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Potentiality of Silver Nanoparticles Prepared by *Ulva fasciata* as Anti-nephrotoxicity in Albino-Rats

Neveen Abdel-Raouf, W.G.M Hozayen[*], M.F. Abd El Neem[#] and Ibraheem B.M. Ibraheem

Botany & Microbiology Department and [*]Chemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef, 62514, Egypt.

THE PRESENT study focused on the biosynthesis of silver nanoparticles using the ethanolic extract of the green alga *Ulva fasciata* (green algae). The synthesized silver nanoparticles were characterized by UV-Vis spectra, TEM (Transmission Electron Microscope) and FT-IR analysis. The absorption spectra of the formed AgNPs was observed at 350 nm, while the FT-IR spectrum revealed a presence of bands at about 2076.96 and 1840.72 cm^{-1}. On the other hand, TEM analysis , exhibited spherical and rod shaped AgNPs with size range of 2 to 200 nm. The chemical constituents, fatty acids, alkaloids, phenolic compounds, terpenoids, aromatic compounds of studied algal extracts were identified by GC-MS. The Gas chromatography-mass spectrometry (GC-MS) analysis of the green alga *Ulva fasciata* ethanolic extract showed a presence of fatty acids, alkaloids, phenolic, terpenoid and aromatic compounds. Potential activity test of the AgNPs formed by *Ulva fasciata* ethanolic extract against the nephrotoxicity on albino rats revealed good positive results indicated the preventive effects of the disease by using of *Ulva fasciata* AgNPs.

Keywords: Silver nanoparticles, Green biosynthesis, *Ulva fasciata*, Gentamicin, Nephrotoxocity.

Introduction

A wide variety of physical and chemical processes have been developed for the synthesis of metal nanoparticles (Kumar & Yadav, 2009), but these methods are expensive and require the use of toxic and aggressive chemicals as reducing and/ or capping agents (Li et al., 2009). Therefore, green chemistry should be integrated into nanotechnologies especially when nanoparticles are to be used in medical applications, which include imaging, drug delivery, disinfection, and tissue repair (Albrecht et al., 2006). The green biosynthesis of nanoparticles can be achieved via the selection of an environmentally acceptable solvent with eco-friendly reducing and stabilizing agents (Jegadeeswaran et al., 2012). Biologically reduction of silver from Ag^{+} to Ag^{0} with green alga *U. fasciata* (as reducing agent) is an effective and ecofriendly method for the synthesis of silver nanoparticles. This alga is a very abundant biomass in nature. Recent findings evidenced that marine algae possess antiviral, antibacterial, antifungal and antitumor-potentials, among numerous others (Ibraheem et al., 2012; Abdel-Raouf et al., 2013 and Ibraheem et al., 2016 & 2017). Marine algae have recently received significant attention for their potential as natural antioxidants, which may be arising from carotenoids, tocopherols and polyphenols. These compounds directly or indirectly contribute to inhibition or suppression of free radical generation (Abirwami & Kowsalya, 2012). Marine macroalgae *Chaetomorpha linum* contain more than 60 elements, macro and micronutrients, proteins, carbohydrates, vitamins, and amino-acids (Kannan et al., 2013) so research on marine algae has been increased considerably for the search of new and effective medicines of natural origin. Synthesis of silver nanoparticles by algal extract is more advantageous than other biological processes. It is cost effective, eco-safe and suitable for human therapeutic use. In the present study we use *Ulva fasciata* for the biosynthesis of silver nanoparticles (AgNPs) and we also investigate the antagonistic activities of the formed AgNPs against the nephrotoxicity effects cussed by application of gentamicin in albino rats.

[#]Corresponding author email: marwa_2060@yahoo.com

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Materials and Methods

The study area

The fresh algal species were collected from the inter-tidal region of Alexandria coast (Fig. 1) on the Mediterranean Sea along the 32 km. located at the north of Egypt during the spring year 2014.

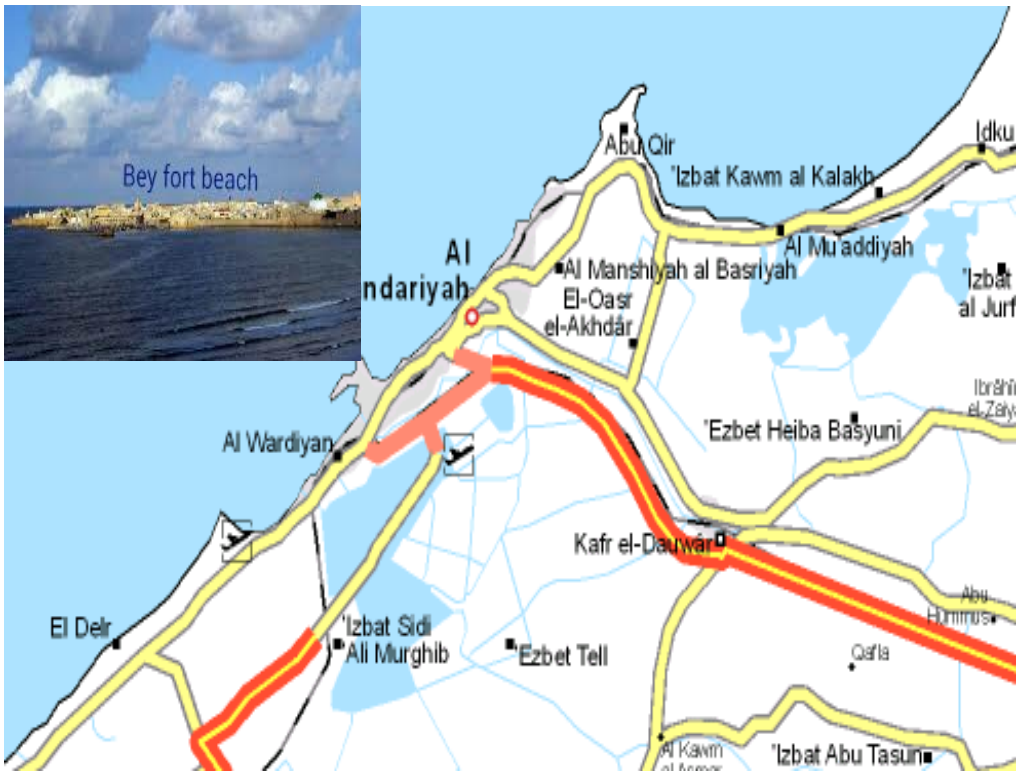

Fig. 1. Map shows the study area ,where samples are collected .

