Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 805-816 | ISSN:2251-6727

Chemical Compositions and Antifungal Activities of Seaweed Extracts Against Fungus Isolated from Groundnut Leaves

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(Received: 07 October 2023 Revised: 12 November Accepted: 06 December)

KEYWORDS

Groundnut leaves, Seaweed extract, Secondary metabolites, Antifungal activity

ABSTRACT:

Numerous fungal diseases of groundnut are known and various fungi are reported to be closely associated with groundnut fruits and seeds. The objective of the study is to report the fungi affected groundnut leaves are isolated and its activity is reduced against seaweed extracts. Marine organisms produce a mixture of compounds with alleviative activities, including anticancer, antimicrobial, antifungal, antiviral, anti-inflammatory and are more effective sources of novel therapeutic agents. The antifungal activity of extracts of different seaweed species was evaluated against fungal strains affected groundnut leaves. The groundnut leaves were collected and fungal strains were isolated by standard agar plate method and identified by 18sRNA sequencing. Based on the sequence alignment analysis four different fugal strains are identified *Colletotrichum siamense, Fusarium equiseti, Lasiodiplodia pseudotheobromae, penicilium oxalicum*. The phytochemical analysis of seaweed shows the presence of various secondary metabolites. The *T.conoides* extract was subjected to LC-MS analysis to identify its active constituents. The chromatogram indicated the presence of 16 bioactive compounds, among which were Mescaline, Quinic acid, Shogaol, and Stachydrine. The minimum inhibitory concentrations (MIC) of seaweed extracts were determined. The result showed that all the seaweed extracts has high potential of MIC against the isolated fungi. The present results suggest that seaweed extracts are capable of preventing fungal infection to a certain extent..

1. Introduction

Peanut *(Arachis hypogaea Linn*.) is one of the main Oil yielding crops grown in Indian, the average yield is approximately 900 kg/ha. It is belonging to the *Fabaceae* family of bean or legume and is captivated throughout the world in an extensive range of forms [1]**.** This legume is as well vital in biogeochemical cycles specially nitrogen which is an essential nutrient for the growth of plant which is increasing the soil fertility**.** The seeds of groundnut contain oil, protein, carbohydrate, fiber with various distributions and furthermore rich in micronutrients such as Boron,Copper,Iron, and Zinc [2].As primary utility of the source is suitable for eating oil, the significance of this crop is prominence the status of "king of oil seed crops". The seeds of Groundnut are boiled or roasted and ingested as a snack and for preparation of soup as agglomeration [3] whereas the hay/haulms are fed to livestock. Peanut is particularly products for complete dietary source, as it is protein rich, oil, and fibers [4].

However, its productivity yields are inhibited by various factors such as pathogens (viruses, bacteria, fungi, and insects) other physical factors like drought, salinity, and unsuitable temperature [5]**.** In addition to abiotic stresses, biotic factors such as insect pests and weeds had been recognized as the foremost factors regulating the groundnut production. Globally the most important factors which reduce the yield of groundnuts are when the plants are attacked by different pathogens [6]**.** Furthermost of the infections of the peanut crop is affected by seed accepted fungi that can effortlessly survive in infested peanut seeds [7]. Though, it was

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JCHR (2023) 13(6), 805-816 | ISSN:2251-6727

described that peanuts are recurrently adulterated by the fungal type species. Approximately fungi-induced diseases of groundnut consist of *Macrophomina*, *Choanephora*, *Colletotrichum*, *Cylindrocladium*, *Drechslera*, *Pestalotiopsis*, *Phomopsis*, Phyllosticta, zonate, early leaf spot, and late leaf spot [8].

