achieving 60–80 % confluency, the medium was aspirated and replaced with 3 mL of fresh medium. Approximately 15 mg of each sample was dissolved in DMSO to achieve a concentration of 15,000 µg/mL and was sterilized through a 0.2 µm filter. The samples were then added to each flask at final concentrations of 50, 100, 200, and 300 µg/mL in triplicate, using a maximum of 0.5 % DMSO as the solvent. DMSO also served as a negative control in the experiment. The flasks were incubated at 37 °C with 5 % CO₂ for 24 h. Following incubation, cells were harvested using 1 mL of 0.2 g/l EDTA solution. Cell morphology was observed under an inverted microscope and compared with the control. Viable cells were detached and counted using Trypan Blue in an automated cell counter (Luna II, UK). The antiproliferative activity was calculated using the equation no. 1 [20].

$$\% \text{ Dead Cells} = \frac{\text{No. of Dead Cells}}{\text{Total No. of Cells}} \times 100 \quad (1)$$

The antiproliferative activity of the test samples on the cells was expressed in IC₁₀₀ values determined by a best-fit curve between % of viable cells to sample concentration.

2.7. Identification and quantification of polyphenols using HPLC

HPLC-DAD was utilized to identify and quantify phenolic compounds in the fraction [21]. Experiments were carried out using a Dionex UltiMate 3000 system equipped with a quaternary rapid separation pump (LPG-3400RS) and a photodiode array detector (DAD-3000RS). Compounds were separated on a 4.6 × 250 mm Acclaim® C18 (5 µm) Dionex column at 30 °C, employing a flow rate of 1 mL/min and an injection volume of 0.5 µL [9]. The mobile phase comprised acetic acid, acetonitrile, and methanol. Post-run, the system was reequilibrated with 5 % A/95 % B for 5 min. The DAD was set to scan from 200 to 700 nm, while the UV detector was programmed at 280 nm for 18 min, 320 nm for 6 min, and 380 nm for the remainder of the analysis. To establish the calibration curve and ensure accurate quantitative analysis of target compounds, a standard stock solution was prepared in methanol with concentrations of 20 µg/mL for GA, VA, CH, EC, PCA, RH, and EA, 8 µg/mL for CA, and 6 µg/mL for QU. The *S. apetala* methanol seed (MS) solution concentration was 5 mg/mL. Prior to HPLC analysis, all solutions were filtered through a 0.20 µm nylon syringe filter (Sartorius, Germany) and degassed in an ultrasonic bath (Hwashin, Korea) for 15 min. Data collection, peak integration, and calibration were conducted using Dionex Chromeleon Software (Version 6.80 RS 10) [22].

2.8. Isolation of compound ASD-1 from methanol extract of *S. apetala* seeds using column chromatography

For the initial fractionation, a 5.0 g methanolic extract from the seeds and pericarp of *S. apetala* was processed using column chromatography. The extract was subjected to TLC analysis, which displayed multiple spots across various solvent systems under UV light and after being sprayed with a detecting reagent on a TLC plate. Notably, the resolution was enhanced when using an n-hexane-dichloromethane mixture as the mobile phase. To prepare the methanolic extract for chromatography, it was first diluted in a minimal volume of ethanol and then adsorbed onto silica gel (Kieselgel 60, mesh 70–230). This mixture was then introduced into a column packed with analytical-grade silica gel. The column, measuring 35 cm in length and 10 cm in diameter, was filled with a slurry of silica gel in n-hexane. After ensuring the adsorbent bed was at the desired height, several hundred milliliters of n-hexane were passed through to achieve proper packing. The adsorbed extract was carefully applied at the top of the column. Elution was performed using solvents of gradually increasing polarity: n-hexane, dichloromethane, a mixture of dichloromethane and ethyl acetate, ethyl acetate, a mixture of ethyl acetate and methanol, and ultimately 100 % methanol. This process yielded eight fractions, ranging from 60 to 100 mL each, which were further purified based on their TLC profiles. From fraction 5 of the VLC methanol extract (collection numbers 1–3), white crystals were isolated by decantation after three days at room temperature. These crystals were then rinsed with a few drops of methanol to yield the compound ASD-1 in its pure form (5.0 mg).

2.9. NMR spectra analysis

At the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, 1D (¹H) NMR spectra were recorded using CDCl₃ solvents on a BRUKER WH 400 MHz NMR spectrometer. All spectra were obtained at room temperature (RT). Chemical shifts are expressed in parts per million (ppm). Prior to use, common laboratory solvents were distilled in glass.