Collection and preparation of alga sample

The algal sample was manually collected from the intertidal region between 1- 3 m depth. Collected algal samples were immediately brought to the laboratory in new plastic bags containing sea water to prevent drying of algal samples. Algal material was washed thoroughly with tap water and filtered seawater to remove extraneous materials and shade-dried for 5 days and oven dried at 60°C until constant weight was obtained, then was grind into fine powder using electric mixer and stored at 0°C for future use. Algal species were identified according to Aleem (1978) and Coppejans et al. (2009) (Plate 1).

Plate 1. *U. fasciata*.

Synthesis of silver NPs by algal ethanolic extract

The synthesis of silver nanoparticles from *Ulva fasciata* , were carried out according to Singaravelu et al. (2007).

Synthesis of silver NPs by algal ethanolic extract

For synthesis of silver NPs by algal ethanolic extract, 10 mg of the dried ethanolic extract of *Ulva fasciata*, were added to 90 ml of 10^{-3} M aqueous $AgNO^{-3}$ (Sigma–Aldrich, city) solution in 500 ml conical flask and kept at room temperature for (5 min). Suitable controls were maintained throughout the experiments.

Characterization of AgNPs

UV–Visible spectroscopy analysis

TThe bio-reduction of $AgNO_3$ was confirmed by sampling the reaction mixture at regular intervals and the absorption maximum was scanned by UV–vis spectra, at the wavelength of 300–700 nm in Perkin Elmer Lamda 25 spectrophotometer (Japan). All the measurements were carried out at room temperature.

TEM analysis of AgNPs

The morphological analysis of the nanoparticles was done with transmission electron microcopy (TEM). A drop of aqueous silver nanoparticle sample was loaded on carbon-coated copper grid and it was allowed to dry completely for an hour at room temperature. The TEM micrograph images were recorded on a JEOL JEM 2100 high resolution transmission electron microscope (Japan). The clear microscopic views were observed and documented in different ranges of magnifications.

FTIR measurement

Fourier Transforms Infrared Spectroscopy (FTIR) was used to identify the possible biomolecules responsible for the reduction of the Ag ions and capping of the bio-reduced silver nanoparticles synthesized by extract *Ulva fasciata.* In order to determine the functional groups and their possible involvement in the synthesis of silver nanoparticles, liquid algal silver nanoparticles analyzed on a Jasco 430 (Japan), in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

GC-MS Data analysis

The crude ethanolic extracts of *Ulva fasciata* was analyzed by Gas chromatography-Mass spectrometry (GC-MS) for determination of active substances in extracts.

Biochemical analyses

Chemicals and drugs

Gentamicin was purchased from Sigma Company (United Kingdom), urea kit from Biomed (Egypt), creatinine kit from Diamond Diagnostics (Egypt) and uric acid kit from spectrum (Egypt).

Experimental animals

White male albino rats (*Rattus norvegicus*) weighing about 110-150 gm were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Opthalmology, El-Giza, Egypt. They were kept under observation for about 10 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at normal atmospheric temperature (25±5^{o}C) as well as 12 h daily normal light periods. Moreover, they were given access of water and supplied daily with standard diet of known composition and consisting of not less than 20% proteins, 5.5% fibers, 3.5% fats and 6.5% ash and were also supplied with vitamins and mineral mixtures. This experiment was conducted under controlled of the animal house supervisor (assistant supervisor), Biochemistry Department, Faculty of Science, Beni-Suef University.

Experimental design

The considered rats were divided into 3 groups containing six animals for each. These groups were:

Group 1: It was regarded as normal animals which were kept without treatments under the same laboratory conditions.

Group 2: The animals in this group were received intraperitoneal nephrotoxic dose of gentamicin for 14 days (80 mg/kg body weight) (Hozayen et al., 2011). This group was considered as control for the remaining groups.

Group 3: (Toxic treated with nanoparticles *Ulva fasciata* aqueous extract): The rats in this group were administrated by nanoparticles *Ulva fasciata* aqueous extract by gastric intubation after gentamicin at dose level of 150 mg/kg b.wt for 14 days (Abirami & Kowsalya, 2015 and Abirami & Kowsalya, 2012).

All the treatments were performed orally and daily between10.00 and 11.00 a.m. By the end of the experimental periods, normal, control groups and treated rats were sacrificed under diethyl ether anesthesia. Blood samples were taken and centrifuged at 3000 r.p.m. for 30 min. The clear non-haemolysed supernatant sera were quickly removed, divided into three portions for each individual animal, and kept at -20 °C till used according to Proverbio et al. (2015).

Assay of kidney functions

Urea concentration in serum was determined according to the method of Vassault et al. (1986) using the reagent kits purchased from Biomed Diagnostics (Egypt). Uric acid concentration in serum was determined according to the method of Tiffany et al. (1972) using reagent kits purchased from spectrum Company (Egypt). Creatinine level in serum was determined according to the method of Henry & Bernard (2001) using the reagent kits purchased from Diamond Diagnostics (Egypt).

Histopathological assessment

After sacrification and dissection, kidneys were immediately excised, cleaned, washed, and rinsed in an ice-cold normal saline solution (0.9% NaCl, pH 7.4) until bleached of all the blood and blotted dry on filter paper sheets to remove blood, then kept in 10% neutral buffered formalin (pH 7.4) for at least 24 h for histopathological investigation at the histology unit, National Cancer Institute, Cairo University, Egypt for preparation. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 µm thicknesses by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin & eosin stain for routine examination through the light electric microscope (Banchroft et al., 1996)

Statistical analysis

The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985) followed by LSD test to compare various groups with each other. Results were expressed as mean ± standard deviation (SD) and values of $P > 0.05$ were considered non-significantly different, while those of $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered significantly, highly and very highly significantly different, respectively.

