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Investigation of Larvicidal Activity and Histopathological Variations of Brown Algae *Spatoglossum asperum* J. Agardh against *Aedes aegypti, Culex quinquefasciatus,* and *Anopheles stephensi*

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ABSTRACT

The purpose of the current study was to determine whether the methanolic extract of *Spatoglossum asperum* has larvicidal properties against *Aedes aegypti, Culex quinquefasciatus*, and *Anopheles stephensi*. Various doses of methanolic seaweed extract (50, 100, 150, and 200 ppm) were applied to chosen larvae in the early 4th instar. Each test was carried out in triplicate, with a control group present simultaneously. The mortality rate was observed at regular 24 hr intervals. FTIR and GC-MS studies analyzed the methanolic extract's functional groups and phytochemical compounds. The standard plate procedure was used to test the antibacterial capacity using clinically isolated pathogens; the extract effectively killed bacteria of all types, according to findings. FTIR peaks confirmed the presence of carbonyl groups, amino acids, hydroxyl groups, and alkynes. The GC-MS study of the seaweed extract found 14 major bioactive compounds, such as citronellol and diethyl phthalate, found to have insecticidal, nematocidal, antibacterial, antioxidant, cytotoxic, antifungal, and hepatoprotective properties. DPPH radical scavenging and nitric oxide assays showed that the efficacy grew as the extract level was raised. Total phenol and flavonoid content of the methanol extract was estimated at 73.2±02 µg/mL and 89.1±0.03 µg/mL, respectively. Against 4th instar *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. stephensi* larvae,

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methanolic extract of *S. asperum* showed LC_{50} values of 2.56 mg/L, 3.32 mg/L, and 2.84 mg/L, respectively. These studies suggest methanolic extracts of *S. asperum* have more potential use as a larvicidal agent against vectors of *Aedes, Culex*, and *Anopheles* mosquitoes.

Keywords: Antibacterial; Histopathological; Larvicidal; Methanol extract; Seaweed

1. Introduction

Mosquitoes are the most common disease-carrying insects, transmitting diseases such as malaria, chikungunya, typhoid, and dengue. The proliferation of these mosquitoes and their global distribution and the diseases they transmit significantly contributed to total mortality rates [1]. *Aedes albopictus, Anopheles, Mansonia,* and *Culex* are important disease vectors. According to the National vector control board in India, 30,627 cases of dengue fever and 15 million cases of malaria have been reported, and deaths range from 19,500 to 20,000 each year as reported by the World Health Organization [2]. Control of mosquitoes is significant in stopping the propagation of vector-borne illnesses. Although many malaria vaccines are being developed, nothing is presently accessible. Using chemical mosquito repellents causes environmental damage, and resistance can harm humans [3]. Syed et al. [4] studied the effect of kidney cell degeneration on albino mice using mosquito coils. They concluded that d-trans allethrin coming from mosquito coils is hazardous and changes creatinine and serum urea levels in 10 weeks of observation. Continuous chemical insecticides are poisonous to humans and harmful to other living organisms. Alternatives now focus on finding environmentally friendly and cost-effective mosquito repellents [5].

Humans have used plants for centuries against these deadly pathogens and other pests. Numerous studies have revealed larvicidal, pest development inhibiting, and deterrent activities of phytochemicals produced by plants [6]. The ocean contains many types of natural resources. The sea's nearly 5,000,000 lifeforms are a practically untapped source of secondary compounds [7]. As a result, experts are exploring how to kill insects with plants and seaweed. Seaweeds are abundant in bioactive substances and compounds with therapeutic properties. They have been shown to affect insect metabolic activity through intoxication, morbidity, growth and development changes, morphological changes, oviposition, and reproduction system changes [8].

Marine algae, known as seaweeds, typically grow on the seafloor between the high and low tide marks. These algae are multicellular, polyphyletic, and oxidative. Seaweeds and other marine algae prefer rocky shores in the ocean's littoral zone for their growth [9]. More than 60 trace elements are present in marine algae at concentrations several times higher than those in higher ecosystems and these elements have shown anxiolytic and antidepressant action [10]. Elements like sodium, potassium, calcium, phosphorus, as well as vitamins are plentiful in marine algae. They contain high concentrations of the nine essential amino acids necessary for optimal wellness [11].

Spatoglossum asperum is a brown seaweed distributed all over the world. Seaweeds have been reported for their antibacterial [12-16], antioxidant [17-20], anticancer [21-25], anti-ulcer [26-30], anti-diabetic actions [31-35], and wound healing [36, 37] activities. Some seaweed-derived bioactive compounds are mosquitocidal as well [8]. *Lobophora variegate, Sargassum wightii, Ulva lactuca,* and *Caulerpa racemosa* are some species that have been reported to display anti-larvicidal activity against mosquitoes.

The brown seaweed *S. asperum* was extracted with methanol for this study to identify the extract's bioactive compounds and functional groups. By using the hole plate process, the resultant extract's antimicrobial

efficacy was examined towards microorganisms. In the larvicidal investigation, *Anopheles stephensi, Culex quinquefasciatus*, and *Aedes aegypti* were targeted. Changes in histology and death rates were also analyzed.

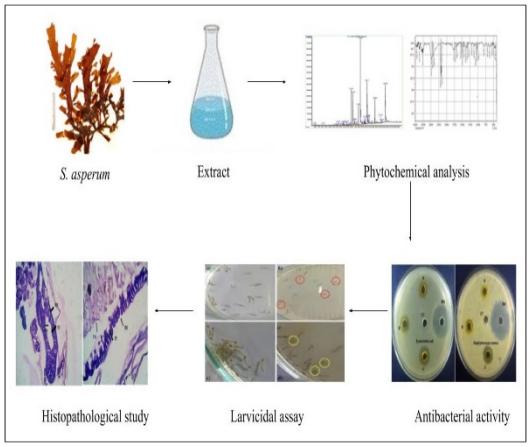


Fig. 1. Research methodology.

