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Subhash R. Yende
 Department of Pharmacology,
 Gurunanak College of Pharmacy, Nari,
 Nagpur, Maharashtra-440026, India

Uday N. Harle
 Clinical Research Consultant,
 Mahalaxmi Nagar, Nagpur,
 Maharashtra-440026, India

Sumit K. Arora
 Department of Pharmacognosy,
 Gurunanak College of Pharmacy, Nari,
 Nagpur, Maharashtra-440026, India

Vipinchandra B. Pande
 Department of Pharmacology,
 Gurunanak College of Pharmacy, Nari,
 Nagpur, Maharashtra-440026, India

Correspondence:
Subhash R. Yende
 Department of Pharmacology,
 Gurunanak College of Pharmacy, Nari,
 Nagpur, Maharashtra-440026, India
 Email: subhashyende[at]gmail.com

Phytochemical screening and anticonvulsant activity of *Sargassum ilicifolium* (brown algae) in mice

Subhash R. Yende*, Uday N. Harle, Sumit K. Arora, Vipinchandra B. Pande

ABSTRACT

Sargassum ilicifolium (SI) is a tropical and subtropical marine macroalgae (brown algae) found in coastal area of India. This study investigated the anticonvulsant activity of SI in maximal electroshock (MES) induced convulsion and pentylenetetrazole (PTZ) induced convulsion in mice. The result of present study indicated that chloroform extract (600 mg/kg) and ethanol extract (400 mg/kg and 600 mg/kg) of SI significantly decreased the duration of tonic hind limb extension in MES model, as well as it significantly increased the latency to onset of convulsions in PTZ model. These results were comparatively similar with the effect of phenytoin (25 mg/kg) and phenobarbitone (20 mg/kg). This activity may be due to the presence of alkaloids, terpenoids, flavonoids, steroids and saponin in chloroform and ethanol extract of *Sargassum ilicifolium*. However, further research will be necessary to investigate the exact mechanism underlying this anticonvulsant activity.

Keywords: Anticonvulsant activity; *Sargassum ilicifolium*; Brown algae; MES induced convulsion; PTZ induced convulsion.

INTRODUCTION

Marine macroalgae or seaweeds are found in the coastal region between high tide to low tide and in the sub-tidal region up to a depth where 0.01 % photosynthetic light is available and can be classified into three classes; Brown algae (Phaeophyta), Green algae (Chlorophyta) and Red algae (Rhodophyta). Marine macroalgae have created a promising significance in the biomedical area, mainly because of their contents of bioactive substances. Polysaccharides, terpenoids, phlorotannins, fucoidans, sterols and glycolipids obtained from marine macroalgae showed wide range of pharmacological properties which includes anticancer, anti-inflammatory, antimicrobial, antiviral, antioxidant, hypoglycaemic, hepatoprotective and neuroprotective activities [1-3]. Also, some marine organism and marine macroalgae showed the potential as a source of new drugs for the treatment of neurological disorders [4, 5]. Many traditional herbs and herbal medicines have been reported for their CNS activities whereas, the marine flora has not explored up to that extent. Hence, we undertook the study to evaluate CNS potential of some marine macroalgae.

Sargassum ilicifolium (Turner) C. Agardh is tropical and sub-tropical brown algae, distributed in intertidal open coast of Gujarat, Maharashtra, Goa, Karnataka and Lakshadweep. Ethyl acetate extract of *Sargassum ilicifolium* has been reported to possess immunomodulatory activities [6], alcoholic extract of this seaweed reported for antibacterial activity [7], analgesic and anti-inflammatory activity [8] and antioxidant and anticancer activity [9]. Furthermore, the antidepressant and anxiolytic activity of *Sargassum ilicifolium* has been previously reported in mice [10, 11]. However, anticonvulsant activity of *Sargassum ilicifolium* has not been investigated. Hence, we evaluated the anticonvulsant activity of chloroform and ethanol extracts of *Sargassum ilicifolium* against seizure induced by maximal electroshock (MES) and pentylenetetrazole (PTZ) in mice.

MATERIALS AND METHODS

Animals

Swiss albino mice (25-30 g) were used for the experiment purpose. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions. The mice were fed with commercial standard diet and water *ad libitum*. The experiments were designed and conducted in accordance with the guideline of CPCSEA and approved by Institutional Animal Ethical Committee (Approval No. GNCP/IAEC/2011-12/P'cology-01).