Results and Discussion

Reduction of $AgNO_3$ was visually evident from the colour change (colorless to brownish-yellow) of reaction mixture after 3 min of reaction (Plate 2). Intensity of brown colour increased in direct proportion to the incubation period. It may be due to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$ (Mulvaney, 1996). Also, the time taken for AgNPs formation very fast (less than 3 min). These result are agree with many investigators (Rajeshkumar et al., 2012 and Abdel-Raouf et al., 2013) who reported that the appearance of dark brown color in solution. Also, Johnson et al. (2015) reported formation of silver nanoparticles in a few mint, and Bhimba et al. (2014) and Devi & Bhimba (2014) who suggested the time formation of silver nanoparticles 10 min at 121ºC. But Rahimi et al. (2013) who reported that the formation of silver nanoparticles in a long time, while in the current work the algal nanoparticals recorded high stability and formed within short time, although Sajidha Parveen & Lakshmi (2016) reported biosynthesis of silver nanoparticles using red algae, *Amphiroa* fragilissima time of reduction of AgNO-3 was visually evident from the colour change (brownish-yellow) of reaction mixtures within 20 min.

Characterization of AgNPs synthesized by U. fasciata

UV-visible spectroscopy analysis

The formation of silver nanoparticles was confirmed by color changes followed by UV–Visible spectrophotometer analysis. The UV–Visible spectrophotometer has proved to be a very useful technique for the analysis of some metal nanoparticles. Absorption spectrum of reaction mixture at different wavelengths ranging between 300-400 nm revealed a peak at 320 nm (Fig. 2). The characteristic absorption peak at 320 nm UV-vis spectrum (Fig. 2) confermed the formation of AgNPs. This is similar to the surface plasmon vibrations with characteristic peaks of AgNPs prepared by chemical reduction (Kong & Jang, 2006). The frequency and width of the surface Plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium (Kasthuri et al., 2009 and Sharmaet et al., 2009).

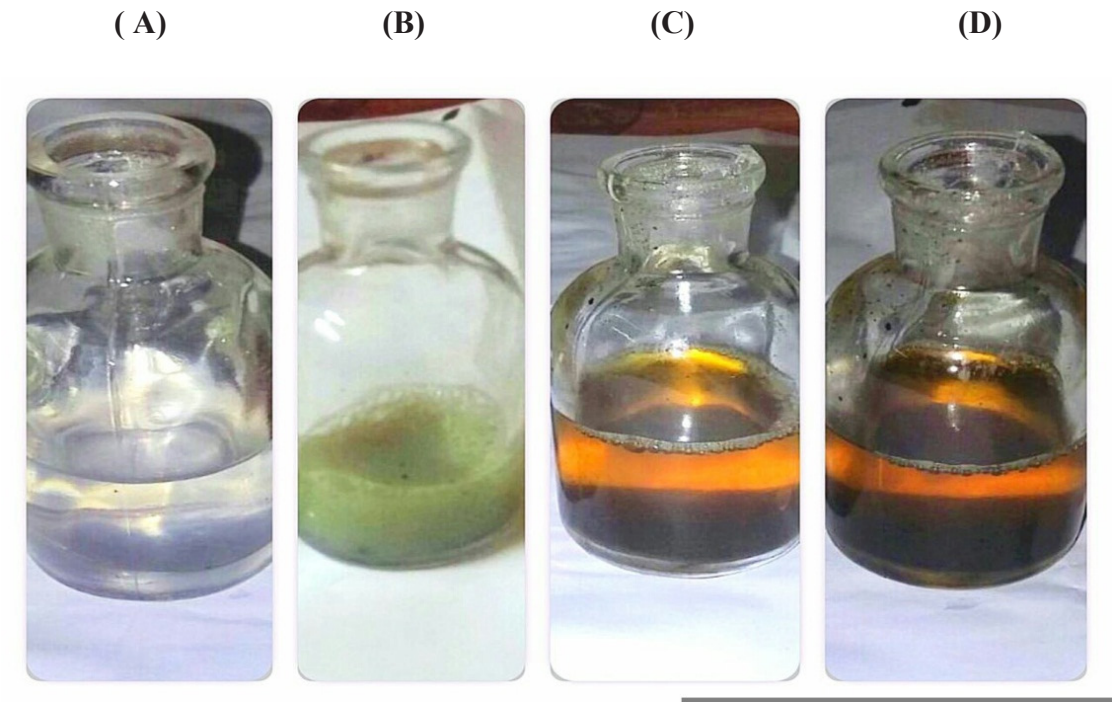

Plate 2. Tube A containing the aqueous solution of 10^{-3} M of silver nitrate with clear color at the beginning of reaction; after adding *Ulva fasciata* ethanolic extract with yellow brown color after 3 min (B), after10 min with brown (C) and after 30 min adding with dark brown color (D).

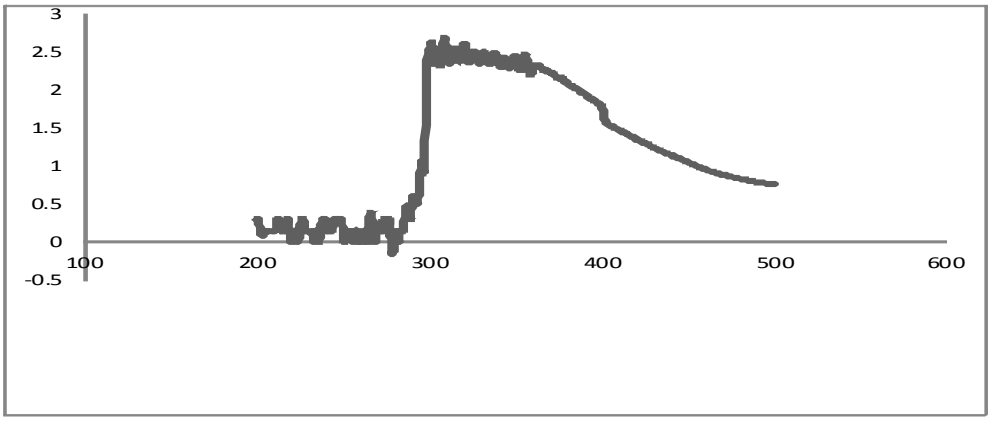

Fig. 2. UV-visible rang spectra of AgNPs synthesized from Plate 2 *U.fasciata* ethanol extract.