2.10. Statistical analysis

All experiments were conducted in triplicate, with results presented as the mean ± standard error of the mean (SEM). The antiproliferative assay assessed the percentage of inhibition at each concentration relative to the negative control. Statistical significance was determined using Dunnett's multiple comparison test following a one-way analysis of variance (ANOVA) conducted in GraphPad Prism version 9.00. Results were considered statistically significant at P values less than 0.01. Data are reported as mean (average) ± standard deviation (SD) for the specified number of observations. A P-value threshold of 0.05 was set as the criterion for statistical significance.

3. Results and discussion

Plants have medicinal properties owing to their distinctive chemical compounds, which have specific physiological effects on the human body. Principal bioactive components available in the investigated plant include alkaloids, anthraquinones, carbohydrates, coumarins, flavonoids, glycosides, steroids, saponins, and tannins. These secondary metabolites also referred to as phytochemicals, are produced during the plant's normal metabolic processes and serve as a means of defense. Consequently, the medicinal efficacy of a plant is not due solely to a single active ingredient but rather to the synergistic effects of multiple compounds contained within the plant. The phytochemical screening results for the qualitative analysis of methanol extracts of *S. apetala* pericarp and seed, presented in Table 2, revealed the presence of carbohydrates, tannins, flavonoids, steroids, alkaloids, glycosides, and terpenoids. Conversely, the extracts from both the pericarp and seed were found to lack saponins and anthraquinones.

The biological activities of carbohydrates encompass antitumor, antioxidant, antidiabetic, antiviral, hypolipidemic, and immunomodulatory effects [23]. Furthermore, plant tannins are recognized for their antioxidant, antibacterial, antiviral, antiparasitic, anti-inflammatory, anti-HIV, and antidiarrheal properties [24]. Saponins are noted for their pharmacological, antibacterial, fungicidal, and insecticidal impacts [25]. Glycosides exhibit a range of activities including laxative, diuretic, antiseptic effects, and actions against microorganisms, insects, and herbivores [26]. Flavonoids are significant for their antimicrobial, hypoglycemic, antidiabetic, anti-inflammatory, antioxidant, antitumor, anticarcinogenic, antimutagenic properties, and free radical-scavenging activities, besides aiding in the reduction of high blood pressure [27–29]. Alkaloids, naturally occurring nitrogen-containing compounds, have antimicrobial capabilities due to their ability to intercalate with microbial DNA [30]. The hypoglycemic effects are also observed with glycosides, flavonoids, and alkaloids. Terpenoids indicate potential cytotoxic properties in the methanol extract [31]. Pharmaceuticals utilize steroids such as corticosteroids, sex hormones, neurosteroids, vitamin D, bile acids, and cardiotonic steroids for therapy [32]. The pharmacological actions of coumarin, including antitumor, anticoagulant, anti-inflammatory, anticancer, antioxidant, anti-HIV, and antibacterial activities, have garnered significant interest [33–36].

The study on *Sonneratia apetala* fruit extracts revealed promising cytotoxic potential against cancer cell lines, notably the lung A549 cells. Utilizing the trypan blue exclusion method, the research demonstrated that the extracts, particularly at a concentration of 300 µg/mL (refer to Figs. 1 and 2 (a,b)), exhibited significant antiproliferative effects, underscoring the potential of *S. apetala* as a source of anticancer compounds. These findings align with the growing body of evidence supporting the use of natural plant extracts in cancer treatment and prevention strategies, suggesting that the bioactive compounds within *S. apetala*, such as polyphenols, may play a critical role in its cytotoxic activity.

3.1. Measurement of polyphenols in *S. apetala*'s seed and pericarp methanol fractions

The chromatographic separation of polyphenols present in MS and MP extracts, as performed by High-Performance Liquid Chromatography (HPLC), is depicted in Fig. 3 (A, B).

The average of five measurements was utilized to calculate the level of each phenolic compound, which is presented in Table 3 along with the respective calibration curves. High concentrations of (–)-epicatechin (ET) were detected in MS and MP (349.29 and 140.39 mg/100 g of fraction, respectively). Catechin hydrate (CH), rutin hydrate (RH), *trans*-ferulic acid (TFA), *trans*-cinnamic acid (TCA), myricetin (MT), and kaempferol (KF) were also identified in lesser amounts (46.65 and 12.72; 5.26 and 33.06; 10.35 and 29.28; ND and 11.93; 10.03 and 7.90; 55.02 and 1.20 mg/100 g of fraction, respectively) for both fractions. Furthermore, the HPLC chromatogram shown in Fig. 3A, B revealed peaks corresponding to simple flavonoid aglycones, anthocyanins, polyphenols, and flavonoid

Table 2
Screening results of extracts from the pericarp and seed of *S. apetala*.

