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QUANTITATIVE ESTIMATION AND LC MS STUDIES OF SOME MEDICINAL PLANTS

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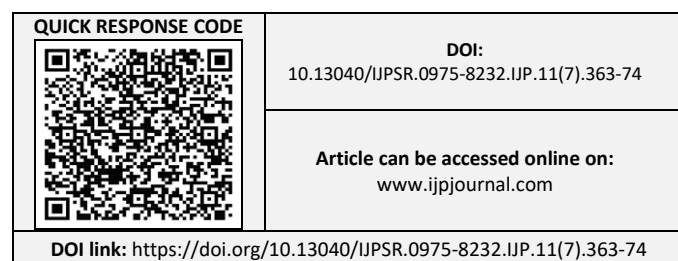
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ABSTRACT: This paper describes the Quantitative estimation and LC MS study of selected plants are *Adenocalymma alliaceum* and *Turbinaria ornata*. Is a plant belonging to the family Bignoniaceae commonly called as 'Garlic vine' and or 'false garlic'. *Turbinaria ornata* (*T. ornata*) is one of the main seaweeds in the marine ecosystem that has been used as a source of medicine among brown seaweeds. These brown algae belong to the family *Sargassaceae*. This review paper discusses in detail study of quantitative estimation and LC MS study. Quantitative determination of *Adenocalymma alliaceum* shows the presence of alkaloid, flavonoid and tannin. Quantitative determination of *Turbinaria ornata* shows the presence of alkaloid, phenol, flavonoid and tannin. LCMS study reveals the presence of 1-hexamine, Apigenin and p-coumaric acid in *Adenocalymma alliaceum* and Tetradecanoic acid, Hydroperoxide and Nonyl trifluoroacetate in *Turbinaria ornata*.

INTRODUCTION: The plants fulfil the variety of human requirements including food, nutraceuticals, medicine and additionally contribute to maintaining the ecosystem. According to the literature search, *Adenocalymma alliaceum* is a species of flowering vine in the Bignoniaceae family that is used traditionally for the treatment of analgesic, anti-inflammatory, antipyretic, insect bite, snake bite and required to prove its traditional claim. Experimental evidence suggests that the most significant medicinal agents are found in seaweeds. Also states that the extracts of seaweeds used to eradicate dreadful diseases including cancer.

A seaweed, *Turbinaria ornata* belongs to the family *Sargassaceae* widely distributed in Indian oceans has a wide variety of health benefits including antioxidant, anti-inflammatory, neuro-protective effects that require scientific investigation for proving its medicinal value.

***Adenocalymma alliaceum* (Mansoa alliacea):** Is a plant belonging to the family *Bignoniaceae* commonly called as 'Garlic vine' and or 'false garlic'. Leaves have pungent garlic smell and flavour when it is crushed but it does not smell if the plant is left alone ¹. Fresh young and soft leaves and stems have been added into the preparation of salads, sandwiches, and other food items ². *A. alliaceum* is widely used in folk medicine treatments for many ailments like cold, as an aid to fertility, commonly added to baths to treat feverish conditions, flu, body aches, cramps, fatigue, mosquito and snake repellent, epilepsy, uterine disorders ³, etc. Whole plant parts should be used in



analgesic, anti-rheumatic and anti-inflammatory. Garlic vine is known for its analgesic, anti-inflammatory⁴, anti-rheumatic and antipyretic properties and is beneficial as herbal medicine⁵.



FIG. 1: *ADENOCALYMMMA ALLIACEUM*

***Turbinaria ornata*:** *T. ornata* is one of the main seaweeds in the marine ecosystem that has been used as a source of medicine among brown seaweeds⁶. *Turbinaria* species, such as *Turbinaria ornata* and *Turbinaria conoides*, have been extensively distributed along the coastal waters of Tamil Nadu. These brown algae belong to the family Sargassaceae⁷. Therapeutic potentials of pure compounds isolated from the Genus *Turbinaria* are extraordinarily promising as antiproliferative, antipyretic, anti-inflammatory, immunostimulatory, anti-diabetic, anti-obesity, antiviral, antimicrobial, cardioprotective, hepatoprotective and hypolipidemic⁸. A wide range of biological properties of this seaweed, including antibacterial, anti-coagulant, anti-inflammatory and antioxidant properties, have been reported, which are used as thickening, gelling, and stabilizing agents in food and drinks, as well as in cosmetics and pharmaceutical products⁹. *Turbinaria ornata* has a wide variety of health benefits and is being researched for pharmaceutical purposes because of its antioxidant, anti-inflammatory, antidiabetic, antiproliferative, and neuroprotective effects on humans¹⁰. *Turbinaria ornata* has the proper compounds to be used as a potential source for reducing postprandial hyperglycemia in humans making it an alternative therapeutic approach in treating diabetes¹¹. *Turbinaria ornata* can be grown and used as a natural alternative wastewater treatment that would reduce untreated dangerous chemicals from being dumped into land and water bodies. Compounds found in *T. ornata* can also be used to restore land

and bodies of water that were previously contaminated by toxic and environmentally destructive chemicals¹².



