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Phytochemical profile of ethanolic extract of *Laurencia pedicularioides* Boergesen

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Abstract
Marine macro algae are one of the important renewable resources in the marine environment. They produce a variety of secondary metabolites with pharmacologically important compounds. The present study was aimed to evaluate the phytochemical constituents of ethanolic extract of *Laurencia pedicularioides* Boergesen. *The preliminary phytochemical compositions were analyzed using qualitative chemical analysis and the chemical constituents were characterized using UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy, High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrophotometry.* The phytochemical analysis revealed the presence of alkaloids, coumarin, diterpenes, emodins, flavonoids, saponins, steroids, tannins and triterpenoids. The UV-Visible spectrum of ethanolic extract of *Laurencia pedicularioides* showed the existence of the compounds separated at the nm of 484.5, 508, 531.5 and 652.5 with the absorption 2.857, 1.452, 1.180 and 3.346 respectively. The FTIR results confirmed the occurrence of functional groups such as cis disubst alkenes, organophosphorus, amines, sulfonyl chlorides, primary amides, δ-lactones, alcohols and phenols. GC-MS spectrum indicated the presence of 11 compounds. The existing compounds were toluene, ethylbenzene, 1,3-cyclopentadiene, styrene, paromomycin, 2-furanmethanamine, 1,3,4-hexatriene, 3-methoxy, isophytol, methyltetradecanoate, n-hexadecanoic acid and citronellyisobutyrate.

Keywords: Marine macro algae, *Laurencia pedicularioides*, Phytochemicals, Ethanolic extract

Introduction
Marine macro algae, the marine plants are important sources of the elements which are useful for metabolic reactions in human such as enzymatic regulation of lipids, carbohydrates and proteins metabolism (Nisizawa *et al.*, 1987) [1]. The total dietary fiber content of Marine macro algae described from previous reports ranges from 33-50% dry weight (Lahaye, 1981) [2]. However, the nutrient compositions of Marine macro algae may vary depend on the species, maturity, environmental growth conditions and seasonal period (Ito and Hori, 1989) [3]. Apart from the nutrient composition, marine macro algae possesses a huge and variety of secondary metabolites with pharmacological activities. Like land plants, marine macro algae contain various inorganic and organic chemical compounds which can benefit human health (Kuda *et al.*, 2002) [4]. Marine macro algae are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with anti-oxidant, anti-viral, anti-fungal and anti-microbial activities have been previously detected in brown, red and green algae (Yuan *et al.*, 2005; Bansemir *et al.*, 2006, Madhusudan *et al.*, 2011) [5, 6, 7]. Hence the present study was undertaken for the characterization of phytochemicals of *Laurencia pedicularioides.*

Materials and Methods
Collection of plant materials
The plant materials for the present study were collected from Hare Island, Thoothukudi, located in Thoothukudi district, in the south east coast of Tamil Nadu, India, during the month of December, 2017. The collection of *Laurencia Pedicularioides* Boergesen belonging to Rhodophyceae (red algae) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. The samples were packed separately in polythene bags in wet conditions and brought to the laboratory, thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. For the preparation of ethanolic extract, the plant specimens were placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. 30g powdered samples were packed in Soxhlet apparatus and extracted with ethanol for 10h (John Peter Paul, 2021) [8].

Characterization of Phytochemicals
Preliminary phytochemical analysis
The ethanol extract of *Laurencia pedicularioides* was tested for alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpenes, emodins, flavonoids, saponins, steroids, tannins and triterpenoids. The preliminary phytochemical screening of the extracts was carried out according to Harborne (1998) [9] method.

UV-Visible spectral analysis
The ethanol crude extract containing the bioactive compound was analyzed spectroscopically for further confirmation. The crude extract of *Laurencia pedicularioides* was scanned in a wavelength ranging from 200-900nm using a Shimazdu spectrophotometer and characteristic peaks were detected.

FTIR analysis

The ethanol crude extract of *Laurencia pedicularioides* was subjected for FTIR analysis using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum (John Peter Paul and Yuvaraj, 2013) [10].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of ethanol extract of *Laurencia pedicularioides* was carried out using GC model Clarus 680, Mass Spectrometer Clarus 600 (EI) Perkin Elmer, Gas Chromatograph equipped and coupled to a mass detector TurboMass 5.4.2 spectrometer with an Elite-5MS, (100% Dimethyl ply siloxane), 30.0mX250µm 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 up to 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 50-600Da. The chemical constituents were identified by GC-MS. 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. Retention time, name of the compound, structure, molecular weight and molecular formula of the compounds of the test materials was ascertained.

Results and Discussion

In the preliminary phytochemical analysis of *Laurencia pedicularioides*, twelve different types of secondary metabolites (alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarin, diterpenes, emodins, flavonoids, saponins, steroids, tannins and triterpenoids) were tested in ethanol extract. Alkaloids, anthocyanin, anthraquinones, diterpenes, saponins and tannins were only found in the ethanol extract of *Laurencia pedicularioides*. The present study showed the absence of cardiac glycosides, coumarin, emodins, flavonoids, steroid and triterpenoids.

