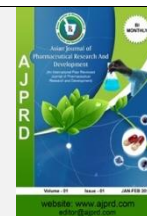


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Research Article

## Screening of Preliminary Phytochemicals and GC-MS Analysis of Chloroform Extract of *Chaetomorpha media* (C.Ag.) Kuetzing

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### ABSTRACT

**Objectives:** The present study was carried out to analyse the chloroform extract of marine green macro alga *Chaetomorpha media* (C.Ag.) Kuetzing, belonging to the family Chlorophyceae.

**Design:** The plant materials were collected from Manapad, located in Thoothukudi district, Tamil Nadu, India. The preliminary phytochemical analysis was carried out by Harborne method and followed by the characterization of biochemicals were predicted using GC-MS analysis.

**Intervention:** Among the various solvent extracts, chloroform extract was intervened in this study

**Main outcome measure:** The main measurement results in this study were to determine the presence of various phytochemicals in the chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing

**Results:** In the preliminary phytochemical analysis of the chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing, seventeen different types of secondary metabolites such as alkaloids, anthocyanin, anthraquinone, catechin, cardiac glycosides, coumarins, flavonoids, glycosides, phenolic groups, phlobatannins, phytosteroids, quinones, saponins, tannins, terpenoids, emodins and diterpenes were present in the chloroform extract. GC-MS spectrum of chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing revealed 18 different major peaks which indicated the presence of eighteen compounds. The prevailing compounds in chloroform extract were 6-Methyloctahydro coumarin, Cyclododecane methanol, 2,3,4-Trimethoxyphenyl acetonitrile, Silicic acid, diethyl bis(trimethylsilyl) ester, Propanamide, 3-bromo-N-(4-bromo-2-chlorophenyl), 2-Ethylacridine, Bis(2-ethylhexyl) phthalate, Silane, trimethyl [5-methyl-2-(1-methylethyl)phenoxy], 1H-Indole, 1-methyl-2-phenyl, Benzo[h]quinoline, 2,4-dimethyl, Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl, Tris(tertbutyl dimethylsilyloxy) arsane, [1,2,4]-Triazolo[4,3-a] [1,3,5]-triazine 5,7-diacetylamino-3-methyl, Cyclotrisiloxane, hexamethyl, Benzo[h]quinoline, 2,4-dimethyl, N,N-Dimethyl-4-nitroso-3-(trimethylsilyl) aniline, 2-Methyl-7-phenylindole, 1,1,1,3,5,5,5-Heptamethyltrisiloxane.

**Conclusion:** The chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing showed the presence of various phytochemicals which can be used as medicine to cure various diseases.

**Keywords:** Marine macro green algae, Phytochemicals, *Chaetomorpha media*, Chloroform extract, GC-MS.

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### INTRODUCTION

Algae are the renewable living resources of the marine environment which act as primary producer of the marine biosphere by supplying oxygen to all the marine organisms. It is known that for the past few

decades, marine macro algae are used as a source of food, fodder, medicine, fertilizer and chiefly for economically important phycocolloids in many parts of the world<sup>1</sup>. These plants are incomparable source of minerals, macro elements and trace metals. Mineral content in marine macro algae compared to land and animal products is generally higher

concentration and the essential minerals and trace elements needed for human nutrition are also present in them. From the literatures it was also observed that marine macro algae are valuable sources of carbohydrates, protein, vitamins and lipids which are essential for human nutrition.

Marine macro algae contain pharmacological and bioactive components like flavonoids, terpenoids, carotenoids, dietary fiber, essential oils and used as a confectionery, textiles, pharmaceutical, dairy and paper industry. The secondary metabolites of the marine macro algae used as a gelling, stabilizing and thickening agents<sup>2</sup>. Bioactive components exhibited significant amount of therapeutic potential and prevent numerous disease infection<sup>3</sup>, anti-peroxidative, anti-hyperlipidaemic activities<sup>4</sup>, antibacterial<sup>5</sup>, anticholesterolemic and anti-tumor activity anti pyritic activity, antidiabetic, anti cancer activity, anti inflammatory<sup>6</sup>, hepatoprotective<sup>7</sup>, CNS depressant<sup>8</sup> and antioxidant activity<sup>9</sup>. Hence the present study was undertaken to screen the phytochemicals present in *Chaetomorpha media* (C.Ag.) Kuetzing, an important marine macro green algae.

## MATERIALS AND METHODS

### Collection of plant materials

The plant materials used in the present study was *Chaetomorpha media* (C.Ag.) Kuetzing belonging to the family Chlorophyceae. The plant materials were collected from Manapad located in Thoothukudi district, Tamil Nadu, India during the month of December, 2018. The marine macro green algae were identified and confirmed using by the book "Seaweeds of the south east coast of Tamil Nadu, India"<sup>10</sup>. The collected plant materials were washed thoroughly with the tap water followed by distilled water. Then they were stored in refrigerator for further use<sup>11</sup>.

