*Muthu Sheeba M and John Peter Paul J. / International Journal of Phytopharmacology.* **7(2), 2016, 80-84.**

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

**International Journal of Phytopharmacology**

Journal homepage: www.onlineijp.com

**IJP**

# DIURETIC ACTIVITY OF METHANOL EXTRACT OF *ENTEROMORPHA LINZA* (L.) J.AG. IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

**Muthu Sheeba M and John Peter Paul J***

Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

**ABSTRACT**

In the present study, the screening of diuretic activity of *Enteromorpha linza* (L.) J.Ag. collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India was evaluated. Dried powder plant materials were subjected to methanol extract at the dose of 200mg/kg and 400mg/kg body weight. The method described by Wiebelhaus *et al.* was employed with modification for the assessment of diuretic activity. Methanol crude extract showed a remarkable significant diuretic activity in both doses from 1 hour to 4 hour as compared to the standard drug furosemide. The present study has been provided the evidence for the diuretic activity of *Enteromorpha linza* (L.) J.Ag. which could partly contribute to its ethno medical uses also.

**Key words:** Green seaweed, Diuretic, *Enteromorpha linza*, Furosemide, South east coast of Tamil Nadu.

## INTRODUCTION

The ocean environment contains over 80% of world's plant and animal species and with more than 150,000 seaweeds found in the intertidal zones and tropical waters of the oceans, it is a primary source of natural products (Jha and Zi-rong, 2004 and Falcao, 2006). On the basis of the pigmentation, seaweeds are categorized into three groups namely green seaweed (Chlorophyceae), brown seaweeds (Phaeophyceae) and red seaweeds (Rhodophyceae). Seaweeds or marine algae constitute one of the commercially important renewable marine living resources. Seaweeds are the source of the production of phytochemicals, confectionary, pharmaceutical, dairy, textile, paper, paint and varnish industries. The potential areas in India for luxuriant growth of several species of green, brown and red algae are the south east coast of Tamil Nadu from Mandapam to Kanyakumari covering 21 islands in the Gulf of Mannar, Gujarat coast, Lakshadweep and Andaman Nicobar Islands. Seaweeds are a valuable food resource which contains low calories and are rich in vitamins, minerals, proteins, polysaccharides, steroids and dietary fibers (Darcy, 1993). Since as early as 3000 BC, seaweeds were also considered important as traditional remedies (Smit, 2004).

The unsaturated lipids afford protection against cardiovascular pathogens (Vallinayagam *et al.,* 2009). Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites (Faulkner, 2002). The marine macro algae synthesize a variety of compounds such as carotenoids (Paniagua *et al.,* 2009), terpenoids, xanthophylls, chlorophylls, vitamins, saturated and polyunsaturated fatty acids, amino acids (Cen *et al.,* 2010 and Klisch and Hader, 2008), acetogenins (Pallela *et al.,* 2010), antioxidants such as polyphenols, alkaloids (Souza *et al.,* 2009 and Guven *et al.,* 2010) and halogenated compounds (Cabrita *et al.,* 2010 and La Barre *et al.,* 2010). Seaweeds and can act as antimicrobial, antifouling and herbivore deterrents or as ultraviolet screening agents (Ianora et al., 2006).

Corresponding Author

**John Peter Paul J.**
Email: johnarock2008@yahoo.com

Compounds with cytostatic, antiviral, antihelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman *et al.,* 2003 and Lindequest and Schweder, 2001). Seaweeds are also used by the pharmaceutical industry in drug development to treat diseases like cancer, acquired immune-deficiency syndrome (AIDS), inflammation, pain, arthritis, infection for virus, bacteria and fungus (Deig *et al.,* 1974). Literature review revealed that no pharmacological studies especially diuretic activity has been carried out using an important green seaweed *Enteromorpha linza* (L.) J.Ag. Based on these details, the present study was aimed to evaluate the diuretic activity of methanol extract of *Enteromorpha linza* (L.) J.Ag. collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India.

## MATERIALS AND METHODS

### Collection of Plant Sample

*Enteromorpha linza* (L.) J.Ag. (Figure 1) is green seaweed belonging to Chlorophyceae member showed much attention in the present study for diuretic activity. *Enteromorpha linza* (L.) J.Ag. was collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India. The collected plant materials were rinsed with marine water to remove debris and epiphytes. The entire epiphytes were removed using soft brush. The plants were brought to the laboratory. In the laboratory, the plants were once again washed with freshwater and stored in refrigerator for further analysis (John Peter Paul and Shri Devi, 2014).

