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Gc-Ms Analysis of Soil Application Of Extract Of *Hypnea Musciformis* Grown Leaves Of *Amaranthus Tristis* L.

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ABSTRACT

The present investigation was carried out to determine the phytocomponents of methanol extract of soil application of extracts of *Hypnea musciformis* (Wulfen) J.V. Lamouroux grown leaves of *Amaranthus tristis* L. by using GC-MS analysis (Gas Chromatography - Mass Spectrum). The analysis revealed the Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). There were nine chemical compounds identified in leaves of control and 10 compounds in treated of which 4-Methoxy benzoic acid, methyl ester, 3-Hydroxyhexanoic acid, Deoxynivalenol, n-heptane and Napthazoline were present in both control and treated. Soil application of *H. musciformis* induced the synthesis of five different chemical components viz., Neopenthylhydroxyacetate, 11-Dodecenoicacid, -hydroxy-,methyl ester, Benzene,1-[(dimethoxymethyl)-1-ethyl]-4-methoxycarbonyl-1-ethyl,1,2-benzenedicarboxylic acid, disooctyl ester and (-)-Spathulenol which were not found in control. From the present study, it is concluded that seaweed induced new phytocomponents which were not present in control and these compounds correspond to various medicinal properties that can be exploited for the treatment of many diseases.

KEY WORDS: Phytocomponents, methanol extract, Gas Chromatography-Mass Spectrum,

Hypnea musciformis, *Amaranthus tristis*

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INTRODUCTION

Green leafy vegetables are regarded as 'natures antiaging wonders' due to its medicinal properties beyond essential sustenance[1]. They are the major component in traditional diet because of the presence of more phytochemicals with potential antioxidant properties[2]. They are commonly consumed by lower economic group people than the comfortable counterparts[3]. *A. tristis* is a common edible plant that contains amarantin, isoamarantin, betaine, aminoacids and sterols[4]. It has a great medicinal value, as it is used as an astringent in dysentery, diarrhoea, and hemorrhagic colitis, cough and bronchitis. Several species of *Amaranthus* are often considered as weeds, people around the world worth amaranths as leaf vegetables, cereals and ornamentals[5]. The pharmacological properties of amaranth products are considered of vital importance[6]. For reducing tissue swelling the leaves are well thought-out to be constructive, and they have a cleansing effect too. The leaves of amaranthus are used for the treatment of intestinal bleeding, excessive menstruation, diarrhoea and other related problems[7]. Though few reports are available with respect to the pharmacological properties of this plant, this may be the first time to identify the phytoconstituents of methanolic extract of *Hypnea musciformis* grown leaves of *Amaranthus tristis* using GC-MS analysis. Hence, the aim of the present study is to scientifically validate the phytochemical content of *A. tristis*.

MATERIALS AND METHODS

Collection of seaweed

H. musciformis (Wulfen) J.V. Lamouroux was collected during low tide, at Hare Island, Thoothukudi from November 2016 to February 2017. 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 extract for soil application

Seaweed extract (SWE) was prepared by adopting the standard method with certain modifications[8]. 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 2017 at Plant Research Centre, St. Mary's College Campus, Thoothukudi, Tamil Nadu, India. The pots were filled with 3kg of garden soil. 50 seeds of *A. tristis* were sown in each pot. After the emergence of seedlings, they

were thinned to ten plants per pot and allowed to grow for a period of 30 days. Weeding and watering were done at regular intervals throughout the experimental period. 1% SWE was applied in soil (along with 100ml of distilled water in the ratio of 1: 10) after expansion of first leaf and was continued for twenty days. 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[9]. 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^{o}C. The oven temperature was programmed from 110^{o}C (isothermal for 2 minutes) with an increase of 100C/minutes to 200^{o}C, then 50C/minutes to 280^{o}C, ending with a 9 minutes isothermal at 280^{o}C. Mass spectra were taken at 70 eV; 200^{o}C 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.