FIG. 2: *TURBINARIA ORNATA*

MATERIALS AND METHODS:

Quantitative Estimation of Phytoconstituents:

Quantitative estimation refers to the measurement of specific chemical constituents in plant extracts. It helps identify and quantify various secondary metabolites present in medicinal plants. These include alkaloids, flavonoids, phenolic compounds, saponins, and tannins.

Estimation of Phenols¹³:

Principle: The principle of the F–C assay is the reduction of the Folin–Ciocalteu reagent (FCR) in the presence of phenolics resulting in the production of molybdenum–tungsten blue which is measured spectrophotometrically at 750 nm and the intensity increases linearly with the concentration of phenolics in the reaction medium.

Procedure: From 10mg/ml stock solution 300µl of test sample was pipetted out and the volume in each tube was made up to 3.0 ml with distilled water. Folin-Ciocalteu reagent (0.5ml) and 2mL 20% Na₂CO₃ were added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 750nm in a spectrophotometer against a reagent blank. Standard gallic acid solutions (2.5-100µg/ml) were also treated as above.

Estimation of Flavonoids¹⁴:

Principle: Total flavonoid content was measured by the aluminum chloride colorimetric assay. The basic principle of Aluminium chloride colorimetric method is that Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones

and flavonols. In addition, it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids.

Procedure: Total flavonoid content was measured by the aluminum chloride colorimetric assay. The reaction mixture consists of 1mg of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5% sodium nitrite was treated and after 5 minutes, 0.3 ml of 10% aluminum chloride was mixed. After 5 minutes, 2ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water.

A set of reference standard solutions of Quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as µg of QE/ mg of extract.

Estimation of Alkaloid ¹⁵:

Principle: Total alkaloid is estimated based on the reaction with Bromocresol Green (BCG). In this method alkaloid and bromocresol green (BCG) reacts to form a yellow complex and is easily extractable using chloroform. The principle is that certain organic solvent can extract the colored complex (ion pair) quantitatively, which is the combination of an acid dye and a salt ion formed by the reaction of alkaloids and hydrogen ions under some acidic conditions. Chloroform is the most commonly used organic solvent for the determination of alkaloids, having the advantages of forming hydrogen bonds with ion pair easily, high extraction rate, good selectivity and poor aqueous solubility.

Procedure: The plant extract (1mg) was dissolved in 1ml dimethyl sulphoxide (DMSO), added 1ml of 2N HCl and filtered. This solution was transferred to a separating funnel, 5ml of bromocresol green solution and 5ml of phosphate buffer were added.

The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100µg) were prepared in the same manner as described earlier. The absorbance for test and standard

solutions were determined against the reagent blank at 470nm with an UV/Visible spectrophotometer.

Estimation of Tannins ¹⁶:

Principle: Tannins like compounds reduce phosphotungsto molybdic acid in alkaline solution to produce a blue colour complex and the colour intensity is proportional to the concentration of tannin and measured at 700nm.

Procedure: Content of tannins in sample was determined by Folin-Ciocalteu method. Colorimetric estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungsto molybdic acid by tannin like compounds in alkaline medium. One mg/1ml of extract and standard solution of tannic acid (20-100µg) was made up to 7.5mL with distilled water. Then 0.5mL of Folin-Ciocalteu reagent and 35% 1mL sodium carbonate solution were added. The volume was made up to 10mL with distilled water and the absorbance was measured at 700nm.

Estimation of Terpenoid ¹⁷:

Principle: Terpenoids, also known as isoprenoids, are a class of chemical compounds produced from isoprene. Triterpenes are ubiquitous secondary metabolites present in plants. They can be found in both forms, as genins or conjugated as glycosides. The method is known as the vanillin- acid assay because the basic principle of the method is the reaction of sulphuric acid-oxidised triterpene saponins with vanillin, which gives a distinctive red-purple colour measured at wavelengths ranging from 473 to 560 nm using a spectrophotometer.

Procedure: A volume 200µL of extract solutions in methanol (1 mg/mL) was first mixed with 1 mL of perchloric acid and 300µL vanillin/glacial acetic acid (5% w/v) solution. 5 mL of glacial acetic acid was then added to it and the absorbance was measured at 548nm with a UV-Visible spectrophotometer. Linalool (µg/ml) in methanol was used as standard. Results were expressed as mg Linallol equivalents.

Estimation of Glycosides ¹⁸:

Principle: Cardiac glycosides develop an orange red colour complex with Baljet's reagent (Picric acid in alkaline medium). The intensity (absorbance) of colour produced is proportional to

the concentration of glycosides. Cardiac glycosides were quantitatively determined according to Solich *et al.* by some modifications.

Reagents: Standard digitoxin: 0.02% digitoxin is prepared in chloroform: methanol (1:1). Baljet's reagent: Freshly prepared 95ml 1% picric acid + 5ml 10% NaOH are mixed immediately before use and filtered through a sintered glass funnel.

Procedure: 1ml of the extract and 1ml of Baljet's reagent are taken and allowed to stand for one hour. Then dilute the solution with 2ml distilled water and mix. Read the intensity of the colour obtained against blank at 495nm using a spectrophotometer. Standard graph can be prepared using varying concentration of standard digitoxin (2-14µg/ml).

