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PHYTOCHEMICAL EVALUATION AND ANALYSIS OF ULVA INTESTINALIS USING GC-MS TECHNIQUE AND ITS PROTECTIVE EFFECTS AGAINST ETHYLENE GLYCOL-INDUCED UROLITHIASIS IN RATS

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Abstract
Seaweed has a huge potential as an alternative source for high-quality and healthier food products that have inspired researchers in the last few years. Despite considerable progress in medical therapy, there is no satisfactory drug to permanently treat kidney stones. Therefore, in the current study, the effects of oral administration of hydroalcoholic extract of *Ulva intestinalis* on calcium oxalate urolithiasis have been investigated. Various phytochemicals were evaluated by gas chromatography coupled mass spectrometry technique. The GC-MS analysis of the methanolic extracts identified 31 metabolites in *Ulva intestinalis* belonging to different chemical classes. Administration of hydroalcoholic extract of *Ulva intestinalis* significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. The serum levels of creatinine, uric acid, and BUN were also reverted to optimum suggesting the attainment of the normal kidney physiology. Consequently, the anti-urolithiatic activity found in the Ulva indicates its promising role in future perspectives as a treatment option for patients with calcic urolithiasis.

Key words: Ulva intestinalis, marine alga, GCMS, kidney stones, ethylene glycol, calcium oxalate

1. Introduction

One of the most prevalent illnesses in the developing world is urolithiasis. It is estimated that the lifetime risk of kidney stones is 3–5% for women and 10-15% for men. Situated at the centre of the Afro-Asian stone belt, Pakistan's population has a 10–15% prevalence of stone sickness. The process of stone production is complex and multifaceted, involving a series of events such as the nucleation, growth, and aggregation of crystals that form stones, as well as the retention of these aggregates in the renal tubules. The reactive oxygen species, or ROS, may play a part in the production of stones, according to an increasing number of researches. It has been demonstrated that elevated oxalate and calcium oxalate crystal levels cause damage to renal cells, which raises ROS levels (Yasir et al 2018). Even though this condition has been recognized since antiquity, there aren't many effective

urolithiasis preventives on the market, and it is still unclear how kidney stones form. The past two decades have seen the development of minimally invasive management techniques like percutaneous nephrolithotomy; however, these procedures have serious side effects, including bleeding and renal fibrosis. For this reason, it is of critical importance to identify substances that can effectively prevent crystal deposition and the development of urolithiasis in the healthcare setting (Zhu et al 2014).

A few composite herbal drugs and plants, the majority of the remedies in traditional medical systems, such as Ayurveda, were derived from plants and were shown to be effective, though the reasoning behind their use has not been thoroughly established through systematic pharmacological and clinical studies. According to reports, these plant-based treatments have no negative side effects and are useful in reducing the recurrence rate of renal calculi (KVSRG et al 2007). Microalgae are thought to be a potent source of biofuels and bioactive molecules, such as pigments, carotenoids, vitamins, proteins, and phenolic compounds, which have a wide range of applications in the manufacturing of medicines, cosmetics, animal feed additives, nutraceuticals, and vitamins. The marine natural products have long been used to prevent and treat a wide range of illnesses, making them excellent candidates for developing anticancer, antibacterial, and antiviral medications as well as treating other conditions like diabetes (Smith-Warner et al 2000).

The *Ulva* genus of green seaweed may offer a promising source of bioactive ingredients to be used as functional foods. Although sulphated polysaccharides, one of *Ulva*'s bioactive components, have garnered considerably greater attention recently for their potential medical uses, the plant has long been utilized for food consumption in both fresh and dried forms due to its high nutritional content. *Ulva intestinalis* was found useful in accumulating a higher amount of metals such as copper (Cu), chromium (Cr), Zinc (Zn), cadmium (Cd), and lead (Pb) in comparison to other marine alga (5). Numerous researches suggest a diversified bioactivity of *Ulva intestinalis*, however, so far, no scientific study has been reported regarding the antiurolithiatic property of the extract of *Ulva Intestinalis*. In this study, we investigated the protective effect of the hydro-alcoholic extract of *Ulva Intestinalis* against ethylene glycol-induced urolithiasis and its possible underlying mechanisms.