2. Materials and Methods

2.1 Collection and identification of seaweed

S. asperum, brown algae, was gathered from the Gulf of Mannar's Mandapam shorelines. The obtained seaweed was identified by Dr. Palanisamy, Scientist 'E' Botanical Survey of India, West Bengal. The herbarium of the collected sample is stored at Thiruvalluvar University (Fig. 1.).

2.2 Extract preparation

The gathered material was washed with sterile water and dried for 15 days in the shade. To grind the dehydrated specimens, a mechanical mixer was used, followed by a sieve. Next, 10 grams of the obtained powder were macerated with 50 mL of methanol for 48 hrs under a continuous stirrer. After that, Whatman No. 1 filter sheet was used to filter the samples. Phytochemical analysis was performed on the filtrate and its anti-bacterial and larvicidal activities were observed.

2.3 Phytochemical analysis

The goal of this common testing procedure was to detect the existence of several botanical constituents. The methanolic extract of *S. asperum* was previously submitted for preliminary phytochemical screening [38].

2.4 FTIR analysis

The FTIR spectrum of the methanolic seaweed extract of *S. asperum* was analysed to learn more about the functional groups found in the seaweed. When undiscovered chemicals are discovered in plants, spectroscopy can be a valuable tool for elucidating their structures. The crude methanolic extract was subjected to FTIR analysis, and thus the extract was loaded in an FTIR spectroscope (FTIR-8400S, Shimadzu) with an analysis range from 500 to 4000 cm^{-1}.

2.5 GC-MS analysis

GC-MS analysis of methanolic *S. asperum* extract was performed using GCMS-QP 2010 Plus Shimadzu system. Identification and interpretation of phytocompounds on mass spectrum GC-MS were conducted using the NIST and WILEY8 Archives. The spectra of the unknown constituents were contrasted to spectra of known compounds in the NIST collection. The compounds which were spotted were given names, molecular formulas, and structures.

2.6 Total phenolic and flavonoid content

Employing standards (gallic acid and quercetin), the total phenolic content (TPC) and total flavonoid content (TFC) was established. The procedure from Singelton et al. [39], with some minor alterations, was applied to determine the TPC of various concentrations of seaweed extract (20 µg/mL-100 µg/mL) which were made using sterile water. A mixture of 3.4 ml of 30% methanol, 0.15 mL of $NaNO_2$ (0.5 M), and 0.15 mL of $AlCl_3.6H_2O$ (0.3 M) were combined with 0.3 mL of sample. After 5 minutes, 1 mL of NaOH (1 M) was added. The solution was thoroughly mixed, then absorption was read at 506 nm and compared to blank reagent. TFC was computed using a modified version of Park et

al.'s [40] formula. In a tube, 0.1 mL of test samples (20 µg/mL-100 µg/mL) were added together with 1.9 mL of distilled H_2O, 1 mL of Folin-Ciocalteu's reagent, and 1 mL of 100 g/L Na_2CO_3 to stop the process. The mixture's absorbance was gauged at 765 nm after the reaction mixture had been set aside for 2 hrs at 25°C.

2.7 Antioxidant activity

2.7.1 DPPH assay

The antioxidant capacity of extracts was estimated by UV spectrophotometer against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. For this task, the technique of Prieto, 1999 [41] was applied. In methanol, 0.1 mM solution of DPPH was added, then 1 mL of the resulting mixture was mixed with 1 mL of extract at different levels (100-1000 µg/mL). After stirring, the liquid was left alone for 30 minutes at room temperature. The absorbency was obtained at 517 nm. Ascorbic acid was used as a baseline. Higher energy is indicated by lower absorbance levels.

To determine the % of antioxidant production, the following calculation was employed.

$$\%\ inhibition = \left(\frac{A_C - A_E}{A_C} \right) \times 100, \qquad (2.1)$$

where A_C is the efficiency of the control and A_E is the efficiency of the tested sample.

2.7.2 Nitric oxide scavenging activity

Phosphate buffer (pH 7.4) was used to prepare 10 mm sodium nitroprusside. 1 mL of leaf extract at various concentrations was combined with 0.5 mL of sodium nitroprusside solution. The reaction mixture was incubated for 180 minutes at 25ºC. Then, 0.5 mL of the incubated sample was combined with 0.5 mL of Griess reagent (1% sulphanilamide, 2% H_2PO4, 0.1% N-1-Naphthyl Ethylenediamine Dihydrochloride). As a control, the reagent without extract was used. The absorbance was measured at 546 nm. Ascorbic acid was used as a standard. The formula (Patel, 2010) [42] was used to figure out the % obstruction.

$$\%\ Scavenging\ activity = \left(\frac{A_C - A_t}{A_C} \right) \times 100, \ (2.2)$$

where A_C stands for the control's efficiency and A_t for the test's efficiency.

2.7.3 Reducing power activity

Srinivasan et al.'s [43] technique was used to find the reducing power. Several concentrations of extract (100-1000 µg/mL) were combined in 1 mL of distilled water, 2.5 mL of phosphate buffer (pH 6.6), and 2.5 mL of potassium ferricyanide (1% w/v). For 20 mins, the resulting mixture was left to stand at 50ºC. After 20 mins, 2.5 mL of 20% Trichloroacetic acid was added to halt the process. For 10 mins, the mixture was mixed at 3,000 rpm. 2.5 mL of sterile water and 0.5 mL of ferric chloride (0.1%) were combined with 2.5 mL of the mixed sample, and intensity at 700 nm was obtained. Enhanced mixture absorbance suggested higher reducing power. Ascorbic acid was utilized as a reference. As a control, phosphate buffer (pH 6.6) was utilized.