Estimation of Steroids ¹⁹:

Principle: Steroids react with ferric chloride in the presence of concentrated sulphuric acid to give a pink colour. Cholesterol gives a reddish pink red colour with ferric chloride and polar sulphuric acid. The intensity of colour developed is directly proportional to the amount of steroids present and it is read at 540 nm in a calorimeter.

Procedure: One mg/ml of extract was taken in a clean test tube. Cholesterol was used as standard and was taken at varying concentrations of (1-10µg/ml) in test tubes. To the standard and test samples, 5ml of ferric chloride reagent and 4ml of concentrated sulphuric acid were added. The reaction mixtures were incubated at room temperature for 30 minutes and OD was read at 540nm. A standard graph was plotted from which the unknown value of steroid in the test sample was determined.

LC-MS ²⁰: Spectroscopic and chromatographic methods are one of the basic and reliable methods to identifying pharmaceutically active biomolecules from the natural sources. LC-MS (Liquid chromatography -mass spectroscopy) are the sophisticated instruments which are used to screening of bioactive secondary metabolites from the natural sources. These methods are simplest, fastest and more acceptable methods for the identification of bioactive molecules from crude extract of the medicinal plants and which only needs a small amount of plant extract. In order to detect and identify the phytochemical components

present in the medicinal plant, the LC-MS technique was used in the current investigation.

Procedure ²¹: *Adenocalymma alliaceum* extract phytochemical analysis was studied by using the LC-MS. The chemical constituents of the extract were determined using LC-MS. LC-MS analysis was performed using Mariner Bio spectrometry equipped with a binary pump.

The HPLC was interfaced with a Q-TOF mass spectrometer fitted with an ESI source. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140°C. HPLC ²² column Phenomenex 5µ C8, (150 × 2 mm i.d.) was used for the analysis. Solvent was methanol with 0.3% formic acid. Solvents were delivered at a total flow rate of 0.1 mL/min. The solvent was run by isocratic elution. The MS spectra were acquired in the positive ion mode.

Procedure ²³: *Turbinaria ornate* extract phytochemical analysis was studied by using the LC-MS. The chemical constituents of the extract were determined using LC-MS. LC-MS ²⁴ analysis was performed using Mariner Bio spectrometry equipped with a binary pump.

The HPLC was interfaced with a Q-TOF mass spectrometer fitted with an ESI source. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140°C. HPLC column Phenomenex 5µ C8, (150 × 2 mm i.d.) was used for the analysis. Solvent was methanol with 0.3% formic acid. Solvents were delivered at a total flow rate of 0.1 mL/min. The solvent was run by isocratic elution. The MS spectra were acquired in the positive ion mode.

RESULT AND DISCUSSION:

Quantitative Estimation of Phytoconstituents: Estimation of Phenols (Gallic Acid Equivalent Method):

TABLE 1: ESTIMATION OF PHENOL

Standards	Concentration of gallic acid (µg/ml)	OD at 750 nm
S1	5	0.123
S2	10	0.183
S3	20	0.304
S4	40	0.522
S5	80	0.998
S6	100	1.242

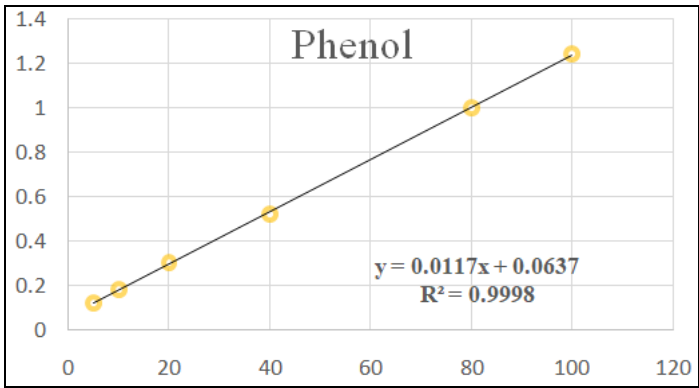


FIG. 3: ESTIMATION OF PHENOL

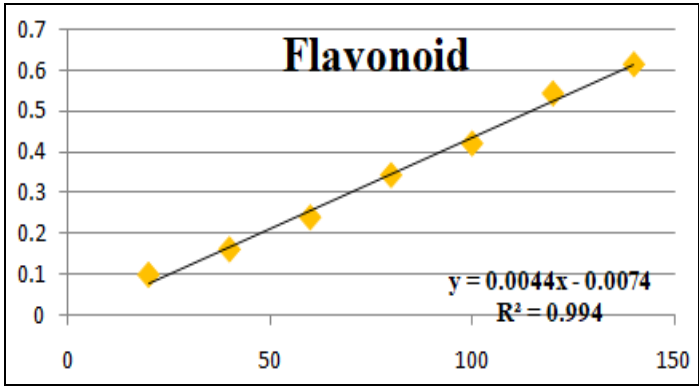
TABLE 2: ESTIMATION RESULT OF PHENOL

S. no.	Sample name	Absorbance at 750 nm	Concentration of phenol (µg/ml)
1	Adenocalymma alliaceum	0.871	69.00
2	Turbinaria ornata	0.225	13.79