UV-Visible spectrum analysis

The UV-Visible spectrum of *Laurencia pedicularioides* was selected at the wavelength of 200nm to 900nm due to the sharpness of the peaks and proper baseline. The UV-Visible spectrum of ethanol extract of *Laurencia pedicularioides* was presented in Table 1 and Fig. 1. The spectrum was noted the presence of the compounds separated at the nm of 484.5, 508, 531.5 and 652.5 with the absorption 2.857, 1.452, 1.180 and 3.346 respectively.

Table 1: UV-Visible spectrum of ethanol extract of *Laurencia pedicularioides* Boergesen

Laurencia pedicularioides	
Nm	**Abs**
652.5	3.346
531.5	1.18
508	1.452
484.5	2.857

Nm: Nanometer **Abs: Absorbance**

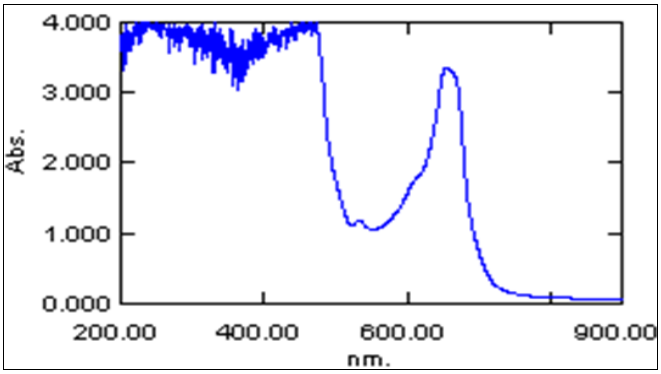

Fig 1: UV-Visible spectrum of ethanol extract of *Laurencia pedicularioides* Boergesen

FTIR analysis

The FTIR spectrum was used to identify the functional group of the active components present in the ethanol extract of *Laurencia pedicularioides* based on the peak value in the region of infra red radiation. The FTIR results of ethanol extract of *Laurencia pedicularioides* showed different peaks at 686.61, 1045.35, 1228.57, 1383.83, 1627.81, 1740.64 and 3403.16cm^{-1}. It was confirmed the presence of functional groups such as cis disubst alkenes (CH out-of-plane deformation), organophosphorus (P-O-C antisym stretch), amines (C-C-N bending), sulphonyl chlorides (SO_2 antisym stretch), primary amides (C=O stretch and NH_2 deformation), δ-lactones (C=O stretch) and Alcohols and phenols (OH stretch) respectively (Table 2 and Fig. 2)

Table 2: FTIR spectrum of ethanol extract of *Laurencia pedicularioides* Boergesen

Peak value	Functional group	Assignment
3403.16	Alcohols and Phenols	OH stretch
1740.64	δ-lactones	C=O stretch
1627.81	Primary amides	C=O stretch and NH_2 deformation
1383.83	Sulfonyl chlorides	SO_2 antisym stretch
1228.57	Amines	C-C-N bending
1045.35	Organophosphorus	P-O-C antisym stretch
686.61	Cis disubst alkenes	CH out-of-plane deformation