2.8 Antibacterial activity of the extract

The methanolic extract of *S. asperum* was assessed for its antimicrobial efficacy against Gram-positive *Staphylococcus epidermidis, Bacillus subtilis, Micrococcus luteus, Streptococcus pneumoniae,* and Gram-negative *Escherichia coli, Klebsiella aerogenes, Salmonella typhimurium,* and *Vibrio parahaemolyticus.* These microbes were collected from Christian Medical College (CMC), Vellore. 20 mL of Muller Hinton Agar (MHA) (Hi-Media, Mumbai) was put into sterilized Petri dishes. Various concentrations of sample (25, 50, 75, and 100 mg/mL) were loaded in wells. Streptomycin (10 µg/disc) was used as a positive control. The loaded wells were left for 30 mins at room temperature for compound diffusion. For 24 hrs, the dishes were maintained at 37°C. The circumferences

of the zones of inhibition were assessed, and the mean values for every organism were ascertained [14]. The experiment was repeated in triplicate.

2.9 Larvicidal study

Laboratory strains of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus* were received from ICMR, VCRC, at Madurai. All procedures for larvicidal assays followed the standard WHO protocol. In a laboratory setting, the larvae were raised on plastic crates and served yeast and dog biscuits in a 1:2 ratio. Young 4th instar mosquito larvae were delivered in sets of 25 into 100 mL water-filled disposable cups. Various concentrations (50, 100, 150, and 200 ppm) of methanolic seaweed extract were introduced. Cups without any methanol extract added were used as a control. The setup was observed for 72 hrs. The mosquito death rate was recorded after 24 hrs of treatment. All experiments were done in triplicate under the precise temperature of 25°C± 2°C and relative moisture level of 75%−85%. Dead larvae were identified by lack of movement.

2.10 Histopathological analysis

The midgut epithelial cell changes were observed after the treatment. The treatment and control of mosquito larvae of *Cx. quinquefasciatus, Ae. Aegypti*, *and An. Stephensi's* 4th instars were preserved in 10% formalin. After being fixed, the samples were dehydrated and mounted in paraffin. Using a microtome, the prepared slides were sectioned. Eosin and hematoxylin were used for staining. The slides were cleaned with xylene, the pathological alterations of the sections were observed under light microscopic, and the changes were photographed. The obtained results were compared with the control group.

2.11 Statistical analysis

The information is displayed as average and standard deviation. The paired-sample t-test was implemented to establish the significance of the data. The comparisons were evaluated at various times using ANOVA with repeated measurements. We conducted our data analysis using SPSS® version 20. *P* values ≤ 0.05 were considered significant. The LC_{50} values were measured using Probit analysis tool.

3. Results and Discussion

3.1 Phytochemical analysis

The secondary compounds found in the methanolic extract of *S. asperum* are given in Table 1. The presence of tannins, saponins, flavonoids, alkaloids, and carbohydrates in the present study of *S. asperum* is supported by several others. Andrews et al. [44] reported that the methanolic leaf extract of *S. asperum* contains tannins, phlobatannins, saponin, flavonoids, terpenoids, alkaloids, proteins, and polyphenols. *S. marginatum* has therapeutically active ingredients, as shown by the phytochemicals present in methanolic extract such as tannins, flavonoids, steroids, terpenoids, triterpenoids, and alkaloids [45]. Rajakumari and Kaleeswari [46] found substantial phytochemical substances in the methanolic extract of marine *Sargassum wightii* such as carbohydrates, proteins, phenol, saponins, glycosides, steroids, terpenoids, and alkaloids, and they concluded that it must substantiate the assertions of the health care sector and indigenous medicine that these brown seaweeds exhibit biomedically significant activities. The methanol extract of *Padina boergesenii* contains flavonoids, saponins, terpenoids, carbohydrates, phenolics, and tannins [47]. The brown seaweed *Spatoglossum squarrosum* was extracted with methanol, and phytochemical testing confirmed the presence of alkaline substances, terpenoids, polyphenolic compounds, tannins, steroids, and reducing carbohydrates [48].

Table 1. Phytochemical screening of methanolic extract of *S. asperum.*

Test	Reagents used	Result
Tannins	Ferric chloride	+
Phlobatannins	1% Aqueous HCl	-
Saponin	Distilled water	+
Flavonoids	Sodium hydroxide	+
Steroids	Phytochemical screening of methanolic extract of S. asperum the test steroid Salkowski test	-
Alkaloids	Dragendroff's reagent	++
Protein	Millon's reagent	-
Carbohydrate	Benedict's reagent	+
Terpenoids	Chloroform Con. Sulphuric acid	-