Generally Peanut contamination is carried up by microbes such as micro fungi. *Cercospora arachidicola* fungi, plant pathogen that causes Early light brown leaf spots and *Phaeoisariopsis personata* fungus, causes late darken leaf spot in groundnut leave (*Puccinia arachidis L*.) have been reported along with the evidence for serious financial crop damage in groundnut worldwide [9], [10], [11]. Mostly, leaf spots can cause skirted by 50-70% yield losses in West Africa [12] and up to 50% yield loss universal [13], [14]. Aflatoxin is stated to be extremely poisonous and hazardous secondary metabolites of distress in food protection [15]. The fungal species are vulnerable substrates for aflatoxin production and numerous research studies have described heavy adulteration of aflatoxins in peanut species [16]. Infectious contaminations differ from each region due to changed environmental circumstances based on their topographical locations. Fungal contamination illustrations suggestion of a problematic degree of assortment in tropical than moderate regions [17], and few are contaminated through transport or packing of peanut meals. One of the key purpose of diminish seed feasibility of the peanut seeds in storage condition [18]**.** Thus, it is vital in recognizing and measuring fungal species found in raw peanuts since some of this fungal species discharge harmful compound. Conferring to Bennett and Klich report, particular fungal strains yield mycotoxins are capable of affecting humans and animals. This will warrant the eminence of the peanut-based products traded in the local or international market.

In order to control or prevent the pest growth, the existing method is chemical pesticides. The usage of fungicides can diminish the rigorousness of leaf spots and upturn crops [19], [20]. Nonetheless chemical insect killer had an important role in shoot up the manufacturing of groundnut, their random usage of the pest control had headed to numerous environmental difficulties such as growth of resistance in pests to pesticides, excess of pesticides and the devastation of valuable creatures like vermin and beast of prey.

Extreme use of chemical composts is detrimental to fitness and spoils the atmosphere. Based on the recent year result data's voluminous vegetal source of extracts played role in agriculture. The practice of microalgae as a fertilizer has supported and gradually overwhelmed the conventional chemical fertilizers [21]. The megascopic oceanic algae are otherwise called as "Seaweeds". They are intended to be applied as pabulum for human as well cattle, alternate form of chemical fertilizer and used as numerous porous chemicals with high degree of purity. Apart from that, they had been used in production of agronomic things like gelatinous substances. Subsequently, they have numerous growth regulators with several nutrients necessary for growth and development of plant, and they also had been used as fertilizers in liquid form. It supports in increasing the useful soil microorganisms [22], thus improves the restraint capacity against environmental stress [23], intensifications of nutrient acceptance from soil [24], [25] and boosts levels of phytonutrients [26]. Based on the erstwhile research evidence comprehended to assess the grade and find the solution for the leaf spot diseases in groundnuts by using seaweeds. In this research, we aimed to isolate, identify the fungal species concomitant with groundnut leaves and resolute the anti-fungal activity of different seaweed species extracts against identified fungus along with the analysis of phytochemicals constituent of the extracts.

2. Materials & Methods

2.1 Collection of sample source and Authentication Number:

Infected Groundnut leaves were collected from various place of Tindivanam, Villupuram district, Tamil Nadu, India. These leaves were stored in tight container and brought to the Greensmed lab, Chennai for further studies.

2.2 Isolation and identification of the fungi associated with groundnut leaf spots

The infected groundnut leaves (Figure No: 1) were examined for the separation and identification of fungi. The fungi were isolated using Potato Dextrose Agar (PDA) medium. The isolated fungi were sub cultured to get pure cultures and were kept in the freezer

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JCHR (2023) 13(6), 805-816 | ISSN:2251-6727

for further studies live identification of various species of fungi. [27]

2.2.1 Preparation of media: To recognize the fungi, potato dextrose agar was used as growth media. PDA media was prepared by weighing of required quantity of agar powder and transferred to a flask containing one liter of distilled water and boiled until it dissolved completely. Subsequently, the solution was steam sterilized at 121ºC for 15mins and permissible to cool. To this few gm of chloramphenicol antibiotics were added and the 20 ml of medium were distributed to each petri-dish.

2.2.2. Pure Culture of Infected Leaves: Pure culture of potato dextrose agar was alternatively prepared and permitted to solidify (Figure No 2). From this, a slight portion of different colony were provoked out and placed in the middle of PDA plates and allowed to grow at 25°C.

2.2.3. Identification of Fungi:

To isolates the fungi, pure culture was grown on the media (PDA) at 25°C at least for a week. A clean glass slide was prepared for microscopic identification of fungi. Few drops of iodine solution placed on the center of slide, sterilized inoculating needle was used to cut small portion of fungi culture. The cut piece was placed on the slide and viewed under the microscope with the magnification of 40x.