2. Materials and Methods

2.1. Chemicals and Apparatus:

Ethylene glycol and Ammonium chloride were obtained from Merck Ltd. Germany. All other chemicals and reagents used were of analytical grade and procured from approved chemical suppliers. Apparatus such as the metabolic cages (Tecniplast, Italy), semi-auto analyzer (Metrolab, 1600-DR), *cold centrifuge (Remi Instruments, C-30BL)*, UV-spectrometer (Shimadzu Scientific Instruments, UV-3600) were used in the study.

2.2 Sample Collection and Preparation.

The samples were taken from sandy bays, large and shallow sand-bottom flats, and distinct places along Karachi coast during low tide. A voucher specimen was placed in the University of Karachi, Pakistan's Department of IMS Herbaria. Using a soft bristle brush, collected seaweed samples were cleared of any unwanted items such as sand particles, epiphytes, shells, and stones. They were then completely cleaned with seawater.

These samples were divided into two groups. One group was sent for taxonomic identification and placed in the herbarium. Cross-referencing taxonomic identification was carried out through taxonomic publications, monographs, and reference herbaria. The samples were properly cleaned in the lab using tap water and then distilled water. They were then frozen in a deep freezer (Thermo Scientific, USA) for an entire night at -80°C. After that, a laboratory-scale blender was used to grind all of the dried seaweed samples into a fine powder, and a 250 µm-sized sieve was used to filter them. 120 grams of fine sample powder were extracted using methanol in a Soxhlet device and concentrated on a rotary evaporator. Following the procedure by (Shaheen et al 2000), the methanolic extract (35.56 g) was separated using water, hexane, dichloromethane, and ethyl acetate in that order (Habtemariam, 2019). At room temperature of 30 minutes, the final solution was extracted by

sonication using an ultrasonic water bath (Power Sonic 505, Korea). After that, Whatman No. 1 filter paper was used to filter the resultant solvent extract. The filtered extracts were combined and dried with a rotary evaporator kept at 40°C. After that, the concentrated extracts were kept for later use in a freezer in amber bottles.

2.3. GC-MS analysis of *Ulva intestinalis*:

The seaweed extracts were subjected to GC-MS analysis to identify and measure the bioactive compounds present. With a few minor adjustments to the methodology used by (Han et al. 2009), methanolic extracts were quantitatively analyzed using GC-MS on an Agilent Technologies 7890A/7000 GC-MS Triple Quad system. 3.5 µL of the material was separated on an AGILENT DB-35MS 360 °C: 30 m x 320 µm x 0.25 µm column during the experimental process. A one-minute purging time was used for the split-less injection. Helium was the carrier gas, and the flow rate was 0.5 milliliters per minute whereas, a 1µl injection volume of the sample was used. The oven temperature was programmed initially at 50°C for 5 min, then increased to 200°C for 15 minutes at a rate of 5°C, and then programmed to increase to 300°C at a rate of 10°C ending with a 10 min. The total run time was 70 min whereas, the inlet and the detector temperatures were set at 250°C, the MSD transfer line was maintained at 260°C, and the solvent cut time was set at 4.50 minutes. The component spectrums were compared to the database of known component spectrums kept in the GC-MS library.

2.4. Animals Selection

For this experiment, 18 male Wistar albino RATs weighing between 180 and 200 grams at 7-8 weeks of age were employed. They were purchased from the ICCBS Animal Center University of Karachi in Pakistan. The RATs were kept on a 12-hour light and 12-hour dark cycle and were acclimated to regular laboratory settings (temperature: 25±2 ◦C). The animals were given regular rat chow and drinking water ad libitum supplied by ICCBS University of Karachi Pakistan and weighed daily. The study protocol was approved by the Institutional Animal Ethics Committee (Ref. No: IBC KU 412/2024) University of Karachi, Pakistan.