### Preparation of chloroform extract

The plant specimen washed thoroughly and spread out on the blotting paper in room temperature. After drying the plant sample were grounded to fine powder by using tissue blender and the powdered sample stored in refrigerator for further use. 30g of powdered sample were packed in Soxhlet apparatus and extracted with chloroform for 8 h separately<sup>12</sup>.

### Preliminary phytochemical analysis

The chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing was tested for the presence of alkaloids, anthocyanin, anthraquinones, catechin, cardiac glycosides, coumarin, flavonoids, glycosides, phenols, phlobatannins, phytosteroids, quinones, saponins, tannins, terpenoids, emodins and diterpenes. Phytochemical screening of the extracts was carried out according to the standard method<sup>13</sup>.

### Test for alkaloids

1ml of 1% HCl was added to the 2ml of extract in a test tube and was treated with few drops of Mayer's reagent. A creamy white precipitate indicates the presence of alkaloids.

### Test for anthocyanin

1ml of 2N HCl was added to the 1ml of extract and was treated with NH<sub>3</sub>. Pink red colour turns blue violet.

### Test for anthraquinones

2ml of extract was mixed with 1ml of benzene and 1ml of 10% ammonia solution was added. The presence of a pink, red or violet color indicates the anthraquinones.

### Test for catechin

2ml extract was mixed with Ehrlich's reagent and few drops of Conc. HCl. Formation of pink colour indicates the presence of catechin.

### Test for cardiac glycosides

0.4ml of glacial acetic acid was added with 1ml extract and trace amount of FeCl<sub>3</sub> and 0.5ml Conc. H<sub>2</sub>SO<sub>4</sub>. Blue colour indicates the presence of cardiac glycosides.

### Test for coumarins

1ml of extract was added with 1ml of 10% NaOH. Formation of yellow colour indicates the presence of coumarins.

### Test for flavonoids

A few drops of 1% NH<sub>3</sub> solution was added to 2ml of extract in a test tube. Yellow coloration indicates the presence of flavonoids.

### Test for glycosides

2ml of 50% H<sub>2</sub>SO<sub>4</sub> was added to 2ml of extract in a boiling tube. The mixture was heated in boiling water bath for 5 min. 10ml of Fehling's solution was added and boiled. A brick red precipitate indicates the presence of glycosides.

### Test for phenolic groups

To 1ml extract, add 2ml distilled water followed by few drops of 10% Ferric chloride. The formation of blue or black colour indicates the presence of phenolic groups.

### Test for phlobatannins

1ml extract was added with 1% aqueous HCl and then boiled. Red precipitate indicates the presence of phlobatannins.

### Test for phytosteroids

1ml of extract added to 1ml CHCl<sub>3</sub> and few drops of Conc. H<sub>2</sub>SO<sub>4</sub>. Golden red colour or Brown colour indicates the presence of phytosteroids.

### Test for quinones

1ml of plant extract added with 1ml of alcoholic KOH. Red to blue colour indicates the presence of quinones.

### Test for saponins

2ml of extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

### Test for tannins

To 2ml extract, 1ml of distilled water and 1-2 drops of ferric chloride solution was added and observed for brownish green or a blue black coloration indicates the presence of tannins.

### Test for terpenoids

2ml extract was mixed with 2ml of CHCl<sub>3</sub> in a test tube. 3ml Conc. H<sub>2</sub>SO<sub>4</sub> was added carefully along the wall of the

test tube to form a layer. An interface with a reddish brown coloration confirms the presence of terpenoids.

### GC-MS spectrum analysis

The GC-MS analysis of the chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing were carried out using GC model Clarus 680, Mass Spectrometer Clarus 600 (EI) Perkin Elmer, Gas Chromatograph equipped and coupled to a mass detector Turbo Mass 5.4.2 spectrometer with an Elite-5MS, (100% Dimethyl ply siloxane), 30.0m  $\times$  250 $\mu$ m df capillary column. The instrument was set to an initial temperature of 60°C and maintained at this temperature for 2min. At the end of this period, the oven temperature was raised upto 300°C, at the rate of an increase of 10°C/min and maintained for 6min. Injection port temperature was ensured as 250°C and Helium flow rate as 1ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass Spectral condition solvent delay 2min, transfer temperature 240°C, source temperature 240°C and scanning range was set at 50-600 Da. The chemical constituents were identified by GC-MS<sup>14</sup>.

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, retention time, molecular weight and molecular formula of the components of the test materials were ascertained.

