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Preliminary Phytochemical Screening, GC-MS, FTIR, and Antimicrobial Activity of *Commelina forskaolii* Vahl Leaves

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Abstract: The *Commelina forskaolii* Vahl (Commelinaceae) is an edible herb with wide therapeutic value. The present study aimed to explore the preliminary phytochemical screening, GC-MS spectrometry analysis, FTIR, and evaluate the antimicrobial activity of *C. forskaolii* Vahl leaves using various solvent extracts with different polarities tested for their inhibitory property against most prevalent aquatic pathogenic bacteria (Gram-positive and Gram-negative) and fungi by the agar diffusion method. The results of phytochemical and GC-MS analysis of the different solvent extracts revealed the occurrence of secondary metabolites and totally 35 biologically active compounds. The FTIR results confirmed the presence of alkanes, amines, alcohol, aryl disulfides, ethers, hydroxy group, vinylidene, chloro, bromo, and iodo compounds. The *in vitro* antimicrobial activity of five solvent extracts of *C. forskaolii* Vahl was investigated against *Streptococcus agalactiae* and *Streptococcus iniae*, *Aeromonas hydrophila, Pseudomonas aeruginosa*, *Vibrio cholerae*, *Aspergillus niger* and *Aspergillus flavus.* Among all extracts, aqueous and chloroform extract showed maximum antibacterial activity against all bacterial pathogens based on the different concentrations, which inhibited in the range of 11 mm to 18 mm (aqueous), and 11 mm to 27 mm (chloroform) followed by inhibition range of other extracts 9 mm to 12 mm (ethanol), 11 mm to 23 mm (methanol), and 12 mm to 16 mm (acetone). In addition, the highest antifungal activity was reported in chloroform extract against *Aspergillus niger* and aqueous extract against *Aspergillus flavus* with inhibition of 16 mm followed by 17 mm at 75 µl concentration. In the present investigation, *C. forskaolii* Vahl leaves were examined and reported for the first time and the study suggests that leaves of *C. forskaolii* Vahl could be potentially used as a natural source of antimicrobial agents in aquaculture and fisheries industries.

Keywords: *Commelina forskaolii* Vahl, Antibacterial, Antifungal, Antimicrobial, Fish pathogens, Phytochemicals

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Introduction

Medicinal plants are major sources of bio-active chemical constituents having rich therapeutic value and are used mostly in drug development. Alkaloids, flavonoids, glycosides, saponins, tannins, phenols, cardiac glycosides, and terpenoids are secondary metabolites with abundant biological properties (Shahidi *et al.*, 2008). GC-MS is a compatible common technique used to identify and quantify biologically active phytocompounds present in the plant (Uma and

Balasubramaniam, 2012). FTIR is the analytical tool for identifying various functional groups present in different compounds (Ronald, 1997).

The *Commelina forskaolii* Vahl (*C. forskaolii* Vahl) is an edible perennial or an annual herb that belongs to the family Commelinaceae and distributed in Arabian Peninsula, India, Madagascar, Southeast Mediterranean, tropical Africa, and Western Asia. They are chiefly found as weeds of cultivated ground or along roadside ditches and swamps. It is good fodder for animals and suited for the preparation of silage. The leaves of *C. forskaolii* Vahl are edible as a vegetable (Kariuki *et al*., 2013). This plant is widely used to have different ethnomedicinal properties including indigestion (Poornima and Jeyam, 2016), smoothening of sore, feed for goat and cattle, and also the whole plant to make a charm that is used during tribal cleansing rituals (Abbas *et al*., 2020).

Generally, fishes are one of the main food components for humans due to their high nutritional value. Fish products have much economic activity all over the world. However, bacterial and fungal diseases constitute major challenges for sustainable aquaculture and fisheries production. Globally, the efficacy of various plant extracts using different solvents on microbes has been studied.

To the best of our knowledge, no work has been done on the preliminary phytochemical screening, GC-MS analysis, FTIR, and antimicrobial activity of aqueous, ethanol, methanol, acetone, and chloroform extract of *C. forskaolii* Vahl leaves. Hence, the present investigation was carried out to determine the phyto-constituents using primary phytochemical analysis, GC-MS, FTIR as well as antimicrobial activity of *C. forskaolii* Vahl leaves and elucidate their efficiency on aquaculture and fisheries industries by testing against fish pathogens via their inhibitory effect against them.