2.5. Antiurolithiatic activity of hydro-alcoholic extract of *Ulva intestinalis*

Hyperoxaluria and CaOx deposition in the kidney was induced by ethylene glycol (EG) in the drinking water to a final concentration of 0.75%, with 2% ammonium chloride NH4Cl to accelerate lithiasis (Karadi et al 2006). Thirty animals were randomly divided into five groups (Groups I, II, III, IV, and V) each containing six animals. Groups I, II, and III served as normal control served as vehicle-treated control, and maintained regular rat food and drinking water ad libitum. pathological control RATs received 0.75% ethylene glycol in the drinking water for 8 weeks, and standard drug received cystone (750 mg/kg body weight) from the 1st day to the 28th day of calculi induction., respectively. Groups IV and V were treatment groups administered with 15 and 30 mL/kg body weight hydroalcoholic extract of *U.intestinalis* by gavage (intragastric administration) respectively. Groups IV and V served as the treatment group and received extract at doses of 15 and 30 mL/kg respectively from the 15th day to the 28th day of calculi induction. To induce calcium oxalate deposition in the kidneys of groups 2–5, the RATs were provided with 0.75% ethylene glycol and 2% ammonium chloride in the drinking water, initially for 3 days, and afterward with 0.75% ethylene glycol only.

2.5.a. Collection and analysis of urine:

All animals were housed in individual metabolic cages and 24 h urine samples were collected on 0, 7, 14, 21, and 28th day of calculi induction treatment with 0.02% sodium azide as a preservative (Figure 1). The urine volume, pH, and calcium content of urine were measured. Urine was also analyzed for calcium, phosphate, and oxalate (Blqand et al, 20021).

2.5.b. Serum analysis:

After the experimental period, the blood was collected from the retro-orbital sinus under anesthetic condition and serum was separated by centrifugation at 10,000 g for 10 min. The serum was then analyzed for creatinine, uric acid, and urea nitrogen (Pachla et al, 2020).

2.5.c. Statistical analysis:

All the data were analyzed by SigmaPlot ver.12.0. The results were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using a one-way analysis of variance (ANOVA) followed by Dunnett's comparison test Differences between the data were considered significant at $P < 0.05$.

3. Results and Discussion

3.1. Taxonomy of *Ulva intestinalis*:

The species of *Ulva intestinalis* Linn. were collected from the bay of the Arabian Sea at the coast of Karachi (Figure 2). The thallus was dark green to light green, tubular with wave, erect, terete, unbranched, hollow, attached to substratum with minute discoid holdfast. On cross-section, the thallus is monostromatic with chloroplast filled throughout the cell. The cells are square in shape 10-15µm x 10-15µm in size and thickening of the walls up to 1µm (Figure 3).The species of *Ulva* have been extensively analysed for their chemical constituents useful as food, fertilizer, and medicine. Various phytochemicals extracted from *Ulva* exhibit antimicrobial, antiviral, antioxidant, anticoagulant, anti-inflammatory, and anti-cancer activities. The extract of *Ulva intestinalis* and *Ulva lactuca* were also reported to show antiprotozoal and antimycobacterial activity (Spavieri et al, 2010)

Figure 1. Acclimation of rat metabolic cages for urine collection

Figure 2. Morphology of *Ulva intestinalis Linn.*

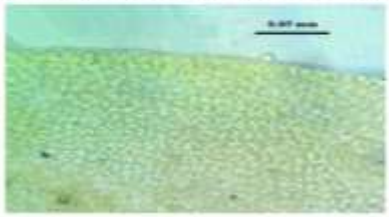

Figure 3. Surface View of *Ulva intestinalis Linn.*

Figure 4. Hydroalcoholic extract of *Ulva Intestinalis Linn.*

3.2 Result of GC-MS analysis in *Ulva intestinalis*:

The results of GC-MS profiling indicated the presence of 31 different compounds which were interpreted using the NIST library (Figure 4 & Table 1).