### RESULTS & DISCUSSION

In the preliminary phytochemical analysis of the chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing, seventeen different types of secondary

metabolites such as alkaloids, anthocyanin, anthraquinone, catechin, cardiac glycosides, coumarins, flavonoids, glycosides, phenolic groups, phlobatannins, phytosteroids, quinones, saponins, tannins, terpenoids, emodins and diterpenes were predicted.

### GC-MS spectrum of chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing

GC-MS spectrum of chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing revealed 18 different major peaks which indicated the presence of eighteen compounds. The prevailing compounds in chloroform extract were 6-Methyloctahydro coumarin (0.95%), Cyclododecane methanol (2.25%), 2,3,4-Trimethoxyphenyl acetonitrile (3.42%), Silicic acid, diethyl bis(trimethylsilyl) ester (4.83%), Propanamide, 3-bromo-N-(4-bromo-2-chlorophenyl)- (1.57%), 2-Ethylacridine (3.31%), Bis(2-ethylhexyl) phthalate (9.59%), Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]- (4.27%), 1H-Indole, 1-methyl-2-phenyl- (10.73%), Benzo[h]quinoline, 2,4-dimethyl- (2.73%), Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl- (8.80%), Tris(tert-butyl)dimethylsilyloxy)arsane (3.71%), [1,2,4]-Triazolo[4,3-a][1,3,5]-triazine 5,7-diacetylamino-3-methyl- (4.72%), Cyclotrisiloxane, hexamethyl- (12.40%), Benzo[h]quinoline, 2,4-dimethyl- (10.21%), N,N-Dimethyl-4-nitroso-3-(trimethylsilyl)aniline (6.22%), 2-Methyl-7-phenylindole (5.22%), 1,1,1,3,5,5,5-Heptamethyltrisiloxane (4.38%). The spectrum profile of GC-MS confirmed the presence of eighteen major components with retention time of 13.055 min, 13.178min, 16.365min, 16.582 min, 16.610 min, 16.677min, 16.828min, 16.913 min, 17.083min, 17.149min, 17.272min, 17.414min, 17.471min, 17.754 min, 18.520 min, 19.192min, 21.035min and 21.489min respectively (Figure-1&Table-1).

