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Insecticidal, antibacterial and dye adsorbent properties of *Sargassum muticum* decorated nano-silver particles

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ABSTRACT

Marine algae contain many bioactive constituents. In the current research, the brown seaweed *Sargassum muticum* was collected from Red Sea and its extract used as a capping agent for the formulation of biocompatible stable silver nanoparticles (AgNPs). The seaweed-fabricated AgNPs were studied through UV–vis, TEM, XRD, EDX, FTIR and Zeta potential analyses. The chemical constituents of *S. muticum* extract were evaluated by RP-HPLC and ^{1}H NMR. Both seaweed extract and AgNPs had mosquito larvicidal and adulticidal activities. The seaweed-fabricated AgNPs induced high larval mortality against mosquitoes from both Indian and Saudi Arabian strains when compared to the seaweed extract. The LC_{50} values of AgNPs against the Indian strains of *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* were 43.9, 35.9 and 27.0 μg/ml, respectively; those against the Saudi Arabian strains of *Ae. aegypti* and *Cx. pipiens* were 110.4 and 126.2 μg/ml, respectively. In adulticidal experiments, the LC_{50} values of *S. muticum*-fabricated AgNPs against Indian strains of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* were 42.3, 34.3 and 29.7 μg/ml, respectively, whereas those against the Saudi Arabian strains of *Ae. aegypti* and *Cx. pipiens* were 86.4 and 120.0 μg/ml, respectively. Minimal doses of seaweed-fabricated AgNPs were highly effective in inhibiting the growth of *Bacillus subtilis* (11.25 mm inhibition zone), *Escherichia coli* (13.35 mm), *Klebsiella pneumoniae* (14.24 mm) and *Salmonella typhi* (12.23 mm). Additionally, the seaweed extract was highly efficient to adsorb methylene blue (MB) and methyl orange (MO) dyes. In conclusion, the *S. muticum* extract revealed to be an effective capping agent to fabricate AgNPs to be industrially used for the control of deadly mosquito vectors and as an antimicrobial and dye adsorbent agent.

1. Introduction

Sargassum muticum (Yendo) Fensholt (Ochrophyta, Phaeophyceae), popularly known as Japanese wire weed, is a fast-growing brown marine seaweed which can tolerate high variations of salinity and temperature. This invasive species, first identified in Britain in 1944, is widely distributed from Norway to Portugal, Spain and North African coast of Morocco (Elatouani et al., 2016) and has spread over north Pacific and, north Atlantic it has also been recorded in Australia and Indo-west Pacific (Tseng et al., 1985). *S. muticum* grows mainly on the hard substrata along the half tidal towards the infra-littoral zones. This species is capable of removing cadmium and nickel from the aquatic environment (Lodeiro et al., 2006).

Insect pests are becoming increasingly resistant to chemical pesticides and these chemicals are also causing severe damage to the natural ecosystem through its detrimental negative impact on other living entities (Murugan et al., 2015a; Benelli et al., 2019). Synthesized metal nanomaterials represent an eco-friendly alternative to chemical pesticides (Panneerselvam et al., 2016; Athanassiou et al., 2018; Aziz et al., 2020). Seaweeds and marine based nanoparticles can serve as a valuable source for future bio-nanotechnology (Ramkumar et al., 2016; Singh et al., 2015; Vijayan et al., 2014). For example, *S. muticum* mediated zinc nanoparticles reduced angiogenesis and induced apoptosis, thereby creating interest as a potential anticancer drug (Sanaeimehr et al., 2018). *S. muticum* extracts have a high polyphenol content and anticancer potential (Namvar et al., 2013). *Sargassum* seaweeds are used in the traditional Chinese

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medicine to treat a variety of ailments including thyroid disease (Liu et al., 2012). Therapeutic benefits of *Sargassum* species include anti-viral, anti-allergic and anti-tumor properties (Yende et al., 2014). *S. muticum* fabricated silver nanoparticles (AgNPs) showed effective insecticidal properties against the butterfly *Ariadne merione* (Cramer) (Moorthi et al., 2015). *S. wightii* Greville synthesized NPs showed inhibitory activity against bacterial pathogens (Shanmugam et al., 2014). *S. polycystum* C. Agardh synthesized AgNPs exhibited high larval toxicity on *Aedes aegypti* and *Culex quinquefasciatus* (Vinoth et al., 2018). *S. wightii* aqueous extract and ZnO NPs showed larvicidal activity against *Ae. aegypti* (Ishwaryaa et al., 2018). *S. wightii* fabricated zirconia NPs had bactericidal properties (Kumaresan et al., 2018).

Mosquitoes act as vectors for several deadly diseases like Zika virus disease, malaria, dengue, filariasis, yellow fever, chikungunya and West Nile Fever. Controlling the spread of these mosquitoes is a global concern (Pavela et al., 2019). Dengue virus is spreading in several countries through the bite of *Ae. aegypti* causing risk to about four billion people (Brady et al., 2012). *Ae. aegypti* mosquitoes act also as a vector of Zika virus disease. The recent upsurge of Zika virus disease has been a serious concern for health workers and WHO. Chikungunya and yellow fever are also vectored through *Aedes* mosquitoes (Roni et al., 2015). Malaria vectored by *Anopheles* mosquitoes is another disease of global concern and a major burden for developing nations, particularly in the African continent. Filariasis, Japanese encephalitis and West Nile fever are vectored by *Culex* mosquitoes (Murugan et al., 2015a). In this study the efficacy of the seaweed *S. muticum* as a capping agent to fabricate AgNPs with potential against major mosquito vectors and microbial pathogens was investigated. Furthermore, the dye removal capability of the produced AgNPs was evaluated.

2. Materials and methods

2.1. Seaweed collection and extraction

S. muticum was collected from the Red Sea coast of Haql (29° 20′25.4″N, 34°56′53.51″E), Tabuk region, Saudi Arabia. The seaweeds were shade-dried at room temperature and thoroughly ground into a fine powder using an electrical blender. The aqueous extraction of *S. muticum* was achieved by dissolving 10 g of finely powdered *S. muticum* into 100 mL of double distilled water according to the method of Murugan et al. (2015a).