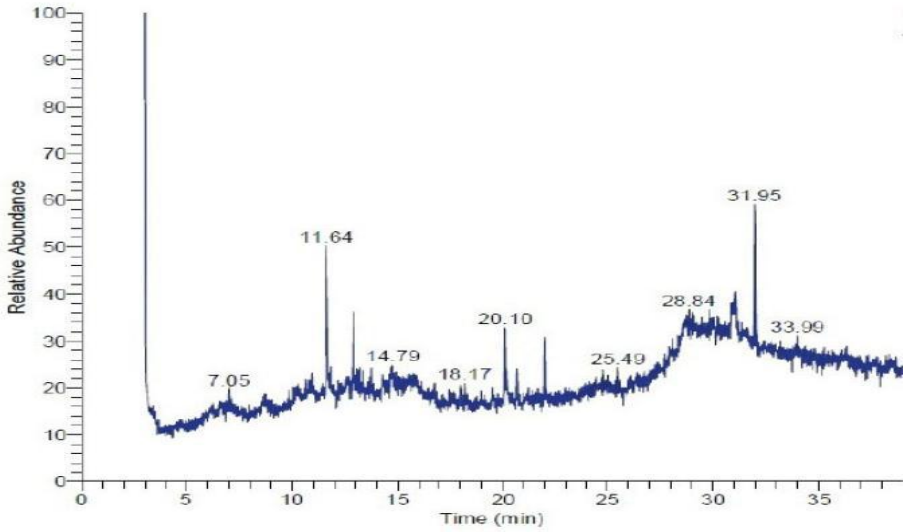


Figure 1: GC-MS spectrum of methanolic leaf extract of *A. tristis* (control)

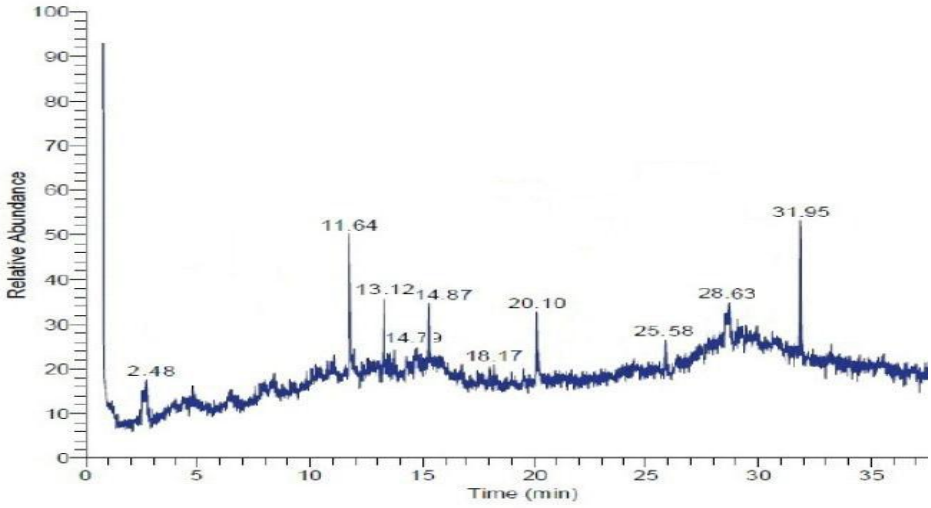


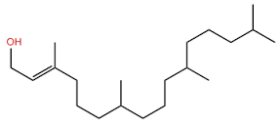
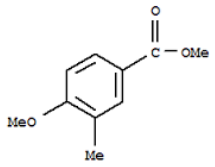
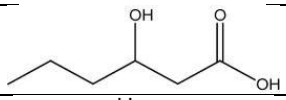
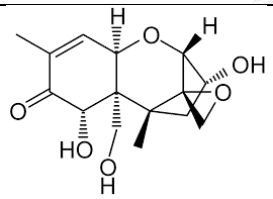
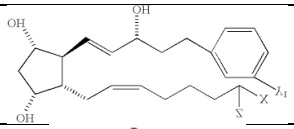
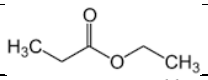
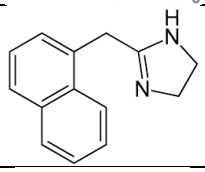
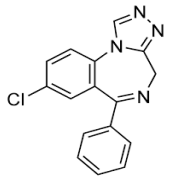
Figure 2: GC-MS spectrum of methanolic extract leaf extract of *A. tristis* (Treated)

Table No. 1: Phytocomponents detected in the methanolic extract of *A. tristis* (control) by GC-MS analysis