Drugs and Chemicals

Pentylentetrazole (Sigma-Aldrich, USA), Phenytoin (Epsolin Inj., Zydus Cadila Ltd.) and Phenobarbitone (Abbott India Ltd.) were used in this study. The drug was diluted with distilled water before use.

Seaweed Collection and Extracts Preparation

The brown seaweeds, *Sargassum ilicifolium* (SI) was collected from inter-tidal rocky shore of Bhatkarwada, Ratnagiri coast in Nov-Dec 2011. The seaweed species was identified by Professor B. B. Chaughule, Emeritus Professor, Department of Botany, University of Pune, Pune (India). The fresh samples was washed with sea water followed by fresh water to remove salts, epiphytes, microorganisms and other suspended materials, and dried at room temperature. The air-dried and coarsely powdered sample was extracted using Soxhlet apparatus by Petroleum Ether, Chloroform and Ethanol.

Phytochemical Analysis and High Performance Thin Layer Chromatography (HPTLC) study

The Chloroform and ethanol extracts of SI was analyzed for the presence of phytochemicals by qualitative analysis [12, 13]. Further, the analysis of major phytoconstituents was carried out by TLC and HPTLC using pre-coated silica gel G plates. Different solvent systems were used, to obtain better separation of the components of extracts. The developed plate was observed under visible as well as UV light (254 nm and 356 nm) using CAMAG TLC Scanner (Switzerland). Rf value of each spot was calculated as $R_f = \text{Distance travelled by the solute} / \text{Distance travelled by the solvent}$ [14].

Acute toxicity studies

The Acute toxicity was determined on Swiss albino mice as per OECD-423 guidelines [15]. The overnight fasted animals were administered extracts orally at the dose level of 2000 mg/kg body weight and were continuously observed for 2 h to detect changes in the autonomic or behavioural responses and then, monitored for any mortality for the following 7 days.

MES induced convulsion

Animals were divided into six groups consisting of five mice in each group. Group I (Vehicle) was treated with 5% Tween-80, Group II (PHT 25) was serve as standard and treated with Phenytoin (25 mg/kg); groups III and IV (CSI 400 & CSI 600) were treated with 400 and 600 mg/kg of chloroform extract of SI; Group V and VI (ESI 400 & ESI 600) were treated with 400 and 600 mg/kg of ethanol extract of SI. All the extracts were administered orally for seven day. 60 min. after administration of last dose seizure was induced using electroconvulsimeter (INCO, Ambala, India). An electric shock (45 mA, 0.2 s) was applied through ear-clip electrode, and duration of tonic hind limb extension (THLE) and % protection was recorded [16].

PTZ induced convulsion

Animals were divided into six groups consisting of five mice in each group. Group I (Vehicle) was treated with Tween-80 (5%), Group II (PB 20) was serve as standard and treated with (25 Phenobarbitone (20 mg/kg); groups III and IV (CSI 400 & CSI 600) were treated with 400 and 600 mg/kg of chloroform extract of SI; Group V and VI (ESI 400 & ESI 600) were treated with 400 and 600 mg/kg of ethanol extract of SI. All the extracts were administered orally for seven day.

60 min. after administration of last dose, PTZ (80 mg/kg, ip) was administered. The onset of clonic convulsion and % protection was noted [16, 17].

Statistical analysis

Results were expressed as Mean \pm SEM. The data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test and $P < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical analysis

The qualitative phytochemical analysis revealed the presence of steroids, terpenoids, flavonoids, and glycosides in chloroform extract and steroids, terpenoids, flavonoids, alkaloids, glycoside and saponin in ethanol extract of SI (Table 1). A number of solvent systems were tried for all the extract. The solvent system, which gave best resolution, was considered valid and useful. CSI and ESI showed best resolution in toluene: Methanol (Table 2; figure 1 & 2).