TEM of AgNPs

Transmission electron microscopy (TEM) analysis, measurement were applied to characterize the nanoparticles size and morphology. TEM micrographs of representative silver nanoparticles synthesized by *U. fasciata* ethanol extract are shown in Plate 3. These result showed that ethanol extract of *U. fasciata* strongly affected the size and shape of the silver nanoparticles were not uniform in size and shape. The nanoparticles were large and small spherical particles and small percentages of rod. TEM analysis of particles size also showed maximum particles in size 2 to 200 nm. These results indicated that the ethanol of *U. fasciata* strongly affected the size and shape of the silver nanoparticles. We hypothesize that the bioactive constituents of the studied alga play a pivotal reduction and controlling role in the formation of AgNPs in the solution.

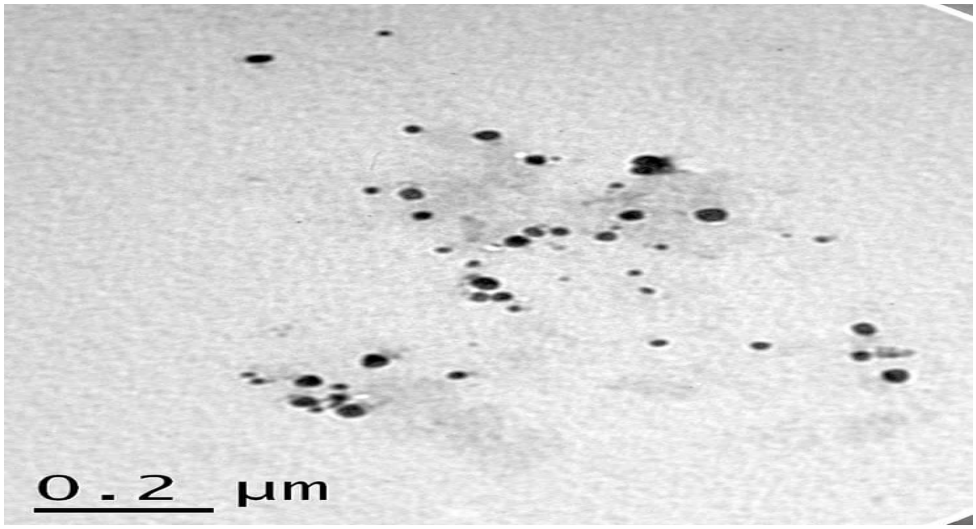

Plate 3. Representative TEM micrograph silver nanoplates synthesized by the reduction of $AgNO^{-3}$ ions in an *Ulva fasciata* ethanolic extract.

FT-IR analysis of AgNPs

FT-IR spectroscopy measurements were carried out to identify the possible biomolecules present in *U. fasciata* which are responsible for reduction and capping of silver NPs. The control spectra (Fig. 3) showed a number of peaks thus reflecting a complex nature of the *U. fasciata* ethanol. In order to determine the functional groups on *U. fasciata* ethanol extract was performed. Figure 3 revealed the band intensities in different regions of the spectrum test sample were analyzed. Graph that there was a shift in the following peaks: 2076.96 and 1840.72 cm^{-1}. The IR bands at 2076.96 and 1840.72 cm^{-1} are characteristic of the Aromatic C – H

Gc-Ms analysis of the ethanolic extract of U. fasciata

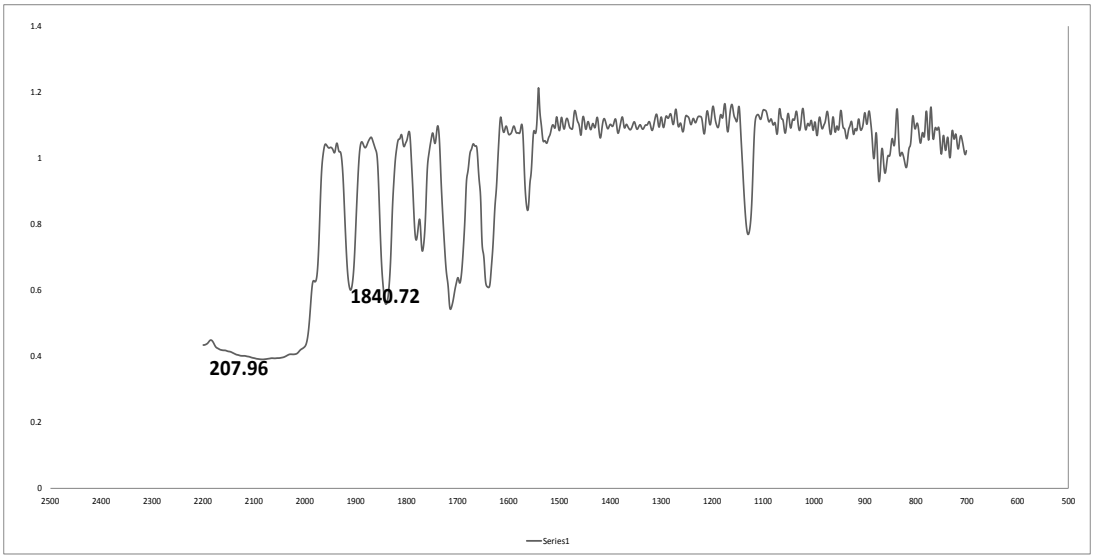

Fig. 3. FT- RI spectrum of silver nanoparticles synthesis by Ulva fasciata ethanolic extract.

Marine macroalgae, or seaweeds as they are more commonly known, are one of nature's most biologically active resources, as they possess a wealth of bioactive compounds. Compounds isolated from marine macroalgae have demonstrated various biological activities (Babu et al., 2014). The GC–MS analysis of ethanolic extracts of *U. fasciata* revealed many components, phytochemical screening of the algae showed the presence of fatty acid e.g., Butyric, Strearic, Myristic acid, Palmitoleic acid, Palmitic acid. Terpenese as agood antioxidante. Esters is used in medicine. Hydrocarbons used as Antibacterial, antimicrobial .Ketones, Aldehydes, Sulfur compounds and Phenolics (Table 1).

