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GC-MS ANALYSIS OF FOLIAR APPLICATION OF EXTRACT OF *ULVA RETICULATA* GROWN FRUIT OF *CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.

T. Kumareswari*

Department of Botany, Kamaraj College, Thoothukudi, Tamil Nadu, India.

***Corresponding Author: T. Kumareswari**
Department of Botany, Kamaraj College, Thoothukudi, Tamil Nadu, India.

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ABSTRACT
Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) is widely distributed in tropical and subtropical areas across the world. The presence of diverse phytoconstituents has been reported from beans of this plant. However, there has been not much information available on phytochemical components and their biological activity of effect of extract of *Ulva reticulata* grown fruit of Cluster bean. The present investigation was carried out to determine the foliar application of *Ulva reticulata* induced the synthesis of three different chemical components viz., 4,4,5,8-tetramethyl chroman-2-ol, 3-Hexen-2-one, 3,4-dimethyl and 5(2H)-Oxazolone-4-(phenylmethyl)- by Gas Chromatography-Mass Spectrophotometric (GC-MS) analysis. These compounds were referred to have antioxidant, anti-inflammatory, anti-tumor, anticancer, antimutagenic and antibacterial.

KEYWORDS: Cluster bean, phytoconstituents, *Ulva reticulata* Forsskal, antioxidant, anti- inflammatory.

INTRODUCTION
Phytochemicals are present in a variety of plants and are utilized as important component of both human and animal diets. These include fruits and vegetables.[1] Screening of active compounds from plants has lead to the invention of new medicinal drugs, which have efficient protection and treatment roles against various diseases including cancer and Alzheimer's diseases.[2,3] Cluster bean (*Cyamopsis tetragonoloba* L. Taub) commonly known as guar is a native plant of India principally grown for its green fodder and pods that are used for food and feed. It grows well in arid to semiarid areas on a wide range of different soil types, preferably in fertile, medium-textured and sandy loam soils. Guar is one of the most important and potential vegetable cum industrial crop, grown mainly for its tender vegetable pod purpose. Green pods are rich source of protein, minerals and vitamins. As a rich source of nutritional value, cluster bean offer several health benefits in vegetable form. *C. tetragonoloba* is a well-known in folklore medicine, it acts as an appetizer, cooling agent, laxative and is useful in dyspepsia and anorexia. In addition antiulcer, anti-secretory, cytoprotective and hypolipidemic effects have also been reported.[4] Also, Guar beans are potentially high sources of different natural compounds.[5] For the pharmacological as well as pathological discovery of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemicals screening of plant extracts.[6] The aim of the present study is to find out the phytocomponents of extract of *Ulva reticulata* Forsskal grown fruit of *Cyamopsis tetragonoloba* L.using GC-MS analysis.

MATERIALS AND METHODS
Collection of seaweed
Green alga (*U. reticulata* Forsskal) was collected during low tide, at Hare Island, Thoothukudi from November 2014 to February 2015. The sample was washed thoroughly with seawater followed by fresh water to remove sand particles and macroscopic epiphytes. After draining, the seaweed was shade-dried, powdered, sieved and used for the preparation of seaweed concentrate.

Preparation of seaweed liquid fertilizer for foliar application
Seaweed extracts (SWEs) were prepared by adopting the standard method with certain modifications.[7] About 20g dried seaweed powder with 200ml distilled water was heated to 60^{o}C and maintained at the temperature for 24 hr in a hot air oven. The extract was filtered and then centrifuged at 10000 rpm to remove suspended impurities. The filtrate was stored in air tight bottles at 4^{o}C (100% seaweed concentrate) for further use.

Experimental design
A pot culture experiment was conducted during February to April 2015 at Plant Research Centre, St. Mary's College Campus, Thoothukudi, Tamil Nadu, India. The pots were filled with 3kg of garden soil. 20 seeds of *C. tetragonoloba* were sown in each pot. After the emergence of seedlings, they were thinned to ten plants

per pot and allowed to grow upto fruiting stage. Weeding and watering were done at regular intervals. 1% SWE was applied as foliar spray (along with 100ml of distilled water in the ratio of 1: 100) after expansion of first leaf and was continued till fruiting stage. Enough replicates were maintained.

Determination of chemical compounds by Gas Chromatography Mass Spectrophotometric analysis (GC-MS)

25 g leaf sample was extracted with 250 ml of methanol in the ratio of 1:10 in soxhlet apparatus. The extract was allowed to dry and the residue thus obtained was analysed.