2.2. RP-HPLC and ^{1}H NMR analysis

Bruker Advance III spectrometer was used to acquire ^{1}H NMR spectra operating at 400 MHz. Spectra were elaborated by ADC software. Dried *S. muticum* (200 mg) was extracted with deuterated methanol (2 mL) and sonicated (10 min). The solution was centrifuged (5 min, 25 °C, 13,000 rpm) and used for NMR analysis. For chromatographic analysis, samples were dissolved in methanol (50 mg/mL) (Scharlau, Italy cat no-ME03152500). Analyses were performed on an Agilent Infinity series 1200 chromatograph equipped with diode array (1260 series) and Agilent/Varian 500MS mass spectrometer. Eclipse XDB-C18, C18 3.5 μm, 3 × 150 (Agilent technology) was used as column and flow rate was maintained at 400 μL/min. The solvent system was water 0.1% formic acid (Scharlau, Italy cat no-AC10085100) and acetonitrile (Scharlau, Italy Cat. No- AC03292500). The gradient elution started with 10% acetonitrile and changed to 100% over 30 min. The chromatograms were studied at 214, 230, 250, 254, 280, 330, 350 and 545 nm. The same extract was also chromatographed with the same system but using an Agilent PoroshellHilic-Z (3 × 100 mm) column as stationary phase. The mobile phase was 98% acetonitrile, 2% water, 1% formic acid, with gradient starting with 98% acetonitrile and in 10 min reached 85% acetonitrile. Flow was 400 μL/min.

2.3. Seaweed synthesis and characterization of AgNPs

$AgNO_3$ (1 mM) was mixed with 100 ml of *S. muticum* extract and incubated at room temperature until the color of the solution changed to brown-yellow, indicating the production of AgNPs. The *S. muticum* fabricated AgNPs were characterized through UV Vis spectroscopy (UV-3600 spectrometer, Shimadzu, Japan) for the determination of the maximum absorption of AgNPs with optical density ranging from 300 to 700 nm. The shape and size of the *S. muticum*-fabricated AgNPs were examined through TEM (JEOL 1200 EX, Japan) by operating at 120 Kv. EDX analysis (EDX-JEOL mode 6390, Japan) was used to detect metals on the surface of the samples. FTIR (Perkin-Elmer spectrum 2000 FTIR, USA) was adopted to determine the functional groups within the spectral range 500–4000 cm^{-1}. The crystalline nature of AgNPs was achieved by XRD (X'PERT PROP Analytical, Philips, USA) and the stability was confirmed by Zeta potential (Zetasizer Nano ZS-90, Malvern Instrument, Malvern, UK).

2.4. Toxicity on mosquito larvae

Following the method described by Suresh et al. (2018), five species of mosquito life stages (IV instar larvae and adults) were maintained and cultured under laboratory conditions. Indian strains (*Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*) were originally collected and cultured for more than 6 years in Coimbatore, Tamil Nadu, while Saudi Arabian strains (*Ae. aegypti* and *Cx. pipiens*) where obtained and cultured for more than 3 years in Tabuk. The larval toxicity assay of seaweed extract (100, 200, 300, 400 and 500 μg/ml) and AgNPs (20, 40, 60, 80 and 100 μg/ml) against IV instar larvae of all five mosquito species was conducted following the method described by Murugan et al. (2015b). Twenty mosquitoes were maintained in each dose with five replicates and the control was deionized water. After 24 h post treatment the percentage of larval mortality was calculated.

2.5. Toxicity on mosquito adults

The adulticidal bioassay was conducted according to the WHO (1981) with minor modifications by Subramaniam et al. (2016), Twenty newly emerged adults of all five species were exposed to different concentrations of seaweed extract (150, 250, 350, 450 and 550 μg/ml) and AgNPs (25, 50, 75, 100 and 125 μg/ml), applied on Whatman filter paper n.1 (size 12 × 15 cm^2) lining a glass holding tube (diameter 30 mm; length 60 mm). Five replicates for each concentration were maintained where each replicate had 20 adults. Deionized water and $AgNO_3$ were used as control.

2.6. Antibacterial activity

The antibacterial activity of seaweed extracts and AgNPs were tested on *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhi* according to the procedure designed by Dinesh et al. (2015). Tested bacterial strains were originally obtained from Microbial Type Culture, Sector 39A, Chandigarh 160,036 (India). The antibacterial activity of AgNPs was tested against the selected bacteria species by disk diffusion method (DDM) as reported by Dinesh et al. (2015). The selected bacteria species were grown in nutrient broth: peptone (5 g/L), hydrolyzed yeast extract (1.50 g/L), beef extract (1.50 g/L), NaCl (5 g/L) were used to culture the bacteria species with a final pH of 7.4. Thirteen g of nutrient broth were suspended in 100 mL double distilled water. Twenty-five mL of broth were poured in each of the four conical flasks and autoclaved for 15 min at 121 °C (15 psi). Later, all tested bacteria species were

inoculated and incubated for 24 h at 37 °C. After 24 h incubation, 2×10^6 cfu/mL of the culture was used for antibacterial assays. To compare the efficacy, commercial antibiotic (Tetracyclin) was used in separate experiments. The zone of inhibition was observed by using Photomicroscope (Leica ES2, Germany) (Rai et al., 2009). The antibacterial activity assay was conducted three times.