Estimation of Flavonoids (Aluminium Chloride Assay Method):

TABLE 3: ESTIMATION OF FLAVONOIDS

Standards	Concentration of Quercetin (µg/ml)	OD at 510 nm
S1	20	0.101
S2	40	0.163
S3	60	0.241
S4	80	0.344
S5	100	0.421
S6	120	0.543
S7	140	0.614



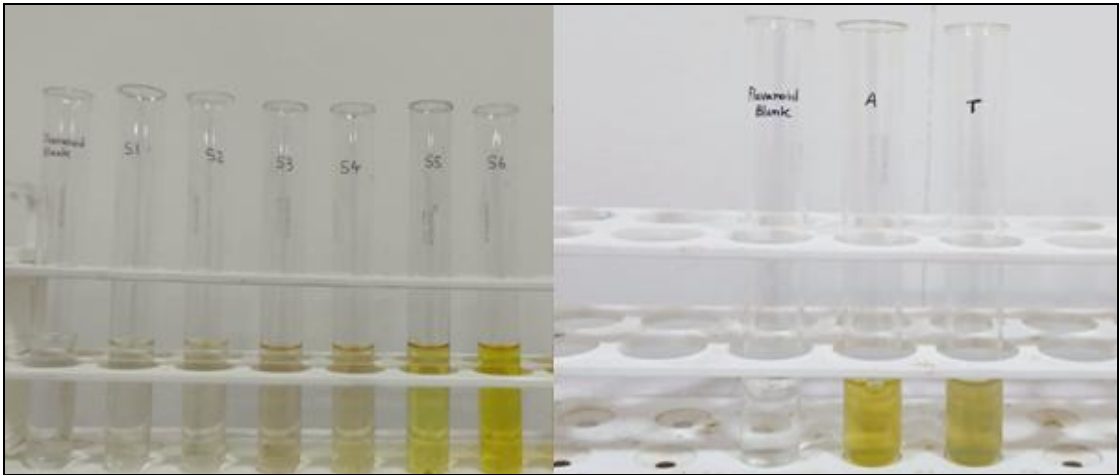


FIG. 4: ESTIMATION OF FLAVONOIDS

TABLE 4: ESTIMATION RESULT OF FLAVONOIDS

S. no.	Sample name	Absorbance at 510nm	Concentration of Flavonoid (µg/ml)
1	<i>Adenocalymma alliaceum</i>	0.269	62.82
2	<i>Turbinaria ornata</i>	0.545	125.55

Estimation of Alkaloid (UV Spectrophotometric Method):

TABLE 5: ESTIMATION OF ALKALOID

Standard	Concentration of Atropine (µg/ml)	OD at 470 nm
S1	20	0.036
S2	40	0.063
S3	60	0.084
S4	80	0.112
S5	100	0.135

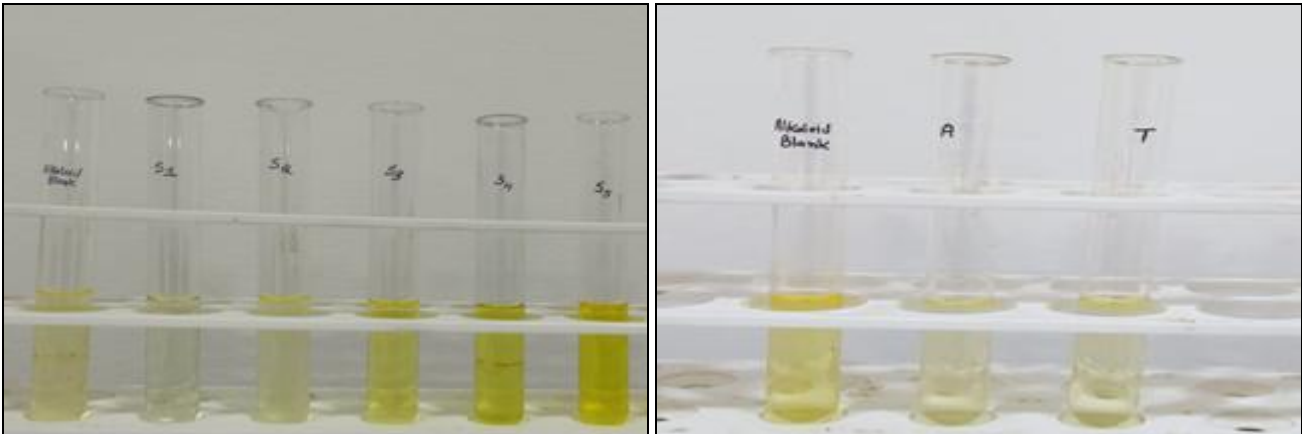
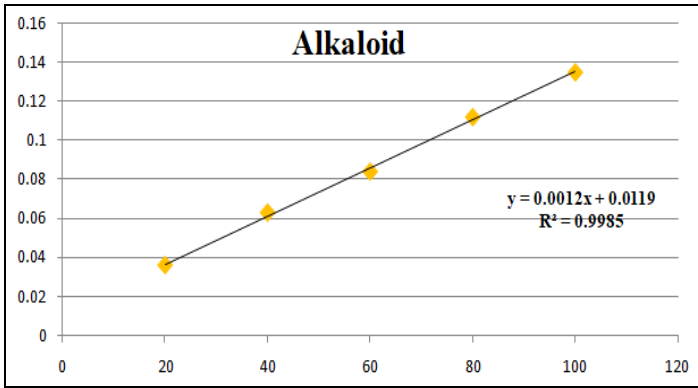