GC-MS analysis was performed using a Perkin-Elmer GC Clarus 500 system and Gas Chromatography interfaced to a Mass Spectrophotometer (GC-MS) equipped with a Elite-5MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm) composed of 5% diphenyl / 95% dimethyl polysiloxane.[8] For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml /min and an injection volume of 2 µl was employed; split ratio of 10:1; injector temperature 250^0C. The oven temperature was programmed from 110^0C (isothermal for 2 minutes) with an increase of 10^0C/minutes to 200^0C, then 5^0C/minutes to 280^0C, ending with a 9 minutes isothermal at 280^0C. Mass spectra were taken at 70 eV; 200^0C of inlet line source temperature, a scan interval of 0-2 minutes and mass scan from 45 to 450 (m/Z). Total GC running time was 36 minutes. The relative % amount was calculated by comparing its peak area to the total areas. Software adopted to handle mass spectra and chromatogram was Turbomass (Version 5.2).

Interpretation of the mass spectrum was done using the database of National Institute of Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST Library (Version, 2005). The name, molecular weight and structure of the components of the test materials were ascertained.

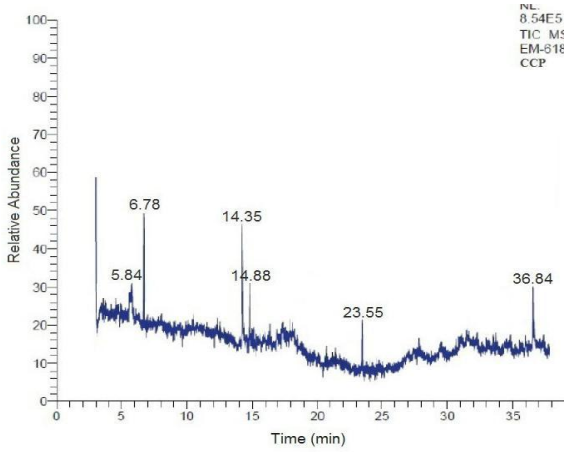


Fig. 1 GC-MS spectrum of methanolic fruit extract of *C. tetragonoloba* (Control).

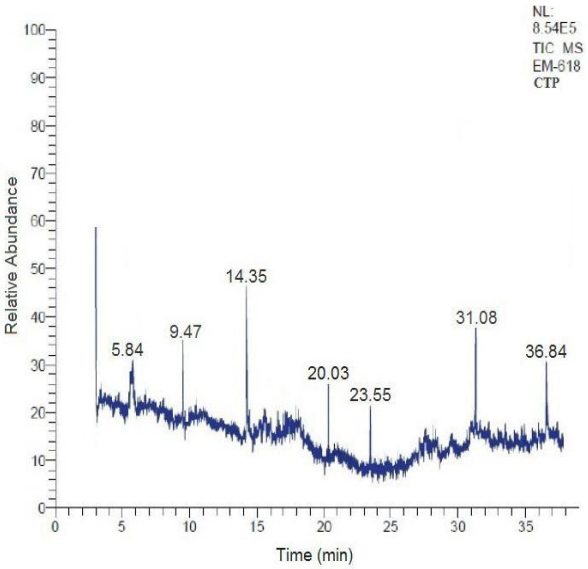


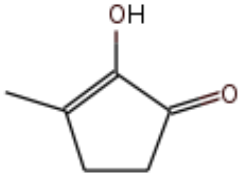
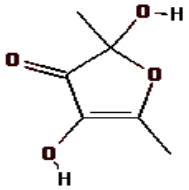
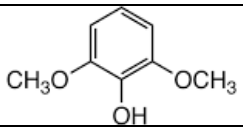
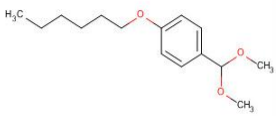
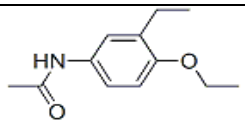
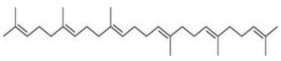
Fig. 2 GC-MS spectrum of methanolic fruit extract of *C. tetragonoloba* (Treated).

Table 1a. Phytocomponents detected in the methanolic extract of *C. tetragonoloba* fruit (control) by GC-MS analysis.