2.7. Adsorption studies

Methylene blue (MB) and methyl orange (MO) (10 mL of 20 mg/L) were introduced into 25 mL amber bottles containing 0.3 g of *S. muticum* powder. The bottles were kept in a shaker-incubator at 26.5 °C and 160 rpm for 23 h. The mixture was separated using Whatman filter paper and the final concentration of the filtrate was determined by UV–vis spectrophotometer (Jenway, 6800/UV, UK) with maximum wavelength of 617 nm for MB and 464 nm for MO. The percentage removal of each dye was determined by the following equation:

$$\% \text{ removal of dye} = C_f/C_i X\ 100$$

Where, C_i and C_f are the initial and final concentration of dyes

2.8. Statistical analysis

SPSS 16.0 software was used for all experimental data analysis. In laboratory mosquito assays, 24 h post treatment, the dead mosquito larvae and adults were recorded separately and LC_{50} and LC_{90} were obtained by using Probit analysis (Finney, 1971). Mosquito larval and adult mortality data and bacterial growth zone inhibition data were analyzed by ANOVA with two factors (i.e., tested dose and bacterial species or mosquito life stages) followed by Tukey's HSD test ($P < 0.05$).

3. Results and discussion

3.1. ^{1}H NMR and RP-HPLC analysis of seaweed extract

NMR was used as a general technique to assess the composition of the seaweed extract and provided information on the most abundant constituents using a simple sample preparation. Extracting *S. muticum* with methanol resulted in a spectrum showing the presence of fatty acid derivatives and carbohydrate (Fig. 1). In these conditions signals ascribable to phenolics were not detectable indicating a limited amount of these compounds in *S. muticum*.

In order to assess composition of less abundant constituents, two different types of HPLC analysis were performed. Reverse phase chromatography was used to assess the presence of small phenolics and potential glycosides. The main compounds were rutin, 10-O-[(*E*)-*caffeoyl*]-geniposidic acid and dihydrokaempferol (Table 1a, Fig. 2). Small amounts of thiamine were also detected from *S. muticum* extract (Table 1b) (De Roeck-Holtzhauer et al., 1991). Rutin is a flavonoid which is found in fruits like orange, banana and has antioxidant activity (Panche et al., 2016). 10-O-[(*E*)-*caffeoyl*]-geniposidic acid is an iridoid glucoside which was isolated from the mangrove *Avicennia marina* (Forssk.) Vierh. (Shaker et al., 2001).

By using a HILIC-Z column a different selectivity towards seaweed metabolites was obtained, and it was possible to observe signals ascribable to phlorotannins polymers (Table 1c). The fragmentation of the parent peaks in both the positive and negative mass spectrometry mode provided a more comprehensive analysis of the phlorotannins present (Table 1c). The identified compounds showed similar fragmentation patterns supporting the fuco-phloroethol structures. Typical features showed losses of water molecules (−18 Da, −36 Da), phloroglucinol units (−126 Da), and phloroglucinol and methyl groups (−126 Da and −14 Da). Thus, the seaweed material contained different fucophloroethol derivatives and isomers with different degrees of polimerization. Fucophloroethols are phlorotannins formed by fucols and phloroethols, showing aryl and ether linkage between the phloroglucinol units. Due to similarity of structures and lack of isolated reference compounds it was not possible to differentiate the possible isomers; so, compounds were indicated as isomers and by their degree of polimerization (Table 1c). Diacylglycerylhydroxymethyl-N, N, N-trimethyl-β-alanine (DGTA) was also present (Fig. 3); these betaine lipids are non-phosphorous acylglycerolipids containing an ether linked between a quaternary amine alcohol and a diacylglycerol moiety. Such compounds are well known constituents of brown seaweeds (Li et al., 2017).

The chemical analysis revealed the presence of several other constituents that are expected in such material. Sulfated polysaccharide and fucans occur in several species of brown algae (Wijesinghe and Jeon 2012; Dore et al., 2013) and *S. muticum* contained α and γ-tocopherol (Farvin and Jacobsen 2013), fucoxanthin (Kumar et al., 2013) and apo-9′-fucoxanthinone (Chae et al., 2013; Yang et al., 2013). These latter constituents may be in large part lost during drying procedures while they can be more present in fresh materials due to their easy oxidable nature (Badmus et al., 2019).

3.2. UV–vis spectroscopy

The AgNPs were fabricated from *S. muticum* within 24 h by adding the seaweed extract to the $AgNO_3$ solution. UV–vis absorption peak of synthesized AgNPs was detected at 400 nm (Fig. 4). Similarly, *S. wightii* synthesized zirconia NPs had an absorption peak at 277 nm (Kumaresan et al., 2018); *S. wightii*-synthesized AgNPs exhibited an absorption peak at 439 nm (Shanmugan et al., 2014); *S. muticum* synthesized AgNPs had an absorption peak at 420 nm (Azizi et al., 2013; Madhiyazhagan et al., 2015).

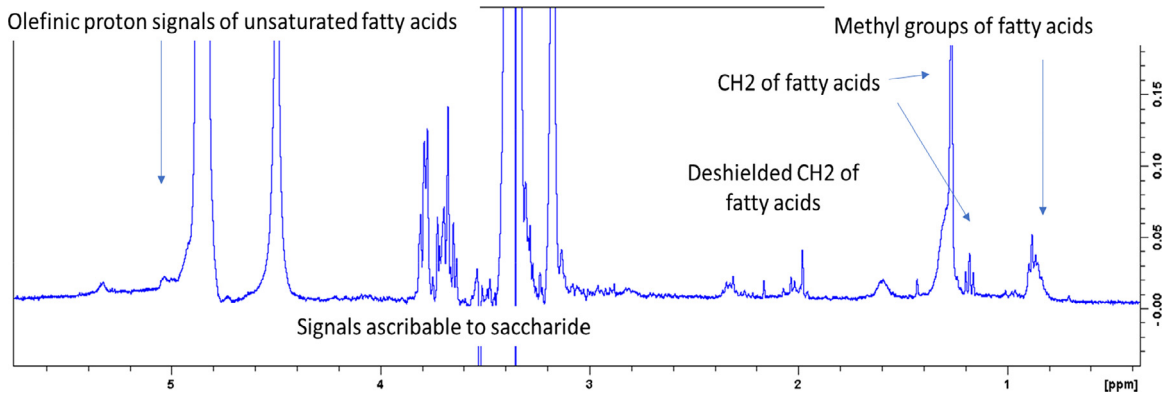

Fig. 1. Typical ^{1}H NMR of the *Sargassum muticum* methanol extract.