### Preparation of methanol extract

For the preparation of methanol extract of *Enteromorpha linza* (L.) J.Ag., the collected plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 3g powdered sample was packed in Soxhlet apparatus and extracted with methanol for 8h separately. The excess amount of methanol was evaporated and fine methanol crude powder was prepared and stored in the refrigerator for the antipyretic activity (John Peter Paul and Yuvaraj, 2013).

### Experimental Animals

Wistar albino rats (160-200g) and Swiss albino mice of either sex were procured from Venkateswara Enterprises, Bangalore, Karnataka, India. The selected animals were acclimatized for 7 days under standard husbandry conditions, i.e. room temperature 35±1°C, relative humidity 45-55% and light/dark cycle 12/12h. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% *Arachis* oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conduct between 10.00 and 17.00h and were in accordance with the ethical guidelines of the International Association for Study of Pain (Zimmerman, 1983). All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Acute toxicity test

Acute oral toxicity study was performed as per OECD-423 guidelines (Ecobichon, 1997). Albino mice (n=6) of either sex selected through random sampling technique was used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract (50% methanolic extract) was administered orally at the dose level of 5 mg/Kg body weight by gastric intubation and observed for 14 days. If mortality is observed in 2 out of 3 animals, then the dose administered would be assigned as toxic dose. If mortality is observed in 1 animal, then the same dose would be repeated again to confirm the toxic dose. If mortality is not observed, the procedure would be repeated for further higher doses such as 50, 300 and 2000 mg/Kg body weight. According to the results of acute toxicity test, the doses were chosen for experiments.

### Diuretic activity

The method described by Wiebelhaus *et al.* 1965. It was employed with modification for the assessment of diuretic activity. Healthy albino rats of either sex (160-200g) were divided into four groups of six animals each. They were fasted 18h prior to the test with free access to water. On the day of the experiment, Group I animals were given 5ml/Kg of body weight normal saline orally and served as control group. Groups II, III and IV were treated with standard drug (Furosemide 5mg/Kg p.o.), methanolic crude extract (200mg/Kg) and methanolic crude extract (400mg/Kg) respectively. Standard drug and crude extracts were administered orally (p.o.). Immediately after dosing, the rats were placed in the metabolic cages with special provision to collect faeces and urine.

Animals were kept at room temperature of 35±1°C throughout the experiment. Urine excreted for the next 4h from 15min was collected and the total 4h urine volume for each rat was compared with the volume of urine produced after the administration of normal saline. The volume of urine excreted during 4h for each animal in the group was expressed as the percent of the liquid (normal saline) administered.

This percentage gave a measure of urinary excretion independent of the animal weight. The ratio of urinary excretion in the test group to urinary excretion in the control group was used as a measure of the diuretic action for the given dose of the drug. The diuretic activity of the crude extract was compared to that of the standard drug in the test group (Mukherjee, 2002).

## RESULTS AND DISCUSSION

Screening of diuretic activity of methanol crude extract of *Enteromorpha linza* (L.) J.Ag. was analyzed by determining the effect on albino rats. The methanol extract of *Enteromorpha linza* (L.) J.Ag. showed the highest obvious diuretic activities which was dose dependent on albino rats. Acute toxicity studies showed that the methanolic extracts did not cause any mortality up to 2000 mg/Kg and were considered as safe (Ecobichon, 1997). The present study examined the diuretic potential of *Enteromorpha linza* (L.) J.Ag.

The results expressed that both the methanol crude extracts (200mg/kg and 400mg/kg) increased urine output up to 4h following its administration which compared to control group. The amount of urine collected from standard fruosemide (group II), 200mg/kg methanol crude extract (group III) and 400mg/kg methanol crude extract (group IV) was found to be 6.95ml, 7.7ml and 1.38ml respectively as compared with control (group I) as presented in Table. 1.

Diuretic activity of 200mg/kg methanol crude extract was found to be highest in the present study. The urinary electrolyte content following the administration of the methanol crude extracts was presented in Table 2. The dose of 200mg/kg methanol crude extract produced a significant increase in $Na^+$ (from 76.30meq/L to 89.34meq/L), $K^+$ (from 48.54meq/L to 63.89meq/L), $Cl^-$ (from 84.22meq/L to 79.48meq/L) excretion and pH showed a small change (from 7.1 to 6.35) compared with the control group ($P<0.01$). The dose of 400mg/kg methanol crude extract produced a significant increase in the $Na^+$ (from 76.30meq/L to 119.75meq/L), $K^+$ (from 48.54meq/L to 67.32meq/L) $Cl^-$ (from 84.22meq/L to 81.82meq/L) excretion and pH showed decreased state from 7.1 to 6.64 compared with control group ($P<0.001$).