S.No.	Rention Time	Name of the compound	Molecular Formula	Molecular Weight	Peak Area (%)
1.	7.05	3,7,11,15-Tetramethyl-3-hexadecan-1-01	$C_{20}H_{40}O$	296	4.54
2.	11.46	4-Methoxy benzoic acid, methyl ester	$C_{12}H_{30}O_2$	178.14	0.91
3.	14.79	3-Hydroxyhexcanoic acid	$C_6H_{12}O_3$	132.15	0.51
4.	18.17	Deoxynivalenol	$C_{15}H_{20}O_6$	296.32	0.43
5.	20.10	n-heptane	C_7H_{16}	100.21	0.74
6.	25.49	Cyclopentane, 1-methyl 1-212- propyl trans	C_9H_{18}	126.23	2.76
7.	28.84	Ethyl propinonate	$C_5H_{10}O_2$	102.13	0.24
8.	31.95	Napthazoline	$C_{14}H_{14}N_2$	246.73	0.76
9.	33.99	Estazolam	$C_{12}H_{11}C_1N_4$	294.7	3.20

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

Table No. 1a: Chemical nature and activity of phytocomponents identified in the methanolic extract of *A. tristis* (Control) by GC-MS analysis

S. No.	Name of the compound	Structure of the compound *	Nature of the compound	Activity*
1.	3,7,11,15-Tetramethyl-2-hexadecan-1-ol		Terpene Alcohol	Anti microbial, Anti-inflammatory, Anti oxidant,
2.	4-Methoxy benzoic acid, methyl ester		Methyl ester	Anti-inflammatory
3.	3-Hydroxyhexanoic acid		Fatty Acid	-
4.	Deoxynivalenol		Cyanide containing substance.	*In vitro* study (porcine ovarian granulosa cells), Anti-tumour.
5.	n-heptane		Straight line Alkane	Not specified
6.	Cyclopentane, 1-methyl-2,1,2-propyl trans		-	Not specified
7.	Ethyl propionate		Carboxylic Acid Esters	Anti microbial
8.	Napthazoline		Corticosteroids	Antiinflammatory, Vasoconstrictor
9.	Estazolam		Aromatic Heteropolycyclic Co mpounds	Anxiolytic, anticonvulsant, sedative and skeletal muscle relaxant properties

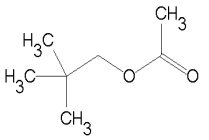
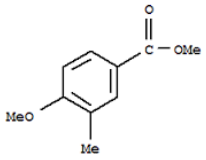
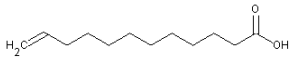
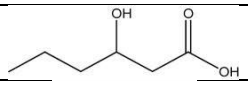
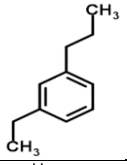
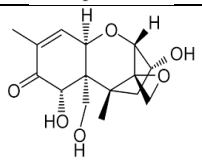
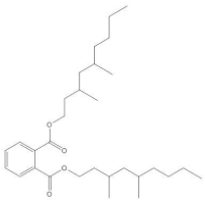
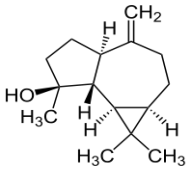
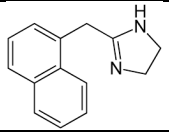
*Name of the compound was identified from the database stored in the National Institute of Standard Technology (NIST-version 2005). Leaf extract was used for this analysis. Control = Plants were irrigated with water.

Table No. 2: Phytocomponents detected in the methanolic extract of *A. tristis* (Treated) by GC-MS analysis

S.No.	Rention Time	Name of the compound*	Molecular Formula	Molecular Weight	Peak Area (%)
1	2.48	Neopenthylhydroxyacetate	$C_7H_{14}O_3$	146.00	98.41
2	11.64	4-Methoxy benzoic acid, methyl ester	$C_{12}H_{30}O_2$	178.14	0.91
3	13.12	11-Dodecnoic acid, 10-hydroxy-, methyl ester	$C_{13}H_{24}O_3$	228.00	1.46
4	14.79	3-Hydroxyhexcanoic acid	$C_6H_{12}O_3$	132.15	0.51
5	14.87	Benzene, 1- [(dimethoxymethyl)-1-ethyl]-4-methoxycarbonyl -1-ethyl	$C_{15}H_{22}O_4$	266.00	0.62
6	18.17	Deoxynivalenol	$C_{15}H_{20}O_6$	296.32	0.43
7	20.10	n-heptane	C_7H_{16}	100.21	0.74
8	25.58	1,2-benzenedicarboxylic acid, disooctyl ester	$C_{24}H_{38}O_4$	390.00	23.00
9	28.63	(-)-Spathulenol	$C_{15}H_{24}O$	220.35	20.76
10	31.95	Napthazoline	$C_{14}H_{14}N_2$	246.73	0.76

*Name of the compound was identified from the database stored in the National Institute of Standard Technology (NIST-version 2005). Leaf extract was used for analysis. Treated = *H. musciformis* extract (1%) was applied through soil.