Materials and Methods

Collection and Identification of Plant Material:

Fresh leaves of *Commelina forskaolii* Vahl were collected from Coimbatore district, Tamil Nadu, India. The plant was taxonomically identified and authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu. The voucher specimen was retained in our laboratory for further reference. (Voucher ID: BSI/SRC/5/23/ 2021/ Tech).

Preparation of leaf extract:

The leaves were washed with distilled water and shade dried for 7 days at room temperature (28±2 C). The dried material was homogenized to obtain a coarse powder and stored in air-tight bottles. About 20 g of the powdered material was subjected to 5 different extracts like chloroform, acetone, ethanol, methanol, and aqueous which were used as the solvents for the preparations of plant extracts, and for the further study, filtrates were used. The dried leaves were put in a Soxhlet apparatus (Borosil Glass Workers Ltd, Mumbai, India) and extracts were prepared by chloroform, acetone, ethanol, methanol, and aqueous [(Loba Chemie Pvt. Ltd., Mumbai, India. 99% purity) (concentration of chloroform, acetone, ethanol, methanol, and aqueous was 100%, extraction period 72 h and the temperature were maintained 30-40 C)]. The yield extract was evaporated to dryness in a rotary vacuum evaporator and the dried residues obtained were stored in airtight bottles in a refrigerator for further analysis.

Preliminary Phytochemical screening:

The preliminary phytochemical analysis of different solvent extracts of *C. forskaolii* Vahl leaves were carried out using standard methods as described by Harborne (1973) and Trease and Evans (1989). The phytochemicals were examined to distinguish the presence or absence of the specific phytochemical groups.

Gas chromatography and mass spectroscopy (GC-MS) analysis and Components Identification:

The Clarus 680 GC was used in the analysis which employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector

temperature was set at 260 C during the chromatographic run. The 1µl of extract sample was injected into the instrument and the oven temperature was as follows: 60 C (2 min); followed by 300 C at the rate of 10 C min^{-1}; and 300 C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 230 C; ion source temperature 230 C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The Identification of compounds was done based on the retention indices, molecular structure, molecular mass spectra, and calculated fragmentation patterns with those stored on the computer library and also with published literature. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library and WILEY9 (Van Den Dool and Kratz, 1963) online library source was also used for matching the identified components.

Fourier Transform Infrared Spectrophotometer (FTIR) analysis:

The dried leaves powder was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of cm^{-1}. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined (Cakmak *et al.,* 2006).

Collection of bacterial and fungal culture:

The fish pathogenic bacteria were obtained from the Microbiology Laboratory at CMFRI, Cochin, India. The fish pathogenic fungus was obtained from TRI-Biotech, Trichy, India. The cultures of five bacteria made up of three Gram-negative bacteria are *Aeromonas hydrophila, Vibrio cholerae* and *Pseudomonas agalactiae* and *Streptococcus iniae* were the gram-positive bacteria used and then two fungus are *Aspergillus niger and Aspergillus flavus* were used for the antimicrobial screening in the Laboratory of Department of Zoology, Nirmala College for Women, Coimbatore, India.

Antibacterial activity:

Antibacterial activity of leaf extracts was tested using the standard agar well diffusion method with slight modifications. The fish pathogenic bacteria were cultured on the Nutrient Agar prepared by dissolving 28 g in 1000 ml of distilled water and sterilized in an autoclave at a pressure of 15 psi and 121 C for 15 min. The media were poured to sterilize the Petri plate and allowed for solidification. After solidification, 70 µl of the bacterial suspension of *Aeromonas hydrophila, Streptococcus agalactiae, Streptococcus iniae, Pseudomonas aeruginosa,* and *Vibrio cholerae* were swabbed. Cork borer was used to make well and each sample was poured (25 µl, 50 µl, 75 µl), amoxyclav (10 mcg) was used as a positive control. After placing the samples, plates were incubated at 37 C for 24 h and the zone of inhibition was measured in mm (Johney *et al*., 2017).