2.3. Extraction of microorganisms from scratches found on leaves: Leaves with more mildew lesions, were selected randomly and washed with sterilized water, then impregnated with 0.5% of sodium hypochlorite solution for 2 minutes, and the left-over of fumigant was removed with sterile distilled water. Germ-free leaves were retained on Petri dishes holding 20 ml of agar as a base and incubated at various temperatures. After couple of days, the thread like fungi formed on the leaves with lesions was observed with the dissecting microscope (40x) and the characteristic colonies of different genera were identified, with the reference of [28]. Later, spores were scuffled off the plate with a glass rod, put off in 2 ml of 0.01%, of Tween 20. Altogether, 4 spore suspensions were found and, from each suspension 50 ml aliquots were transferred on Petri dishes holding 20 ml of culture medium. As the suspension were dispersed on the surface of the medium using sterile glass rod and the plates were incubated at various temperatures for the form of fungal colonies.

2.3.1 Identification of Isolated fungi by 18SRNA Sequencing:

The isolated fungal species from the infected groundnut leaves were morphological identified by based on sequence alignment analysis over NCBI BLAST server, the species Nomenclature and percentage of similarity were identified by 18s RNA Sequencing.

2.4 Collection of seaweeds sources and Authentication Number: Macroalgae or seaweeds was collected from Rameswaram, called Gulf of Mannar, Tamil Nadu, India more specifically from Olakuda, Nallupani, Thangachimadam, Mandapam, Thonithurai & Kilakkarai**.** For our work, 10 different sources of seaweeds such as four Red algae, four brown algae and two green algae were collected, the seaweeds were safely taken to the research laboratory in separate plastic bags holding seawater to avoid dryness of the sources. The seaweeds were identified and authenticated by the Algologist by referring to the deposited Herbarium. The details of the species and authentication number of microalgae are given in table 1.

The seaweed material was washed with distilled water for several times and was subjected to air drying under the shade. After drying they were ground by an electrical mixer until they became a powder. Then the sample was stored in a sterile place, and subjected to extraction method. Extraction of seaweed sample was done using aqueous ethanol. Aliquots of the sample were soaked in 250 ml of the solvent for 72 h. Later the soaked sample was homogenized in an electric blender along with the solvent at room temperature, filtered, and concentrated under reduced pressure using a rotary evaporator and keep stored vacuum desiccator.

2.4.1 Phytochemicals Analysis of Extraction of different microalgae species

Test for alkaloids: *Mayer's* Test: To all the extracts, 2 ml of Mayer's reagent was added formation of reddish brown precipitate indicates the presence of alkaloids.

Test for saponins: Take 1 ml from each extracts, 5 ml of water was added in all the tubes and the tubes was shaken vigorously. Copious lather formation indicates the presence of saponins.

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Test for tannins: All the extracts were treated with ferric chloride for the formation of a dark blue or greenish black color which indicates the presence of tannins.

Test for cardiac glycosides: *Keller-Killani* test: 1ml of each extracts was treated with 2 ml of glacial acetic acid having a drop of $FeCl_3$. To this, equal volume of concentrated sulphuric acid was added slowly along the sides of the tube. Formation of brown color ring indicates the presence of cardiac glycosides.

Test for flavonoids: *Alkaline reagent* test: All the extracts were treated with few ml of 10% of sodium hydroxide solution this results in the formation of intense yellow color which indicates presence of flavonoid.

Test for phenols: *Lead acetate* test: Few ml of each extracts were treated with 3 ml of 10% lead acetate solution and noted the formation of bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids: 1 ml of each extracts was being dissolved in 10 ml of chloroform & equal volume of concentrated sulphuric acid was added along the side of test tube. The presence of steroids was indicated by two distinguish layer such as the upper layer turns red and the lower layer turns yellow with fluorescent green.

Test for terpenoids: Salkowski test: 5 ml of each extracts were mixed well with few ml of chloroform followed by few ml of concentrated sulphuric acid was carefully added to get a layer. Formation of reddish brown color at the junction of the two interfaces indicates the presence of terpenoids.

Test for Quinones: Few ml of all the extracts were treated separately with alcoholic potassium hydroxide solution. The presence of quinones was indicated by the appearance of color turns from red to blue.

Test for Proteins: Ninhydrin test: All the extracts were treated with few drops of Ninhydrin reagent (freshly prepared) and heated until it turns the color either pink or purple color indicates the presence of proteins, peptides or amino acids.

