Biomedical & Pharmacology Journal, March 2025. Vol. **18**(1), p. 581-591

Assessment of Antimicrobial, Cytotoxic and Antioxidant Potentials of n-Hexane, Chloroform and Ethyl Acetate Extracts of Sonneratia apetala Banks Fruits

Israt Jahan Bulbul*, Sabiha Ferdowsy Koly, Yesmin Begum, Nisrat Jahan, Nusratun Nahar and Md. Siddiqul Isalm

Pharmacy Department, Southeast University, Tejgaon, Dhaka, Bangladesh.
*Corresponding Author E-mail:israt.bulbul@seu.edu.bd

https://dx.doi.org/10.13005/bpj/3110

(Received: 19 January 2025; accepted: 06 March 2025)

This study evaluated the antioxidant, antimicrobial and cytotoxic activities of different fractions from the ethanolic crude extract of Sonneratia apetala fruits (Family: Lythraceae). Antimicrobial activity was assessed using the disc diffusion method with ciprofloxacin and fluconazole as reference standards. Among the fractions, the chloroform fraction demonstrated mild to moderate antimicrobial activity (8-20 mm zone of inhibition), with the highest activity against Pseudomonas aeruginosa (20 mm) and a minimum inhibitory concentration (MIC) of 32 μg. The lethality test for brine shrimp was used to assess cytotoxicity, showing LC50 values of 22.41 μg/ml (n-hexane), 20.53 μg/ml (chloroform), and 24.60 μg/ml (ethyl acetate), compared to vincristine sulfate (8.66 μg/ml). Antioxidant activity, determined via DPPH radical scavenging, revealed IC50 values of 42.03 μg/ml (n-hexane), 13.76 μg/ml (chloroform), and 49.99 μg/ml (ethyl acetate), with ascorbic acid as the standard (IC50 45.47 μg/ml). The highest amount of total phenolic content was detected in the chloroform fraction (26.88 mg GAE/g of extract). These findings suggest the chloroform fraction of S. apetala possesses significant antioxidant and cytotoxic potential, alongside moderate antimicrobial activity.

Keywords: Antimicrobial; Antioxidant; Cytotoxic; Pharmacological Activity; Sonneratia apetala.

Research on medicinal plants is essential to finding novel therapeutic phytochemicals that can be used to manage, alleviate or cure different diseases and health condition. Over time, nonconventional therapies have gained enhanced consideration due to the negative effects of traditional medicines and the growing demand for more toxin-free natural items. These plants, which are a rich source of bioactive chemicals, have also been employed for millennia in traditional medicine. [1-3] This choice is probably due to the fact that natural antioxidants are abundant in plants which are inexpensive, have few or no negative side effects, and can help people live longer while efficiently treating a variety of conditions.[4] Toxic effects of drugs can cause significant medical issues and even death. Most drugs with toxicological characteristics have thus been removed from the market. Due to their therapeutic qualities, affordability, and purity, plant-based therapies and herbal remedies have become increasingly significant in recent times.

Modern pharmacology continues to explore these natural remedies, often leading to the

development of new drugs that are more effective and safer than conventional one. Additionally, the conservation of medicinal plants is crucial for sustaining biodiversity and ensuring future research opportunities. This research supports global health initiatives by providing affordable and accessible treatment options, particularly in developing countries. [5-6]

Research on plant extracts' antimicrobial properties is vital for discovering natural antibiotics and combating antibiotic resistance. Plants produce bioactive compounds that can inhibit or eliminate infections, offering a safer, eco-friendly alternative to conventional drugs. These plant-based antimicrobials target pathogens effectively, reducing adverse effects and environmental impact. This research is crucial for expanding our arsenal against infectious diseases while promoting sustainable and less harmful treatments.

As plant extracts have the ability to fight against oxidative stress, it is essential to investigate their antioxidant properties. Chronic illnesses such as diabetes, cardiovascular disease, and cancer are mostly triggered by oxidative stress. Antioxidants from plants can neutralize free radicals, thereby protecting cells from damage. These natural compounds are often safer and more effective than synthetic alternatives. Understanding the antioxidant capacity of various plants can lead to the discovery of new, potent sources of natural antioxidants. Moreover, This study might help in generating of nutraceuticals and functional food and pharmaceuticals. In addition, antioxidant studies can promote sustainable agriculture by encouraging the cultivation of plants with high antioxidant potential. Lastly, this research fosters innovation in natural product development, aligning with the rising consumer demand for natural and health-promoting products.