S. No.	Retention Time	Name of the compound*	Molecular Formula	Molecular weight	Peak area %
1	5.84	2-Cyclopenten-1-one, 2-hydroxy	$C_5H_6O_2$	98	0.9748
2	6.78	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	8.1055
3	14.35	Phenol, 2,6-dimethoxy	$C_8H_{10}O_3$	154	0.5251
4	14.88	Benzene, 1-[(dimethoxymethyl)-1-ethyl]-4-methoxycarbonyl-1-ethyl	$C_{15}H_{22}O_4$	266	2.3316
5	23.55	Acetamide, N-(4-ethoxy-3-hydroxyphenyl)-	$C_{10}H_{13}NO_3$	195	0.9822
6	36.84	Squalene	$C_{30}H_{50}$	410	1.6386

** Compounds were identified from the database stored in the National Institute of Standard Technology (NIST – Version, 2005). Fruit extract was used for analysis. Control = Plants were irrigated with water.*

Table 1b. Chemical nature and activity of phytocomponents identified in the methanolic extract of *C. tetragonoloba* fruit (control) by GC-MS analysis.

Molecular formula	Name of the Compound*	Structure of the compound	Nature of the compound	Activity*
$C_5H_6O_2$	2-Cyclopenten-1-one, 2-hydroxy		Methyl cyclopentenlone	Antioxidant and antimicrobial activities
$C_6H_8O_4$	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one		Furaneol	Antioxidant activity and DNA breaking activity
$C_8H_{10}O_3$	Phenol, 2,6-dimethoxy		Phenolic	Antioxidative antimutagenic, and antibacterial activity
$C_{15}H_{22}O_4$	Benzene, 1-[(dimethoxymethyl)-1-ethyl]-4-methoxycarbonyl-1-ethyl		-	Not specified
$C_{10}H_{13}NO_3$	Acetamide, N-(4-ethoxy-3-hydroxyphenyl)-		Acetamide derivatives	Anti-inflammatory and analgesic Activity
$C_{30}H_{50}$	Squalene		Un saturated hydrocarbon	Antioxidant, antibacterial, antitumor, cancer preventive, lipoxygenase-inhibitor and cosmetic dermatology.

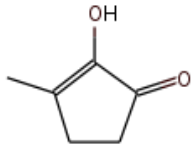
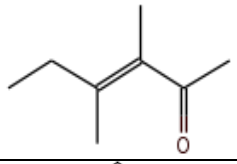
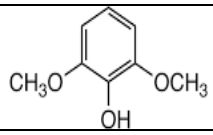
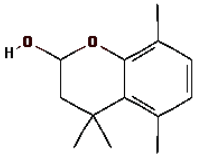
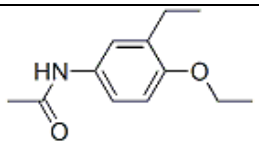
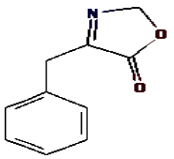
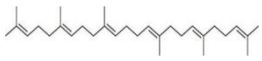
** Compounds and their activities were identified from the database stored in the National Institute of Standard Technology (NIST – Version, 2005). Fruit extract was used for analysis. Control = Plants were irrigated with water.*

Table 2a. Phytocomponents detected in the methanolic extract of *C. tetragonoloba* fruit (Treated) by GC-MS analysis.

S. No.	Retention Time	Name of the compound*	Molecular Formula	Molecular weight	Peak area %
1	5.84	2-Cyclopenten-1-one, 2-hydroxy	$C_5H_6O_2$	98	0.9748
2	9.47	3-Hexen-2-one, 3,4-dimethyl	$C_8H_{14}O$	126	0.8912
3	14.35	Phenol, 2,6-dimethoxy	$C_8H_{10}O_3$	154	0.5251
4	20.03	4,4,5,8-Tetramethylchroman-2-ol	$C_{13}H_{18}O_2$	206	2.7872
5	23.55	Acetamide, N-(4-ethoxy-3-hydroxyphenyl)-	$C_{10}H_{13}NO_3$	195	0.9822
6	31.08	5(2H)-Oxazolone, 4-(phenylmethyl)-	$C_{10}H9NO_2$	175	6.9731
7	36.84	Squalene	$C_{30}H_{50}$	410	1.6386

** Compounds were identified from the database stored in the National Institute of Standard Technology (NIST – Version, 2005). Fruit extract was used for analysis. Treated = Extract of U. reticulata (1%) was applied as foliar spray.*

Table 2b. Chemical nature and activity of phytocomponents identified in the methanolic extract of *C. tetragonoloba* fruit (Treated) by GC-MS analysis.