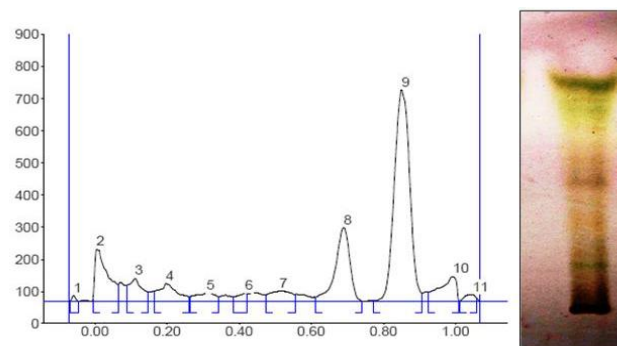
Table 1: Phytochemical constituents of extracts of *Sargassum ilicifolium*

Plant Constituents	CSI	ESI
Tannins	-	-
Steroids	+	+
Terpenoid	+	+
Flavonoids	+	+
Alkaloids	+	+
Glycosides	+	+
Carbohydrates	+	+
Saponin	-	+

+ positive; - negative

Table 2: Mobile phase for HPTLC study of extracts

Sr. no.	Test extracts	Solvent system
1	CSI	Toluene: Methanol (93:7)
2	ESI	Toluene: Methanol (86:14)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.07	0.2	-0.06	18.4	1.33	-0.05	0.1	141.2	0.31	unknown *
2	-0.01	4.2	0.00	163.8	11.88	0.08	52.9	4679.9	10.12	unknown *
3	0.09	49.4	0.11	71.4	5.17	0.15	29.5	2138.3	4.62	unknown *
4	0.16	31.8	0.20	56.2	4.07	0.26	15.5	2288.3	4.94	unknown *
5	0.26	15.6	0.31	25.9	1.88	0.34	14.4	1158.5	2.51	unknown *
6	0.39	13.9	0.42	25.7	1.86	0.42	23.8	534.0	1.15	unknown *
7	0.47	20.8	0.51	32.9	2.38	0.56	20.6	1603.7	3.47	unknown *
8	0.61	12.8	0.69	229.6	16.62	0.74	0.0	7496.6	16.21	unknown *
9	0.77	1.4	0.85	659.0	47.71	0.91	25.3	22868.8	49.46	unknown *
10	0.93	29.6	0.99	77.7	5.63	1.01	1.6	2835.6	6.13	unknown *
11	1.01	0.5	1.05	20.6	1.49	1.06	9.7	495.0	1.07	unknown *

Figure 1: HPTLC studies of chloroform extract of *Sargassum ilicifolium* (CSI)

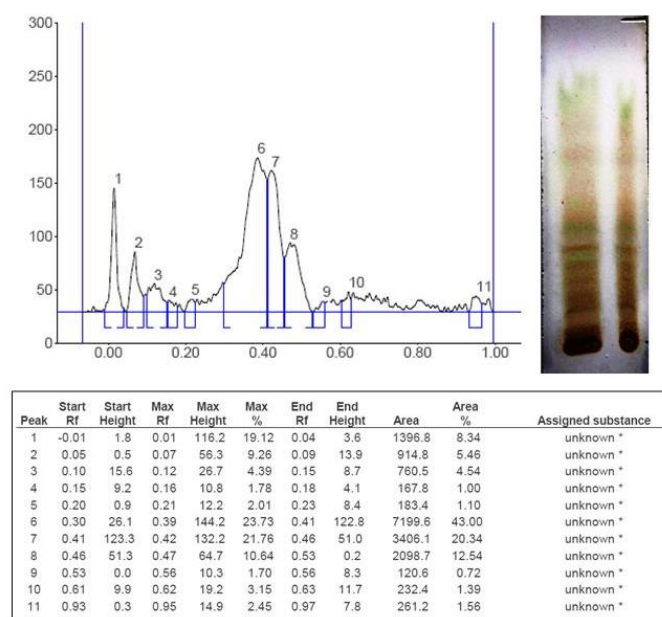


Figure 2: HPTLC studies of ethanol extract of *Sargassum ilicifolium* (ESI)

Acute toxicity studies

Since no observable toxic effects and mortality was observed at 2000 mg/kg. Therefore doses of 400 and 600 mg/kg of SI extracts were selected for further study.

MES induced convulsion

Administration of Phenytoin (25 mg/kg), CSI (600 mg/kg), ESI (400 mg/kg) and ESI 600 (mg/kg) significantly decreased the duration of THLE to 2.72 ± 1.72 s, 12.57 ± 0.75 s, 12.60 ± 1.12 s and 11.97 ± 1.10 s, respectively. However, CSI (400 mg/kg) did not significantly modify the duration of THLE as compared with the vehicle treated group. Furthermore, treatment with SI extract showed 80 % protection, while phenytoin treatment showed 100 % protection against MES induced seizures (Table 3).