Studying the cytotoxic activity of plant extracts is vital in drug discovery, as plants are plentiful sources of bioactive phytoconstituents with potential anticancer properties. Many studies help identify extracts that selectively kill cancer cells while sparing healthy ones, aiding the development of safer, effective treatments. They also reveal mechanisms of action, contributing to understanding therapeutic potential and identifying novel compounds for drug development. Additionally, such research highlights alternative treatments for diseases with limited conventional therapies, promoting the sustainable preservation and utilization of medicinal herbs. Overall, In order to improve patient care and advance medical science, cytotoxicity studies are essential. *S. apetala*, also called belongs to the Sonneratiaceae family of plants that grows across coastal areas of multiple regions, including Bangladesh, Malaysia, India, New Guinea, China, Myanmar.[7-8] Additionally, because of its pharmacological benefits, locals commonly utilize it as medication to treat a variety of illnesses, such as bruising, diarrhea, and hepatitis. Furthermore, *S. apetala* is recommended as an anti-inflammatory medication by traditional healers in Bangladesh to lessen gastrointestinal issues such diarrhea, dysentery, and cramping in the stomach.[9-10] Research has shown that *S. apetala*'s fruits and bark can effectively treat fevers, hemorrhages, wheezing, swellings, ulcers, and sprains. Studies have demonstrated that fruit extracts from *S.* apetala shows a diverse biological fuctions, such as antioxidant, antibacterial, anti-diabetic, anti-cancer, and and antibacterial effects.[11] Major bioactive compounds present in this mangrove plant are flavonoids (Apigenin, Luteoline), carbohydrates, tannins (Catechin, Epicatechin), polyphenols (Sonneradon A, Ellagic acid, Gallic acid, Betulinic acid, Caffeic acid among others), and polyphenols.[12-13] These substances also help the plant exhibit antioxidant activity.

MATERIALS AND METHODS

Plant collection

The fresh fruits of *Sonneratia apetala* were collected in the month of September 2020 from the area of mangrove forest, Kolagachia, Shyamnagar, Satkhira. Identification of the gathered plant pieces was done at the Bangladesh National Herbarium, located at Mirpur 1, Dhaka, Bangladesh. The plant's fresh fruits were first washed with water to remove any dirt that stuck to them, then they were chopped into small pieces and let to sun-dry for seven days. Following thorough drying, the entire quantity was crushed to a coarse powder using a grinder and preserved in an airtight container for later use.

Plant materials extraction and fractionation

The powdered 300 gm of the fruits of *S.*

apetala was extracted three times using ethanol over the course of seven days, with sporadic shaking and stirring. Next the extracts underwent for filtering using cotton and filter paper. The extract was concentrated under at 50ÚC and it provided a greenish mass which was stored for further analysis. The final mass of the ethanolic extract was 2.85g.

Partition with *n*-Hexane

Water was added to the concentrated ethanol extract to create a slurry. After placing the slurry in a separating funnel, 40 milliliters of n-hexane were added. After giving the funnel a good shake and letting it stand for a few minutes, the top layer of n-hexane was collected. The procedure was carried out twice. The combined *n*-hexane extract was concentrated.

Partition with Chloroform

Following n-hexane extraction, 40 milliliters of chloroform were mixed to the aqueous solution, and the bulk was vigorously shaken in a separating funnel. For the layers to completely separate, the funnel was then left to stand for a few minutes. The lower, organic layer was gathered. Two repetitions of the procedure were made. The combined extract of chloroform was concentrated.

Partition with Ethyl Acetate

The aqueous layers left after chloroform extraction was again extracted with ethyl acetate in the same procedure as chloroform extraction. The combined ethyl acetate extracts was concentrated.

Antibacterial assay

Eleven different bacteria were screened for antibacterial activity using the disc diffusion bioassay method. The test samples were measured and dissolved in a determined volume of solvents to create solutions with known concentrations (mg/ml). Filter paper discs (6 mm in diameter) that had been dried and sanitized were then applied with known concentrations of the test chemicals using a micropipette. On nutrient agar medium that had been evenly inoculated with the pathogenic test microorganisms, discs holding the test materials were put. Blank discs (impregnated with solvents) and standard antibiotic discs (Ciprofloxacin 30ìg/disc) served as positive and negative controls, respectively. For a whole day, these plates were then maintained at a low temperature (4°C) to maximize diffusion. For 24 hours, the plates were then placed for incubation at 37°C to get maximum proliferation of the organisms. The components of plants fruit extracts' antibacterial properties prevented the microbes from growing, and a distinct and transparent zone of inhibition was observed around the medium. The test agent's antibacterial activity was estimated by measuring the zone of inhibition.[14-16]

Cytotoxicity screening

A common bioassay for bioactive chemicals is brine shrimp lethality. Here, *Artemia salina*, a test organism, served as a practical screening monitor. Brine shrimp eggs were procured from pet shop in Dhaka, Bangladesh, and these eggs were incubated for 48 hours in lab made simulated seawater (3.8% NaCl solution) to produce nauplii. For the assay, test samples (extracts) were dissolved in dimethyl sulfoxide (DMSO) and then diluted with artificial seawater to prepare concentrations of 5, 10, 20, 40, and 80 µg/ml. The control group consisted of a vial containing 50 µl of DMSO which is then diluted to 5 ml with 3.8% NaCl solution. Vincristine sulfate served as the standard in this assay. Each vial, including the control and test vials, was loaded with 10 brine shrimp nauplii.[17] Upon completion of incubation time at room temperature, the test tubes were checked for counting and the number of surviving nauplii was recorded. The mortality percentage was calculated for each concentration.[18-19]

Screening for antioxidant activity

The antioxidant effect of *S. apetala* fruit extracts in ethyl acetate, n-hexane chloroform and was assessed using both qualitative and quantitative assays considering the capability to scavenge the stable DPPH free radical.[20]

Qualitative assay

To identify the polar and non-polar substances of the extracts, TLC plates (pre-coated with silica gel) were spotted with appropriate diluted stock solutions and then kept in solvent systems of varying polarity (polar, medium polar, and non-polar). 0.02% DPPH in ethanol was sprayed on the plates after they had been let to dry at room temperature. After 10 minutes of DPPH bleaching, the identified band's changes of color (yellow on a purple backdrop) were seen.