2.4.2. High Resolution Liquid Chromatography and Mass Spectrometry (HR-LCMS) analysis:

The TC extract underwent qualitative analysis using HR-LCMS at the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Mumbai. Methanol was utilized for sample preparation, and the analysis was conducted with Agilent's High-Resolution Liquid Chromatography and Mass Spectrometry model-G6550A to generate chemical fingerprints with 0.01% mass resolution. The MS acquisition method ranged from a minimum of 50 (m/z) to a maximum of 1000 Da (m/z) at a scanning rate of one spectrum per second. Column 18 (100 × 1.0 mm, particle size 1.8 µm; Waters) was employed for chromatographic separation. The ejection speed and ancillary speed were set at 100 µl/min, with an injection volume of 8 µl, and 5 µl for the flush-out factor during HR-LCMS. Solvent A, consisting of methanol: acetonitrile (90:10), and Solvent B, a 10 mM ammonium acetate solution in water, were used in HR-LCMS. The mass spectra generated were interpreted using the SAIF database, which encompasses more than 62,000 patterns. The seaweed extract's spectrum was identified by comparing it with the spectral patterns available in the SAIF library

2.4.3 Anti-fungal Activity:

The Anti-microbial activity of seaweeds was assessed by Minimum inhibitory concentration method. The extracts were added against isolated fungi species & the efficacies are determined.

Minimum inhibitory concentration (MIC):

The minimum inhibitory concentration was determined through the broth dilution method. Fungi were first grown in the Potato Dextrose broth for 24 hrs and then the inoculums were diluted for five times because to control its vigorous growth. Then each test tube was added with 4800 µl of potato dextrose broth and different concentrations of seaweed extracts (6.25 to 200µg/ml) separately followed by inoculation of 200µl of respective fungi and kept at 37°C for 48hrs. The tubes were examined for visual turbidity and compared with positive control (Tebuconazole).

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3. Results & Discussion:

3.1 Isolation and identification of the fungi from groundnut leaves:

The present stages in our research work, four different fungal species had been isolated based on the observation of pure culture inoculated plates after 5 days of inoculation and incubation period for morphological characterization. The appearance of fungal colonies helps to categorize the microorganism belongs to the genus were observed from the figure no 1 &2.

Figure - 1: Infected Groundnut leaves

Similarly in one of the previous research findings five fungal species were identified from raw groundnut seeds obtained from four major markets in Port [28]**.**

Noteworthy to mention that our research findings characteristics are similar to that of various research works had given us suggestions to predict the kind of microorganism. Based on the observation of fungal colony plates the isolated samples morphological, microscopic characteristic and possible organisms were depicted in the table 1.

The Morphological & Microscopic characteristic of isolated fungus illustrate the microorganism belongs to the family of *Fusarium, Colletotrichum, Penicillium, Lasiodiplodia.* Further to identify the species of the organism the samples were analyzed in BLAST database sequence.

3.1.1 Isolated Fungal Species Identification using BLAST database sequence:

According to the previous database report several fungal species affect the peanut plant. Thereby microbial identification of infected groundnut leaves isolated species was analyzed by Sanger sequence using 18S RNA method the result data of the species, the gene bank number and similarity of the sample were portrayed in the Figure 3.

Based on the study literature, all the fungi predicted to infect peanut causing different diseases in the field & also during the storage. Once the peanut contaminated by the fungal species obviously will diminish germination capability of peanut seeds at the time of storage [26]. study clarifies occurrence of *Lasidioplodia theobromae* in peanut seeds causing lesion and leads to collar rot disease followed by the another species such as *Fusarium, Rhizopus, Mucor & Penicillium* were also most bountiful fungi confront in groundnut seeds contamination. In one more research work of affected groundnut collected from different localities in Pakistan isolated fungus are *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Macrophomina phaseolina and Rhizoctonia solani*.