Effect of Ulvta fasciata nanoparticles on Urea, Uric acid, Creatinine levels in rats

Data in Table 2 indicated that blood urea nitrogen, uric acid and creatinine activities were significant ($P < 0.001$) elevated in gentamicin in toxicated rats in comparison to control group. The recorded data were (1.15 ± 0.04mg/dl), gentamicin + Ulva fasciata rats comparison to gentamicin group (0,69± 0.03mg/dl) for uric acid, gentamicin intoxicated rats in comparison to control group, The recorded data were (60.2± 3.0 mg/dl), gentamicin+ Ulva fasciata rats comparison to gentamicin group (38.5 ± 1.4 mg/dl) for Urea, and Gentamicin intoxicated rats in comparison to control group, The recorded data were (1.10±0.09 mg/dl), gentamicin+ Ulva fasciata rats comparison to gentamicin group (0,36 ± 0.03mg/dl) for creatinine along the (14 day), respectively. Otherwise, administration of gentamicin-intoxicated rats with control group the percentages of change were (0.408%) for Uric acid (-0,700%) for urea, and (-1.44%) for creatinine. Respectively nanoparticles of Ulva fasciata at daily doses of (150 mg/kg b.wt, orally) showed significant ($P < 0.001$) decrease of these levels as compared with their gentamicin-intoxicated rats, the percentages of change were (-0.400%) for Uric acid, (-0,360%) for urea and (-0.672%) for creatinine (Fig. 4,5 and 6)

Gentamicin induced toxic effects in the kidney (Hozayen et al., 2011). The renal dysfunction due to gentamicin treatment was manifested by a very highly significant increase in serum urea, creatinine and uric acid levels as compared to the normal group of rat. This is in agreement with the results of Hozayen et al. (2011). It was reported that treatments with gentamicin produces nephrotoxicity as a result of reduction in renal functions which was reflected in an increase in serum creatinine and serum urea level accompanied by impairment in glomerular functions. Serum creatinine level was more significant than the urea levels in the earlier phase of the renal damage. In the present study, it was shown that treatment with gentamicin alone to rats caused nephrotoxicity, which was correlated with increased creatinine, and urea levels in plasma (Hozayen et al., 2011). Algae are a good source of compounds with a positive impact in human health. *Ulva* organic components had been identified in algae of *Ulva* genus.

Simple bromophenols, especially 2, 4, 6-tribromophenol, lipid components, dimethylsulfoniopropionate (DMSP) had been largely described for this genus (Mendes et al., 2010). It was reported that a sulfated heteropolysaccharide from *Ulva rigida*, which belongs to the same family as Enteromorpha, could markedly activate macrophages (Leiro et al., 2007). It was also found that over sulfated fucoidan inhibited the growth of Lewis lung carcinoma and B16 melanoma in mice, and increasing numbers of sulfate groups in the fucoidan contributes to the effectiveness of its anti-angiogenic and antitumor activities (Koyunagi et al., 2003).

Our result showed that the treatment of gentamicin intoxicated rats with nanoparticles algae extracts cause decreasing in serum creatinine, serum uric acid and serum urea level due to its ability to treatment nephrotoxicity. This is in agreement with the results of Hozayen et al. (2011). Urea is the end product of protein catabolism, and the presence of some toxic compounds might increase blood urea and decrease plasma protein (Varely et al., 1987). Enhanced catabolic proteins and accelerated amino acid deamination for gluconeogenesis is possibly an acceptable postulate to interpret the elevate urea levels (Bishop et al., 2005). Uric acid is the end product of nucleic acid catabolism in tissues, and the increment in its concentration might be due to degradation of purines or by either over production or inability of excretion. Elevated serum creatinine is indicative of renal injury and associated with abnormal renal function, particularly as it relates to glomerular function (Bennett, 1996 and Bishop et al., 2005). Upon treatment of intoxicated rats with nanoparticles *U. fasciata* improve and normalize the levels of urea, creatinine and uric acid. These results indicated that these extracts may protect against gentamicin induced renal toxicity.

TABLE 1. Gc of - Ms analysis of the ethanolic extract of *Ulva fasciata*.

s	Compound (IUPAC-Name)	Common name	Peak area %	Activity
	Fatty acids			
1	Tetradecanoic acid	Myristic	0.18	Antioxidant
	Hexadecanoic acid	Palmitic acid	15.03	Muscle weakness, Tetany, Anemia, Diarrhea, Pulmonary edema, Respiratory failure. Antiviral, antibacterial, antioxidant activities
	9- Octadecenoic acid, (E)	Elaidic acid	2.93	
	9- Octadecenoic acid, (Z)	Oliec acid	0.73	antiviral,antibacterial ,antioxidant activities
	9,12,15-Octadecenoic acid	Linoliec acid	1.23	
	Esters			
2	-Tetradecanoicacid, methyl ester	Methyl tetradecanoate	0.3	Muscle weakness, Pulmonary
	-Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	11.03	edema, Anemia, Respiratory
	-9- Hexadecanoic acid, methyl ester		1.34	failure, Drowsiness, Diarrhea
	-Hexadecanoic acid, ethyl ester	Palmitic acid, ethyl ester	0.73	Muscle weakness, Pulmonary
	-Octadecenoicacid, methyl ester	Strearic acid, methyl ester	0.94	edema, Anemia, Respiratory
	10,13- Octadecenoicacid, methyl ester		1.31	failure, Drowsiness, Diarrhea.
	-9- Octadecenoicacid, methyl ester		1.84	
	-9,12,15-Octadecenoic acid, methyl ester	Linoliec acid, methyl ester	1.71	Sleep disturbance, Tetany.
	- Carbamic acid, methyl ester	Urethylane	1.61	antiviral,antibacterial ,antioxidant activities
	- Phthalicacid, di(2-propylpentyl) ester		0.38	Anesthetic, Antioxidant, catalase activator, Antioxidant

TABLE 1. Cont.