Quantitative assay

S. apetala leaf extract's antioxidant capacity was assessed using 1, 1-diphenyl-2-picrylhydrazyl reactive species free radical

scavenging test. For titrating the oxidizable groups of synthetic or natural antioxidants, DPPH provides a quick and precise technique. The solution of DPPH was made in 95% methanol. 5 mg 50 mL^{-1} was the stock solution produced by mixing the raw extracts of *S. apetala* with 95% methanol. 100ìg mL^{-1} was selected as the concentration of the sample solutions. By diluting the stock solution with methanol, the test samples were made, achieving concentrations of 20 g/ml, 40 g/ml, 60 g/ml, 80 g/ml, and 100 g/ml, respectively. *S. apetala* leaf extracts were placed in each of these test tubes with freshly made DPPH solution, and after 20 min, at 517 nm the absorbances were taken. The positive control that was employed was ascorbic acid. The control solution was the DPPH solution without the sample solution. A blank of 95% methanol was utilized. In order to calculate the percentage of DPPH radical scavenging stated below:

$$\text{DPPH free radical inhibition} = [1-(Abs/Abc)] \times 100$$

Here, Abc stands for the control's absorbance and Abs for the sample solution's.

After that, the percentages of inhibitions were plotted against the corresponding concentrations, and the IC_{50} was computed from the graph. [21-22]

RESULTS

Antibacterial Activity

The partitionates of fruits of *S. apetala* were examined for their antimicrobial effect on various gram positive, gram negative bacterial and fungus species by disc diffusion method. Ciprofloxacin (30 µg/disc) standard antibiotic discs were utilized as standard for bacterial species and Fluconazole was used (30 µg/disc) standard antibiotic discs were utilized as standard for fungal species For study against Bacteria Agar medium was used. The zone of inhibition (mm) can be used to calculate the results. The activity of the applied sample increases with the size of the zone of inhibition.

The result of antimicrobial activity for different extracts against several microorganisms, including gram-positive and gram-negative bacteria and fungus are shown below in tables.

Minimum Inhibitory Concentration (MIC)

Any biological or chemical substance that causes death or inhibits the growth of microorganisms is referred to as an antimicrobial agent. These antimicrobial agents are used to save the life of the peoples from ancient time to present. Plant is a potent source of these antimicrobial agents. The activity of a plant extract as an antimicrobial agent can be determined qualitatively by antimicrobial test which is discussed in the above portion of this paper. But by performing the lowest effective conc. the activity of a plant extract as an antimicrobial agent can be determined quantitatively.

The lowest effective concentration of a drug or substance that may inhibit an organism's growth is known as the minimum inhibitory concentration, or MIC. Here MIC test is performed by Serial tube dilution technique or turbid metric assay. The serial dilution assay quantities the anti microbial activity of the crude extract by providing the MIC value of the drug for specific organism. It is an important consideration for further development of bioactive compounds.

In this method a large number of autoclaved test tubes containing sterile nutrient broth medium were used. A 10µl suspension of test organisms was taken in the test tubes and thoroughly mixed, and test samples were employed in a range of concentrations (in serial dilution from 1-256µg/ml). Here the MIC test of only the chloroform extract of *S. apetala* was performed against Gram-negative bacteria *Pseudomonas aeruginosa*.

Three control test tubes designated as C_S, C_M and C_I were used to perform antimicrobial test which contains test sample and nutrient broth medium, only nutrient broth medium, and inoculums and nutrient broth medium respectively. Then the test tubes were incubated at 37.5ºC for 24 hrs.

After complete incubation of 24 hrs, growth of organism was observed only in the C_I test tube among the control test tubes. The C_S and C_M showed no growth of organisms. The other test tubes were compared with them and the concentration up to which no growth of organism

was observed was determined. This concentration is the Minimum Inhibitory Concentration of the test sample against the specific organism.

The result of that test is shown in following table.

Where NG, G means-

NG = No growth

G = Growth

The minimum inhibitory concentrations (MIC) of Chloroform extract of fruits of *S. apetala* was found to be 32µg which indicates that 32µg extract will be the minimum dose of antimicrobial activity for *Pseudomonas aeruginosa*.[23-24]

Cytotoxicity Activity Test

Brine Shrimp Lethality Bioassay is an important technique in the determination of cytotoxicity of bioactive compounds obtained

Table 1. Antimicrobial activity of *S. apetala* against gram positive bacteria

Test organism	Diameter of Zone of inhibition			
	n-Hexane extract (500µg/disc)	Chloroform Extract (500µg/disc)	Ethyl Acetate extract (500µg/disc)	Ciprofloxacin/ Fluconazole (30µg/disc)
Gram positive bacteria				
Sarcina lutea	-	13	7	25
Staphylococcus aureus	-	9	6	30
Bacillus subtilis	-	13	6	30
Bacillus megaterium	-	11	6	32
Bacillus cereous	-	13	7	28
Gram negative bacteria				
Salmonella paratyphi	5	14	0	32
Vibrio parahemolyticus	-	9	6	32
Shigella dysenteriae	-	11	6	28
Pseudomonas aeruginosa	7	20	8	30
Escherichia coli	-	8	7	33
Fungi				
Saccharromyces cerevaceae	-	9	6	30
Candida albicans	5	14	6	32
Aspergillus niger	-	10	6	28