Antifungal activity:

The potato dextrose agar medium was prepared by dissolving 40 g of potato infusion, 4 g of dextrose, and 3.5 g of agar in 200 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121 C for 15 min. The autoclaved medium was mixed well and poured onto 100 mm Petri plates (25-30 ml/plate) while still molten. The anti-fungal agent present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition was uniformly circular as there was a confluent lawn of growth. The diameter of the zone of inhibition was measured in millimeters. Petri plates containing 20 ml potato dextrose agar medium was seeded with 72 h culture of fungal strain (*Aspergillus niger* and *Aspergillus flavus*) wells were cut and different concentration

(25, 50, and 75 µl/ml) of samples were added. The plates were then incubated at 37 C for 48-72 h. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B (100 units) was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

Results

Primary phytochemical screening of C. forskaolii Vahl:

This is the first study for the preliminary phytochemical screening and GC-MS analysis of *C. forskaolii* Vahl leaves extract using various solvents such as chloroform, acetone, ethanol, methanol, and aqueous which revealed the presence of various primary and secondary bioactive compounds such as proteins, reducing sugar, alkaloids, phenols, flavonoids, tannins, saponins, glycosides, triterpenoids, and steroids.

In the present study, the chloroform leaf extract of *C. forskaolii* Vahl showed the presence of reducing sugar and flavonoids. Acetone leaf extract of *C. forskaolii* Vahl showed the presence of proteins and 4 bioactive compounds including phenols, flavonoids, tannins, and steroids. Ethanol leaf extract of *C. forskaolii* Vahl showed the presence of triterpenoids and methanol leaf extract of *C. forskaolii* Vahl showed the presence of 5 compounds namely, alkaloids, phenols, flavonoids, tannins, and steroids as compared with other solvent extracts. Aqueous leaf extract of *C. forskaolii* Vahl showed the presence of five bioactive compounds such as proteins and 4 bioactive constituents including phenols, saponins, triterpenoids, and steroids. Among the five solvent extracts, maximum Phyto-compounds were present in the acetone, methanol, and aqueous leaf extract of *C. forskaolii* Vahl (Table 1).

GC-MS analysis:

35 phyto-components have been identified from all the different solvent extracts from the *C. forskaolii* Vahl leaves after a comparison of the mass spectra with the NIST library. In the present investigation, GC-MS analysis of the chloroform extract of *C. forskaolii* Vahl *leaves* and the results are shown in Table 2.1. The GC-MS chromatogram of 20 compounds was detected as shown in Figure 1.1. The first peak was determined to be Phytol. The second peak indicated to be 3,7,11,15-Tetramethyl-2-hexadecen-1-ol. The next peaks were considered to be Pentadecanoic acid, 2-Piperidinone, N-[4-bromo-n-butyl]-, Octadecanal, 1-Octadecyne, Octadecanal, 2-bromo, cis-9,10-Epoxyoctadecan-1-ol, 17-Pentatriacontene, 14-Heptadecenal, Hexatriacontane, 2,2-Dibromo-cholestanone, 1-Heptatriacotanol, Tetradecane, 1-chloro, Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.). Meanwhile, the GC-MS analysis of acetone extracts of *C. forskaolii* Vahl leaves have identified the presence of 18 different important compounds and these include Oxirane, tetradecylo, 1-Octadecyne, 1-Eicosanol, Pentade-canoic Acid, n-Nonadecanol-1, Octadecanal, 9-Octadecenal, n-Nonadecanol-1, Octadecanal, Hexadecanal, Octadecane, 1-(ethenyloxy)-, Hexatri-acontane, cis-9,10-Epoxyoctadecan-1-ol, 1-Heptatriacotanol, 1-Octadecanesulphonyl chloride, and 2,2-Dibromocholestanone, respectively (Table 2.2; Fig. 1.2). The ethanol extract of *C. forskaolii* Vahl contains 16 different secondary compounds namely, Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitroso, Tetrahydro-3-furanmethanol, 1,1-Dodecanediol, diacetate, n-hexadecanoic acid, Pentadecanoic Acid, Dodecanal, Hexadecanal, 2-Methyl-6-methylene-octa-1,7-dien-3-ol, Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-, Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3.beta.,17.beta.)-, Ethyl iso-allocholate, Cholest-8-en-3-ol, 14-methyl-, (3.beta.,5.alpha.)-, and Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.), respectively (Table 2.3; Fig. 1.3). For methanol extract, 11 active Phyto-constituents were successfully detected from *C. forskaolii* Vahl that included 2-Butanone, 4-(acetyloxy)-, Hexadecanal, 1,1-Dodecanediol, diacetate, 3-Acetoxydodecane, n-hexadecanoic acid, Pentadecanoic Acid, Octadecanal, Pentadecanoic Acid, Hexadecanal, and Decanal (Table 2.4; Fig. 1.4). The GC-MS analysis of all the different solvent extracts such as