S	Compound (IUPAC-Name)	Common name	Peak area %	Activity
3	**Hydrocarbons**	Tetradecene	0.81	as a solvent for perfumes andflavors and in medicine.
	n- Tetradecane	Hexadecane	1.29	
	n- Hexadecane	Octadecane		
	n- Octadecane	Dodecane	0.58	
	n-Dodecane	1-Nonadecene	0.34	Antibacterial, antimicrobial
	Nonadec-1-ene		0.20	
4	**Terpenese**			Antioxidant
	-(-)Loliolide	-(-)Loliolide	1.00	
	Neophytadiene	Neophytadiene	11.3	antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant
5	**Ketones**		0.58	anticancer
	Methyl propyl ketone	2-Pentanone		
	2-Pentadecanone,6,10,14-trimethyl-	Hexahydrofarnesyl acetone	0.67	
6	**Aldehydes**			
	2- Furancarboxaldyde	Fufural	9.52	Antifungal, antimicrobial
7	**Sulfur compounds**		21.27	Antibacterial, antifungal,anticancer, as dietary supplement
	Dimethyl Sulfoxide	DMSO		
8	**Phenolics**			
	1-(Methylphenyl)-2,7-dimethylant		0.31	anticancer

TABLE 1. Cont.

s	Compound (IUPAC-Name)	Common name	Peak area %	Activity
9	Cyclohexasiloxane, dodecamethy		0.37	Antifungal
10	Benzene, 1-ethyl-4-methyl	Toluene,p-ethyl	1.69	antiviral, antibacterial, antioxidant activities
11	4-Butyl-indan-5-ol		0.37	anti-inflammatory and Antituberculosis
12	Cyclohexasiloxane,tetradecamet		0.3	
13	Dihydroactinidiolide		١.43	
14	3-Ethyl-4-(phenylsulfonyl)isoxazolin		١٦.1	
15	8-Heptadecene	(8E)-8-Heptadecene	3.31	Pulmonary edema, Irritation,Tetany, Diarrhea, Anemia, Respiratory failure
16	m-Nitrobenzaldehyde dimethylhydrazone		1.30	
17	2-Pentadecanone,6,10,14-trimethyl		0.67	
18	Methyl4,7,10,13-hexadecatetren		٠.٤٥	
19	Anthracene,9,10-dihydro-9,9,10trimethyl		0.64	Anti timer, Anticancer
20	1-(Methylphenyl)-2,7-dimethylanthracene		0.31	

TABLE 2. Effect of *Ulva fasciata* nanoparticles on uric acid, urea and creatinine level in rats.

	Uric acid (mg/dl)	% Change	Urea (mg/dl)	% Change	Creatinine (mg/dl)	% Change
Normal	0.68 ± 0.1[b]	-	35.4 ± 0.9[b]	-	0.45 ± 0.04[b]	-
Gentamicin	1.15 ± 0.04[a]	0.408	60.2 ± 3.0[a]	0.700	1.10 ± 0.09[a]	1.44
Gentamicin + *Ulva*	0.69 ± 0.03[b]	-0.400	38.5 ± 1.4[b]	-0.360	0.36 ± 0.03[b]	-0.672
F- Probability	< 0.001		< 0.001		< 0.001	
LSD at 5 % level	0.102		6.622		0.175	
LSD at 1 % level	0.139		9.031		0.239	

- Data are expressed as mean ± standard error.
- Number of animals in each group is six.
- Means, shared only superscript symbol (s) are not significantly different.
- Percentage changes (%) were calculated by comparing gentamicin (is it gentamicin or gentamycin? be consistent!) Unintended group with normal, gentamicin treated groups (gentamicin + Ulva) were compared with gentamicin group.

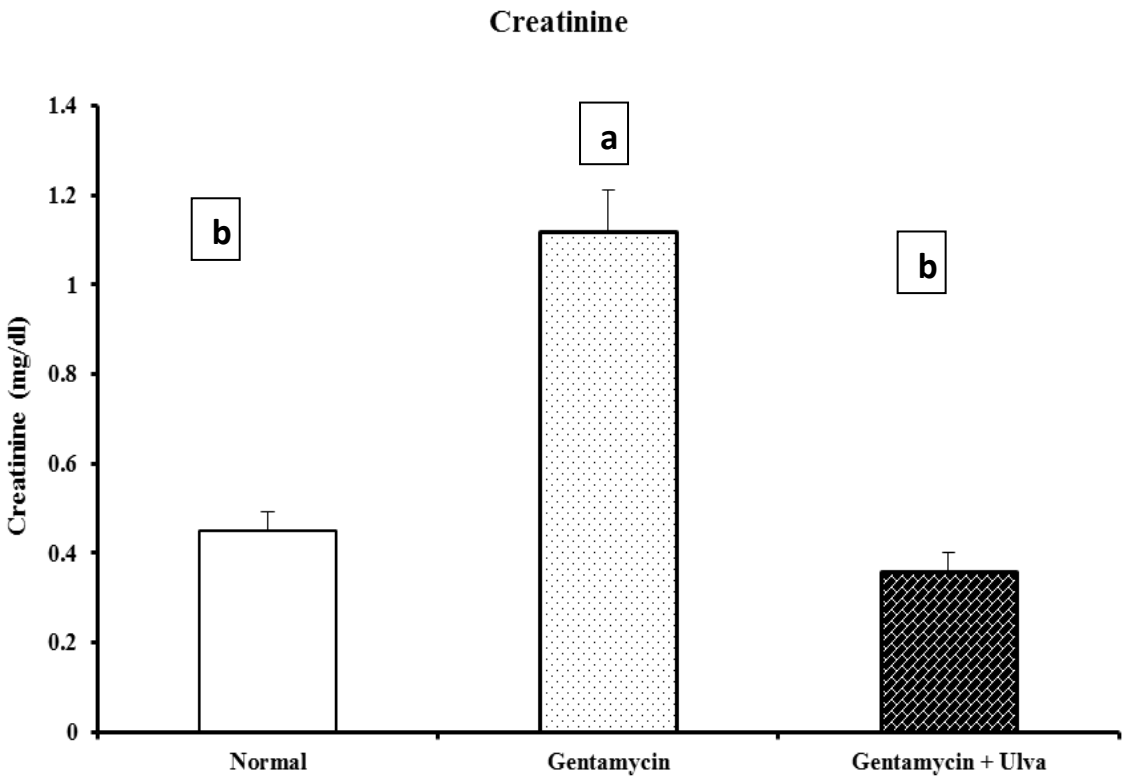

Fig. 4. Effect of *Ulva fasciata* nanoparticles on serum. Creatinine level.