Table 2. Minimum Inhibitory Concentration of Chloroform extract of *S. apetala* against *Pseudomonas aeruginosa*

Serial No. of test tubes	Nutrient broth medium added (ml)	Concentration of chloroform of plant (µg/ml)	Inoculums added	Observation
1	1	256	10^7	NG
2	1	128	10^7	NG
3	1	64	10^7	NG
4	1	32	10^7	NG
5	1	16	10^7	G
6	1	8	10^7	G
7	1	4	10^7	G
8	1	2	10^7	G
9	1	1	10^7	G
C_S	1	256	00	NG
C_M	1	00	00	NG
C_I	1	00	10^7	G

from plant extract. Both toxicity and a variety of pharmacological property (such as anticancer, antiviral, insecticidal, and pesticidal properties) of the compounds are indicated. High dosages of bioactive chemicals are nearly always harmful. Here, brine shrimp nauplii, was given in vivo lethality as a handy way to screen the fractionations in order to find new bioactive natural compounds.

The extracts demonstrated positive results in this assay, suggesting that the chemicals exhibited biological activity. The results of this experiment demonstrated that lethality rates at various concentrations varied among the test samples. After a 24-hour exposure period, the lethality of *S. apetala*'s n-hexane, chloroform, and ethyl acetate fractions to brine shrimp was

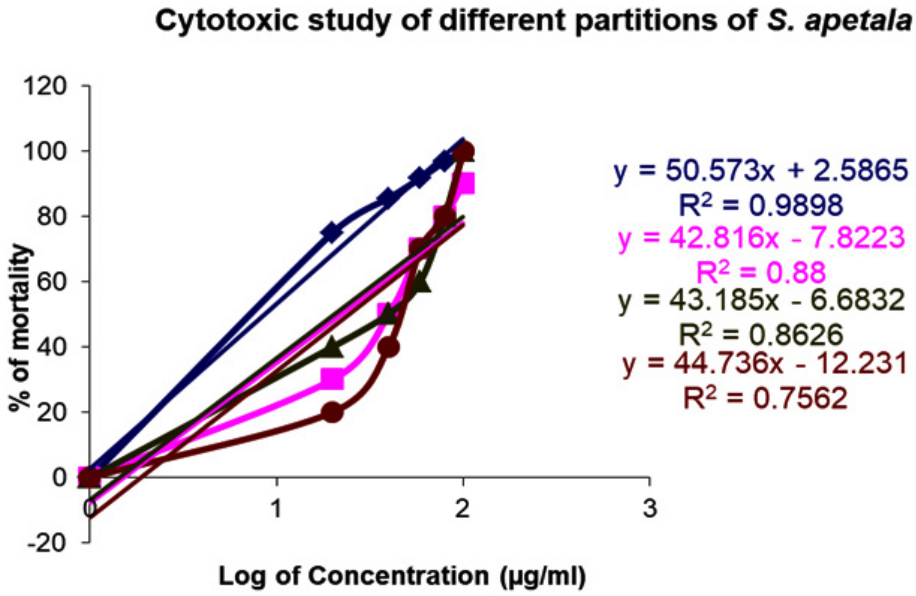

Fig. 1. LC_{50} values calculation for different crude extracts and standard vincristine sulphate of *S. apetala* fruits using a linear relationship between concentration logarithms and mortality percentage

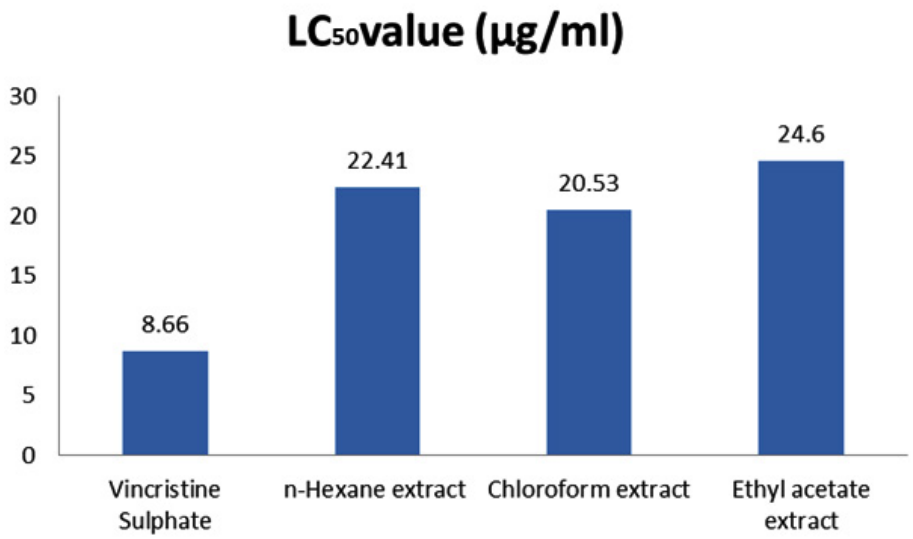

Fig. 2. LC_{50} data of test samples of *Sonneratia apetala*

assessed using vincristine sulphate as the reference. Increases in sample concentration were shown to increase the death rate of brine shrimp, and a graph showing the log of concentration vs percent mortality revealed a roughly linear relationship between the two. LC_{50} is the point where 50% of brine shrimp nauplii die and was calculated for the majority of the samples based on this graph. [25-26]

From the above graph the LC_{50} value of diiferent extracts of *S. apetala* and LC_{50} value of standard vincristine sulphate were obtained (Figure: 02).