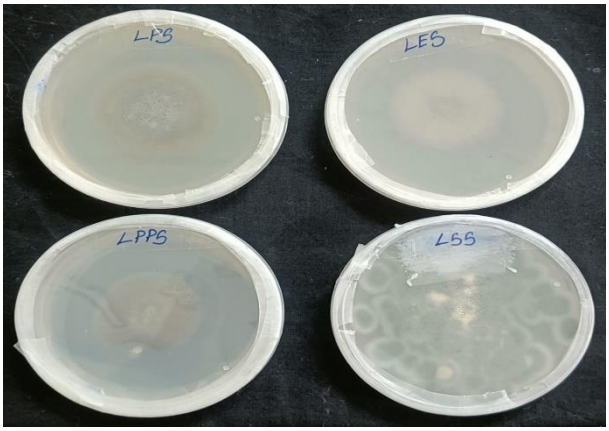

Figure -2 : Pure culture plates of fungi obtained from infected groundnut leaves.

i) LPS : Leaf part - I ii) LES : Leaf Extract iii) LPPS: Leaf Part - II iv) LSS: Leaf Spore

An Identification and DNA sequence of isolated fungi from the infected groundnut leaves and periphery of lesions was carried out for morphologically report and genomically aligned nucleotides [27]. The speculative fungal species are found to be *Ramularia, colletotrichum, Cladosporium and Alternaria* respectively. Likewise in our research findings of infected groundnut leaves we founded isolated fungal species are i) **LPS**: *Fusarium equiseti* ii) **LES:** *Colletotrichum siamense* iii) **LPPS:** *Penicillim oxalicum* iv*)* **LSS:** *Lasiodiplodia pseusothebromaes.*

Further the sample species were founded for its similarity with data base sequence in BLAST genbank. The Blast data are sorted by E-value of each species, when the E

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value is smaller than 0.01 it hits for better match. Notably, our isolated species E-value data's are 0.0 this value is more significant consider for a good hit of identification of homology matches. The similarities of isolated species are more than 97% and the accession numbers were also strongly confirmed to identify the species and its sequences. The nucleotide sequence of the isolated species were found to be 539 length with 743 bits for *Fusarium equiseti* in LPS sample, 521 length with 819 bits for *Colletotrichum siamense* in LES sample, 601 length with 933 bits for *Penicillium oxalicum* in LPPS sample, 1134 length with 1975 bits for *Lasiodiplodia pseusothebromaes* in LSS sample.

Table - 1: Species determined from BLAST searches of Internal Transcribed Spacer based on 18s rRNA gene sequences

Isolated Sample ID	Putative taxonomic affinity (Gene bank no)	% of Similarity
LPS	*Fusarium equiseti* **(MK611672.1)**	**>97%**
LES	*Colletotrichum siamense* **(MH298866.1)**	**>97%**
LPPS	*Penicillium oxalicum* **(MT597864.1)**	**>97%**
LSS	*Lasiodiplodia pseusothebromaes* **(NG_062746.1)**	**>97%**

Comparably in another research work the determination of fungal species related with *Arachis hypogaea* in groundnut were carried using molecular techniques. The nucleotide lengths of the species genes were founded as follows 445 base pairs, 507 base pairs, 523 base pairs, 562 base pairs 580 base pairs, and 620 base pairs for *Lasidioplodia iranensis, Penicillium citrinum, Aspergillus oryzae, Aspergillus tamarii, Macrophomina phaseolina* and *Aspergillus penicilliodes* correspondingly**.**

3.1.2 Phylogenetic Analysis of Isolated Species.

The phylogenetic analysis was carried based on the maximum parsimony method for isolated species and the result was depicted in Figure 3. The phylogenetic tree exhibits the organism are most closely related to our isolate species on GenBank. The vertical line indicates the distance between the branches of species *i.e*. isolates of the sample. Based on the phylogenic concept, when the distance is more the isolates are also more far apart in evolution which means the length of the vertical line is more then it will be directly proportional to difference between the isolates.

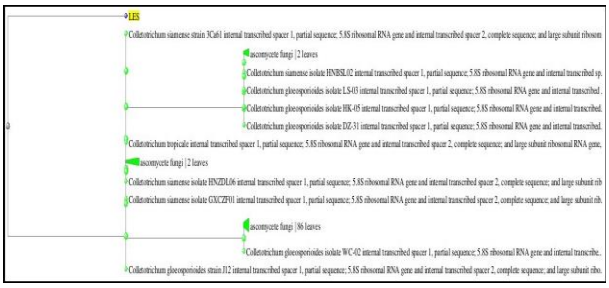

Figure 3. phylogeny tree was construct for 18SrRNA gene sequences by maximum parsimony method which indicates the relationship between LES isolate species and fungi in GenBank.