However, only the 200 mg/kg of the methanol crude extract produced a significant decrease in $Na^+$ from 128.32meq/L to 89.34meq/L, $K^+$ from 78.87meq/L to 67.32meq/L), $Cl^-$ from 82.63meq/L to 81.82meq/L) excretion and pH showed a small change from 6.5 to 6.64 compared with the standard drug fruosemide (5mg/kg p.o.). The dose of 400mg/kg methanol crude extract produced a significant decrease in the $Na^+$ (from 128.32meq/L to 119.75meq/L), $K^+$ (from 78.87meq/L to 67.32meq/L) excretion followed by increase in $Cl^-$ (from 82.63meq/L to 81.82meq/L) excretion and pH remained unchanged 6.64 compared with standard drug fruosemide (5mg/kg p.o.).

**Table 1. Diuretic activity of methanol extract of *Enteromorpha linza* (L.) J.Ag**

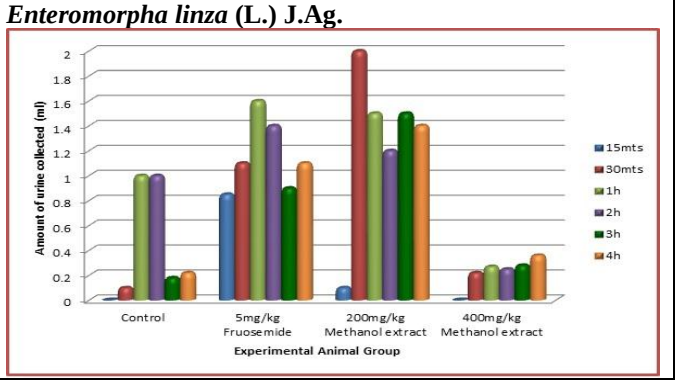
| Group | Drugs | Total Amount of Urine collected | | | | | | Total Volume |
|---|---|---|---|---|---|---|---|---|
| | | **15mts** | **30mts** | **1hr** | **2hr** | **3hr** | **4hr** | |
| **I** | Normal Saline (5mg/kg p.o.) | 0ml | 0.10ml | 1ml | 1ml | 0.18ml | 0.22ml | 2.50ml |
| **II** | Fruosemide (5mg/kg p.o.) | 0.85ml | 1.1ml | 1.6ml | 1.4ml | 0.9ml | 1.1ml | 6.95ml |
| **III** | Methanol extract (200mg/kg p.o.) | 0.1ml | 2ml | 1.5ml | 1.2ml | 1.5ml | 1.4ml | 7.7ml |
| **IV** | Methanol extract (400mg/kg p.o.) | 0ml | 0.22ml | 0.27ml | 0.25ml | 0.28ml | 0.36ml | 1.38ml |

**Table 2. Effect of Methanol extract of *Enteromorpha linza* (L.) J.Ag. on electrolyte excretion and pH on rat**

| | Electrolyte Concentration (meq/L) | | | | |
|---|---|---|---|---|---|
| **Group** | **Drugs** | **$Na^+$** | **$K^+$** | **$Cl^-$** | **pH** |
| **I** | Normal Saline (5mg/kg p.o.) | 76.30 ± 0.48 | 48.54 ± 1.23 | 84.22 ± 1.06 | 7.1 ± 0.2 |
| **II** | Standard Fruosemide (5mg/kg) | 128.32 ± 0.61 | 78.87 ± 0.93 | 82.63 ± 0.57 | 6.5 ± 0.12 |
| **III** | Methanol crude extract (200mg/kg) | 89.34 ± 0.11 | 63.89 ± 0.17 | 79.48 ± 0.45 | 6.35 ± 0.09 |
| **IV** | Methanol crude extract (400mg/kg p.o.) | 119.75 ± 0.04 | 67.32 ± 0.21 | 81.82 ± 0.88 | 6.64 ± 0.39 |

**Figure 1. Natural Habit of *Enteromorpha linza* (L.) J.Ag.**

**Figure 2. Diuretic activity of methanol extract of *Enteromorpha linza* (L.) J.Ag.**




## CONCLUSION

From the above results, it can be concluded that the methanolic extract of *Enteromorpha linza* (L.) J.Ag. possesses significant diuretic activity by increasing the total urine output and increased excretion of Sodium, Potassium and Chloride levels. Among the various concentration of methanolic extracts analyzed, 400mg/kg showed more diuretic activity compared to 200mg/kg. However, the activity was not comparable in terms of quantitative activity elucidated by standard drug. This may be due to the methanolic extract of *Enteromorpha linza* (L.) J.Ag. Hence the isolation and characterization of active principles will be advantages to produce novel bioactive constituents from methanolic extracts which may possess more significant activity.

## REFERENCES

Cabrita MT, Vale C and Rauter AP. Halogenated compounds from marine algae. *Mar. Drugs,* 8, 2010, 2301-2317.