Tests for	Test Name	Pericarp extracts	Seed extracts
Saponins	a) Frothing	b	b
	b) Emulsification	b	b
Flavonoids	a) Alkaline reagent test	a	a
	b) Lead acetate test	a	a
Alkaloids	a) Mayer's reagents	a	a
	b) Hager's test	a	a
	c) Wagner's test	a	a
Carbohydrates	Molisch's test	a	a
Anthraquinones	Borntrager's test	b	b
Steroids	a) Salkowski test	a	a
	b) Liebermann-Burchard's test	a	a
Terpenoids	a) Salkowski test	a	a
	b) Liebermann-Burchard's test	a	a
Tannins	a) Gelatin-salt block test	a	a
	b) Lead acetate test	a	a
Glycosides	a) Sodium hydroxide reagent	a	a
	b) Test for glycosides with glucose as the glycine	a	a
Coumarins	MeOH test	a	a

^a = present.
^b = absent.

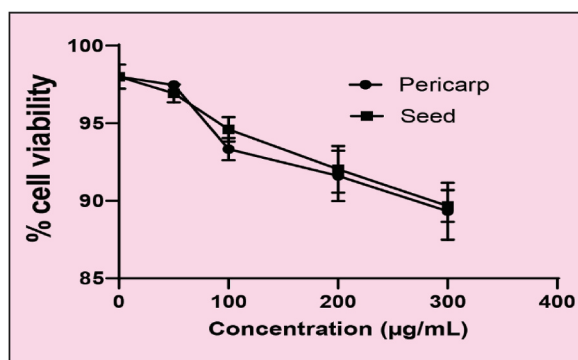


Fig. 1. Inhibition percent of lung A549 cells by pericarp and seed extracts.

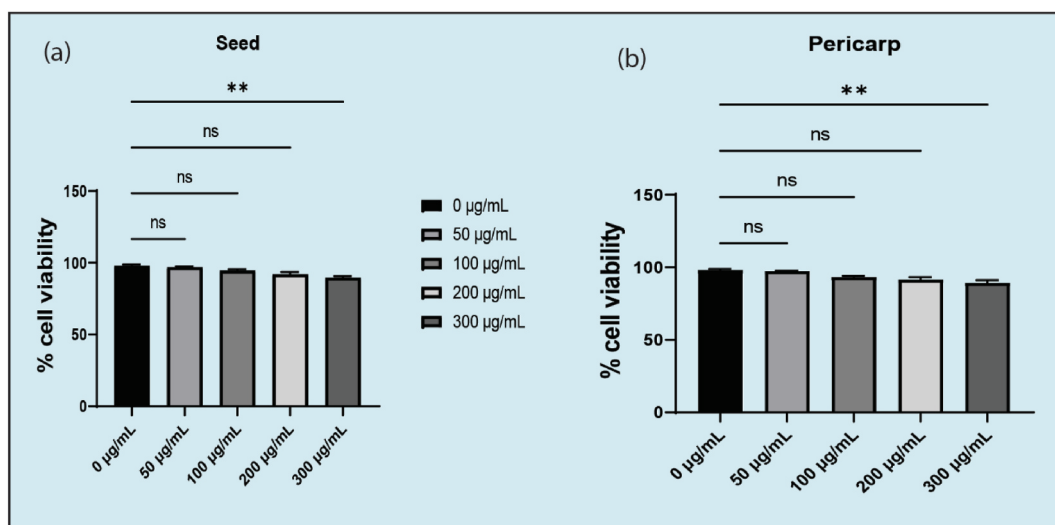


Fig. 2. Inhibition percent of lung A549 cells by seed extract (a) $P < 0.001$ at 300 µg/mL; (b) $P < 0.001$ at 300 µg/mL.

glycosides.