Antioxidant Activity Test

One common free radical used to test a compound's or plant extract's first radical scavenging capability is DPPH.

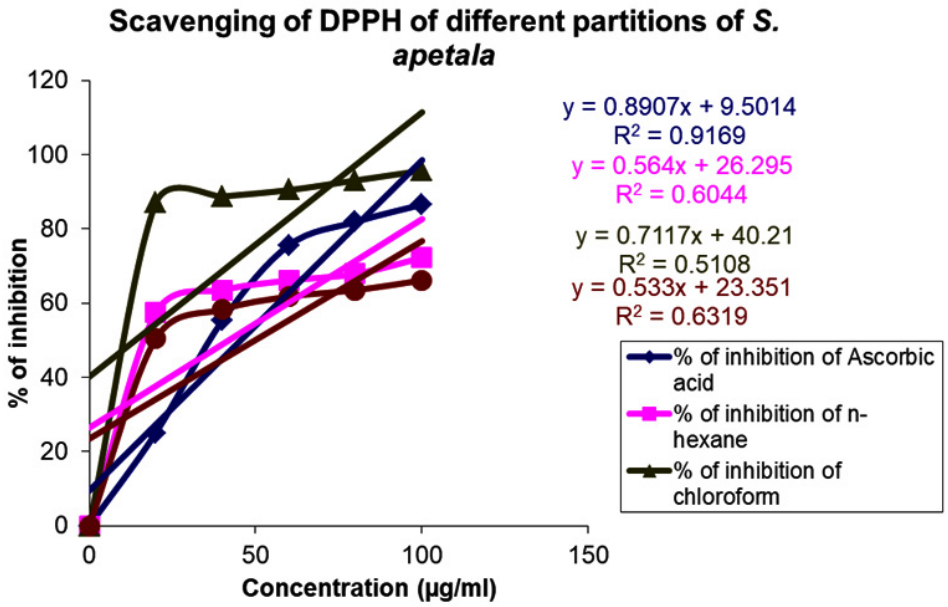

Fig. 3. IC_{50} value calculation for standard and different crude extracts of *S. apetala* fruits using a linear relationship between concentration (µg/ml) and the percentage of DPPH inhibition or scavenging

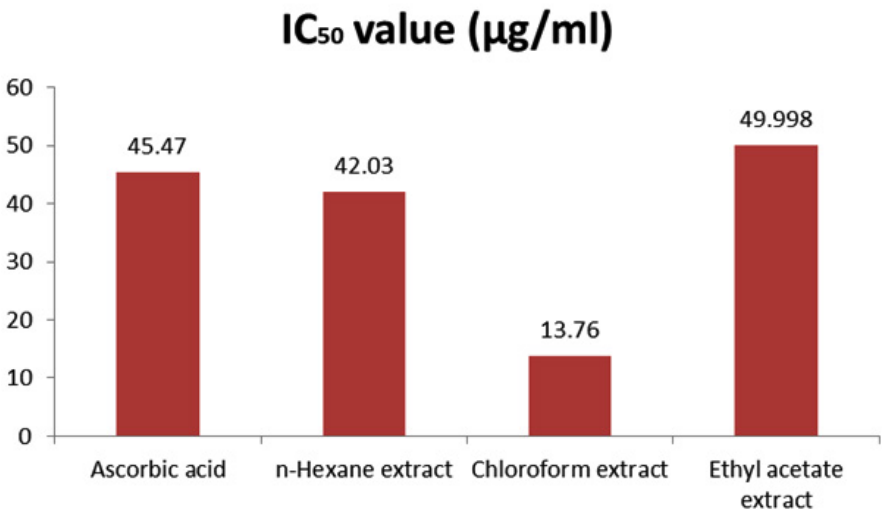

Fig. 4. IC_{50} data of of *S. apetala* (fruits) extracts and ascorbic acid

Table 3. Total phenolic content of *S. apetala* (fruits)

Serial No.	Extractives	Phenolic compound concentration
01	*n*-Hexane extract of plant fruits.	9.41
02	Chloroform extract of plant fruits.	26.88
03	Ethyl acetate extract of plant fruits.	13.92

The antioxidant activity of different partitionates of *S.apetala* is measured from the IC_{50} value. It is the value which indicates half of the maximum inhibitory concentration. IC_{50} value of an extract is usually calculated from the percentage of DPPH quenching by the respective extract at different concentrations.

Percentage (%) scavenging of the DPPH free radical quenching was calculated.

The following graph shows this.

From the above graph the IC_{50} value of the standard ascorbic acid and the *n*-hexane, chloroform, and ethyl acetate fractions of fruits of *S. apetala* can be obtained in the following figure.

The different partitionates of *S.apetela* fruits were subjected for the determination of the total phenolic content. The amount of total phenolic compounds in plant extracts was estimated using the Folin-Ciocalteu reagent (FCR). A sample's reducing capacity is measured by the FCR.