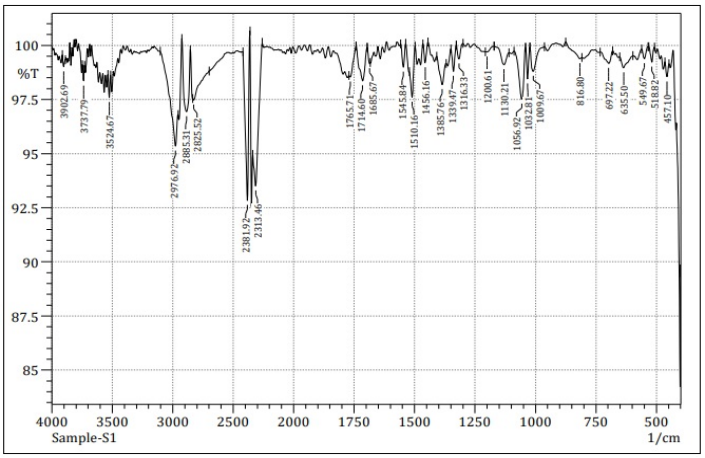


Fig. 2. FTIR investigation of methanolic extract of *S. asperum.*

3.2 FTIR analysis

FTIR outcomes revealed the occurrence of various functional groups in the methanolic extract of *S. asperum* (Fig. 2). The peak at 3524.67 cm^{-1} was assigned to OH-stretching of vibration presence of alcohols and phenols. The rise at 2885.92 cm^{-1} was given to C-H stretching of alkanes, 2976.92 cm^{-1} was given CH and CH_2 trying aliphatic group, the peak range at 1685.67 cm^{-1} -C-C indicated the presence of alkanes, the peak at 1510.16 cm^{-1} represented polyphenol skeletal structure, the peak at 1456.16 cm^{-1} indicated C-C stretching of aromatics, the peak value at 1200.61 cm^{-1} signified C-O extension of alcohols, carboxylic esters, and ethers, the range at 697 cm^{-1} assigned -C (triple bond) C-H: C-H band presence of alkynes. The methanol extract of *S. asperum* had a weak band at 3902.69 cm^{-1}, 3737.79 cm^{-1}, and 1545.81 cm^{-1}, which indicated the presence of O-H stretching of alcohols. According to Yu et al. [8], FTIR investigations on algae and seaweed compounds indicated that the walls of algae had hazardous reaction sites of carbonyl groups, amino acids, and hydroxyl groups.

3.3 GC-MS analysis

A methanol derivative of *S. asperum*'s 14 physiologically active components have been determined by GC-MS. Retention time, molar mass, chemical formula, and biological properties of the identified compounds are given in Table 2 and Fig. 3. The most critical phytoconstituents present in the extracts were dimethyl sulfoxide (0.71), nonadecane (0.20), cyclododecane (0.21), eicosane (0.88), diethyl phthalate (4.94), pentadecanoic acid (0.59), gamolenic acid (1.67), phytol (2.44), vaccenic acid (17.03), octadecanoic acid (2.07), hexadecenoic acid (1.81), citronellal (0.65), and fucosterol (18.39). All of the bioactive

compounds were found to possess nematocidal, insecticidal, repellent, antibacterial, antifungal, cytotoxic, antioxidant properties, and properties for treating skin disorders. Citronella oil derived from citronellol is the most commonly used mosquito repellent. Diethyl phthalate and octadecanoic acid have nematocidal, insecticidal, and pesticidal activities. As reported by Manh et al. [49], the essential oil of *Cymbopogon winterianus,* also called Citronella oil, was tested against 3rd instar dengue parasite *Ae. aegypti*, and the LC_{50} value was found to be 120.6 ppm. The toxicity effect of citronella oil was evaluated against the 3rd stage of *Ae. aegypti* by Cansian et al. [50], and the LC_{50} value was 86.30 µg mL^{-1}; they reported that the tested samples can be used as bioinsecticides. Adsul et al. [51], derived phthalate from the extract of *Ipomoea carnea*, and the isolated compound was found to be lethal to the 4th instar stages of *Ae. aegypti* and *Cx. quinquefasciatus* with the LC_{50} found to be 81.43 and 109.64 ppm, respectively.

Table 2. GC-MS investigation of methanolic extract of *S. asperum.*

S. No	Retention time	Compound name	Molar mass	Chemical formula	Biological properties
1	5.766	Dimethyl sulfoxide	78.13 g/mol^{-1}	C_2H_6OS	Antibacterial, antifungal
2	11.630	Nonadecane	268.518 g/mol	$C_{19}H_40$	Antimicrobial
3	12.956	Cyclododecane	168.319 g/mol	$C_{12}H_{24}$	Antibacterial
4	14.776	Eicosane	282.5475 g/mol	$C_{20}H_{42}$	Antifungal, antipyretic, analgesic
5	15.975	Diethyl phthalate	222.24 g/mol	$C_{12}H_{14}O_4$	Antiviral, antioxidant, insecticidal, cytotoxic, antihyperglycemic, antibacterial, antifungal activity
6	17.542	Pentadecanoic acid	242.3975 g/mol	$C_{15}H_{30}O_2$	Antioxidant
7	19.021	Gamolenic acid	278.43 g/mol	$C_{18}H_{30}O_2$	Atherosclerosis, anti-cancer, diabetic complications, skin disorders
8	19.734	Phytol	296.53 g/mol	$C_{20}H_{40}O$	Antibacterial, anxiolytic, metabolism-modulating, Anticancer, radical scavenging activity, phagocytosis and cell death-inducing, antinociceptive, anti-allergic, immune-modulating, and neurotoxic effects
9	20.371	Oleic acid	282.47 g/mol	$C_{18}H_{34}O_2$	Antibacterial, antiviral, antifungal, and antioxidant
10	20.371	Vaccenic acid	282.461 g/mol	$C_{18}H_{34}O_2$	Antibacterial
11	20.656	Octadecanoic acid	284.48 g/mol	$C_{18}H_{36}O_2$	Antioxidants, hypocholesterolemic, nematicide, and pesticide
12	24.079	Hexadecenoic acid	254.414 g/mol	$C_{16}H_{30}O_2$	Antioxidant, anti-inflammatory, hypo-cholesterolemic, and cancer prevention activities
13	25.455	Citronellol	154.25 g/mol	$C_{10}H_{18}O$	Insect repellent, antifungal, anti-inflammatory
14	25.455	Fucosterol	412.69 g/mol	$C_{29}H_{48}O$	Cytotoxic, antidiabetic, antioxidant, hepatoprotective, antihyperlipidemic, antifungal, antihistaminic, anticholinergic, antiadipogenic, anti-photodamaging, anti-osteoporotic,