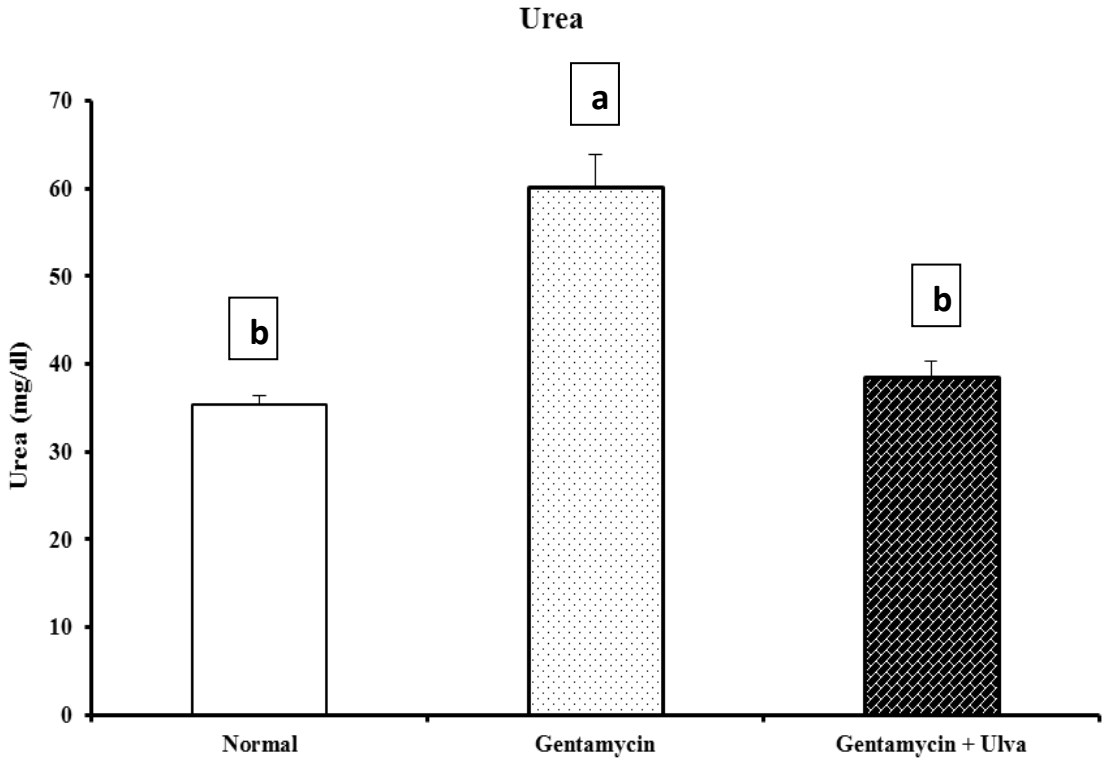

Fig. 5 Effect of *Ulva fasciata* nanoparticles on serum urea level.

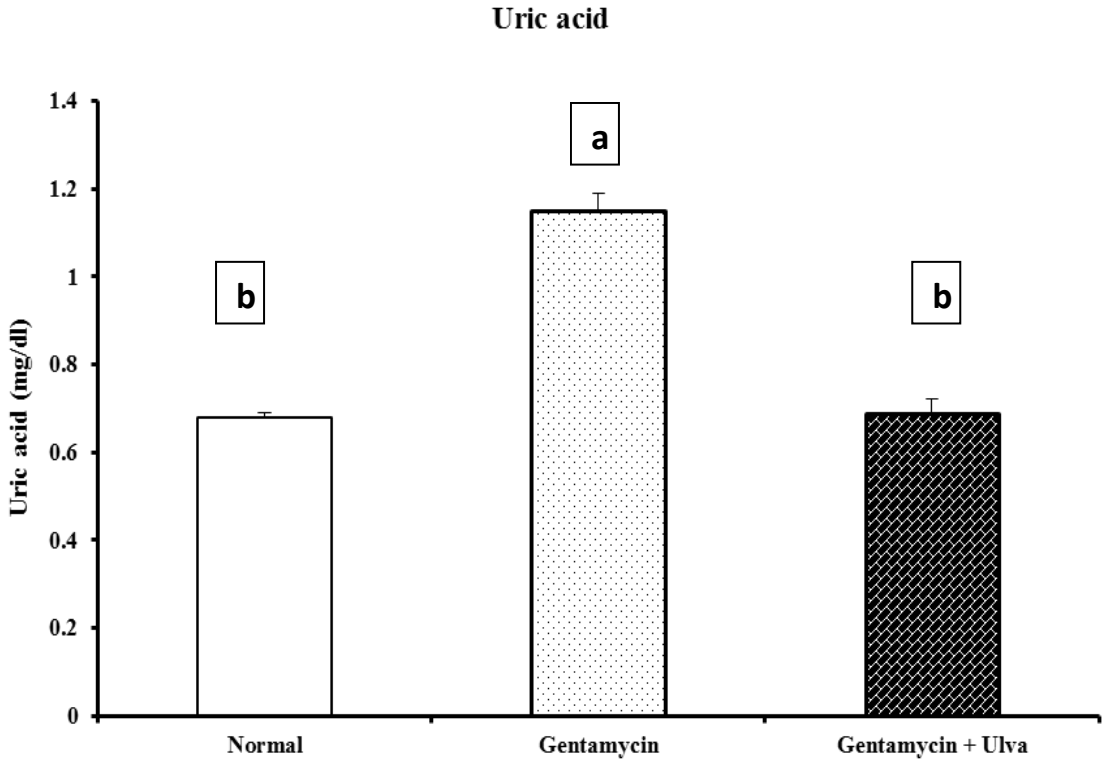

Fig.6. Effect of *Ulva fasciata* nanoparticles on serum uric acid level.

These results are agreement with Mahmoud & Hussein (2014) who demonstrated that aqueous extract of *Ulva lactuca* reduced the nephrotoxicity against N-nitrosodiethylamine and Phenobarbital. It was reported that treatments with gentamicin produces nephrotoxicity (Atessahin et al., 2003) as a result of reduction in renal functions which was characterized by an increase in serum creatinine and serum urea level accompanied by impairment in glomerular functions. Serum creatinine level was more significant than the urea levels in the earlier phase of the renal damage.

Uric acid, Urea and Creatinine levels decreased significantly in the groups 3 with tested nanoparticles algal extracts in comparison with the group 2.

These results indicated that these extracts may protect against gentamicin induced renal toxicity due to these extract contain on Sulfur compounds and active antioxidant constituents, fatty acids, esters, terpenese and sterols. This is in agreement with the results of Mahmoud & Hussein (2014) who demonstrated that oral supplementation of *Ulva lactuca* extract reduces nephrotoxicity by N-nitrosodiethylamine and Phenobarbita.