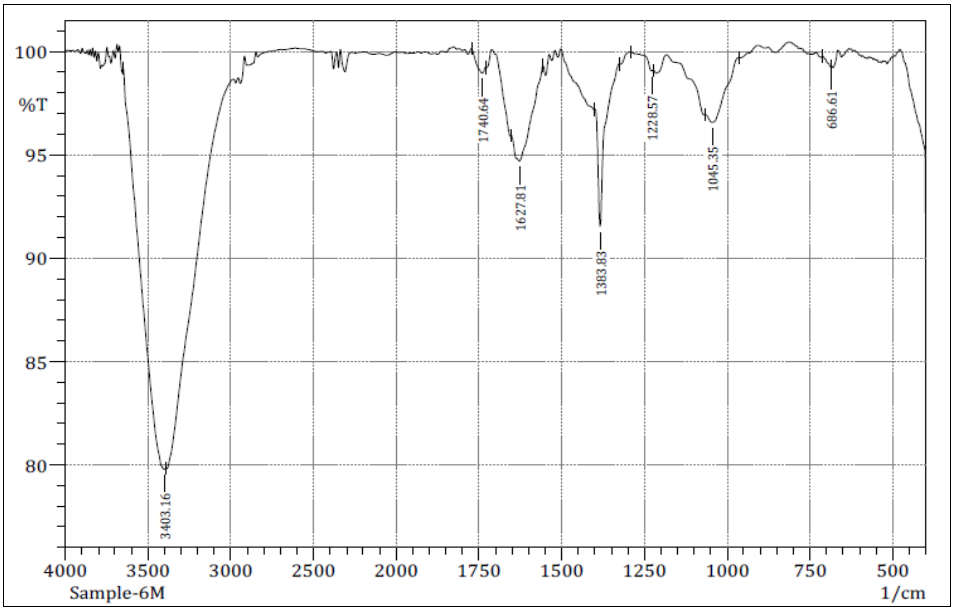

Fig 2: FTIR spectrum of ethanol extract of *Laurencia pedicularioides* Boergesen

Gas Chromatography-Mass Spectroscopic spectra analysis

The compounds present in the ethanol extract of *Laurencia pedicularioides* were identified after the comparison of the mass spectra with NIST library by GC-MS analysis. The active principles with their retention time (RT), name, structure, molecular formula and molecular weight were presented in the tables and figures. Table 3 and Fig. 3 indicated the GC-MS spectrum of ethanol extract of *Laurencia pedicularioides* with 11 different major peaks which indicated the presence of 11 compounds. The existing compounds in ethanol extract were toluene (1.937min), ethylbenzene (2.476min), 1,3-cyclopentadiene (2.523min), styrene (2.693min), paromomycin (12.905min), 2-furanmethanamine (13.765min), 1,3,4-hexatriene, 3-methoxy (14.030min), isophytol (14.210min), methyltetradecanoate (14.635min), n-hexadecanoic acid (14.966min) and citronellyisobutyrate (16.460min).

Table 3: GC-MS spectrum of ethanol extract of *Laurencia pedicularioides* Boergesen

Retention Time (min)	Name of the compound	Molecular Formula	Molecular Weight
1.937	Toluene	$C_6H_5CH_3$	92.14
2.476	Ethylbenzene	C_8H_{10}	106.16
2.523	1,3-Cyclopentadiene	C_8H_{10}	106.16
2.693	Styrene	$C_6H_5CHCH_2$	104.15
12.905	Paromomycin	$C_{23}H_{45}N_5O_{14}$	615.63
13.765	2-Furanmethanamine	C_5H_7NO	97.11
14.030	1,3,4-Hexatriene, 3-methoxy	C_6H_8	80.12
14.210	Isophytol	$C_{20}H_{40}O$	296.53
14.635	Methyltetradecanoate	$C_{15}H_{30}O_2$	242.40
14.966	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
16.460	Citronellyisobutyrate	$C_{14}H_{26}O_2$	226.35

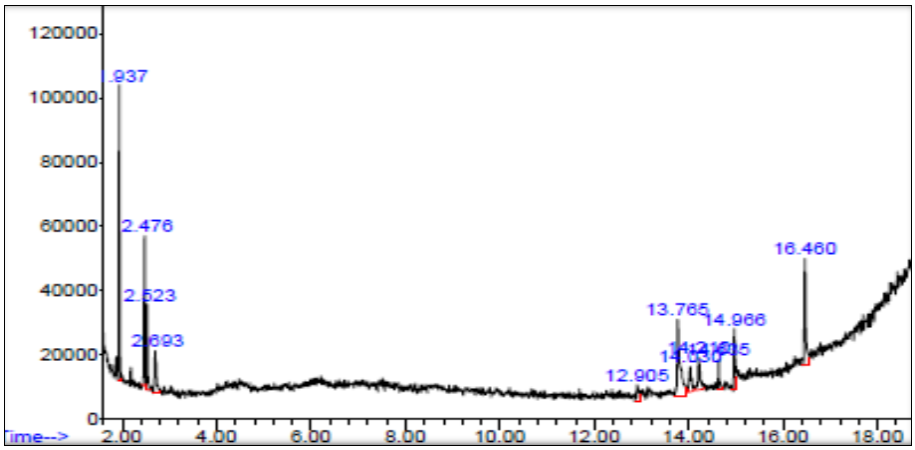

Fig 3: GC-MS spectrum of ethanol extract of *Laurencia pedicularioides* Boergesen

Conclusion

From the present study, it was concluded that the phytochemical analysis revealed the presence of alkaloids, coumarin, diterpenes, emodins, flavonoids, saponins, steroids, tannins and triterpenoids. The UV-Visible spectrum of ethanolic extract of *Laurencia pedicularioides* showed the existence of the compounds separated at the nm of 484.5, 508, 531.5 and 652.5. The FTIR results confirmed the occurrence of functional groups such as cis disubst alkenes, organophosphorus, amines, sulfonyl chlorides, primary amides, δ-lactones, alcohols and phenols. GC-MS spectrum indicated the presence of toluene, ethylbenzene, 1,

3-cyclopentadiene, styrene, paromomycin, 2-furanmethanamine, 1,3,4-hexatriene, 3-methoxy, isophytol, methyltetradecanoate, n-hexadecanoic acid and citronellyisobutyrate.

Conflict of interest

The authors declare that they have no conflict of interest.

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