Table 1. Chemical composition of *Ulva intestinalis* identified by GC-MS

No.	Name of the compound	Rt (min)	Area (%)	Mol. Formula
Carboxylic Acid:				
1	Isoxazolidine-3,5-dicarboxylic acid, dimethyl ester	6.8	1.25	C7H11NO5
Aldehyde:				
2	l-Gala-l-ido-octose	5.1, 8.1	20.03	C8H16O8

3	E-14-Hexadecenal	16.3	2.05	C16H30O
Ether:				
4	Octadecane, 1-(ethenyloxy)-	8.2	1.66	C20H40O
Alcohol:				
5	1-Butanol, 3-methyl-, acetate	5.3	0.34	C7H14O2
6	Acetic acid, pentyl ester	6.6	21.09	C7H14O2
7	Hexahydrofarnesol	25.3	1.17	C15H32O
Ketone:				
8	2-Pentadecanone, 6,10,14-trimethyl-	18.7	0.62	C18H36O
Esters:				
9	4-Methyl-2-pentyl acetate	7.3	3.65	C8H16O2
10	E-11-Methyl-12-tetradecen-1-ol acetate	30.1	3.14	C17H32O2
11	7-Methyl-Z-tetradecen-1-ol acetate	34.2	0.12	C17H32O2
12	Di-n-octyl phthalate	55.4	3.72	C24H38O4
Fatty Acids:				
13	17-Octadecynoic acid	30.2	1.03	C18H32O2
14	n- Hexadecanoic acid	32,5	0.19	C16H32O2
15	Oleic Acid	34.7, 37	4.13	C18H34O2
Phenols:				
16	Phenol, 3,5-bis(1,1-dimethylethyl)-	15.1	0.83	C14H22O
Aromatic Hydrocarbons:				
17	Benzene, 1,2,4-trimethyl-	9.4	0.67	C9H12
Aliphatic Hydrocarbons:				
18	Tetradecane, 2,6,10-trimethyl-	10.2	1.81	C17H36
19	Octadecane, 6-methyl-	10.2, 13.5	0.34	C19H40
20	1-Octadecene	18.9	0.29	C18H36
Sulfated Sugars:				
21	Desulphosinigrin	8.2	0.62	C10H17NO6S
Fatty acid methyl/ethyl esters:				
22	12,15-Octadecadiynoic acid, methyl ester	7.7	0.31	C19H30O2
23	11,14-Octadecadiynoic acid, methyl ester	9.4	0.27	C19H30O2
24	Hexadecanoic acid, methyl ester	33.2	21.54	C17H34O2
25	17-Octadecynoic acid, methyl ester	34.2	2	C19H34O2
26	9-Octadecenoic acid (Z)-, methyl ester	37.1	5.78	C19H36O2
Others:				
27	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	26.3	0.61	C19H38O4
28	9-Octadecenamide, (Z)-	27.1	0.31	C18H35NO
29	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	34.1	0.45	C19H36O3

These substances fall under different chemical classes, and the majority are said to have significant biological activities. The crude methanolic extract revealed the higher peak intensity of the compounds and the predominant presence of Hexadecanoic acid, methyl ester (21.54%), Acetic acid, pentyl ester (21.09%), l-Gala-l-ido-octose (20.03%), 9-Octadecenoic acid (Z)- and methyl ester (5.78%). There was also the moderate presence of compounds like Oleic Acid (4.13%), Di-n-octyl phthalate (3.72%), 4-methyl-2-pentyl acetate (3.65%), and E-11-Methyl-12-tetradecen-1-ol acetate (3.14%). These compounds exhibit activities like antioxidant, cancer-preventive, hypercholesterolemic, nematicide, antifungal, and antimicrobial. The advantage of GC-MS is its high accuracy in the identification of derivatized compounds with abundance in the sample (Figure 5).