Table 1a
Identified compounds in *S. muticum* aqueous extract.

S. No.	Retention Time (Min.)	Compound name	[M-H]-	MS2	MS3	MS4	mg/g
1	7.98	Rutin	609.5	301.45	271.47	227.7	0,060 ± 0,001
2	10.14	10-O-[(E)-*caffeoyl*]-geniposidic acid	519.45	313.5	269.4	164.64	0.120

Table 1b
RP-HPLC MS analysis of *S. muticum* extract in negative ion mode.

No.	Compound	[M-H]- and fragments	mg/100 g
1	Thiamine	265 233 147	0.092
2	dihydrokaempferol	287 241	0.1101
3	Rutin	609 301 255	0.1350
4	10-O-[(E)-*caffeoyl*]-geniposidic acid	519.45	0,132

Table 1c
RP-HPLC MS analysis of *S. muticum* extract in positive ion mode.

No.	Compound	[M + H] + and fragments	mg/g
1	Phlorotannin pentamers	521 504 395 (sodiated ion)	16,21
2	Phlorotannin pentamers	521 504 395	3,82
3	Phlorotannin heptamers	871 521 504 395	42,41
4	Phlorotannin heptamers	871 521 504 395	7,25
5	DGTA various isomers	739 500 474 236 144	3,5

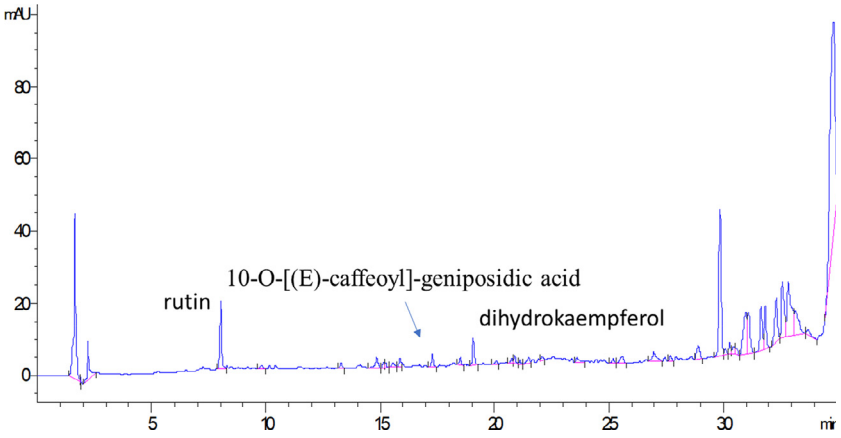

Fig. 2. RP-HPLC chromatogram, with indication of identified compounds.

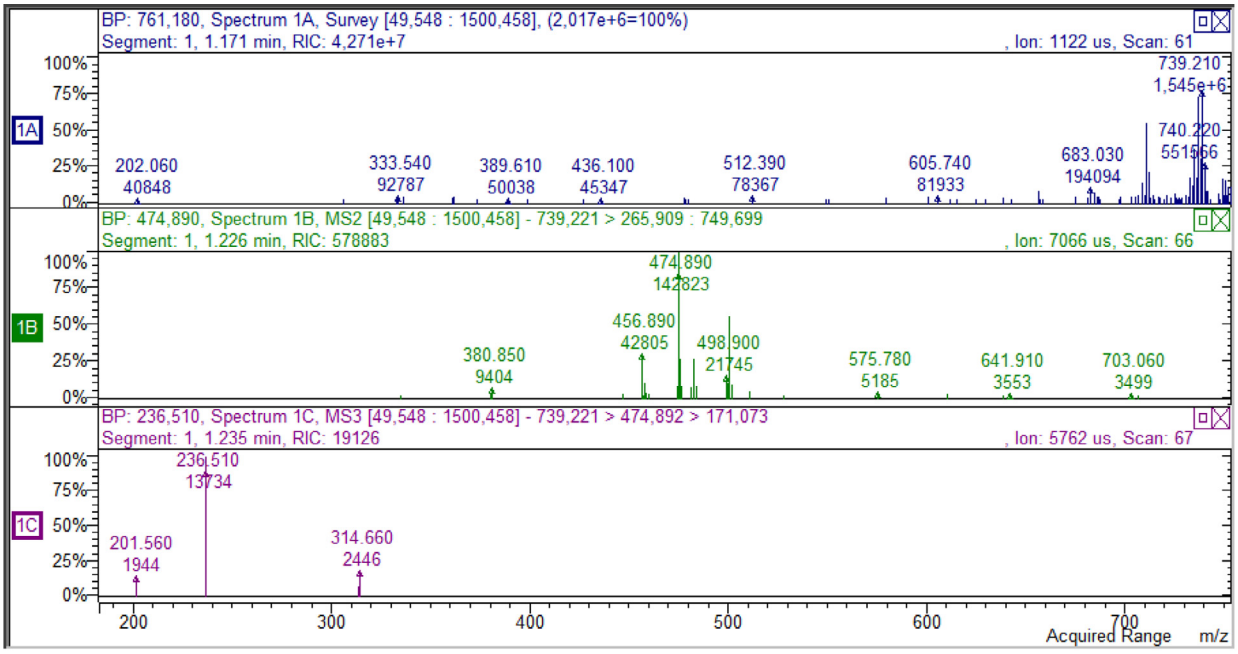

Fig. 3. MS spectrum of peaks ascribable to DGTA.