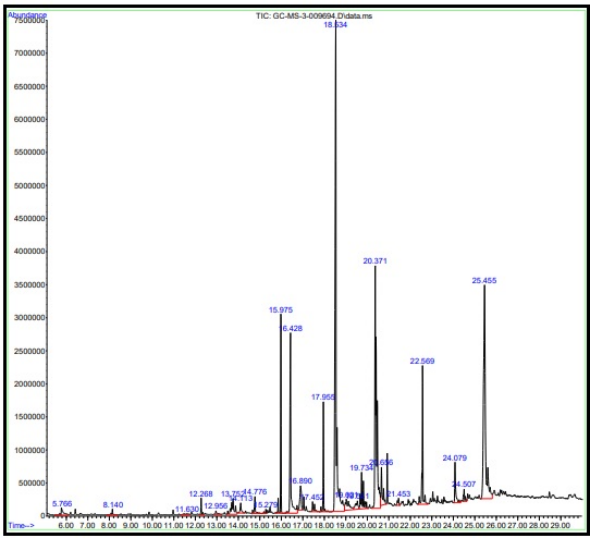


Fig. 3. GC-MS investigation of a methanolic extract of *S. asperum.*

3.4 Total phenol and flavonoid content

The numerous beneficial biological impacts of flavonoids and phenolic compounds have led to substantial research on these compounds [52]. According to the quantitative examination of plant chemicals, *S. asperum* methanol extract is rich in flavonoids and phenolics. In Table 3, it is shown that the methanolic extract of *S. asperum* has an elevated phenol and flavonoid profile. Methanol's capacity to block polyphenol oxidase activity, which is responsible for the oxidation of polyphenols, makes it an ideal solvent for the extraction of polyphenolic substances [53]. The methanolic algal extract had a flavonoid content of 89.1±0.03 mg QE/g and the phenol content was 73.2±0.2 mg GAE/g. Tanna et al. [54], estimate the phenol and flavonoid content of the brown algae *S. asperum* and the TPC and TFC quantities were 15±3 mg/mL−1, 340±60 mg/mL^{-1} they concluded that *S. asperum*, brown algae, has a higher total phenolic content and total flavonoid content than other brown marine algae. *Sargassum, Turbinaria* and *Padina* species were examined for their total phenolic content by Sinjel et al. [55] and the result shows that *Padina Spp* 0.135 mg GAE/g had the greatest total phenolic content, followed by *Sargassum Spp* 0.084 mg GAE/g and *Turbinaria Spp* 0.004 mg GAE/g with the lowest.

Table 3. Total phenol and flavonoid content of methanolic extract of *S. asperum.*

	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
Flavonoid	45.1±0.03	52.1±0.05	68.73±0.23	77.5±0.04	89.1±0.03
Phenol	20.2±2.9	46.0±3.6	50.4±0.6	66.4±0.5	73.2±0.2

3.5 Antioxidant activity

3.5.1 DPPH scavenging activity

DPPH assay represents one of the best ways to assess the effectiveness of antioxidants. In the current research, the *S. asperum* methanol extract was found to have potent antiradical action at therapeutic doses. Fig. 4 shows the result of methanolic extract of *S. asperum*. The antioxidant activity of *S. asperum* was lower than the standard gallic acid. A stable non-radical form of DPPH is produced when a DPPH solution is combined with a substrate that acts as a hydrogen atom donor, and the violet hue simultaneously changes to pale yellow. The methanolic extract of *S. asperum* showed the greatest efficacy of 57% at 400 µg [54]. The highest DPPH activity was 79.1±1.21% in the brown seaweed *S. wightii*, which is relatively high when compared to red and green algae. Chakraborty

et al. [57] studied the DPPH activity of the brown seaweed *T. ornata* and an efficacy of 64.14% was recorded. Airanthi et al. [58] examined the DPPH activity of two brown seaweeds, *S. horneri* and *C. Hakodatensis,* from coastal regions of Japan. The marine algaes were extracted with methanol and the DPPH activity of *S. horneri* and *C. hakodatensis* were 28.50 ± 0.75 µg/mg and 65.32 ± 3.95 µg/mg, respectively.

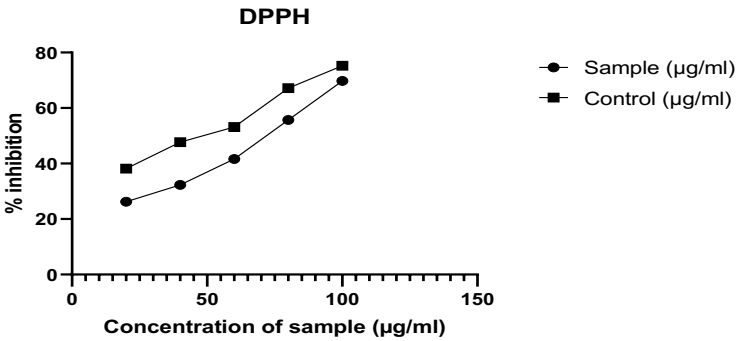


Fig. 4. DPPH activity of methanolic extract of *S. asperum*.