Molecular formula	Name of the compound*	Structure of the compound	Nature of the compound	Activity*
$C_5H_6O_2$	2-Cyclopenten-1-one, 2-hydroxy		Methyl cyclopentenlne	Anti oxidant and Anti microbial activities
$C_8H_{14}O$	3-Hexen-2-one, 3,4-dimethyl		-	Not specified
$C_8H_{10}O_3$	Phenol, 2,6-dimethoxy		Phenolic	Antioxidative, antimutagenic, and antibacterial activity
$C_{13}H_{18}O_2$	4,4,5,8-Tetramethyl chroman-2-ol		-	Antioxidant
$C_{10}H_{13}NO_3$	Acetamide, N-(4-ethoxy-3-hydroxyphenyl)-		Acetamide derivatives	Anti-inflammatory and analgesic activity
$C_{10}H9NO_2$	5(2H)-Oxazolone, 4-(phenylmethyl)-		-	Not specified
$C_{30}H_{50}$	Squalene		Un saturated hydrocarbon	Antioxidant, antibacterial, antitumor, cancer preventive, lipoxygenase-inhibitor and cosmetic dermatology.

** Compounds and their activities were identified from the database stored in the National Institute of Standard Technology (NIST - Version, 2005). Fruit extract was used for analysis. Treated = Extract of U. reticulata (1%) was applied as foliar spray.*

RESULTS

The results revealed that chemical compounds were present in pods of test and control plants as shown in Table (1a) and (2a); Fig. 1, Fig. 2. It was noted that 2-Cyclopenten-1-one, 2-hydroxy, Phenol, 2,6-dimethoxy, Acetamide, N-(4-ethoxy-3-hydroxyphenyl)- and Squalene were found in fruit extract of both test and control. These compounds were referred to have antioxidant, antimicrobial, anti-inflammatory, anti-tumor, anticancer, antimutagenic, antibacterial, lipoxygenase inhibitory and antianalgesic activity (Table 1b and 2b). The data obtained by this study established that foliar application of *U. reticulata* induced the synthesis of three different chemical components viz., 4,4,5,8-tetramethyl chroman-2-ol, 3-Hexen-2-one, 3,4-dimethyl and 5(2H)-Oxazolone-4-(phenylmethyl)-. Among these three compounds, 4,4,5,8-tetramethyl chroman-2-ol was referred to have antioxidant activity (Table 2b). While for the other two methylated compounds, the activity was unable to be identified. The new compounds deduced need further confirmation and the results evidenced the implication of SWE in metabolism of plants. Thus, the result of this present study showed that the seaweed extracts are complex and diverse in nature and offer novel biological mechanisms to exploit.

DISCUSSION

Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic,

antiviral, anti-helminthic, antifungal and antibacterial activities have been detected in green, brown and red algae.[9,10] SWEs also used as organic fertilizer for improving various crop plants due to contain organic matter, macro and microelements, vitamins, fatty acids and growth regulators such as auxin, cytokinins, gibberellins. Earlier reports revealed that they were responsible for promoting the growth of different crops such as tomato, wheat, soybean and amaranths.[11, 12, 13, 14] *C. tetragonoloba* is also used as a home remedy for the treatment of many diseases due to their secondary metabolites. The leaves of the plant have been reported to have anti-asthmatic activity and to cure night blindness.[15] Many researchers have reported that the relationship between legume consumption and health benefits, such as protection from cardiovascular diseases,[16] breast cancer,[17] colon cancer and diabetes,[18] anti-inflammatory and arthritis.[19,20] Additionally,[21] studied the beans of *C. tetragonoloba* are potentially high sources of supplementary phytochemicals. Similarly,[22] also reported that the ethanolic extract of *C. tetragonoloba* pods were found to be rich in various phytochemicals and showed good *in vitro* antioxidant potential. No research work has been done so far in this aspect. This may be the first attempt to identify the new phytocomponents of foliar application of extract of *U. reticulata* grown fruit of *C. tetragonoloba* using GC-MS analysis.

CONCLUSION

From this result it is concluded that SWE induced new compounds and they need further confirmation as source of useful drugs.

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