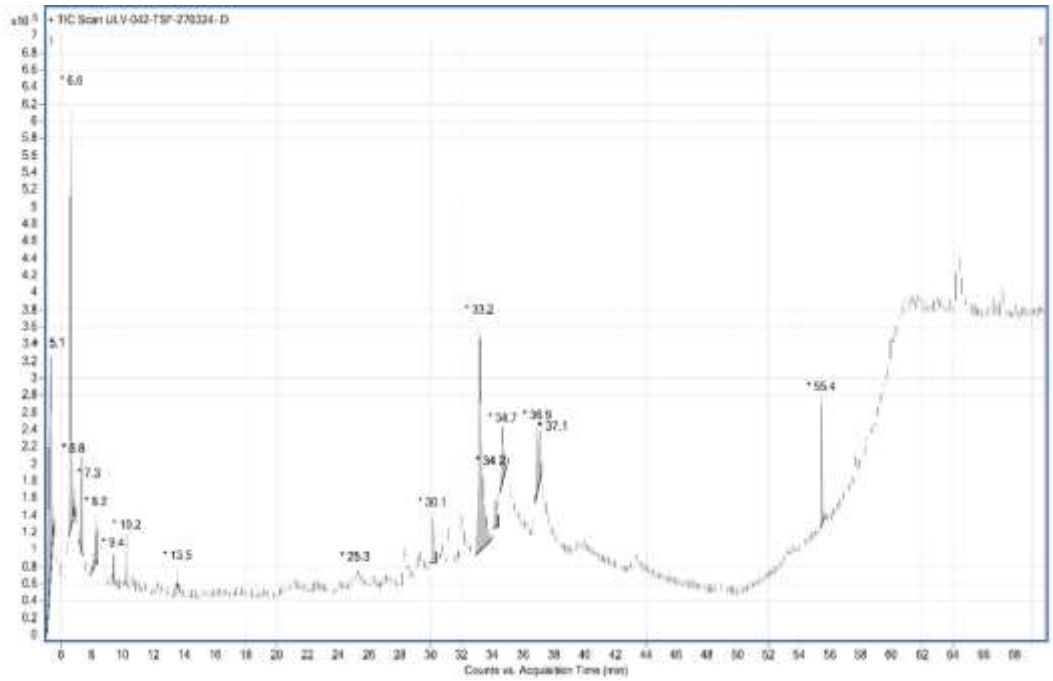

Figure 5. GC-MS spectrum of Ulva intestinalis.

In the present study, the antiurolithiatic activity of the methanolic extract was related to the occurrence of a high number of bioactive compounds including n-hexadecanoic acid, 12 & 15 octadecanoic acid, methyl ester, Di-n-octyl phthalate and phenol. The compound n- n-hexadecanoic acid is a common fatty acid that has been found in abundance in algae. Previous studies reported that n-hexadecanoic acid selectively inhibits DNA topoisomerase-I and thus prevents the proliferation of human fibroblast cells (Harada et al, 2002).

3.2 Result of antiurolithiatic activity of hydro-alcoholic extract From *Ulva intestinalis*:

The present study was conducted to explore the 3.2 Result of GC-MS analysis in *Ulva intestinalis.* During the current study, it has been observed that chronic oral administration of 0.75% (v/v) ethylene glycol aqueous solution to male Wister RATs resulted in hyperoxaluria (Schladt et al. 1998). Male RATs resemble that of humans, and earlier studies have shown that the amount of stone deposition in female RATs was significantly less (Vermeulen, 1962). The urinary supersaturation from stone-forming constituents is generally considered one of the causative factors in calcuogenesis.