The total phenolic content in three different fractions were determined using the measured absorbances and the regression equation of the standard curve for gallic acid is-

$$y = 0.0162x + 0.0215$$

The total phenolic contents of the different extracts of the *S. apetala* are given in below table.

DISCUSSION

The partitionates of crude extracts of fruits of *S. apetala* were investigated against thirteen human pathogenic organisms to check antimicrobial activities by disc diffusion method. Here Ciprofloxacin (30 µg/disc) disc was used as reference standard for antibacterial test. The *n*-hexane partitionates of fruits part possess no significant antimicrobial activity on most of the gram positive, negative bacteria and fungi. The extract has shown antimicrobial activity against fungi of *Candida albicans* with zone of inhibition of 6 mm. The chloroform crude fraction of fruits part of *S. apetala* possessed antimicrobial activity with average zone of inhibition 9-20 mm against gram positive, negative bacteria and fungus. The maximum antibacterial activity was found against *Pseudomonas aeruginosa,* a gram negative bacteria. A moderate antibacterial activity was observed against *E.coli* (8 mm), *S. paratyphi* (14 mm) *S. boidii* (11 mm) and *V. paramolyticus* (9 mm).

Moderate activity with 13 mm zone of inhibition was observed against the gram positive S. *lutea, B. subtilis* and *B. cereus* while for *B. megaterium* zone of inhibition was 11 mm and for *S. aureus* it was 9 mm.The antifungal activity for the extract was mild to moderate with 9 mm to 14 mm as zone of inhibition. A mild activity with 5 mm-8 mm zone of inhibition was possessed by the ethylacetate partitionate of *S. apetala* fruit. A 14 mm zone of inhibition was showed against *Candida albicans*. A minimum antifungal activity against *Aspergillus niger* with zone of inhibition of 10 mm was showed by this chloroform extract.

The cytotoxic activity of leaves of *S. apetala* was tested by brine shrimp lethality toxicity. Here it was found that it possessed potent cytotoxic activity. The study showed that the LC_{50} value of leaves of *S. apetala* was 5.206 ± 0.093 mg/ml. Here in this study *n*-hexane, chloroform, ethyl acetate extract of *S. apetala* has shown the LC_{50} value of 22.41µg/ml, 20.53 µg/ml, 24.60 µg/ml respectively which was significant in comparison of standard LC_{50} value of vincristine sulphate which was 8.66 µg/ml.[27]

Here, Ascorbic acid was used as a standard in the antioxidant assay (by DPPH method) of *S. apetala*. The absorbances of three different partitionates of *S. apetala* fruits were taken at 517nm and their percentages of inhibitions were

measured by using the absorbance of ascorbic acid as a standard or control. IC_{50} value for standard ascorbic acid and crude *n*-hexane, chloroform, and ethyl acetate fractions of fruits of *S. apetala* were determined by using percentages of inhibition of different extracts from linear correlation between concentration (µg/ml) versus percentage of inhibition or scavenging of DPPH free radical .[28-29]

The IC_{50} value of ascorbic acid was observed 45.47µg/ml. The Crude extract of n-Hexane and ethylacetate fractiones have shown antioxidant value with IC_{50} value 42.03µg/ml and 49.998µg/ml respectively. *S. apetala*, has been identified to possess high antioxidant activity in all its parts. IC_{50} value for chloroform extract of *S. apetala* was obtained 13.76 µg/ml which represented an excellent antioxidant activity. [30-33]

The total phenolic content varies in amount in *S. apetala* fractions and the content varied from 9.41 mg to 26.88 mg GAE / gm of extractives. The chloroform partition exhibited the highest phenolic content (26.88 mg GAE/g). A substantial compound was also present in the ethyl acetate fraction (13.92 mg GAE/g). [34-36]

CONCLUSION

The present study highlights the therapeutic potential of *Sonneratia apetala* in antimicrobial, cytotoxic, and antioxidant applications. The research underscores the efficacy of its extracts in addressing bacterial infections, oxidative stress, and the need for safer cancer therapies.

The results of the antimicrobial screening showed that, although not as effective as common antibiotics like ciprofloxacin, the chloroform extract shown significant inhibitory effects against a variety of Gram-positive and Gram-negative bacteria and fungi. Specifically, the extract demonstrated a minimum inhibitory concentration (MIC) of 32 µg/ml against the bacterium *Pseudomonas aeruginosa*, indicating its potential as a supplemental treatment.

Significant activity was confirmed by cytotoxicity evaluation using the brine shrimp lethality assay; the chloroform extract's LC50 value of 20.53 µg/ml suggested that it could be a promising source of anticancer drugs. Despite being less effective than vincristine sulfate (LC50: 8.66 µg/ml), it nonetheless emphasizes the necessity of additional optimization and other combinatorial methods.

Antioxidant assays demonstrated significant free radical scavenging activity, particularly in the DPPH assay, indicating the extracts' potential as natural antioxidants. Identified bioactive compounds, such as polyphenols, tannins, and flavonoids, suggest their role in preventing oxidative stress-related diseases like cancer, diabetes, and cardiovascular disorders. These findings align with the demand for natural antioxidants in functional foods, nutraceuticals, and pharmaceuticals.