3.5.2 Nitric oxide scavenging assay

The greatest amount of suppression was seen in the methanolic extract of *S. asperum*, at 51.1±0.03. It was shown that the action was dose-dependent. Vitamin-C was used as a standard. Nitrite formation is inhibited by algae because they use oxygen for their own cellular processes instead of allowing it to interact with nitric oxide. The structure and function of numerous cellular components might be altered as a result of these chemicals. Chia YY et al. [53] evaluated the antioxidant activity of the brown macro algae *T. ornata* and found that the methanolic extract had the most significant degree of restriction, at 54.96±0.41%. The brown algae *S. wightii* collected from the Mandapam coastline, was subjected to methanolic extraction and tested for its antioxidant activity. The results showed it exhibited 60.22±0.52% inhibition [59]. Sodium nitroprusside (SNP) produces NO, which interacts immediately with oxygen to form nitrite. Fig. 5 shows the results of the methanol extract of *S. asperum.*

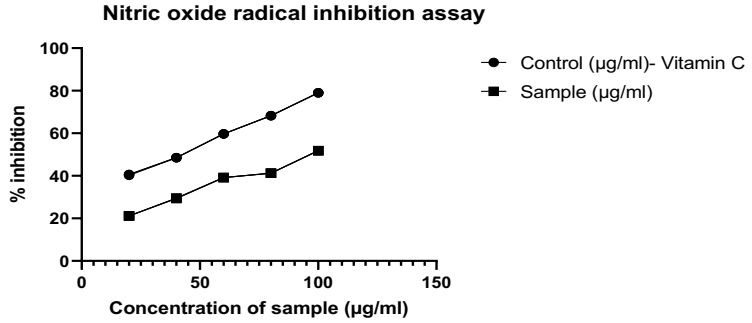


Fig.5. Nitric oxide scavenging activity of methanolic extract of *S. asperum.*

3.5.3 Reducing power activity

Reducing power is a measure of an element's ability to donate an electron, and substances with this property can serve as both primary and secondary antioxidants by neutralizing the oxidized precursors produced during the lipid peroxidation processes [60]. Fig. 6 depicts the decreased activity of the

methanolic *S. asperum* extraction. Chandhini et al. [61] investigated the reducing power of the brown algae *Sargassum marginatum, Padina tetrastomatica,* and *Turbinaria conoides.* The seaweeds were extracted with methanol. With rising concentrations, the reducing power of crude methanolic extract increased. *T. conoides* and *P. tetrastomatica* have greater reducing power than the standard scavenger a-tocopherol. The methanolic extracts of *Turbinaria ornata* and *Sargassum polycystum* had high levels of reducing power. Devi et al. [62] investigated the antioxidant abilities of green, red, and brown algae and found that among them, the brown algae *T. conoids* exhibited the highest antioxidant potential and it was lower than that of the standard. Rabecca and Doss [63] evaluated the reducing power of brown and green seaweeds and found that the methanolic extract of the brown seaweed *Lobophora variegate* displays higher reducing power compared to the green algae *Codium tomentosum*.

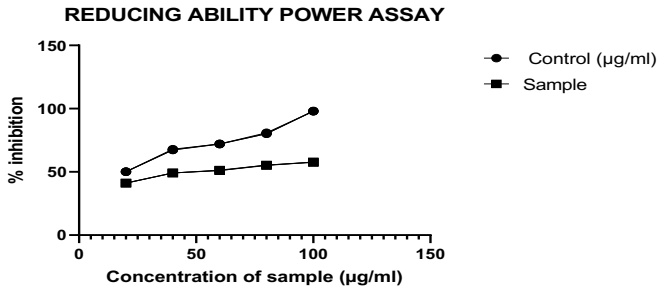


Fig. 6. Reducing power activity of methanolic extract of *S. asperum*.

3.6 Antibacterial activity

In this investigation, the methanolic extract of *S. asperum* showed sensible activity against both gram-negative and gram-positive bacteria. The zone of inhibition of different concentrations of extract used on the gram-negative and gram-positive bacteria is given in Tables 4-5 below. In gram-negative microbes, the solvent has maximum activity against nosocomial and pathogenic *K. aerogenes* 16±1.7 mm and gram-positive pneumococcal pathogen *S. pneumoniae* 18±1.0 mm. Hasan et al. [64] measured the antibacterial activity of the brown seaweeds *Turbinaria conoides, Padina gymnospera,* and *Sargassum tenerrimum.* They found that the methanol extract of all the selected seaweed had moderate activity against *E. coli, S. epidermidis, V. Parahemolyticus,* and *B. Subtilis.* Further, they concluded that the strongest antibacterial activity was displayed by the methanol extract among seven solvents used in their study. Taskin et al. [65] investigated the antimicrobial properties of *Cystoseira barbata*, against gram-negative *E. coli, E. aerogenes* and gram-positive *Micrococcus luteus* and found it exhibited the highest activity against the tested pathogens. The crude extract of *Gracilaria edulis* inhibits the progress of *E. aerogenes* and *E. coli* [66]. The methanolic extract of *Padina australis* shows maximum inhibition towards gram-*positive B. subtilis, S. aureus* and less activity towards gram-negative *E. coli.* According to the current study's outcomes, gram-positive bacteria responded to the extracts more readily than gram-negative bacteria did. A possible explanation is a hydrophobic lipopolysaccharide found in the outer membrane of gram-negative bacteria, which defends against many chemicals by possibly preventing the active compounds from entering the cell [67]. *Sargassum latifolium* and *Sargassum platicarpum* were extracted with methanol and were shown to have good action towards *S. xylosus*, *E. Coli,* and *Salmonella Spp* [68]. Pandithurai et al. [69] reported that the methanolic extract of *S. asperum* has moderate

action against *S. aureus* and *K. pneumoniae.* Two seaweed species, *Hypnea musciformis* and *Enteromorpha intestinalis* were tested against human pathogens *Klebsiella Spp* and *S. aureus* and compared with gentamycin. Methanol has consistently been the greatest active solvent for retrieving antimicrobial properties from the chosen seaweeds. *Sargassum vulgare* and *Sargassum fusiforme* were tested against multidrug-resistant clinically isolated gram-negative and gram-positive bacteria. The extract showed moderate activity against *S. aureus* and *K. pneumoniae.* Morphological changes in bacterial strains have been observed through transmission electron microscopy, indicating diminished cell size and cell wall separation [70]. The methanol-based extracts of *Sargassum wightii* and *Stechospermum marginatum* were efficient against *E. coli, E. faecalis, S. epidermidis, M. luteus, P. aeruginosa, S. aureus,* and the human fungus *Candida albicans*, as determined by the well plate technique [71].