Table 3: Effect of chloroform and ethanol extract of *Sargassum ilicifolium* on THLE and % protection on MES induced convulsion

Groups	Treatment	Tonic Hind limb Extension in Sec. (Mean ± SEM)	% Protection
I	Vehicle	17.18 ± 0.97	40
II	PHT 25	2.72 ± 1.72 **	100
III	CSI 400	13.35 ± 0.51	80
IV	CSI 600	12.57 ± 0.75 *	80
V	ESI 400	12.60 ± 1.12 *	80
VI	ESI 600	11.97 ± 1.10 **	80

The results were expressed as Mean ± SEM. (n=5); Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. * p < 0.05, ** p < 0.01, significantly differ from vehicle.

PTZ induced convulsion

Treatment with phenobarbitone (20 mg/kg), CSI (600 mg/kg) and ESI 600 (mg/kg) significantly increased (P < 0.01) the latency to onset of clonic convulsion while, CSI (400 mg/kg) and ESI (400 mg/kg) did not significantly increase the latency to onset of clonic convulsion as compared with the vehicle treated group. Furthermore,

phenobarbitone (20 mg/kg), CSI (600 mg/kg) and ESI 600 (mg/kg) showed 100%, 80% and 80% protection respectively, against PTZ induced convulsion (Table 4).

Table 4: Effect of chloroform and ethanol extract of *Sargassum ilicifolium* on Onset of clonic convulsion and % protection on PTZ induced convulsion

Group	Treatment	Onset of clonic convulsion in Sec. (Mean±SEM)	% Protection
I	Vehicle + PTZ	78.64±5.74	20
II	PB 20 + PTZ	192.55±6.87**	100
III	CSI 400 + PTZ	100.58±9.13	40
IV	CSI 600 + PTZ	151.38±15.43**	80
V	ESI 400 + PTZ	92.86±12.37	60
VI	ESI 600 + PTZ	134.25±6.11**	80

The results were expressed as Mean ± SEM. (n=5); Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. * p < 0.05, ** p < 0.01, significantly differ from vehicle.

DISCUSSION

Epilepsy is a chronic neurological disorder characterized by recurrent derangement of the nervous system due to sudden excessive disorderly discharge from the cerebral neurons [18]. There are a number of synthetic anticonvulsant drugs currently available for management, control and/or treatment of epilepsy. However, most of these synthetic drugs are associated with serious side effects including teratogenicity, chronic toxicity and adverse effects on cognition and behaviour [19, 20]. Therefore, there is a dire need for the development of cheap, effective and safe anticonvulsant agents from plants and other natural sources.

The results of this study showed that chloroform and ethanol extracts of SI significantly decreased the duration of THLE in MES model, which was similar to anti-epileptic activity of phenytoin. Anticonvulsant activity was also assessed by PTZ-induced convulsive model. Chloroform and ethanol extracts of SI showed an increase in the latency to onset of convulsions as compared with the control group. These results were similar to the effect of phenobarbitone in PTZ induced convulsions in mice.

The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures, where these drugs block maximal electroshock-induced tonic extension. Moreover, this tonic extension can be prevented either by drug that inhibits voltage dependent Na+ channels, such as phenytoin, valproate, lamotrigine etc [21]. The PTZ induced convulsion test is a valid model for human generalized myoclonic and absence seizures. PTZ may be exerting its convulsive effect by inhibiting the activity of gamma amino butyric acid (GABA) at GABA_A receptors, the major inhibitory neurotransmitter which is implicated in epilepsy. It has been indicated that PTZ-induced seizures can be prevented by drugs that reduce T-type Ca²⁺ currents, such as ethosuximide, and also by drugs that enhance GABA_A receptor-mediated inhibitory neurotransmission, such as benzodiazepines and Phenobarbital [22, 23].

Furthermore, various classes of phytoconstituents such as alkaloids, triterpenoids, flavonoids triterpenic steroids and triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ [24-26]. In the

present investigation, the anticonvulsant activity may be due to the presence of alkaloids, terpenoids, flavonoids, steroids and saponin in chloroform and ethanol extract of *Sargassum ilicifolium*.

CONCLUSION

In conclusion, the results of the present study suggest that chloroform and ethanol extract of *Sargassum ilicifolium* possesses anticonvulsant activity against MES and PTZ induced convulsion in mice. However, further research will be necessary to investigate the exact mechanism underlying this anticonvulsant activity.

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