FIG. 5: ESTIMATION OF ALKALOID

TABLE 6: ESTIMATION RESULT OF ALKALOID

S. no.	Sample name	Absorbance at 470 nm	Concentration of Alkaloid (µg/ml)
1	<i>Adenocalymma alliaceum</i>	0.107	79.25
2	<i>Turbinaria ornata</i>	0.060	40.08

Estimation of Tannins (Folin Ciocalteu Method):

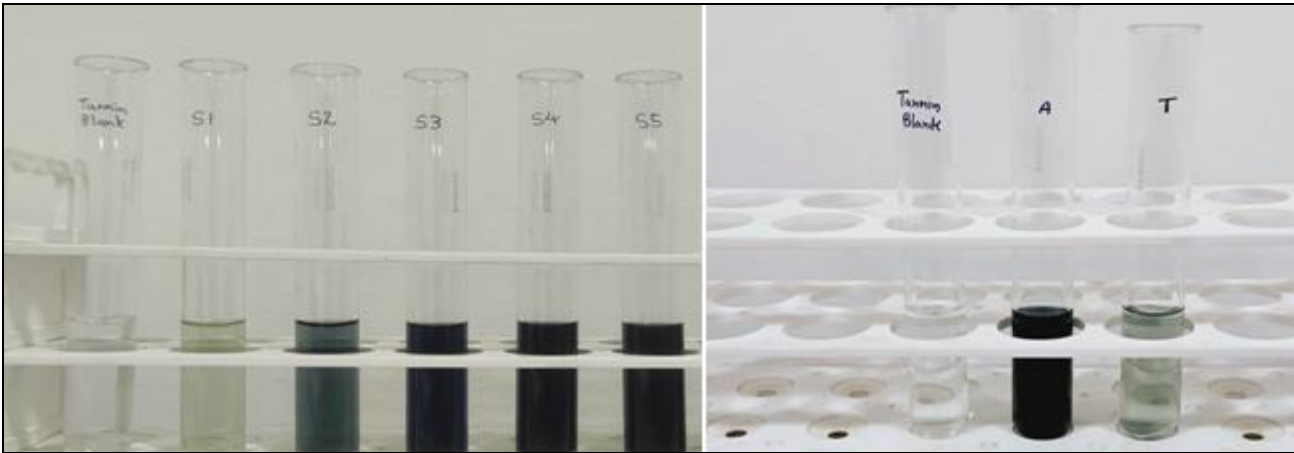
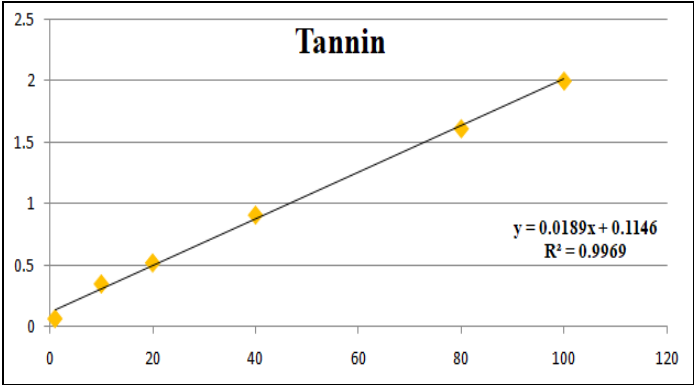


FIG. 6: ESTIMATION OF TANNINS

TABLE 7: ESTIMATION OF TANNINS

Standards	Concentration of tannic acid (µg/ml)	OD at 700 nm
S2	1	0.061
S3	10	0.345
S4	20	0.516
S5	40	0.907
S6	80	1.613
S7	100	2.002

TABLE 8: ESTIMATION RESULT OF TANNINS

S. no.	Sample name	Absorbance at 700 nm	Concentration of Tannins (µg/ml)
1	<i>Adenocalymma alliaceum</i>	1.990	99.23
2	<i>Turbinaria ornata</i>	0.230	6.11

Estimation of Glycosides (UV Spectrophotometric Method):

TABLE 9: ESTIMATION OF GLYCOSIDE

Standards	Concentration of Digitoxin (µg/ml)	Absorbance at 495 nm
S1	5	0.004
S2	10	0.076
S3	15	0.132
S4	20	0.183
S5	25	0.249
S6	30	0.331