Table 1: Phytoconstituents of different solvent extracts of *C. forskaolii* Vahl leaves

Phytochemical Constituents	Test	Chloroform	Acetone	Ethanol	Methanol	Aqueous
Proteins	Xanthoproteic test	-	+	-	-	+
Reducing sugars	Fehlings test	+	-	-	-	-
Alkaloids	Mayer's Reagent	-	-	-	+	-
Phenols	Ferric chloride test	-	+	-	+	+
Flavonoids	Alkaline reagent test	+	+	-	+	-
Tannins	Braymer's reagent	-	+	-	+	-
Saponins	Foam test	-	-	-	-	+
Glycosides	Borntrager's test	-	-	-	-	-
Triterpenoids	Salkowski's test	-	-	+	-	+
Steroids	Liebermann-Burchard reaction	-	+	-	+	+

+ = Present and - = Absent

Table 2.1: GC-MS analysis of chloroform extract of *C. forskaolii* Vahl leaves

S. No.	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	18.375	Phytol	$C_{20}H_{40}O$	296	5.241	871	924
2	18.755	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	6.457	860	934
3	19.110	Phytol	$C_{20}H_{40}O$	296	1.929	801	910
4	20.310	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	6.781	810	906
5	20.716	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}ONBr$	233	3.015	797	921
6	21.051	Octadecanal	$C_{18}H_{36}O$	268	1.478	775	898
7	21.486	Octadecanal	$C_{18}H_{36}O$	268	9.397	779	907
8	22.816	Octadecanal	$C_{18}H_{36}O$	268	1.888	766	901
9	24.892	1-Octadecyne	$C_{18}H_{34}$	250	1.729	743	879
10	25.347	Octadecanal, 2-bromo	$C_{18}H_{35}OBr$	346	1.539	706	881
11	25.963	Cis-9,10-Epoxyoctadecan-1-ol	$C_{18}H_{36}O_2$	284	1.372	685	898

12	26.488	17-Pentatriacontene	$C_{35}H_{70}$	490	4.115	751	911
13	26.913	NV	-	-	3.656	-	-
14	27.393	14-Heptadecenal	$C_{17}H_{32}O$	252	6.359	581	834
15	27.718	Hexatriacontane	$C_{36}H_{74}$	506	7.708	705	936
16	28.244	2,2-Dibromocholestanone	$C_{27}H_{44}OBr_2$	542	10.185	697	895
17	28.899	1-Heptatriacotanol	$C_{37}H_{76}O$	536	6.486	719	863
18	29.154	1-Heptatriacotanol	$C_{37}H_{76}O$	536	3.664	685	868
19	29.404	Tetradecane, 1-chloro	$C_{14}H_{29}Cl$	232	9.399	621	853
20	30.164	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	$C_{28}H_{46}O$	398	7.602	642	798