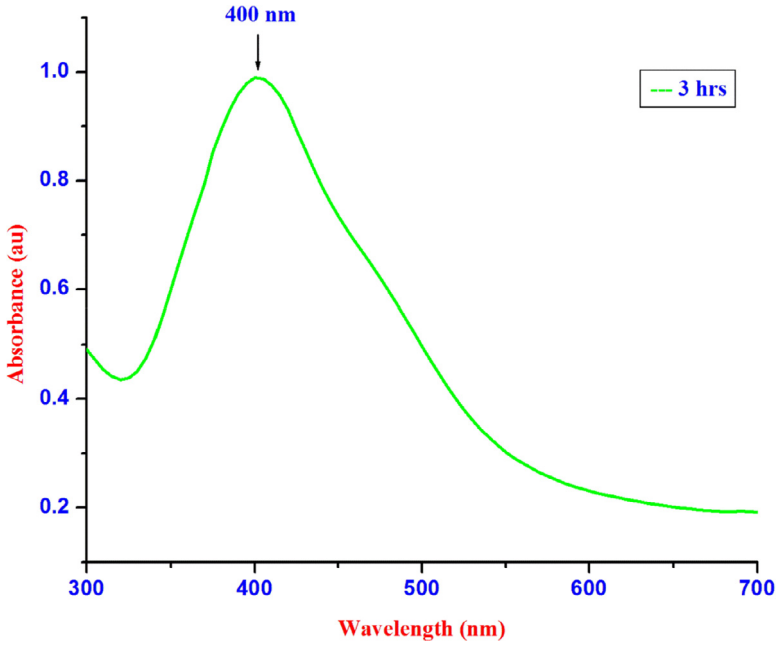

Fig. 4. UV -vis spectroscopy of *Sargassum muticum* fabricated AgNPs at 3 h time interval.

3.3. TEM and EDX studies

The TEM results showed that the *S. muticum*-synthesized AgNPs were mostly spherical in structure and with size ranging from 20 to 54 nm (Fig. 5), which was similar to the particle size of previously synthesized AgNPs (Azizi et al., 2013). The *S. muticum* AgNPs exhibited cubical morphology as resulted from SEM analysis (Madhiyazhagan et al., 2015). In previous studies, the *S. marginatum* based AuNPs were of spherical shape and 40–85 nm in diameter (Rajathi et al., 2012). As a result of EDX analysis, the formation of metallic AgNPs was evidenced by the peak at 3 KeV along with weak oxygen (O) which may have originated from the seaweed aqueous medium (Fig. 6). This is the characteristic peak for AgNPs (Madhiyazhagan et al., 2015; Suresh et al., 2018; Aziz et al., 2020; Alshehri et al., 2020). The long-term stability of the obtained NPs indicates the presence of capping and stabilizing agents in the *S. muticum* extract.

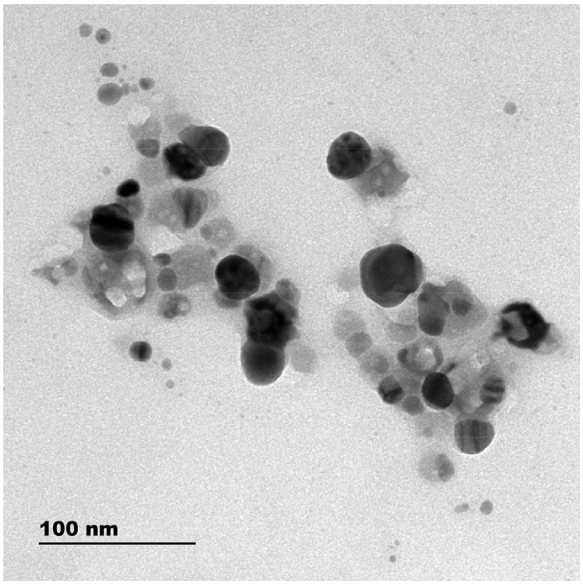

Fig. 5. TEM showing the AgNPs fabricated using the *Sargassum muticum* aqueous extract.

3.4. XRD analysis

The XRD diffraction peaks at 2θ values 32.14°, 46.15°, 54.07°, 57.4°, 67.4° and 76.7° corresponded to 110, 211, 220, 221, 222 and 321 crystalline planes of AgNPs (Fig. 7) and matched peaks reported previously for S. *muticum* (Azizi et al., 2013). The XRD results of seaweed decorated AgNPs corresponded to JCPDS card no: 6568–11. Similarly, there were clear reflections of *S. wightii*-fabricated AgNPs at 45.64, 51.34, 58.06, 66.46, and 71.08 (Madhiyazhagan et al., 2015) and XRD lattice planes when AgNPs were synthesized using the *S. wightii* extract (Shanmugam et al., 2014).

3.5. FTIR analysis

FTIR exhibited a sharp absorption peak of *S. muticum*-fabricated AgNPs at 3420 cm^{-1} (Fig. 8) that was due to the stretching vibration of –OH molecules and the peak at 2920 was assigned to the C—H stretching of aromatic compounds. The peak at 1620 was due to asymmetric carboxylate radical stretches (Song et al., 2017). *S. wightii*-synthesized zirconia NPs had FTIR intense peaks at 3420, 2924 and 1638 cm^{1} (Kumaresan et al., 2018). The peak at 1342 and 1150 cm^{-1} represented the C—N stretching of aromatic amine and C—O stretching vibration, respectively. The peak at 680 cm^{-1} may be attributed to an alkyne C—H bond (Coates, 2006). The presence of various functional groups revealed the successful bio-reduction of Ag^{+} and this helped in stabilization of the silver.

3.6. Zeta potential

The zeta potential analysis of seaweed fabricated AgNPs showed a negative value at –20.3 mV (Fig. 9), indicating the stability of AgNPs, similar to the zeta potential obtained from *Gracilaria birdiae* (E. Plastino & E.C. Oliveira Gurgel, J.N. Norris & Fredericq (formerly *Gracilaria birdiae Plastino & E.C. Oliveira*) (de Aragao et al., 2019) and *Cynara scolymus* L. fabricated AgNPs (Erdogan et al., 2019).