In our study work, the phylogenetic analysis manifest that the isolates are closely related to *Fusarium equiseti*, *Colletotrichum siamense*, *Penicillium oxalicum* and *Lasiodiplodia pseusothebromaes.* These organisms are found to be closely related to species such as *Fusarium pernambucanum, Colleototrichum gleosporioides, penicillium duplex, and Dothidotthia duplex* respectively.

Among the isolates the *LES* sample species, *LPS* sample species, and *LSS* sample species were found to be more strongly connected to each other whereas the LPPS sample species was found to be far from *LSS* sample. More entertainingly the *LES* sample species and *LPS* sample species were found to be very closely associated to each other.

3.2 *In-vitro* studies:

3.2.1 Preliminary phytochemical analysis:

The *in vitro* study of different *seaweed* extracts includes qualitative analysis for phytochemical

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constituents by various suitable methods followed by antimicrobial activity of extracts against isolated fungal species. Since pre literary history seaweeds has been extensively used for cooking as these are the most important source of micronutrients. A range of marine algae they have been used in pharmaceuticals due to their hypothetical health benefits. seaweeds are rich of chlorophyll, which can grow in marine system very well as it belongs to group of multicellular organism it is termed as macro algae [29].

Similarly, extracts and its products are significant nutritional supplements, besides this it has been used to treat certain diseases like anti-microbial, anti-viral, anti-fungal, anti-allergic, anti-coagulant, anti-cancer, anti-fouling and anti-oxidant activities [30]**.** In this study, different *seaweeds* were extracted with water and ethanol.

Table 2: Seaweeds species

S.No	Sample code - Name of Seaweeds	Family
1.	**HA –*Halymenia floresii***	Red Algae
2.	**GE-*Gracilaria edulis***	Red Algae
3.	**GV -*Gracilaria verrucosa***	Red Algae
4.	**GD-*Gracilaria dichotoma***	Red Algae
5.	**PB-*Padina boergesenii***	Brown Algae
6.	**DD-*Dictyota dichotoma***	Brown Algae
7.	**TC-*Turbinaria conoides***	Brown Algae
8.	**SW-*Sargassum wightii***	Brown Algae
9.	**UF-*Ulva fasciata***	Green Algae
10.	**EF-*Enteromorpha flexuosa***	Green Algae

The red algae species and brown algae species was found to be more quantity than the green algae species. Further all the extracts were carried out for identification of preliminary phytochemical constituents.

All over the countries, curiosity has been greater than before in recent times on plant & its derived products, including macro-algae species result of their prospective focused to be used as foods rich in antioxidants and categorize as therapeutic drugs for the better replaced of synthetic analogues [32]**.**

The LC-MS approach was employed to identify semi-polar compounds and active metabolites in the T. conoides extract. A total of sixteen bioactive compounds were discerned in the extract, and Figure-4 depicts the corresponding LC-MS chromatogram. Details such as retention time, molecular weight, and molecular formula for each of the identified 16 compounds are presented in Table-3.