Catechins, phytochemicals found in tea leaves, beans, black grapes, cherries, and cacao, exhibit diverse physiological effects. Research indicates that catechins contribute to health promotion and disease prevention. Antioxidant activity, liver damage prevention, cholesterol reduction, and anti-obesity effects have been established through animal and clinical studies [37]. Foods rich in (–)-epicatechin may help to lower blood pressure, addressing a risk factor for heart and brain complications [38]. Rutin (rutoside) hydrate, a flavonoid present in many plants, possesses anti-inflammatory, antidiabetic, antioxidant, neuroprotective, nephroprotective, hepatoprotective, and amyloid oligomer-reducing properties, and is capable of crossing the blood-brain barrier [39]. *Trans*-ferulic acid (TFA) showcases anti-inflammatory, antibacterial, and antifungal activities through its phenoxy radical scavenging [40]. Cinnamic acid derivatives are employed in the treatment of cancer, bacterial infections, diabetes, and neurological disorders [41]. Myricetin is known for its hepatoprotective, anti-inflammatory, anti-obesity, anti-diabetes, anticancer, and antidiabetic properties [42]. Kaempferol, a flavonol, supports the structural and functional integrity of organs and cells by reducing lipid oxidation. The antioxidant, antibacterial, anticancer, neuroprotective, and hepatoprotective actions of kaempferol have been extensively highlighted in this study [43].

Polyphenols, saponins, flavonoids, tannins, steroids, alkaloids, and other compounds found in mangroves hold significant value for industry and medicine [44]. Polyphenolic compounds contribute to the flavor, color, and taste of foods, vegetables, fruits, and beverages. The methanol seed (MS) and methanol pericarp (MP) extracts displayed high levels of (–)-epicatechin (ET) and lower concentrations of catechin hydrate (CH), rutin hydrate (RH), *trans*-ferulic acid (TFA), *trans*-cinnamic acid (TCA), myricetin (MT), and kaempferol (KF). These compounds are beneficial polyphenols known for their rich antioxidant properties. Most exhibited synergistic effects, with their antioxidant efficacy varying according to structure [45]. It was found that pineapple peels did not exhibit synergistic effects among polyphenols [46]. Fig. 3B displays peaks that remained unidentified due to the use of only nine phenolic standards for quantification in the *Sonneratia apetala* fruit (MS) fraction. These unidentified peaks were observed in MS regions typically associated with simple polyphenols, catechins, anthocyanins, and flavonoid glycosides [47].