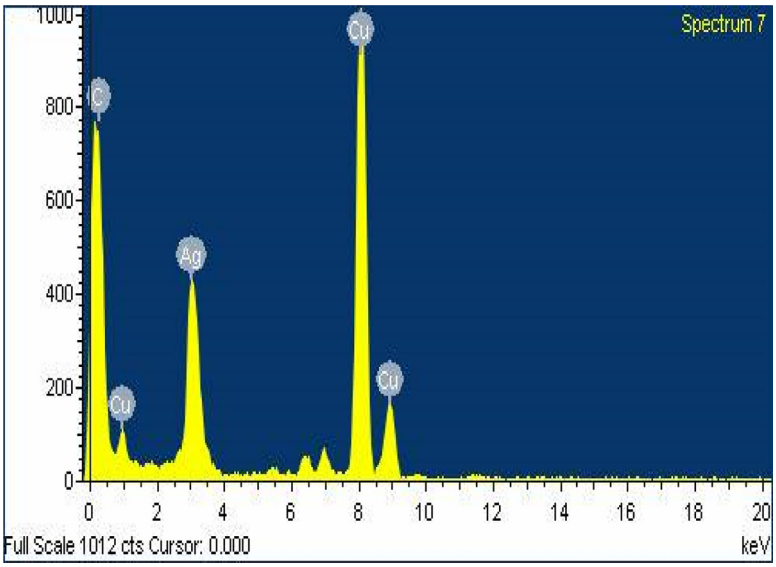

Fig. 6. EDX spectroscopy of AgNPs fabricated using the *Sargassum muticum*.

3.7. Larvicidal and adulticidal toxicity

The larvicidal assays of AgNPs showed a higher toxicity against both Indian (*Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*) and Saudi Arabian (*Ae. aegypti* and *Cx. pipiens*) strains when compared with the seaweed extract (Tables 2 and 3). Consistently, the *S. wightii* mediated ZnO NPs on 3rd instar larvae of *Ae. aegypti* had a LC_{50} value of 49.22 μg/ml (Ishwarya et al., 2018). The seaweed *Hydropuntia edulis* (S.G. Gmelin) Gurgel & Fredericq (formerly *Gracilaria edulis* (S. G.Gmelin) P.G.Silva) (Rhodophyta) synthesized AgNPs were highly toxic on larvae and pupae of *Cx. quinquefasciatus*, with LC_{50} values ranging from 17.146 (I instar) to 29.125 ppm (pupa) (Madhiyazhagan et al., 2017). Mangroves also exhibited significant larval toxicity on *Ae. aegypti* and *An. stephensi* (Alshehri et al. 2020).

S. muticum-fabricated AgNPs were highly toxic against both Indian and Saudi Arabian strains of key mosquito vectors (Tables 4 and 5). Notably, the Saudi Arabian strains of mosquitoes were more resistant than the Indian strains, confirming previous reports (Aziz et al., 2018; 2020). Comparable toxicity rates have been reported for AgNPs synthesized using *Phyllanthus niruri* L. against *An. aegypti* adults (LC_{50}= 6.68 ppm) (Suresh et al., 2015).

3.8. Antibacterial activity

In the present investigation, a maximum zone of inhibition was achieved by AgNPs for the bacteria *B. subtilis* (11.25 mm), *E. coli* (13.35 mm), *K. pneumoniae* (14.24 mm), and *S. typhi* (12.23 mm), respectively (Table 6). Similarly, *S. muticum* extracts inhibited the growth of different bacterial pathogens (Moorthi and Balasubramanian (2015); *S. incisifolium* (Turner) C. Agardh decorated gold and silver NPs had antimicrobial and anticancer properties (Mmola et al., 2016) and *S. wightii* fabricated zirconia (ZrO_2) NPs had antibacterial activity against gram positive and gram negative bacteria (Shanmugam et al., 2014; Kumaresan et al., 2018). These NPs have the capability to interact with the membranes and can enter the bacterial cells, thereby increasing the antibacterial effect (Morones et al., 2005).

3.9. Adsorption studies

MB is a basic dye widely used in the textile industry, while MO, besides being a dye, is also a pH indicator. MB is a thiazine dye that causes severe effects on humans, causing red blood cell breakdown and allergic reactions (Bradberry 2003). In the present research, the

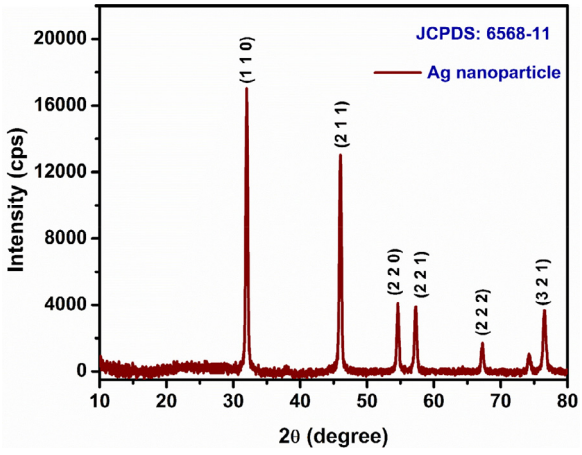

Fig. 7. XRD spectrum of *Sargassum muticum* fabricated AgNPs.

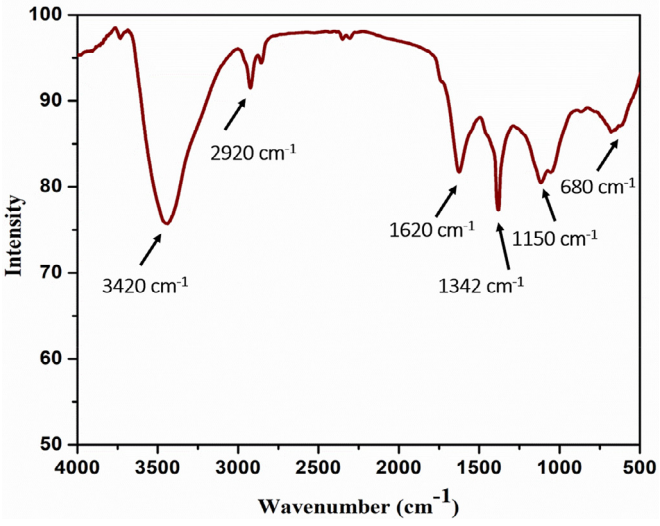

Fig. 8. FTIR spectroscopy of *Sargassum muticum* fabricated AgNPs.