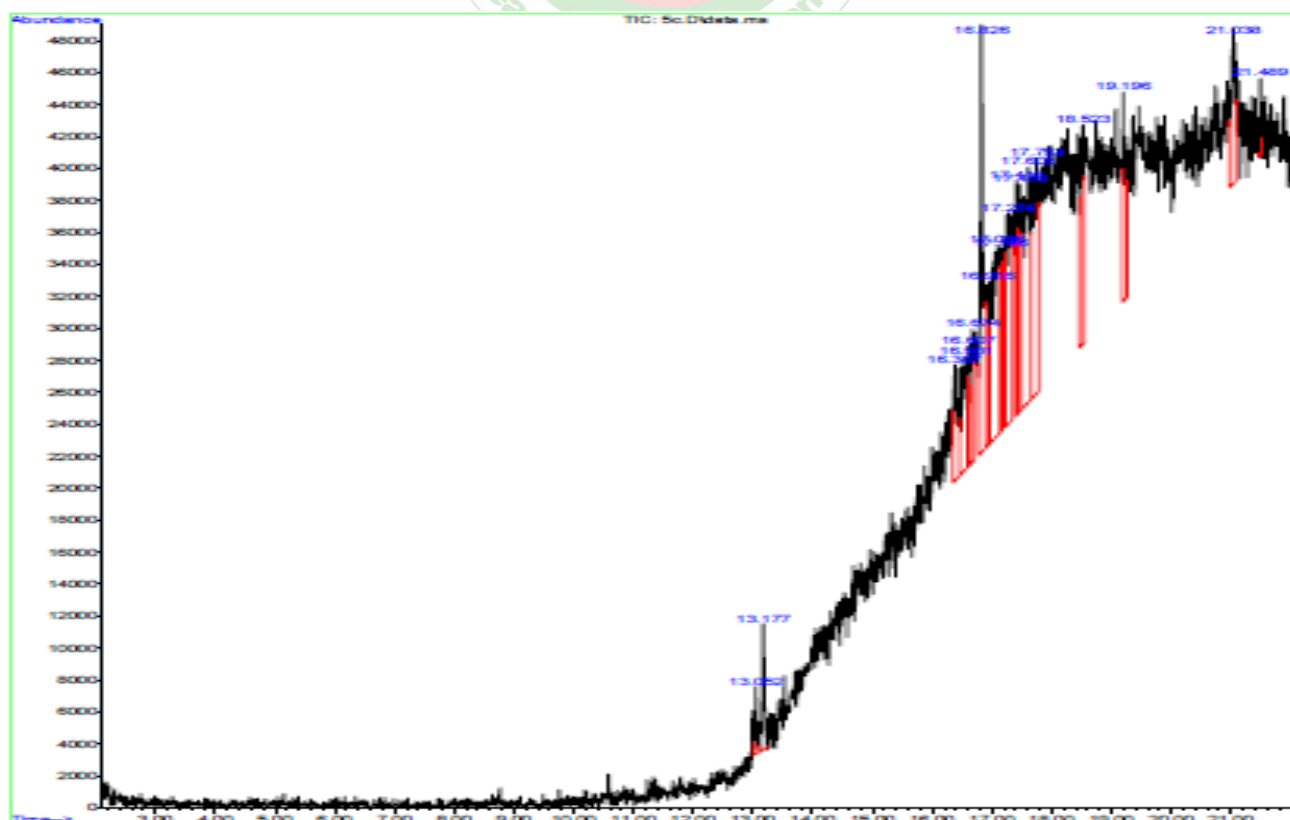


Figure: 1 GC-MS spectrum analysis of chloroform extract of *Chaetomorpha media* (C. Ag.) Kuetzing



**Table: 1** GC-MS spectrum analysis of chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing

S.No	RT	Name of compound	MF	MW	Area %
1.	13.05	6-Methyloctahydro coumarins	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>	168.23	0.95
2.	13.17	Cyclododecane methanol	C <sub>13</sub> H <sub>26</sub> O	198.35	2.25
3.	16.36	2,3,4-Trimethoxyphenyl acetonitrile	C <sub>18</sub> H <sub>19</sub> NO <sub>4</sub>	313.35	3.42
4.	16.58	Silicic acid, diethyl bis (trimethylsilyl) ester	C <sub>10</sub> H <sub>28</sub> O <sub>4</sub> Si <sub>3</sub>	296.58	4.83
5.	16.61	Propanamide, 3-bromo-N-(4-bromo-2-chlorophenyl)	C <sub>9</sub> H <sub>9</sub> BrClNO	262.53	1.57
6.	16.67	2-Ethylacridine	C <sub>15</sub> H <sub>13</sub> N	207.27	3.31
7.	16.82	Bis(2-ethylhexyl) phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.56	9.59
8.	16.91	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-	C <sub>13</sub> H <sub>22</sub> OSi	222.40	4.27
9.	17.08	1H-Indole, 1-methyl-2-phenyl-	C <sub>15</sub> H <sub>13</sub> N	207.27	10.73
10.	17.14	Benzo[h]quinoline, 2,4-dimethyl	C <sub>15</sub> H <sub>13</sub> N	207.27	2.73
11.	17.27	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy)	C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	207.18	8.80
12.	17.41	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	C <sub>11</sub> H <sub>13</sub> NO <sub>3</sub>	207.22	3.71
13.	17.47	Tris(tert-butyltrimethylsilyloxy) arsane	C <sub>18</sub> H <sub>45</sub> SO <sub>3</sub> Si <sub>3</sub>	468.73	4.72
14.	17.75	Cyclotrisiloxane, hexamethyl-	C <sub>6</sub> H <sub>18</sub> O <sub>3</sub> Si <sub>3</sub>	222.46	10.21
15.	18.52	Benzo[h]quinoline, 2,4-dimethyl-	C <sub>15</sub> H <sub>13</sub> N	207.27	6.22
16.	19.19	N,N-Dimethyl-4-nitroso-3-(trimethylsilyl)aniline	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> OSi	222.36	5.21
17.	21.03	2-Methyl-7-phenylindole	C <sub>15</sub> H <sub>13</sub> N	207.27	4.38
18.	21.48	1,1,1,3,5,5-Heptamethyltrisiloxane	C <sub>7</sub> H <sub>21</sub> O <sub>2</sub> Si <sub>3</sub>	221.49	0.70

S. No: Serial Number; RT: Retention Time; MF: Molecular Formula MW: Molecular Weight; Area %: Area Percentage

## CONCLUSION

The present study deals with the screening of phytochemical analysis which shows seventeen different types of secondary metabolites such as alkaloids, anthocyanin, anthraquinone, catechin, cardiac glycosides, coumarins, flavonoids, glycosides, phenolic groups, phlobatannins, phytosteroids, quinones, saponins, tannins, terpenoids, emodins and diterpenes in the chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing. GC-MS spectrum of chloroform extract of *Chaetomorpha media* (C.Ag.) Kuetzing revealed the presence of 18 different major peaks which indicated the presence of eighteen compounds. The present investigation is may lead to ascertain the medicinal quality of this green marine macro algae and brighten the phytochemical profile of it in the field of medicinal value.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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