3.3.a Measurement of urinary variables:

All the groups receiving ethylene glycol lost weight during the days of the experiment ($p < 0.001$). Although the untreated group lost relatively more weight than the treated group of *Ulva* extract, the differences were not statistically significant (Figure. 6).

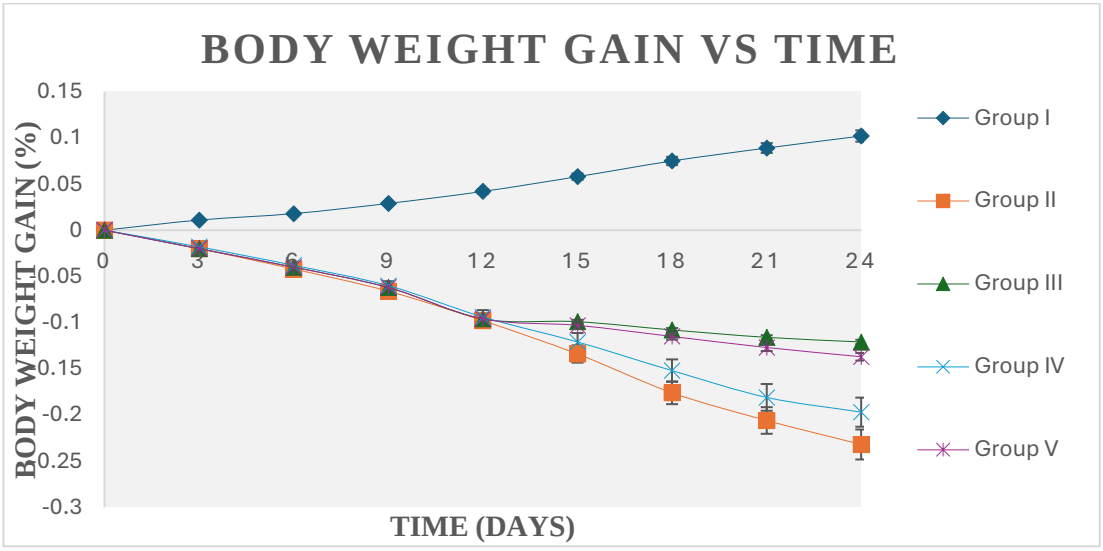

Figure 6. Percentage growth of body weight during treatment (24 days). All values are expressed as mean ± SEM for six animals in each group.

The urinary output was decreased significantly ($p < 0.01$) in calculi-induced RATs. The urinary output of the control group was 4.37 ± 1.20 ml/day/rat on the 28th day, which was decreased to about 35.54% in renal stone-induced Group II. In the standard drug and treatment groups, the urine output was significantly higher than that of the calculi-induced RATs and comparable to that of the controlled treatment RATs. Urinary pH was increased in the pathological control due to the formation of renal stones as compared to the normal and standard drug control (Group II and III) RATs (Table 2). Both the low-dose and high-dose treatment groups (Group IV and V) of RATs resulted in a lowering of pH values.

The deposition of the crystalline components in the renal tissue, namely oxalate, phosphate, and calcium increased very significantly in the stone-forming RATs (Table 2, Group II) when compared to the normal group (Group I). The treatment groups (Groups IV and V) reduced the renal content of these stone-forming constituents at both doses. Although the reduction was non-significant ($P > 0.05$) at low doses but the results were significantly ($P < 0.001$) comparable at higher doses and cystone-treated (Table 2, Group III) animals. Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria, the changes in urinary oxalate levels are relatively more important than those of calcium (Coe and Favus, 1980). Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth (Lemann et al, 1991). However, hydroalcoholic extracts of *Ulva intestinalis* lowered the levels of oxalate as well as calcium excretion.

An increase in urinary phosphate is observed in calculi-induced RATs (Group II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition (Cameron and sakhaee 2007). Treatment of *Ulva intestinalis* extract restores phosphate levels, thus reducing the risk of stone formation.

Table 2. Effect of hydro-alcoholic extract of *Ulva intestinalis* on urine and serum output in urolithiasis induced rats.