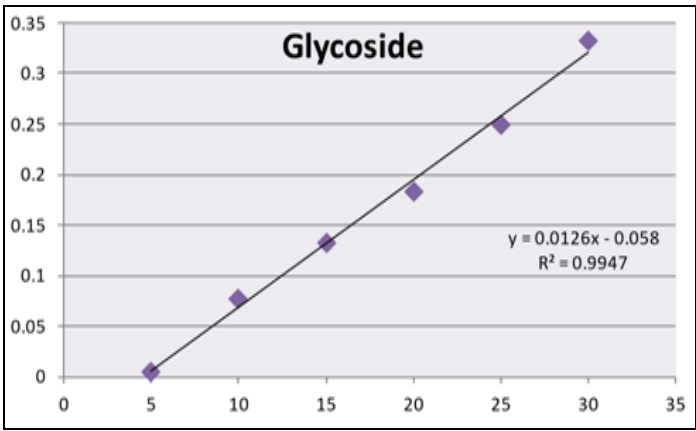


FIG. 7: ESTIMATION OF GLYCOSIDE

TABLE 10: ESTIMATION RESULT OF GLYCOSIDES

Sl. no.	Sample code	Absorbance at 495 nm	Concentration of glycosides (µg/ml)
1	A	0.116	13.81

Estimation of Steroids (UV Spectrophotometric Method):

TABLE 11: ESTIMATION OF STEROIDS

Standards	Concentration Cholesterol (µg/ml)	Absorbance at 540 nm
S1	10	0.062
S2	20	0.138
S3	40	0.221
S4	80	0.405
S5	100	0.495
S6	200	0.966

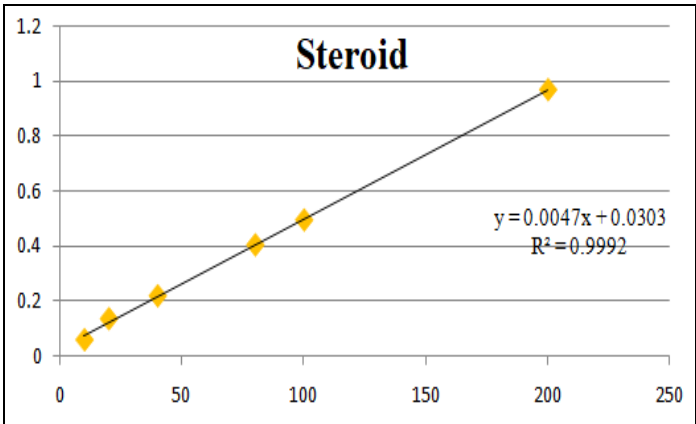




FIG. 8: ESTIMATION OF STEROID

TABLE 12: ESTIMATION RESULT OF STEROID

Sl. no.	Sample code	Absorbance at 540 nm	Concentration of steroid (µg/ml)
1	T	0.105	15.89

Estimation of Terpenoids (UV Spectrophotometric Method):

TABLE 13: ESTIMATION OF TERPENOIDS

Standards	Concentration Linalool (µg/ml)	Absorbance at 548nm
S1	5	0.175
S2	10	0.267
S3	20	0.384
S4	40	0.615
S5	60	0.812
S6	80	1.076
S7	100	1.278

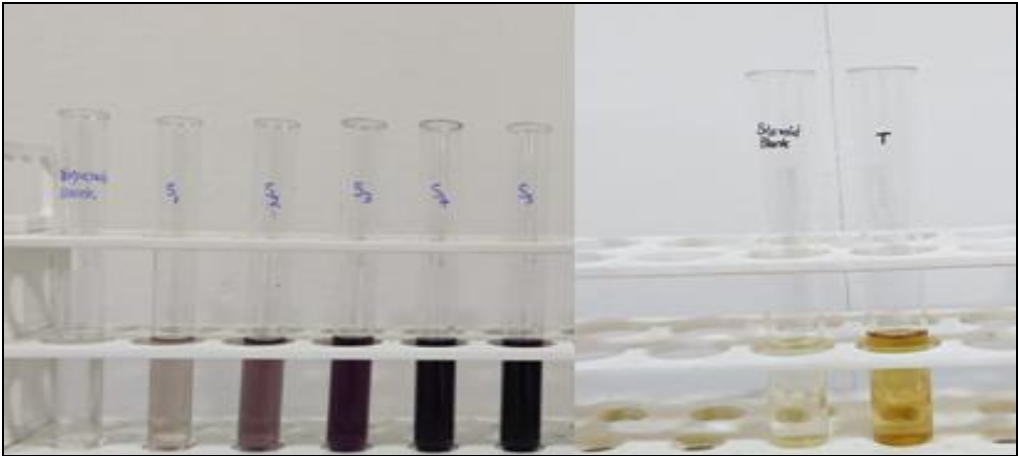
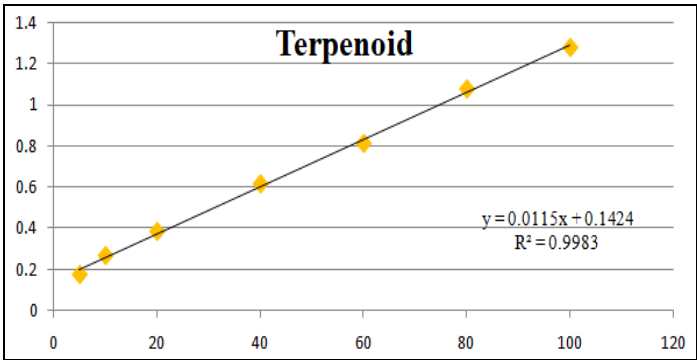


FIG. 9: ESTIMATION OF TERPENOIDS

TABLE 14: ESTIMATION RESULT OF TERPENOIDS

Sl. no.	Sample Code	Absorbance at 548 nm	Concentration of terpenoids (µg/ml)
1	T	0.308	14.4

LC-MS:

LC-MS Sudy of *Adenocalymma alliaceum*: The tentative assignment of compounds detected from the *Adenocalymma alliaceum* extractvia LC-MS analysis are presented. The compounds were detected and were identified by their fragmentation pattern and in conjunction with the PubChem and research reference article. Peak area and retention time were used for the identification of the compounds.