RT- Retention time; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse

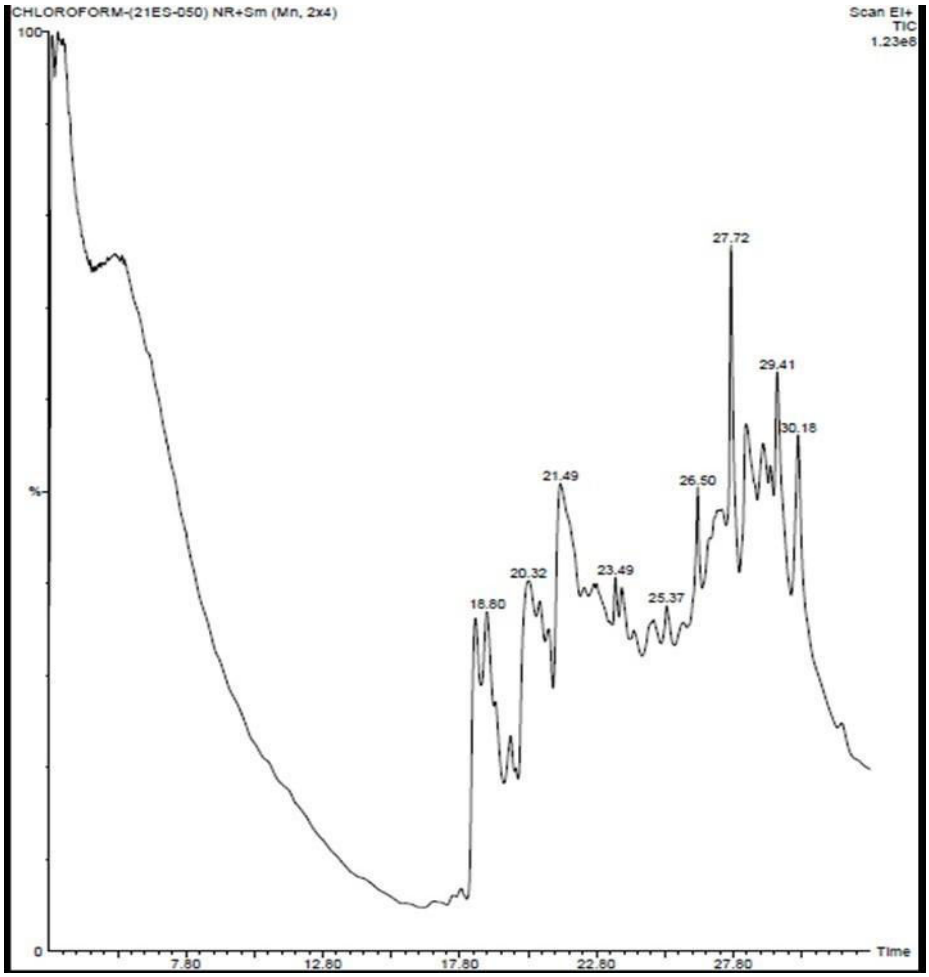


Fig. 1.1: GC-MS Chromatogram of chloroform extract of *C. forskaolii* Vahl leaves.

Table 2.2: GC-MS analysis of acetone extract of *C. forskaolii* Vahl Leaves

S. No.	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	18.215	Oxirane, tetradecyl	$C_{16}H_{32}O$	240	6.126	924	947
2	18.625	1-Octadecyne	$C_{18}H_{34}$	250	3.676	893	936
3	19.455	1-Eicosanol	$C_{20}H_{42}O$	298	2.679	933	973
4	20.155	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	9.840	803	900
5	20.395	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	3.032	833	904
6	20.550	n-Nonadecanol-1	$C_{19}H_{40}O$	284	10.003	859	954
7	21.071	Octadecanal	$C_{18}H_{36}O$	268	2.433	768	893
8	21.471	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	11.167	838	915
9	21.836	9-Octadecenal	$C_{18}H_{34}O$	266	2.502	812	914
10	21.981	n-Nonadecanol-1	$C_{19}H_{40}O$	284	5.980	862	953
11	22.791	Octadecanal	$C_{18}H_{36}O$	268	6.761	756	899
12	25.302	Hexadecanal	$C_{16}H_{32}O$	240	2.038	798	910
13	26.418	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296	2.230	816	924
14	27.588	Hexatriacontane	$C_{36}H_{74}$	506	9.254	767	946
15	28.359	Cis-9,10-Epoxyoctadecan-1-ol	$C_{18}H_{36}O_2$	284	5.836	605	843
16	28.959	1-Heptatriacotanol	$C_{37}H_{76}O$	536	4.119	680	856
17	29.224	1-Octadecanesulphonyl chloride	$C_{18}H_{37}O_2Cl$	352	8.157	722	896
18	29.894	2,2-Dibromocholestanone	$C_{27}H_{44}OBr_2$	542	4.167	648	878