Parameters	Group I	Group II	Group III	Group IV	Group V
Urine					
pH	6.72±0.81	8.04±0.73^{a*}	7.13±0.49^{b*}	6.96±0.72^{b*}	6.83±0.33^{b*}
Volume (ml/24h)	4.37±1.20	2.87±0.91^{a*}	4.41±0.54^{b*}	3.52±0.83^{b*}	3.98±0.39^{b*}
Calcium (mg/24h)	0.95±0.15	3.12±0.33^{a**}	1.11±0.21^{b**}	2.47±0.27^{a*}	1.31±0.06^{b**}
Phosphate (mg/24h)	4.65±1.08	9.34±1.29^{a*}	6.03±1.01^{b**}	7.31±0.21^{a*}	5.53±0.13^{b**}

Oxalate (mg/24h)	0.73±0.08	1.59±0.32[a*]	1.01±0.14[b*]	1.39±0.19[a*]	1.08±0.04[a*b*]
Serum					
Creatinine (mg/dl)	0.9±0.10	1.5±0.19[a*]	1.05±0.08[b**]	1.16±0.21[a* b*]	1.06±0.12[b**]
Urea (mg/dl)	33.45±2.34	98.56±4.12[a*]	41.22±4.03[b*]	49.43±3.45[a*b*]	34.17±3.09[b*]
BUN (mg/dl)	14.3±0.78	38.35±1.07[a*]	17.56±0.35[b*]	21.86±0.20[a*b*]	14.9±0.14[b*]

Values for urine parameters are assessed in the 24-hour urine sample. All values are expressed as mean±S.D. for six animals in each group.

[a] Comparisons are made with Group I.

[b] Comparisons are made with Group II

**p < 0.01 = very significant, *p < 0.05 = significant

3.3.b Measurement of serum variables:

The serum uric acid and BUN levels were remarkably increased in calculi-induced animals (Table 2, Group II) while serum creatinine was only slightly elevated in Group II, indicating marked renal damage. However, *Ulva* extract treatment in both the treatment groups (Group IV and V) significantly ($P < 0.05$) lowered the elevated serum levels of creatinine, uric acid, and BUN.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction of the outflow of urine by stones in the urinary system. Due to this, waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid accumulate in the blood (Ghodkar, 1994). In calculi-induced RATs (Group II), significant renal damage was seen as indicated by the elevated serum levels of creatinine, urea, and BUN. However, the curative treatment with hydroalcoholic extract of Ulva intestinalis may be the cause of diuresis. This hastens the process of dissolving the preformed stones and preventing new stone formation in the urinary system as reported by Atmani et al., 2003. The significantly lowered serum levels of accumulated waste products are attributed to the enhanced GFR and the calculolytic property of Ulva intestinalis (Atmani et al 2003).

Seaweeds are the source of raw materials for many industrial products. Algae are consumed as food in many Asian countries. In conclusion, the present research suggests that administering *Ulva intestinalis* extract to RATs developed urolithiasis by ethylene glycol prevents and halts kidney stone formation. The phytochemical screening of *Ulva intestinalis was* also performed in different solvent extracts. The GC-MS analysis revealed that metabolic with higher medicinal activities such as phenols, sulfate sugars, fatty acid, and steroids were present. Thus the genus *Ulva intestinalis* can be a significant source of important compounds that can be used in the formulation of drugs by the pharmaceutical industries. Further studies are required to investigate the extract of *Ulva intestinalis* for potential pharmacological properties.