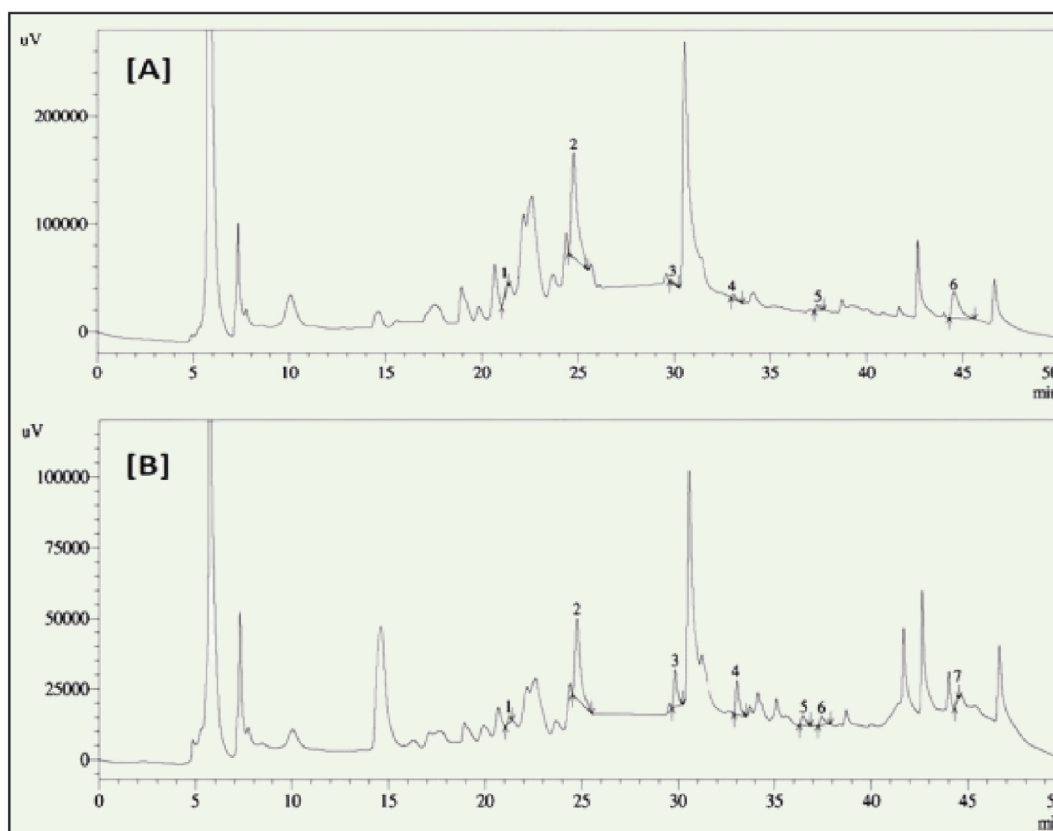


Fig. 3. (A): HPLC chromatogram of MS Peaks: 1.Catechin hydrate (CH); 2. (–) Epicatechin (ET); 3. Rutin hydrate (RH); 4. Trans-Ferulic acid (TFA); 5. Myricetin (MT) and 6. Kaempferol (KF). (B): HPLC chromatogram of MP Peaks: 1. Catechin hydrate (CH); 2. (–) Epicatechin (ET); 3. Rutin hydrate (RH); 4. Trans-Ferulic acid (TFA); 5. Trans-Cinnamic acid (TCA); 6. Myricetin (MT) and 7. Kaempferol (KF).

Table 3

The quantity of polyphenolic compounds in the methanol fraction of seeds and pericarp.

Name of Standard	Pericarp (mg/100 g dry extract)	Seed (mg/100 g dry extract)
Caffeic acid (CA), Gallic acid (GA), Vanillic acid (VA), 3,4 dihydroxy benzoic acid (DHBA), Syringic acid (SA), Rosmarinic acid (RA), p-Coumaric acid (PCA), Catechol (CT) and Quercetin (QU)	ND	ND
Catechin hydrate (CH)	46.65 ± 0.26	12.72 ± 0.52
(–) Epicatechin (ET)	349.29 ± 0.98	140.39 ± 0.55
Rutin hydrate (RH)	5.26 ± 0.43	33.06 ± 0.02
Trans-Ferulic acid (TFA)	10.35 ± 0.37	29.28 ± 0.06
Trans-Cinnamic acid (TCA)	ND	11.93 ± 0.10
Myricetin (MT)	10.03 ± 0.24	7.90 ± 0.20
Kaempferol (KF)	55.02 ± 0.30	1.20 ± 0.02

The methanolic pericarp extract of *Sonneratia apetala* contained five distinct chemicals, as identified by GC-MS analysis (refer to Fig. 4 and Table 4). The structure of the identified compounds (refer to Table 4) was illustrated using ChemDraw software and is shown in Fig. 5. The chromatogram for the methanolic pericarp extract (Fig. 4A) showed five peaks. In contrast, the chromatogram for the seed extract (Fig. 4B) displayed only one peak. Table 4 details the structures of the most prevalent compounds found in the methanolic extract of *S. apetala* fruit. Specifically, the analysis of the methanolic pericarp extract (Fig. 4A) identified five peaks: 2-Methyltetra-cosane with a retention time of 26.830 min and a peak area of 43.96 %, Tetratetracontane with a retention time of 30.306 min and a peak area of 35.81 %, Heptacosane, 1-chloro, with a retention time of 35.194 min and a peak area of 15.95 %, 2-Hexyl-1-octanol with a retention time of 23.871 min and a peak area of 3.44 %, and Phenol, 3,5-bis(1,1-dimethylethyl), with a retention time of 21.184 min. Conversely, the analysis of the methanolic seed extract (Fig. 4B) revealed a single peak corresponding to 1,3-Benzenedicarboxylic acid,