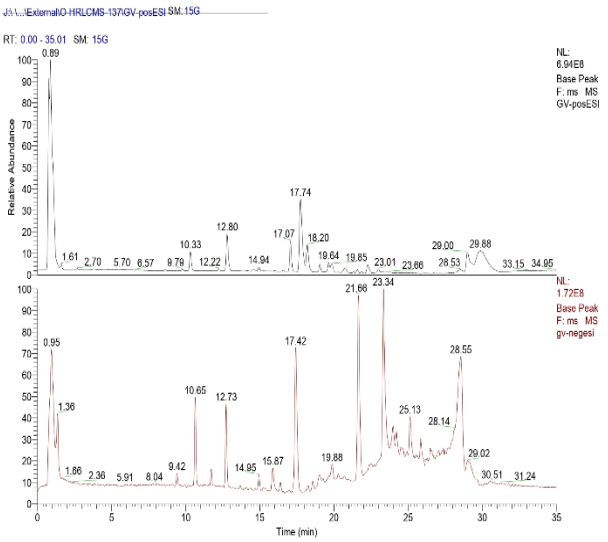

Figure – 4. LCMS chromatogram of seaweed extract

The majority of the bioactive components identification is carried to explore the efficacy of the seaweed extracts. The phytocomponent such as alkaloids, flavonoids, phenol, cardiac glycosides, steroids, terpenoids, quinones and protein were present in all the seaweed extracts, whereas quinones was absent in ***Halymenia floresii*** (Red algae) ***Ulva fasciata*** (green algae) and ***Turbinaria conoides*** (brown algae) extracts. The phenol constituent was found to be absent in ***Halymenia*** (Red algae) and ***Dictyota dichotoma*** (brown algae) species. Similarly, the saponins and tannins were not found in ***Gracilaria edulis*** and ***Gracilaria verrucosa*** of Red algae species. ***Gracilaria dichotoma*** red algae species extract the result shows absence of Alkaloids, flavanoids, saponins and tannins.

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S.No	Name of the compound	Molecular formula	Molecular weight	RT
1.	(9-cis)-Retinal	C20 H28 O	284.2143	20.222
2.	DL-Stachydrine	C7 H13 N O2	143.0947	1.146
3.	Arecoline	C8 H13 N O2	155.0948	1.163
4.	Shogaol	C17 H24 O3	276.1725	17.795
5.	Anacardic acid	C22 H36 O3	348.2666	19.748
6.	9S,13R-12-Oxophytodienoic acid	C18 H28 O3	292.2028	19.082
7.	D-Sphingosine	C18 H37 N O2	299.2825	21.64
8.	Thymine	C5 H6 N2 O2	126.043	1.184
9.	Cholecalciferol	C27 H44 O	384.3392	24.114
10.	6-Gingerol	C17 H26 O4	294.1833	18.059
11.	Mescaline	C11 H17 N O3	211.1211	9.639
12.	Betulin	C30 H50 O2	442.3813	23.647
13.	Lignoceric acid	C24 H48 O2	368.3654	25.723
14.	Azelaic acid	C9 H16 O4	188.105	12.894
15.	Suberic acid	C8 H14 O4	174.0893	10.826
16.	D-(-)-Quinic acid	C7 H12 O6	192.0636	1.183

Table - 3: Phytochemicals determined by LC-MS analysis in *Tubunaria conoides*

The result for ***Padina boergesenii (brown algae)*** found to be absence of Alkaloids, saponins and tannins but presence of all other phytoconstituents. The ***Turbinaria conoides*** & ***Sargassum wightii*** of brown algae species and green algae species of ***Ulva fasciata*** were showed similar result. In case of ***Enteromorpha flexuosa*** green algae species extract contains all the secondary metabolites except flavonoids, saponin and tannins**.** Based on the phytochemical analysis, it depicted that all the macro algae species has curative properties against various diseases.

3.2.2 Determination of Anti-fungal activity of seaweed extracts by MIC method:

The earlier findings in the literature confer great support to explore our aim of study, that alcoholic extracts of seaweed have elevated antimicrobial activity than aqueous extracts [31] (Liu, Q., et al 2017). The antimicrobial activities of ethanolic extracts against each of isolated fungus have been assessed in this study. To explore the efficacy of antifungal activity of ethanol extract of different seaweeds, the tubes containing inoculums of isolated fungi such as *Fusarium equiseti, Colletotrichum siamense, Penicillium oxalicum, Lasiodiplodia pseudotheobromae* were treated with various concentrations of seaweed extracts and observed for minimum inhibitory concentration. The values were noted and illustrated about its effective dosage of inhibitory concentration of extract based on the Table-4.

The results revealed that the ethanol extract of selected seaweeds are efficiently suppressing the growth of fungi with variable potency. The data suggested that the good inhibitory activity of seaweed extracts were found to be at concentration 50 µg/ml for GV extract of red algae species over fungi *Fusarium equiseti*. Similarly, the brown algae extract (PB, SW) and UF green algae extract has found to have MIC at 100 µg/ml whereas the extract of red algae species such as (HA, GE, GD) and brown

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algae species extract such as (TC, DD) has inhibitory capacity at 200 µg/ml against *Fusarium equiseti*.