Table 4. Antimicrobial action of methanolic crude extract against gram-negative bacteria.

Pathogens	Antibiotic	Extract without methanol	25 (mg/mL)	50 (mg/mL)	75 (mg/mL)	100 (mg/mL)
Escherichia coli	20±1.5	17±1.2	15±1.5	16±1.1	18±1.8	19±1.1
Klebsiella aerogenes	22±1.5	15±1.8	16±1.7	16±1.8	17±1.5	19±2.0
Salmonella typhimurium	20±1.5	16±0.9	14±1.5	15±2.0	18±2.5	18±1.5
Vibrio parahaemolyticus	17±1.5	12±1.5	13±1.1	14±0.5	15±0.5	16±0.5

Table 5. Antimicrobial activity of methanolic crude extract against gram-positive bacteria.

Pathogens	Antibiotic	Extract without methanol	25 (mg/mL)	50 (mg/mL)	75 (mg/mL)	100 (mg/mL)
Staphylococcus epidermidis	21±2.0	11±1.8	14±1.5	14±1.0	15±2.5	15±2
Bacillus subtilis	19±1.5	14±1.7	12±0.5	13±0.5	15±1.0	16±1.0
Micrococcus luteus	22±0.2	17±2.8	16±1.5	18±1.5	19±2.0	20±3.1
Streptococcus pneumoniae	21±2.0	16±1.9	18±1.0	18±2.0	19±2.0	21±3.3

Results are represented as average± SD, with results representing the mean of trials carried out in triplicate. The zone of inhibition was measured in mm.

3.7 Larvicidal activity

Tables 6-7 display the insecticidal efficacy of *S. asperum* methanolic extract against *Aedes aegypti, Culex quinquefasciatus,* and *Anopheles stephensi.* Of the three species of mosquitoes studied, only *Ae. aegypti* was significantly affected by the extract. The mortality rate was *Ae. aegypti* (2.56 mg/L), *An. stephensi* (2.84 mg/L), and *Cx. quinquefasciatus* (3.32 mg/L), respectively at the concentration of 200 ppm after 72 hrs of action. The larvicidal activity of *Sargassum microystum* against 4th instar larvae of *Ae. aegypti, C. quinquefasciatus, and An. Stephensi* was studied. It was concluded that the activity may be due to the occurrence of saponins and triterpenoids in the product [6]. The methanolic extract of *S. asperum, S. marginatum,* and *S. wightii* were tested against the 2nd and 3rd instars of *Cx. quinquefasciatus* and *Ae. aegypti* and after 24 hrs, 100% mortality was recorded. The activity was higher in 2nd instar specimens compared with 3rd instar specimens [72]. Hira et al. [73] studied the larvicidal potential of brown seaweeds *S. ilicifolium, S. binderi, S. lanceolatum,* and *S. variabile* against 4th instar *Ae. aegypti.* Among them, *S. binderi* showed a 50% mortality at low concentration. The methanol extract of *Sargassum binderi* caused larvicidal and histological changes in *Aedes Aegypti* with an LC_{50} value of 192.43 g/mL [8]. Extracts *from S. wightii, S. ilicifolium,* and *G. acerosa* have been studied for their larvicidal efficacy towards 4th instar *Ae. aegypti, Cx. quinquefasciatus,* and *An. stephensi.* They explained the way seaweed extracts were tested on Daphnia and how they might have possessed crucial bioactive chemicals that led to the high larval mortality. The outcome shows that it is non-toxic to non-target organisms [74]. *Turbinaria conoids* extract was tested against *Cx. quinquefasciatus* and 80% mortality was observed after 24 hrs of

incubation [75]. *T*he methanol extract of *G. corticate* was tested against the malarial vector *An. stephensi* and the LC_{50} value was 498.91 mg/L [6].

Table 6. Mortality rate of selected mosquito species after being exposed to various concentrations of *S. asperum* methanolic extract for 72 hours.

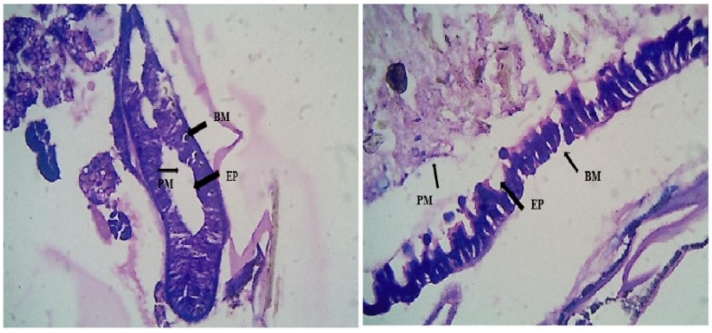
Conc. of *S. asperum* methanol extract	No. of larvae per application (N)	Number of larval mortalities		
		Ae. aegypti	*Cx. quinquefasciatus*	*An. stephensi*
Control	25	0	0	0
50 ppm	25	4	2	5
100 ppm	25	8	7	6
150 ppm	25	14	8	8
200 ppm	25	19	14	13

Table 7. Larvicidal effect of methanolic extract of *S. asperum* against 4th instar larva of *An. stephensi, Cx. quinquefasciatus, Ae. aegypti.*

Name of the species	LC_{50} (mg/L)	Regression equation	R^2
Aedes aegypti	2.56	Y=3.5x-4.7	0.9511
Culex quinquefasciatus	3.32	Y=3.4x-4	0.957
Anopheles stephensi	2.854	Y=2.9x-2.3	0.9428