Histophathological effects (This paragraph is repeated one)

In control group show kidney there was no histopathological alternation and the normal histological structure of the gloimeruli and tubules at the cortex (Fig. 7). But, in group-II – rats treated with 80mg/kg b.w of gentamicin. In treated group compared with control indicated the kidney the renal tubules showed coagulative necrosis in the lining epithelial cells (Fig. 8). While, in group-III –rats treated with 150mg/kg b.w of nanoparticles Ulva fasciata compared with rats treated with gentamicin in the kidney mild congestion in the cortical blood vessels (Fig. 9).

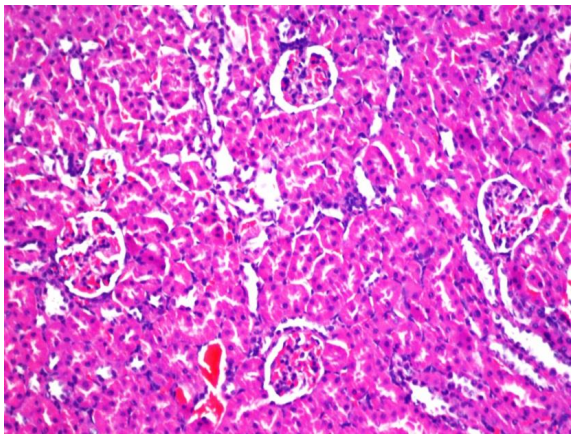

Fig. 7. Kidney of rat showing normal histological structure of the gloimeruli and tubules at the cortex.

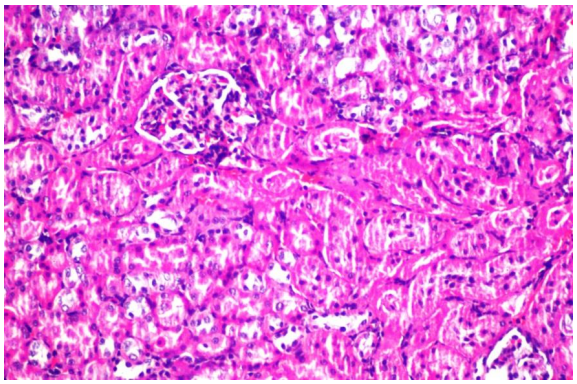

Fig. 8. Showing coagulation necrosis in lining tubular epithelium of the cortical portion

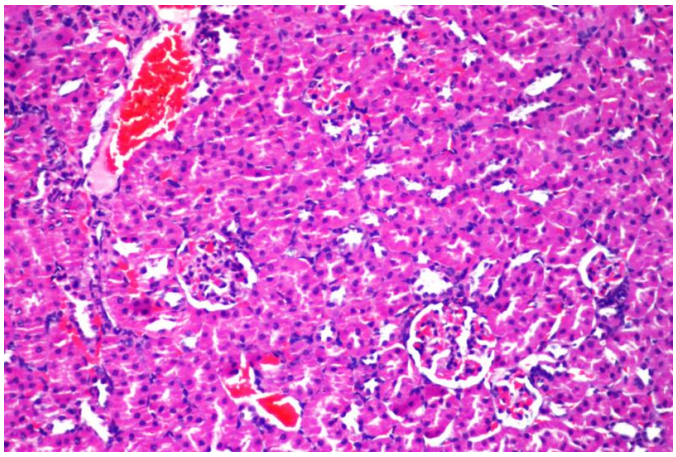

Fig . 9. Showing mild congestion in the cortical blood vessels.

Conclusion

Through the study we have confirmed that the ethanolic extract of seaweed ethanolic extract of green alga *Ulva fasciata* is capable of forming AgNPs by reducing $AgNO^{-3}$ solution and is proved to be an efficient, eco-friendly and simple method. We have characterized the synthesized AgNPs using several techniques. The characteristic absorption peak at 300-400 nm in UV-Visible spectrum . TEM analysis of *Ulva fasciata* synthesized silver nanoparticles exhibited large and small spherical particle's and small percentages of rod. The characteristic peaks in the FTIR spectrum revealed the presence of functional bio active metabolites in seaweed extract which is responsible for the formation of AgNPs. The chemical constituents, fatty acids, alkaloids, phenolic compounds, terpernoids, aromatic compounds of studied algal extracts were identified by GC-MS. The present study concludes that nanoparticles of *Ulva fasciata* has nephroprotective activity. This nanoparticles significantly attenuated the physiological and histopathological alterations induced by gentamicin.

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أمكانية جزيئات النانونية الفضية المستخلصة من طحلب أولفا فاسياتا كمضاد للتسمم الكلوي في فئران التجارب

نيفين عبدالرؤف محمد، ولاء جمال محمود حزين*، مروة فرغلي عبد النعيم و ابراهيم برعي محمد إبراهيم
قسم النبات و الميكروبيولوجي و*قسم الكيمياء - كلية العلوم - جامعة بنى سويف- بنى سويف - مصر.

ركزت الدراسة الحالية على التركيب الحيوي للجسيمات النانوية الفضة باستخدام مستخلص الايثانول من الطحالب الخضراء **أولفا فاسياتا** وقد تميزت جسيمات الفضة النانوية التي تم توليفها بقيس أطياف الأشعة فوق البنفسجية في منطقه 350 نانومتر ، ومن تحليل الميكروسكوب الألكتروني(TEM) حصلنا علي أشكال عصويه وكرويه ومسطحه في حجم من 2 إلى 200 نانومتر. في حين كشف تحليل الطيف (FT-IR) وجود العصابات في حوالي 2076.96 و 1840.72 سم $^{-1}$. تم تحديد المكونات الكيميائية والأحماض الدهنية والقلويدات والمركبات الفينولية والتربينويدات والمركبات العطرية من المستخلصات الطحلبية المدروسة بواسطة تحليل GC-MS. أظهر هذا التحليل على وجود الأحماض الدهنية، قلويدات، الفينولية، تربينويد والمركبات العطرية. اختبار النشاط المحتملة من جزيئات النانو الفضيه التي تم توليفها من مستخلص إيثانول من **أولفا فاسياتا** ضد سمية الكلوية على الفئران البيضاء كشفت نتائج إيجابية جيدة تشير إلى الآثار الوقائية للمرض باستخدام جزيئات النانوالفضيه من طحلب **أولفا فاسيتا**.