Cen PF, Nordstrom L, Souto ML, Martin MN, Fernandez JJ and Daranas AH. Studies on polyethers produced by red algae. *Mar. Drugs,* 8, 2010, 1178-1188.

Darcy VB. Nutritional aspects of the developing use of marine macroalgae for the human food industry. *Int. J. Food Sci. Nutr.*, 44, 1993, 523–535.

Deig EF, Ehresmann DW, Hatch MT and Riedlinger DJ. Inhibition of herpesvirus replication by marine algae extracts. *Antimicrob. Agents Chemother*. 6, 1974, 524-525.

Ecobichon DJ. *The Basis of Toxicology Testing*. CRC press, New York. 1997, 43-86.

Falcao VR. Aspectos moleculares de nitrato redutase da macroalga marinha *Gracilaria tenuistipitata* (Rhodophyta): Sequenciamento do gene estudo da expressao do RNA mensageiro. PhD Thesis, Institute of Chemical, University of São Paulo: Sao Paulo, Brazil, 2006, 1-187.

Faulkner DJ. Marine natural products. *Nat. Prod. Rep*. 19, 2002, 1-48.

Guven KC, Percot A and Sezik E. Alkaloids in marine algae. *Mar. Drugs,* 8, 2010, 269-284.

Ianora A, Boersma M, Casotti R, Fontana A, Harder J, Hoffmann F, Pavia H, Potin P, Poulet SA and Toth G. New trends in marine chemical ecology. *Estuaries Coasts*, 29, 2006, 531-551.

Jha RK and Zi-rong X. Biomedical compounds from marine organisms. *Mar. Drugs,* 2, 2004, 123-146.

John Peter Paul J and Shri Devi SDK. Effect of Seaweed Liquid Fertilizer of *Gracilaria dura* (Ag.) J.Ag. (Red Seaweed) on *Pennisetum glaucum* (L.) R.Br., in Thoothukudi, Tamil Nadu, India. *Indo American Journal of Pharmaceutical Research*, 2014, 4(4), 2183-2187.

John Peter Paul J and Yuvaraj P. Phytochemical analysis of *Padina distromatica* Hauck. *Indo American Journal of Pharmaceutical Research*, 2013, 3(7), 5290-5297.

Klisch M and Hader DP. Mycosporine-like amino acids and marine toxins - The common and the different. *Mar. Drugs,* 6, 2008, 147-163.

La Barre S, Potin P, Leblanc C and Delage L. The halogenated metabolism of brown algae (Phaeophyta), its biological importance and its environmental significance. *Mar. Drugs,* 8, 2010, 988-1010.

Lindequest U and Schweder T. Marine biotechnology, In *Biotechnology*; Rehm HJ, Reed G Eds.; Wliey-VHC: Weinheim, Germany, 10, 2001, 441-484.

Mukherjee PK. Evaluation of diuretic agents. In: *Quality Control of Herbal Drugs*. Business Horizons, New Delhi. 2002.

Newman DJ, Cragg GM and Snader KM. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod*., 66, 2003, 1022-1037.

Pallela R, Na-Young Y and Kim SK. Anti-photoaging and photoprotective compounds derived from marine organisms. *Mar. Drugs*, 8, 2010, 1189-1202.

Paniagua MJ, Capa RW, Olmos SJ and Gutierrez MLE. The carotenogenes is pathway via the isoprenoid-beta-carotene interference approach in a new strain of *Dunaliella salina* isolated from Baja California Mexico. *Mar. Drugs,* 7, 2009, 45-56.

Smit AJ. Medicinal and pharmaceutical uses of seaweed natural products: A review. *J. Appl. Phycol*., 16, 2004, 245-262.

Souza ET, Lira DP, Queiroz AC, Silva DJC, Aquino AB, Mella EAC, Lorenzo VP, Miranda GEC, Araujo-Junior JX and Chaves MCO. The antinociceptive and anti-inflammatory activities of caulerpin, a bisindole alkaloid isolated from seaweeds of the genus *Caulerpa. Mar. Drugs,* 7, 2009, 689-704.

Vallinayagam K, Arumugan R, Ragupathi RK, Thirumaram G and Anantharaman P. Antibacterial activity of some selected seaweeds from Pudumadam coastal regions. *Glob. J. Pharmacol*. 3, 2009, 50-52.

Wiebelhaus VD, Weinstock J, Maass AR, Brennan FT, Sosnowski G, Larsen T. The diuretic and natruretic activiy of triamterene and several related pteridines in the rat. *J. Pharmacol. Exp. Ther.*, 149, 1965, 397-403.

Zimmerman M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain,* 1983, 16, 109-110.