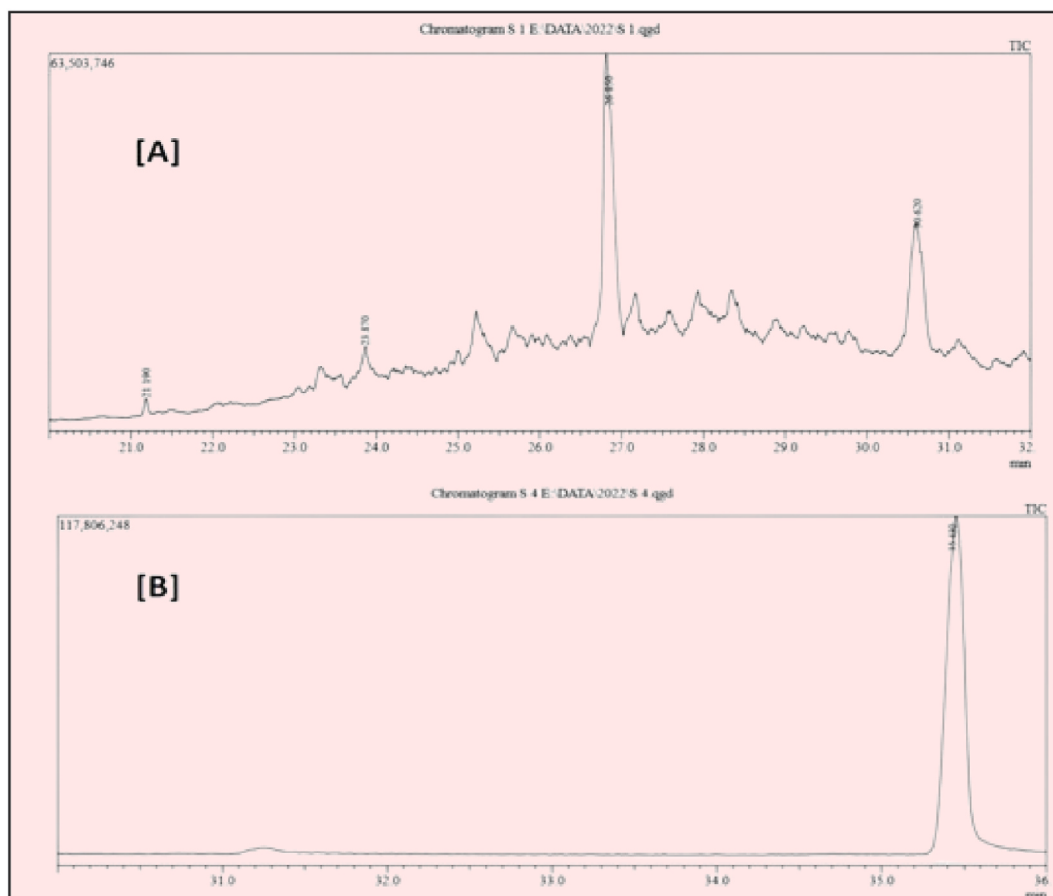


Fig. 4. Chromatogram (GC/MS) of the methanol extract (A) Pericarp and (B) seed of *S. apetala*.

bis(2-ethylhexyl) ester, which accounted for 100 % of the peak area.

Bioactivity tests have demonstrated that the pure compound 1,2-benzene dicarboxylic acid ester exhibits notable anticancer activity [48] and antibacterial properties [48–50]. Additionally, 2-methyltetracosane is known for its free radical scavenging properties [48]. Tetratetracontane has been found to possess antioxidant, hypoglycemic, hypolipidemic [51–53], and antibacterial properties [50]. Phenol, 3,5-bis(1,1-dimethylethyl), is recognized for its antibacterial and anti-inflammatory activities [53,54]. Heptacosane, 1-chloro, exhibits antibacterial and antifungal activities [55]. Hexyl-1-octanol (2-OH) demonstrates antimicrobial properties [56].

3.2. Spectral data of isolated compound (ASD-1)

The isolated compound, designated ASD-1, was obtained from the seed extract. The ^1H NMR spectrum of ASD-1, displayed in Fig. 6, offers insightful information about the molecule's hydrogen atoms. Chemical shifts (δ), expressed in parts per million (ppm), reflect the

Table 4

Tentative compounds identified from the methanol extract of *S. apetala* by GC-MS analysis.

Sl No	Name of compound	Nature	M. weight	M. formula	P. Area	P. area (%)	m/z	RT
01	1,3 Benzendicarboxylic acid, bis (2-ethylhexyl) ester	Ester	390.5561	$\text{C}_{24}\text{H}_{38}\text{O}_4$	85911742	100	57	35.468
02	2methyltetracosane	Fatty acid	352.680	$\text{C}_{25}\text{H}_{52}$	813691133	43.96	57	26.830
03	Tetratetracontane	Chain alkane	619.2	$\text{C}_{44}\text{H}_{90}$	66274922	35.81	57	30.306
04	Heptacosane, 1-chloro	Chain alkane	415.2	$\text{C}_{27}\text{H}_{55}\text{Cl}$	29515430	15.95	57	35.194
05	2-Hexyl-1-octanol	aliphatic alcohol	214.3874	$\text{C}_{14}\text{H}_{30}\text{O}$	6361140	3.44	57	23.871
06	Phenol,3,5-bis(1,1-dimethylethyl)	Phenol	206.3239	$\text{C}_{14}\text{H}_{22}\text{O}$	1560711	0.84	57	21.184