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Conflict of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References:

1. Atmani F, Slimani Y, Mimouni M, Hacht B. Prophylaxis of calcium oxalate stones by Herniaria hirsuta on experimentally induced nephrolithiasis in RATs. 2003;92(1):137-40.
2. Bland JM, Grimm CC, Bechtel PJ, Deb U, Dey MM. Proximate Composition and Nutritional Attributes of Ready-to-Cook Catfish Products. 2021;10(11):2716.
3. Cameron MA and Sakhaee K (2007) Uric acid Nephrolithiasis.*Urologic clinics of North America* **34**:335-346.
4. Coe FL, Favus MJ. Idiopathic hypercalciuria in calcium nephrolithiasis. Disease-a-Month. 1980;26(12):1-36.

5. Ghodkar PJTomlt. Chemical tests in kidney disease. 1994:118-32
6. Habtemariam S. Medicinal foods as potential therapies for Type-2 diabetes and associated diseases: the chemical and pharmacological basis of their action: Academic Press; 2019.
7. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res. 2002;22(5):2587-90.
8. Karadi RV, Gadge NB, Alagawadi KR, Savadi RV. Effect of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in RATs. Journal of Ethnopharmacology. 2006;105(1):306-11.
9. Klongklaew N, Praiboon J, Tamtin M, Srisapoome P. Antibacterial and Antiviral Activities of Local Thai Green Macroalgae Crude Extracts in Pacific White Shrimp (Litopenaeus vannamei). 2020;18(3):140.
10. KVSRG P, Sujatha D, Bharathi KJPR. Herbal drugs in urolithiasis-a review. 2007;1(1):175-8
11. Lemann J, Worcester EM, Gray RW. Hypercalciuria and Stones. American Journal of Kidney Diseases. 1991;17(4):386-91.
12. Mantri VA, Kazzi, Balar NB, Gupta V and Gajaria T (2020). Concise review of green algal Genus *Ulva* Linnaeus. *Journal of Applied Phycology* **32**: 2725:2741.
13. Ning L, Yao Z, Zhu B. Ulva (Enteromorpha) Polysaccharides and Oligosaccharides: A Potential Functional Food Source from Green-Tide-Forming Macroalgae. 2022;20(3):202.
14. Pachla LA, Reynolds DL, Wright DS, Kissinger PT. Analytical Methods for Measuring Uric Acid in Biological Samples and Food Products. Journal of Association of Official Analytical Chemists. 2020;70(1):01-14.
15. Peasura N, Laohakunjit N, Kerdchoechuen O, Vongsawasdi P, Chao LK. Assessment of biochemical and immunomodulatory activity of sulphated polysaccharides from Ulva intestinalis. International Journal of Biological Macromolecules. 2016;91:269-77.
16. Schladt L, Ivens I, Karbe E, Rühl-Fehlert C, Bomhard E. Subacute oral toxicity of tetraethylene glycol and ethylene glycoladministered to Wistar RATs. Experimental and Toxicologic Pathology. 1998;50(3):257-65.
17. Smith-Warner SA, Elmer PJ, Tharp TM, Fosdick L, Randall B, Gross M, et al. Increasing Vegetable and Fruit Intake: Randomized Intervention and Monitoring in an At-Risk Population. Cancer Epidemiology, Biomarkers & Prevention. 2000;9(3):307-17.
18. Spavieri J, Kaiser M, Casey R, Hingley-Wilson S, Lalvani A, Blunden G, et al. Antiprotozoal, antimycobacterial and cytotoxic potential of some british green algae. 2010;24(7):1095-8.
19. Yasir F, Wahab A-t, Choudhary MI. Protective effect of dietary polyphenol caffeic acid on ethylene glycol-induced kidney stones in RATs. Urolithiasis. 2018;46(2):157-66.
20. Zhu W, Xu Y-f, Feng Y, Peng B, Che J-p, Liu M, et al. Prophylactic effects of quercetin and hyperoside in a calcium oxalate stone forming rat model. Urolithiasis. 2014;42(6):519-26.
21. Zhu B, Ni F, Xiong Q, Yao Z. Marine oligosaccharides originated from seaweeds: Source, preparation, structure, physiological activity and applications. Critical Reviews in Food Science and Nutrition. 2021;61(1):60-74.