TABLE 15: PHYTOCHEMICAL ANALYSIS OF ADENOCALYMMMA ALLIACEUM

Sl. no.	Phytochemicals	m/z
1	1,3-Dimethylthiourea	103
2	1,3-Dihydroxyacetonedimer	181.4
3	Aceticacid, [(aminocarbonyl)amino]oxo-	132.1
4	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	144.1
5	1-Hexanamine	101.2
6	p-Coumaric acid	163.4
7	Apigenin	169
8	1,5-Anhydro-d-mannitol	164

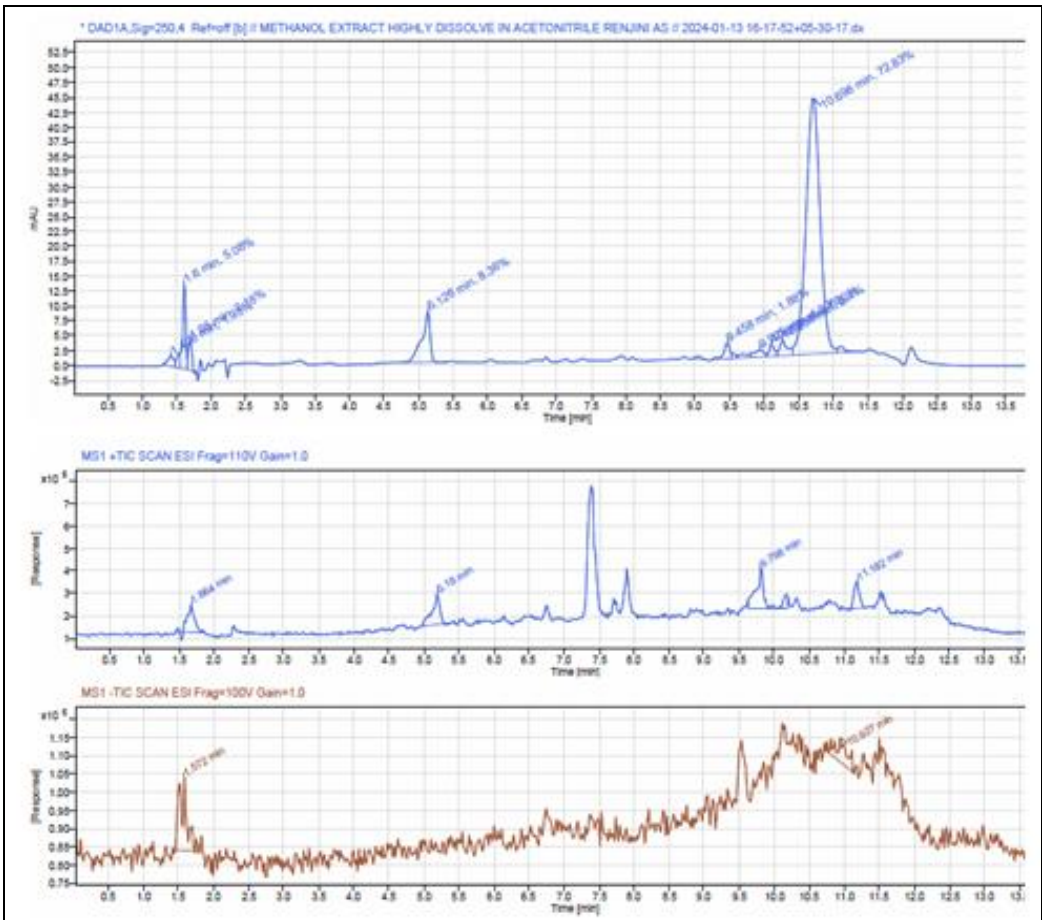


FIG. 10: ADENOCALYMMMA ALLIACEUM EXTRACT LC-MS

LC-MS Study of *Turbinaria ornate*: The tentative assignment of compounds detected from the *Turbinaria ornate*via LC-MS analysis are presented. The compounds were detected and were identified by their fragmentation pattern and in conjunction with the PubChem and research reference article. Peak area and retention time were used for the identification of the compounds

Table 16.