This study emphasizes the importance of conserving *S. apetala* for biodiversity and future research, particularly given the challenges of antibiotic resistance and synthetic drug side effects. The exploration of plant-based therapies offers a promising alternative, particularly for developing regions with limited access to affordable treatments. The findings support integrating traditional medicinal plants into modern pharmacology, paving the way for safer, sustainable, and accessible therapeutic options.

ACKNOWLEDGMENTS

Authors offer sincere gratitude to Department of Pharmacy of Southeast University, Bangladesh for all kind of assistance.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

Israt Jahan Bulbul created concept and supervised study, Yesmin Begum, Nisrat Jahan and Sabiha Ferdowsy Koly conducted the lab experiment, Nusratun Nahar drafted the manuscript, Md. Siddiqul Isalm reviewed the manuscript.

REFERENCES

1. Atanasov A. G., Zotchev S. B., Dirsch V. M., International Natural Product Sciences Taskforce, and Supuran C. T. Natural products in drug discovery: advances and opportunities. *Nat. Rev. Drug Discov*., 2021; 20: 200-216. doi: 10.1038/s41573-020-00114-z.
2. Newman D. J., and Cragg G. M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J. Nat. Prod*., 2020; 83: 770-803. doi: 10.1021/acs.jnatprod.9b01285.
3. Baba S. A., Vahedi M., Ahmad I., Rajab B. S., Babalghith A. O., Irfan S., and Hossain M. J. Crocus sativus L. tepal extract induces apoptosis in human U87 glioblastoma cells. *Biomed. Res. Int*., 2022; 2022: 4740246. doi: 10.1155/2022/4740246.
4. Hu R., Lin C., Xu W., Liu Y., and Long C. Ethnobotanical study on medicinal plants used by Mulam people in Guangxi, China. *J. Ethnobiol. Ethnomed*., 2020; 16(1): 40.
5. Mitra S., Lami M. S., Uddin T. M., et al. Prospective multifunctional roles and pharmacological potential of dietary flavonoid narirutin. *Biomed. Pharmacother*., 2022; 150: 112932.
6. Das R., Mitra S., Tareq A. M., et al. Medicinal plants used against hepatic disorders in Bangladesh: a comprehensive review. *J. Ethnopharmacol*., 2022; 282: 114588.
7. Patra J. K., Das S. K., and Thatoi H. Phytochemical profiling and bioactivity of a mangrove plant, Sonneratia apetala, from Odisha Coast of India. Chin. *J. Integr. Med.,* 2015; 21(4): 274-285. doi: 10.1007/s11655-014-1854-y.
8. Hossain S. J., Pervin T., and Suma S. A. Effects of cooking methods at different time durations on total phenolics and antioxidant activities of fresh and dried-stored fruits of *Sonneratia apetala* (Buch.-Ham.). *Int. Food Res. J*., 2016; 23: 556-563.
9. Shefa A. A., Baishakhi F. S., Islam S., and Sadhu S. K. Phytochemical and pharmacological evaluation of fruits of *Sonneratia apetala*. *Glob. J. Med. Res. B*., 2014; 14(3): 1-6.
10. Teja V. P., and Ravishankar K. Preliminary phytochemical investigation and in vitro antimicrobial activity of ethanolic extract of *Sonneratia apetala* plant. *Int. Res. J. Pharm*., 2013; 4(6): 84-87. doi: 10.7897/2230-8407.04619.
11. Jaimini D., Sarkar C., Shabnam A. A., and Jadhav B. L. Evaluation of antibacterial properties of mangrove plant *Sonneratia apetala* Buch.-Ham. leaf. *World Appl. Sci. J*., 2011; 14(11): 1683-1686.
12. Yi X., Jiang S., Qin M., Liu K., Cao P., Chen S., et al. Compounds from the fruits of mangrove Sonneratia apetala: isolation, molecular docking and antiaging effects using a Caenorhabditis elegans model. Bioorg. *Chem.,* 2020; 99: 103813. doi: 10.1016/j.bioorg.2020.103813.
13. Zahan S., Mowla T. E., Uddin S. M. N., Hossain M. K., Mannan A. B., Rahman M., et al. Evaluation of phytochemical and pharmacological properties of seeds of Momordica charantia. *Avicenna J. Phytomed*., 2020; 10(5): 448-459.
14. Bubonja-Šonje M., Kneževiæ S., and Abram M. Challenges to antimicrobial susceptibility testing of plant-derived polyphenolic compounds. Arh. Hig. Rada *Toksikol*., 2020; 71(4): 300-311. doi: 10.2478/aiht-2020-71-3396.
15. Balouiri M., Sadiki M., and Ibnsouda S. K. Methods for in vitro evaluating antimicrobial activity: a review. *J. Pharm. Anal*., 2016; 6: 71-79. doi: 10.1016/j.jpha.2015.11.005.
16. Matuschek E., Brown D. F., and Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clin. Microbiol. Infect*., 2014; 20: 0255-0266. doi: 10.1111/1469-0691.12373.17.
17. Ntungwe N. E., Domínguez-Martín E. M., Roberto A., Tavares J., Isca V. M. S., Pereira P., et al. Artemia species: An important tool to screen general toxicity samples. *Curr. Pharm. Des.*, 2020; 26(24): 2892-2908. DOI: 10.2174/1381612826666200406083035.
18. Coe F. G., Parikh D. M., Johnson C. A. Alkaloid presence and brine shrimp (*Artemia salina*) bioassay of medicinal species of eastern Nicaragua. Pharm. *Biol.*, 2010; 48(4): 439-445. DOI: 10.3109/13880200903168015.
19. Wakawa H. Y., Fasihuddin B. A. Brine shrimp

lethality bioassay of Abrus precatorius (Linn) leaves and root extract. *Int. J. Pharm. Pharm. Sci*., 2017; 9(1): 179-181.