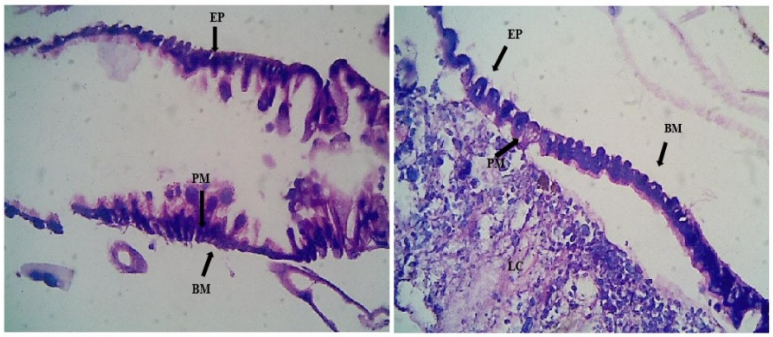
3.8 Histopathological analysis

The midgut of *An. stephensi, Ae. Aegypti,* and *Cx. quinquefasictus* larvae was observed after being exposed to methanolic *S. asperum* extract at various concentrations. Histopathological analysis indicated that the damage was in the midgut, particularly in the corneal epithelium, microvilli, and basal membrane. The histological variations in both untreated and treated larval mosquitoes are shown in Figs. 7-9. Conferring with Rohmah et al. [76], the midgut of larvae was damaged primarily due to several roles occurring in this location, such as the digestive process, nutrition captivation, signal transduction, and osmoregulation. This was triggered by the extract's deadly effect on the columnar epithelium layering the midgut. This disrupted enzyme excretion and the digestive process, leaving larvae with an energy deficit that could lead to death [77]. At 200 ppm, the extract causes severe damage to the peritrophic membrane and epithelial cells. The changes were due to the presence of larvicidal compounds such as citronella and dimethyl phthalate in the extract. According to a report by Yu et al. [8], the occurrence of vesicles of differing shapes, damage to microvilli, and inflammation of cells were observed in the anterior midgut epithelial cells and the posterior intestinal lumen epithelial of control larvae. On the other hand, the epithelium was lined by huge, irregular cells with massive globular bases were observed *Ae. aegypti* larvae treated with *Sargassum binderi* extract.



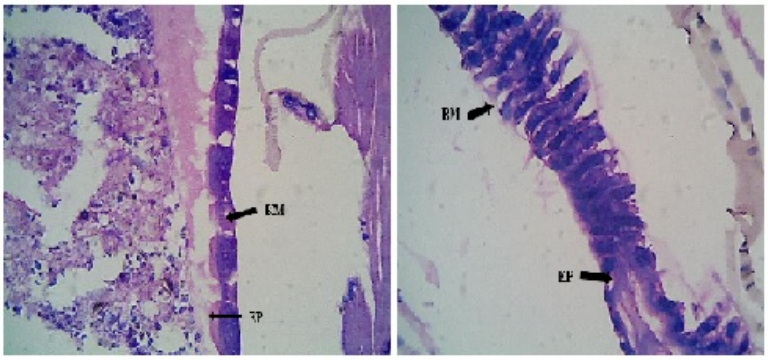
[A] [B]

Fig. 7. Histopathological changes in *An. stephensi* treated with methanolic extract of *S. asperum* [A] Control larvae, [B] Treated larvae, EP (Epithelial cells), BM (Basal membrane), PM (Peritrophic membrane), LC (cell lysis).



[A] [B]

Fig. 8. Histopathological changes in *Ae. aegypti* treated with methanolic extract of *S. asperum* [A] Control larvae, [B] Treated larvae, EP (Epithelial cells), BM (Basal membrane), PM (Peritrophic membrane), LC (cell lysis).



[A] [B]

Fig. 9. Histopathological changes in *Cx. quinquefasciatus* treated with methanolic extract of *S. asperum* [A] Control larvae, [B] Treated larvae, EP (Epithelial cells), BM (Basal membrane), PM (Peritrophic membrane), DC (Destroyed cell), BE (Elongated Brush border).

4. Conclusion

The efficacy of bioactive substances against various insect pests, such as mosquitoes, has been the subject of numerous studies in recent decades. This investigation concluded that methanolic extract of *S. asperum* induced noticeable larval death and showed moderate activity against all the tested organisms. The extract also has good activity against all the verified clinical pathogens. The GC-MS analysis identified 14 biologically active compounds. The methanolic extract of *S. asperum* contains a high quantity of phenol and flavonoids. The DPPH, reducing power, and nitric oxide scavenging assay results from this study are in agreement with the extracts' antioxidant activity shown in other studies. The plant's larvicidal, antibacterial, and antioxidant activities are generally supported by the research. At 200 ppm, methanolic extract of *S. asperum* has high activity against all the tested larvae. After 72 hrs exposure, the highest activity was recorded against *Ae. aegypti* and the LC_{50} rate was 2.56 mg/L. Histopathological damage occurred in the treated groups' peritrophic membrane, cell wall, and epithelial cells compared to control larvae. In accordance with the study's findings, this seaweed could be useful in the development of innovative mosquitocidal formulations in the future and may have beneficial medical applications. Further research is necessary to validate the larvicidal activity, antibacterial, and antioxidant effects, as well as to identify and isolate the active chemicals, and clarify their pharmacological characteristics. This will

include performing in vitro and in vivo studies to understand their antibacterial and antioxidant effects, and to find and isolate the active chemicals, and clarify their pharmacological characteristics. As a result, additional compound fractionation and purification will clarify which compounds are responsible for these effects.

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