TABLE 16: PHYTOCHEMICAL ANALYSIS OF *TURBINARIA ORNATA*

Sl. no.	Phytochemicals	m/z
1	Tetradecanoic acid	227.6
2	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester	278
3	1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester	278
4	Hydroperoxide,1-methylbutyl	104.3
5	Nonyl trifluoroacetate	241.6
6	Dodecyl trifluoroacetate	281.4
7	3 Ethoxy-1,1,1,5,5,5- hexamethyl-3 trimethyl	341.4

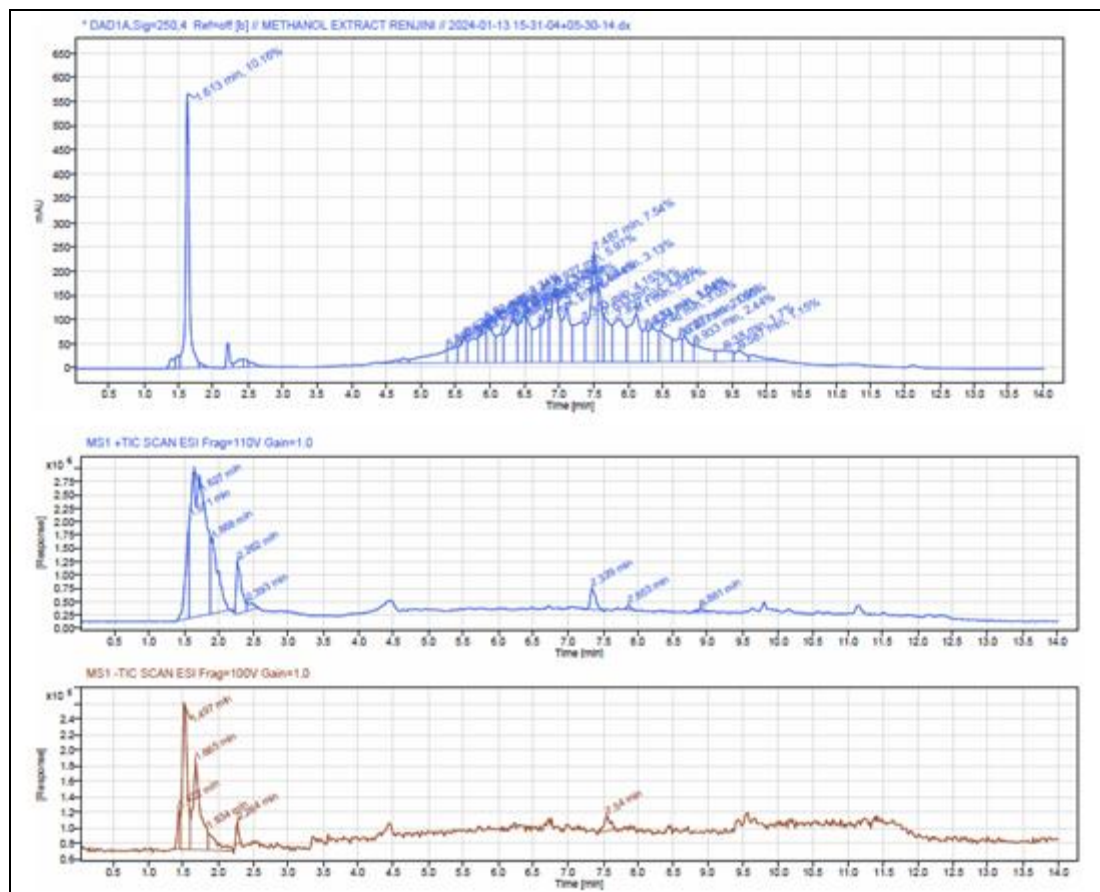


FIG. 11: *TURBINARIA ORNATA* EXTRACT LC-MS

CONCLUSION: In the present investigation, Selected *Adenocalymma alliaceum* plant belonging to terrestrial and *Turbinaria ornata* seaweed from marine source. This review gives information about the quantitative estimation and LC MS study of selected plants. The plant *Adenocalymma alliaceum* used in folk treatments the plant parts are widely used for cold, as an aid to fertility, commonly added to baths to treat feverish conditions, flu, body aches, cramps, fatigue, mosquito and snake repellent, epilepsy, uterine disorders, etc. *Turbinaria ornata* (*T. ornata*) is one of the main seaweeds in the marine ecosystem that has been used as a source of medicine among brown seaweeds. These brown algae belong to the family *Sargassaceae*. Therapeutic potentials of

pure compounds isolated from the Genus *Turbinaria* are extraordinarily promising as antiproliferative, antipyretic, anti-inflammatory, immunostimulatory, anti-diabetic, anti-obesity, antiviral, antimicrobial, cardioprotective, hepatoprotective and hypolipidemic. Quantitative determination of *Adenocalymma alliaceum* shows the presence of alkaloid (79.25µg/ml), phenol (69µg/ml), flavonoid (62.82 µg/ml) and tannin (99.23µg/ml). Quantitative determination of *Turbinaria ornata* shows the presence of alkaloid (40.08µg/ml), phenol (13.79µg/ml), flavonoid (125.55µg/ml) and tannin (6.11µg/ml). LCMS study reveals the presence of 1-hexamine, Apigenin and p-coumaric acid in *Adenocalymma alliaceum* and

Tetradecanoic acid, Hydroperoxide and Nonyl trifluoroacetate in *Turbinaria ornata*.

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