RT- Retention time; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse

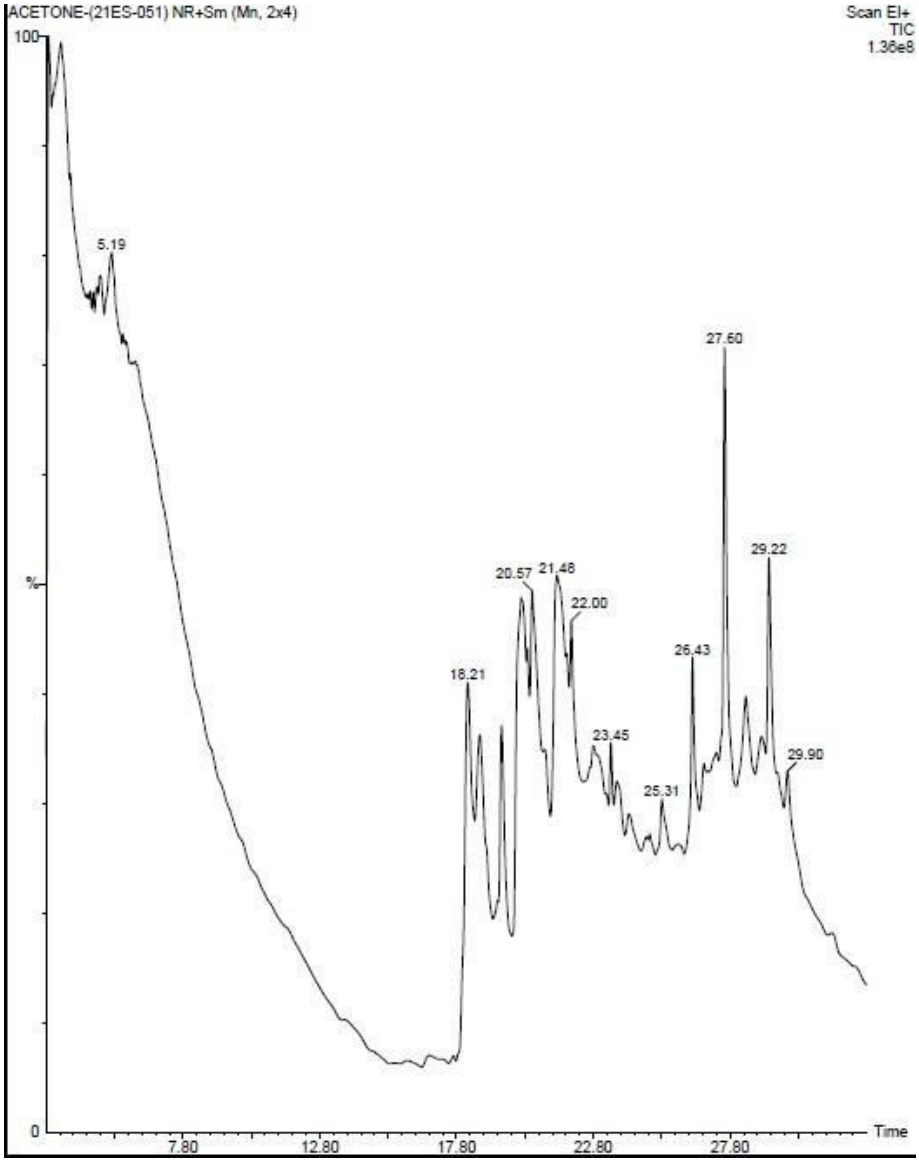


Fig. 1.2: GC-MS Chromatogram of acetone extract of *C. forskaolii* Vahl leaves.

Table 2.3: GC-MS analysis of ethanol extract of *C. forskaolii* Vahl leaves

S. No.	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	14.108	Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitroso	$C_4H_4O_3N_4$	156	13.291	487	819
2	15.919	Tetrahydro-3-furanmethanol	$C_5H_{10}O_2$	102	3.672	692	803
3	18.470	1,1-Dodecanediol, diacetate	$C_{16}H_{30}O_4$	286	1.338	687	853
4	19.775	n-hexadecanoic acid	$C_{16}H_{32}O_2$		20.164	783	910
5	21.236	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	17.474	791	902
6	22.786	Dodecanal	$C_{12}H_{24}O$	184	3.883	644	861
7	23.337	Hexadecanal	$C_{16}H_{32}O$	240	4.954	602	865
8	24.552	Dodecanal	$C_{12}H_{24}O$	184	2.992	670	860

9	25.132	Dodecanal	C12H24O	184	2.887	693	887
10	26.288	2-Methyl-6-methylene-octa-1,7-dien-3-ol	$C_{10}H_{16}O$	152	2.618	541	853
11	27.338	Hexadecanal	$C_{16}H_{32}O$	240	10.347	570	813
12	28.269	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	$C_{28}H_{46}O$	398	4.901	670	876
13	28.824	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3.beta.,17.beta.)-	$C_{22}H_{32}O_2$	328	4.621	670	851
14	29.124	Ethyl iso-allocholate,	$C_{26}H_{44}O_5$	436	1.999	707	837
15	29.414	Cholest-8-en-3-ol, 14-methyl-, (3.beta.,5.alpha.)-	$C_{28}H_{48}O$	400	2.379	647	833
16	29.764	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	$C_{28}H_{46}O$	398	2.479	637	858