Likewise, the better antimicrobial activity of seaweed extracts was found to be brown algae (SW extract) against *Colletotrichum siamense* at 50 µg/ml whereas for red algae species such as (HA, GV extract) and brown algae such as (TB, DD extract) has inhibitory capacity at 100 µg/ml. TC extract of brown algae has capacity of inhibition at 200 µg/ml, and green algae (UF & EF) has antifungal activity at 100 µg/ml against *Colletotrichum siamense.*

Table-4: Determination of Antifungal activity of seaweed species extracts against fungus

Samples	**MIC (µg/ml)**			
	Fusarium equiseti	***Colletotrichum siamense***	***Penicillium oxalicum***	***Lasiodiplodia pseudotheobromae***
HA	200	100	200	200
GE	200	200	200	200
GV	50	100	100	50
GD	200	200	200	200
PB	100	100	100	200
DD	200	100	100	100
TC	200	200	200	200
SW	100	50	100	200
UF	100	100	200	200
EF	200	100	200	200
Tebuconazole	**6.25**	**6.25**	**6.25**	**6.25**

The antifungal property of marine macroalgae extracts against *Penicillium oxalicum* was observed the result data suggest that the extracts have ability to inhibit the growth of microorganism towards the higher concentration. HA, GE, GD of red algae species extracts, TC of brown algae extract, UF & EF of green algae extracts has exhibits its antifungal activity at 200 µg/ml and the other extract exhibits its inhibitory effect at a concentration 100µg/ml.

Remarkably the result data for *Lasiodiplodia pseudotheobromae* fungi was clearly manifest that the extract of red algae (GV species) has found to have inhibition of fungi growth at 50 µg/ml and brown algae species (DD extracts) has inhibitory effect at 100 µg/ml.

Except the above all other macroalgae species has antifungal capacity at 200 µg/ml. Tebuconazole fungicide has showed its antifungal ability at dose of 6.25 µg/ml which is been used as positive control.

In contrast, the investigation of *antibacterial and antifungal activities on Gracilaria* species, [32] Singh and Raadha *et al* 2015 Studies on the antimicrobial potency of the marine algae *Gracilaria corticata*, premeditated the extract of *G. corticata* and found the species might inhibit the growth of human pathogenic bacteria and fungi at a rate of 1000 µL, including species such as *Salmonella*, *E. coli*, *Staphylococcus* and *Candida species.*

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Dayuti et al 2018 [33] research study also suggests the *G. verrucosa* has antibacterial activity against *S. typhimurium* and *E. coli*. It includes that study of the *G. confervoides* extract against fungi and bacteria the result implies that 100 µL of the *G. confervoid* extract could put off the growth of the fertile aerial mycelial of pathogens affects the cucumber plants [34] Stabili L *et al* 2014.

Preceding investigate outcome have recommended that extracts of algae have biological agent to manage or minimize the development of filamentous, mycelium and germination, an augment in intracellular holes (vacuolization), and thereby enhance the interruption of the performance of fungal cells [35] (Manilal A et al 2012). To bring support on our findings the previous study data of antifungal property of *G. persica* confirmed the seaweed species has ability to inhibiting the growth of *A. niger*. Based on our finding result approach, it would be strong evidence to the future researchers to suggest that all the seaweeds species extract has a high potential to inhibit the growth of plant pathogens especially fungi.

4. Conclusion:

Our research study concludes that the infected groundnut leaves will affects the germination and productivity of the plant. Hence the fungi were isolated from infected leaves and identified the fungal species by 18s RNA which would have suggested the strong similarity by phylogenic analysis. These plant pathogen activities have to be inhibited to decrease the impact over the groundnut plants. In marine environment, the macro-algae are the prevalent biomass and symbolize a potential source of novel assorted and inimitable compounds. Hence much responsiveness has been created to develop ground-breaking ideas for utilization of seaweeds in proposes of novel antimicrobial drugs. The *in-vitro* qualitative analysis and antifungal determination of collected seaweeds could afford hopeful bioactive metabolites and good MIC potential over the fungi thereby it can be help in the healing of fungal infectious diseases, also it can be substitute for synthetic antimicrobial agents which are used in crop growing field or even in food industry to attain the profit with no impacts. Futuristic focus of the study to investigate the isolation of compounds for identification of capable algae species, evaluation of their safety in all pharmacological aspects, as well as the assessment of synergism ability among the components towards various ailments with low dose effect.

5.Conflict of Interest

Authors have no conflicts to declare

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