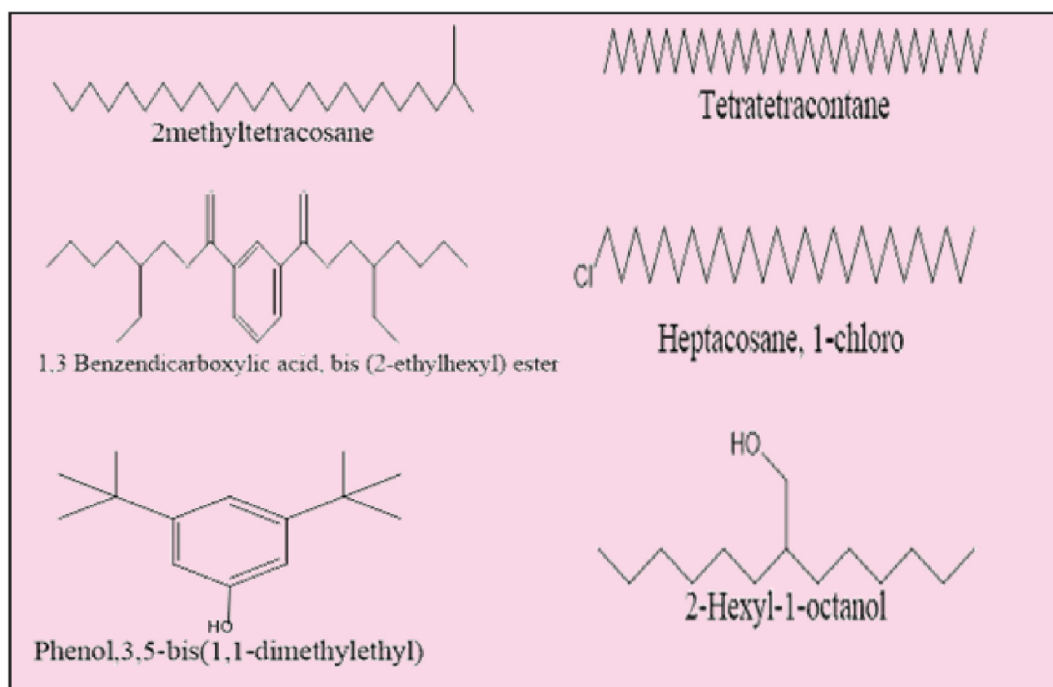


Fig. 5. Chemical structures of the compounds in the methanol extract of the seed and pericarp.

electronic environment surrounding the hydrogen atoms, aiding in the identification of proton types present (e.g., aliphatic, aromatic, hydroxyl, etc.). According to Fig. 5, the observed chemical shifts (δ) values are as follows: δ 8.51 (1H, s, H-5), 4.87 (1H, s, H-4), 4.86 (1H, s, H-2), 4.59 (1H, s, H-8), 4.21 (3H, s, -OCH₃ at C-1), 3.49 (3H, s, -OCH₃ at C-3), 3.32 (3H, s, -CH₃ at C-7), with additional shifts observed at 3.31, 2.70, 2.16, 1.98, 1.29, and 0.90 ppm, respectively.

4. Conclusions

S. apetala is an evergreen pioneer tree species that grows rapidly and is widely distributed along the coast of South Asia. Extracts from the plant components of *S. apetala* have been found to exhibit significant antioxidant and cytotoxic effects. The edible mangrove fruit of *S. apetala* is a natural bounty, rich in bioactive properties and functional phytochemicals. Both the methanol seed (MS) and methanol pericarp (MP) extracts of *S. apetala* demonstrated remarkable antioxidant activity, attributed to the exceptionally high levels of polyphenolic compounds they contain. The various biological properties of the fruit may be partially due to the synergistic effects of identified polyphenols such as (–)-epicatechin (ET), catechin hydrate (CH), rutin hydrate (RH), *trans*-ferulic acid (TFA), *trans*-cinnamic acid (TCA), myricetin (MT), and kaempferol (KF). Furthermore, the seeds of the *S. apetala* plant have the potential to serve as an enhanced source of both hydrophilic and lipophilic functional food components, aiming to improve human physiological health. Additionally, the current HPLC method offers an efficient approach for identifying and quantifying polyphenols in dietary products.