20. Khatun A., Rahman M., Rahman M. M., Hossain H., Jahan I. A., Nesa M. L. Antioxidant, antinociceptive and CNS activities of Viscum orientale and high-sensitive quantification of bioactive polyphenols by UPLC. Front. *Pharmacol.*, 2016; 7: 176. DOI: 10.3389/fphar.2016.00176.
21. Rumzhum N. N., Rahman M. M., Kazal M. K. Antioxidant and cytotoxic potential of methanol extract of *Tabernaemontana divaricata* leaves. *Int. Curr. Pharm. J.,* 2012; 1(2): 27-31.
22. Bulbul I. J. Antibacterial, cytotoxic and antioxidant activities of n-hexane, chloroform and ethyl acetate extracts of *Cucumis sativus* leaves. *PharmaTutor*, 2014; 2(3): 280-284.
23. Bulbul I. J., Khadiza-tul-Kubra, Shimu M. C. Antibacterial, cytotoxic and antioxidant activities of n-hexane, chloroform and ethyl acetate extracts of *Trichosanthes cucumerina* leaves. *PharmaTutor*, 2016; 4(1).
24. Rahmatullah M., Mukti I. J., Haque A. K., Mollik M. A. H., Parvin K., Jahan R., et al. A randomized survey of medicinal plants used by folk medicinal healers of Sylhet Division, Bangladesh. *Adv. Nat. Appl. Sci.,* 2010; 4(1): 52-62.
25. Rahman M. A., Anwar N., Rahman M. A. Evaluation of cytotoxic and antioxidant activity of *Sonneratia apetala* (Buch-Ham.) leaves. *Int. J. Pharm. Pharm. Sci*., 2014; 6(1): 366-369.
26. Apu A. S., Bhuyan S. H., Khatun F., Liza M. S., Matin M., Hossain F. F. Assessment of cytotoxic activity of *Sonneratia apetala* leaves using brine shrimp as an experimental model. *Int. J. Pharm. Sci. Res.,* 2023; 15(3): 1125-1130.
27. Ali M. M., Mahmud M. S., Rahman M. M. Bioactive compounds from Sonneratia apetala and their cytotoxic effects: A brine shrimp lethality approach. *Adv. Tradit. Med*., 2024; 21(1): 57-65.
28. Haq I., Hossain A. B. M. S., Khandaker M. M., Merican A. F., Faruq G., Boyce A. N., et al. Antioxidant and antibacterial activities of different extracts and fractions of a mangrove plant-*Sonneratia alba. Int. J. Agric. Biol.,* 2014; 14: 707-714.
29. Hossain S. J., Iftekharuzzaman M., Haque M. A., Saha B., Moniruzzaman M. M., Rahman H. T. Nutrient compositions, antioxidant activity and common phenolics of *Sonneratia apetala* (Buch.-Ham.) fruit. *Int. J. Food Prop.,* 2015; 19(5): 1080-1092.
30. Linh T. M., et al. Fractionation of phenolic compounds from *Sonneratia apetala* pneumatophores and their bioactivities. *Vietnam J. Biol.,* 2023; 39(4): 451-456. DOI: 10.15625/0866-7160/v39n4.10708.
31. Liu J., et al. The protective effect of *Sonneratia apetala* fruit extract on acetaminophen-induced liver injury in mice. *Evid. Based Complement. Alternat. Med*., 2023; 2023: 6919834. DOI: 10.1155/2023/6919834.
32. Mai N. S., Quyen V. N., Phan V. H. T., et al. The wound-healing potential of *Sonneratia apetala* pneumatophores: A comparative study. *J. Ethnopharmacol*., 2024; 304: 116312. DOI: 10.1016/j.jep.2023.116312.
33. Waghulde, S., Kale, M.K. and Patil, V.R., Brine shrimp lethality assay of the aqueous and ethanolic extracts of the selected species of medicinal plants. *Proceedings.* 2019; 41 (1): 47.
34. Monira S., Islam A., Islam S., Koly S. F., Nesa L., Muhit A. Phytochemical screening and comparative antioxidant activities of fractions isolated from *Sonneratia caseolaris* (Linn.) bark extracts. *Eur. J. Med. Plants*., 2019; 28(4): 1-9.
35. Mukul M. E. H., Hossain M. S., Ahamed S. K., Debnath P., Akter M. Antioxidant and membrane stabilizing activities of bark of *Sonneratia apetala*. *Bangladesh Pharm. J.,* 2016; 19: 147-151.
36. Mukul M. E. H., et al. Phytochemical profiling and bioactivity of a mangrove plant, *Sonneratia apetala*, from Odisha Coast of India. Chin. *J. Integr. Med*., 2023. DOI: 10.1007/s11655-023-3470-x.