Table No. 2a: Chemical nature and activity of phytocomponents identified in the methanolic extract of *A. tristis* (Treated) by GC-MS analysis

S. No.	Name of the compound	Structure of the Compound*	Nature of the compound	Activity
1.	Neopenthylhydroxy Acetate		Ester	No activity was found
2.	4-Methoxy benzoic acid, methyl ester		Methyl ester	Anti-inflammatory
3.	11-Dodecnoic acid, 10-hydroxy-, methyl ester		Fatty Acid	In nutraceutical forms as a food additives and dietary supplements
4.	3-Hydroxyhexanoic acid		Fatty Acid	-
5.	Benzene, 1-[(dimethoxymethyl)-1-ethyl]-4-methoxycarbonyl -1-ethyl		Aromatic compounds/ Aromatic disubstitued compounds	Detergents, drugs and pesticides
6.	Deoxynivalenol		Cyanide containing substance.	*In vitro* study (porcine ovarian granulosa cells), Anti-tumour.
7.	n-heptane		Straight line Alkane	No activity
8	1,2-benzenedi carboxylic acid, disooctyl ester		Plasticizer compound	Anti microbial, Anti oxidant, Cancer preventive, immunostimulant, Chemopreventive, Lipoxygenase,
9	(-)-Spathulenol		Hydroxyl Cyclic compounds/ Non Benzenoid compounds	Antibacterial, antiinflammatory allergy, rheumatism, arthiritis
10	Napthazoline		Corticosteroids	Antiinflammatory and Vasoconstrictor

* Compounds and their activities were identified from the database stored in the National Institute of Standard Technology (NIST - Version, 2005). Leaf extract was used for this analysis. Treated = *H. musciformis* extract (1%) was applied through soil.

RESULTS AND DISCUSSION

National Institute of Standards and Technology library sources were also used for matching the identified chemical components from the methanolic leaves extract of *A. tristis* (Tables 1a and 2a). There were nine chemical compounds identified in leaves of control and 10 compounds in treated of which 4-Methoxy benzoic acid, methyl ester, 3-Hydroxyhexanoic acid, Deoxynivalenol, n-heptane and Napthazoline were present in both control and treated (Figures 1 and 2). In addition, soil application of *H. musciformis* induced the synthesis of five different chemical components viz., Neopenthylhydroxyacetate, 11-Dodecenoicacid,-hydroxy-, methylester, Benzene,1-[(dimethoxymethyl)-1-ethyl]-4 methoxycarbonyl-1-ethyl, 1,2-Benzenedicarboxylic acid, disooctyl ester and (-)-Spathulenol which were not found in control. Among the five compounds, 1,2-benzenedicarboxylic acid, disooctyl ester and (-)-Spathulenol were referred to have anti-inflammatory, antioxidant, cancer preventive, immunostimulant, chemopreventive, antibacterial, anti allergy, antirheumatic properties. While the other two ester and aromatic compounds were valued as nutraceutical, food additives, dietary supplements, detergents and pesticides. Moreover, Neopenthylhydroxyacetate compounds activity was unable to be identified. 1, 2, Benzenedicarboxylic acid, disooctyl ester was reported to increase protein phosphorylation rapidly in HeLa cells via protein kinase C and casein kinase[10]. -(-) Spathulenol was reported to have antimicrobial, Immunomodulatory and antitumor activity[11]. The present study is lined up with my previous report[12] that SWEs induced the synthesis of different chemical components.

CONCLUSION

From this result concluded that twelve phytochemical constituents have been identified from methanolic extract of seaweed grown leaves of *A. tristis* by Gas Chromatogram-Mass spectrometry analysis. The new compounds identified need further confirmation, and the results opened up a new window for comprehensive investigation.

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