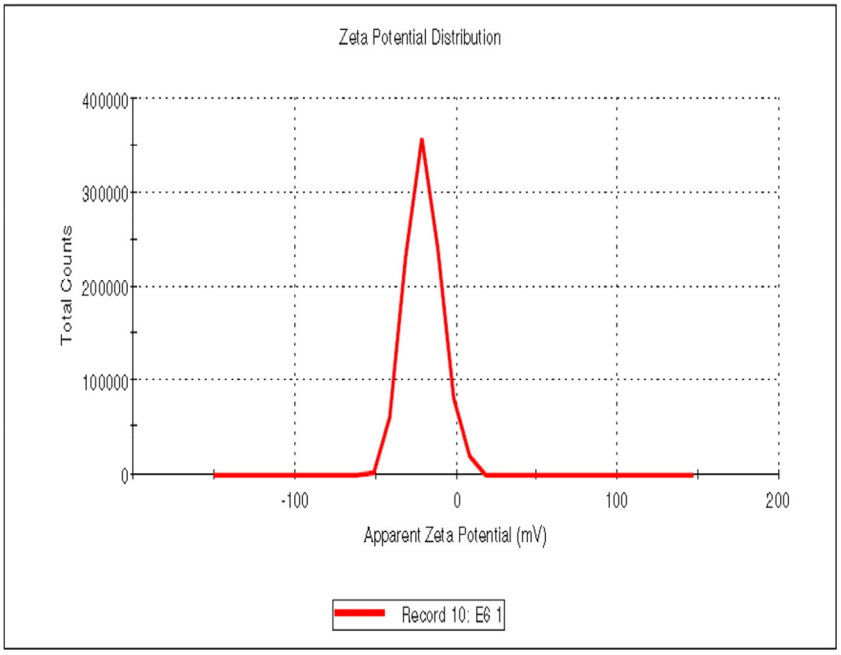

Fig. 9. Zeta potential analysis of *Sargassum muticum* fabricated AgNPs.

Table 2
Biotoxicity of *S. muticum* extract on *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus* (Indian strains), *Ae. aegypti* and *Cx. pipiens* (Saudi Arabian strains).

Mosquito species (strain geographical origin)	LC_{50} (μg/ml) (95% LCL-UCL)	LC_{90} (μg/ml) (95% LCL-UCL)	Regression equation	χ^2 (*d.f* = 4)
Cx. quinquefasciatus (India)	251.631 (220.938–279.159)	563.876 (498.955–624.077)	$y = 1.080+0.004x$	0.135 *n.s*
Ae. aegypti (India)	210.307 (176.123–238.693)	501.664 (455.737–567.474)	$y = 0.925+0.004x$	0.891 *n.s*
An. stephensi (India)	184.082 (148.373–212.736)	460.103 (419.145–517.838)	$y = 0.855+0.005x$	1.409 *n.s*
Ae. aegypti (Saudi Arabia)	281.245 (255.430–306.068)	547.034 (501.433–610.625)	$y = 0.005+1.356x$	1.668 *n.s*
Cx. pipiens (Saudi Arabia)	439.236 (401.824–490.760)	787.680 (692.967–940,057)	$y = 0.004+1.615x$	0.189 *n.s*

Control = no mortality.
LC_{50} = lethal concentration killing 50% of the insects.
LC_{90} = lethal concentration killing 90% of the insects.
χ^2 = chi-square.
d.f. = degrees of freedom.
n.s. = not significant (α=0.05).

Table 3
Biotoxicity of *S. muticum* fabricated AgNPs on *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus* (Indian strains), *Ae. aegypti* and *Cx. pipiens* (Saudi Arabian strains).

Mosquito species (strain geographical origin)	LC_{50} (μg/ml) (95% LCL-UCL)	LC_{90} (μg/ml) (95% LCL-UCL)	Regression equation	χ^2 (*d.f* = 4)
Cx. quinquefasciatus (India)	43.894 (37.515–49.316)	100.111 (91.253–112.643)	$y = 1.001+0.023x$	0.487 *n.s*
Ae. aegypti (India)	35.943 (29.375–41.273)	86.163 (78.880–96.200)	$y = 0.760+0.021x$	0.416 *n.s*
An. stephensi (India)	26.999 (17.242–34.100)	86.842 (78.425–99.027)	$y = 0.578+0.021x$	0.337 *n.s*
Ae. aegypti (Saudi Arabia)	110.390 (102.650–117.901)	184.993 (173.415–199.857)	$y = 0.017+1.896x$	1.626 *n.s*
Cx. pipiens (Saudi Arabia)	126.206 (108.160–142.067)	301.690 (268.823–352.522)	$y = 0.007+0.922x$	1.058 *n.s*

Control = no mortality.
LC_{50} = lethal concentration killing 50% of the insects.
LC_{90} = lethal concentration killing 90% of the insects.
χ^2 = chi-square.
d.f. = degrees of freedom.
n.s. = not significant (α=0.05).

Table 4
Adulticidal activity of *S. muticum* extract on *Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus* (Indian strains), *Ae. aegypti* and *Cx. pipiens* (Saudi Arabian strains).

Mosquito species (strain geographical origin)	LC_{50} (μg/ml) (95% LCL-UCL)	LC_{90} (μg/ml) (95% LCL-UCL)	Regression equation	χ^2 (*d.f* = 4)
Cx. quinquefasciatus (India)	256.006 (215.306–287.830)	588.854 (513.218–678.702)	$y = 1.986+0.004x$	1.308 *n.s*
Ae. aegypti (India)	218.262 (167.656–254.521)	564.207 (507.373–654.421)	$y = 0.809+0.004x$	0.378 *n.s*
An. Stephensi (India)	184.075 (133.756–219.893)	483.877 (441.484–546.44075)	$y = 0.787+0.004x$	1.203 *n.s*
Ae. aegypti (Saudi Arabia)	385.884 (309.086–452.724)	1.095 (977.898–1262.291)	$y = 0.002+0.698x$	1.198 *n.s*
Cx. pipiens (Saudi Arabia)	636.151 (549.558–732.145)	1.604 (1382.897–1963.611)	$y = 0.001+0.842x$	0.623 *n.s*

Control = no mortality.
LC_{50} = lethal concentration killing 50% of the insects.
LC_{90} = lethal concentration killing 90% of the insects.
χ^2 = chi-square.
d.f. = degrees of freedom.
n.s. = not significant (α=0.05).