The fruit could be cultivated in tidal seawater across large tropical coastal areas worldwide, contributing to food security, primary health care, environmental protection, revenue generation, and the development of functional foods or dietary supplements. However, inadequate access to food supplies remains a primary cause of hunger and malnutrition among populations in tropical coastal regions, suggesting the fruit could serve as alternative medicine for deltaic communities. The wood of *S. apetala* has potential applications in various manufacturing processes, including pulping, veneering, hardboard, strand board, and plywood production. Additionally, the fruits could be utilized in fish and animal feed formulations.

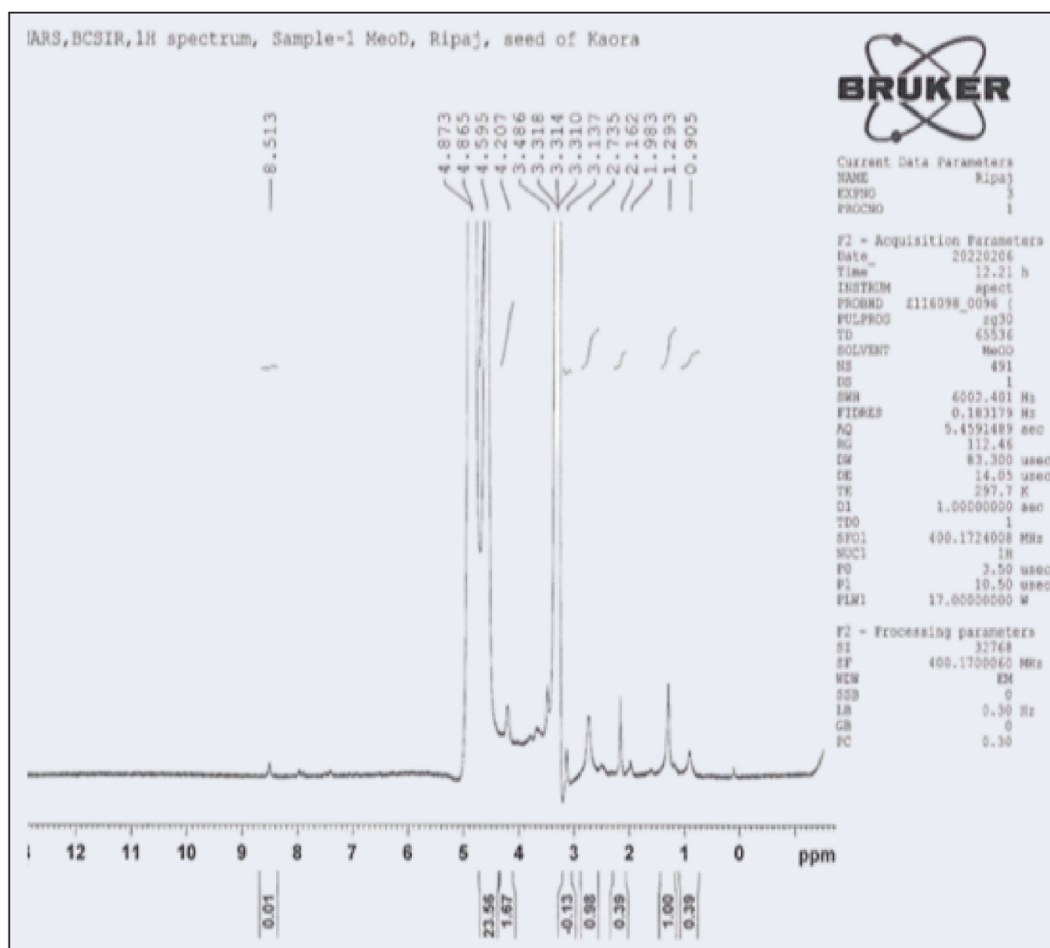


Fig. 6. ^1H NMR of isolated compound (ASD-1).

Data availability

All data are available in the manuscript.

CRediT authorship contribution statement

Md Ripaj Uddin: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fatema Akhter:** Writing – original draft, Methodology. **Md Jainal Abedin:** Writing – review & editing, Supervision. **Md Aftab Ali Shaikh:** Methodology. **Muhammad Abdullah Al Mansur:** Methodology, Formal analysis. **Mohammad Saydur Rahman:** Methodology. **AHM Shofiul Islam Molla Jamal:** Writing – review & editing, Methodology. **Md Ahedul Akbor:** Methodology, Formal analysis. **Md Hemayet Hossain:** Methodology, Formal analysis. **Suriya Sharmin:** Methodology, Formal analysis. **Abubakr M. Idris:** Methodology, Formal analysis. **Mayeen Uddin Khandaker:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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