Table 5
Adulticidal activity of *S. muticum* fabricated AgNPs on *Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus* (Indian strains), *Ae. aegypti* and *Cx. pipiens* (Saudi Arabian strains).

Mosquito species (strain geographical origin)	LC_{50} (μg/ml) (95% LCL-UCL)	LC_{90} (μg/ml) (95% LCL-UCL)	Regression equation	χ^2 (*d.f* = 4)
Cx. quinquefasciatus (India)	42.290 (30.959–50.834)	122.785 (110.524–140.968)	$y = 0.673+0.002x$	1.398 *n.s*
Ae. aegypti (India)	34.284 (20.544–44.033)	118.283 (105.959–136.859)	$y = 0.523+0.015x$	1.677 *n.s*
An. stephensi (India)	29.689 (17.979–38.241)	96.752 (87.795–109.208)	$y = 0.567+0.019x$	2.614 *n.s*
Ae. aegypti (Saudi Arabia)	86.363 (71.505–102.424)	233.832 (196.053–302.202)	$y = 0.009+0.751x$	1.289 *n.s*
Cx. pipiens (Saudi Arabia	129.938 (118.766–142.373)	246.791 (223.685–278.990)	$y = 0.011+1.425x$	1.777 *n.s*

Control = no mortality.
LC_{50} = lethal concentration killing 50% of the insects.
LC_{90} = lethal concentration killing 90% of the insects.
χ^2 = chi-square.
d.f. = degrees of freedom.
n.s. = not significant (α=0.05).

Table 6
Zone of inhibition of *S. muticum* extract and *S. muticum* fabricated AgNPs against the bacteria *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris*.

Species	Inhibition zone (mm)			
	Bacillus subtilis	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus vulgaris*
S. muticum extract	7.17 ± 0.14^{a}	8.12 ± 0.12^{b}	10.28 ± 0.25^{c}	10.41 ± 0.14^{d}
S. muticum fabricated AgNPs	11.25 ± 0.28^{a}	13.35 ± 0.13^{b}	14.24 ± 0.21^{c}	12.23 ± 0.25^{d}
Commercial antibiotic disc	10.26 ± 0.23^{a}	12.25 ± 0.28^{b}	13.38 ± 0.33^{c}	11.33 ± 0.37^{d}

Values are means ± SD of 3 replicates.
Within a row, different letters indicate significant differences (ANOVA, Tukey's HSD, $P<0.05$).
Zone of inhibition in mm indicate the distance from the border of the disc to the edge of the clear zone.

percentage removals for MB and MO were 63.5 and 66.5%, respectively (Fig. 10), thereby indicating that *S. muticum* could remove theses dyes to a high extent. Comparably, the green seaweed *Codium decorticatum* (Woodward) M.A. Howe showed dye removal capacity of crystal violet by 96.9% and Congo red by 89.8% (Oualid et al., 2020), while the removal capability of basic dye by the marine seaweed *Ulva lactuca* L. (Chlorophyta) and *Sargassum* was 96% (Tahir et al., 2008).

4. Conclusion

In this study, *S. muticum*-fabricated AgNPs were obtained and characterized. ^{1}HNMR and RP-HPLC analysis revealed the presence of rutin and 10-O-[(E)-*caffeoyl*]-geniposidic acid as possible capping agents for AgNPs synthesis. Bioassay studies suggested their suitability to be used in the control of mosquito vector control programs. *S. muticum* fabricated AgNPs showed higher antibacterial activity

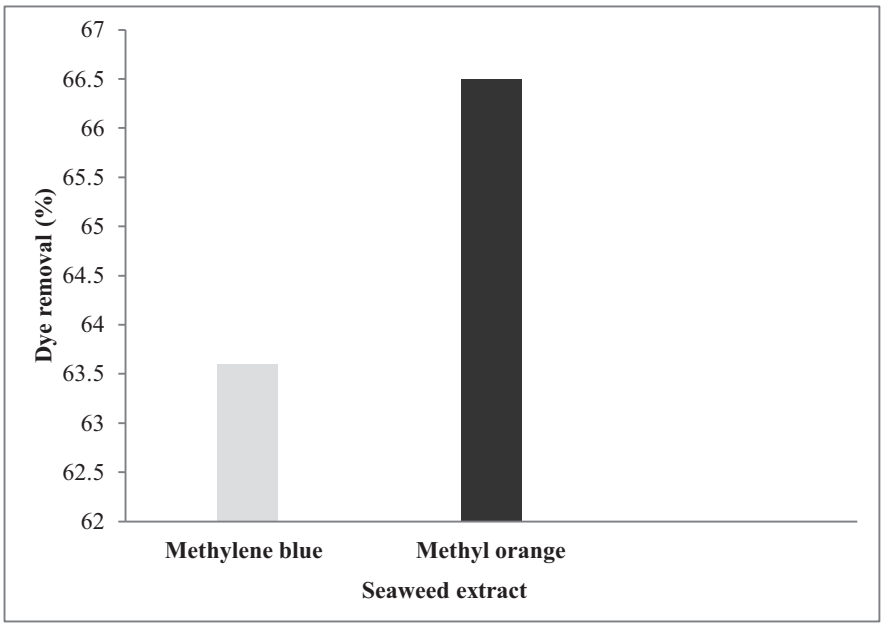

Fig. 10. Dye adsorbent properties of marine algae, *S. muticum* extract. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.).

compared with a commercial antibiotic suggesting its potential use as an eco-friendly antimicrobial agent. Noteworthy, these fabricated AgNPs have the additional ability to remove methylene blue and methyl orange, thereby indicating the possibility to be used as an eco-friendly alternative in the treatment of wastewater.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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