RT- Retention time; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse

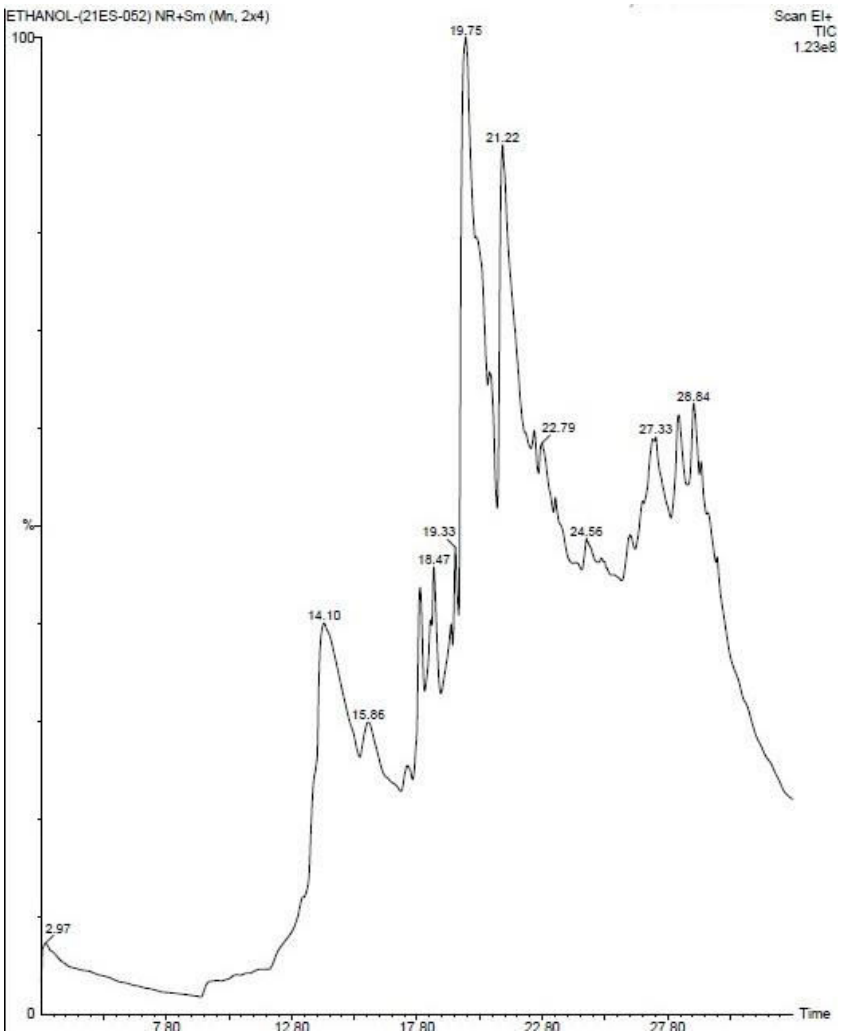


Fig. 1.3: GC-MS Chromatogram of ethanol extract of *C. forskaolii* Vahl leaves.

Table 2.4: GC-MS analysis of methanol extract of *C. forskaolii* Vahl Leaves

S. No	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	13.117	2-Butanone, 4-(acetyloxy)-	$C_6H_{10}O_3$	130	26.583	672	852
2	17.739	Hexadecanal	$C_{16}H_{32}O$	240	6.169	740	873
3	18.240	1,1-Dodecanediol, diacetate	$C_{16}H_{30}O_4$	286	3.132	667	841
4	18.795	3-Acetoxydodecane	$C_{14}H_{28}O_2$	228	3.796	619	876
5	19.505	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.875	733	900
6	19.965	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	12.389	689	864
7	21.021	Octadecanal	$C_{18}H_{36}O$	268	19.981	765	910
8	22.666	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	11.556	698	864
9	25.272	NV	-	-	2.142	-	-
10	26.208	Hexadecanal	$C_{16}H_{32}O$	240	1.233	575	801
11	27.118	Decanal	$C_{10}H_{20}O$	156	3.144	614	862

RT- Retention time; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse

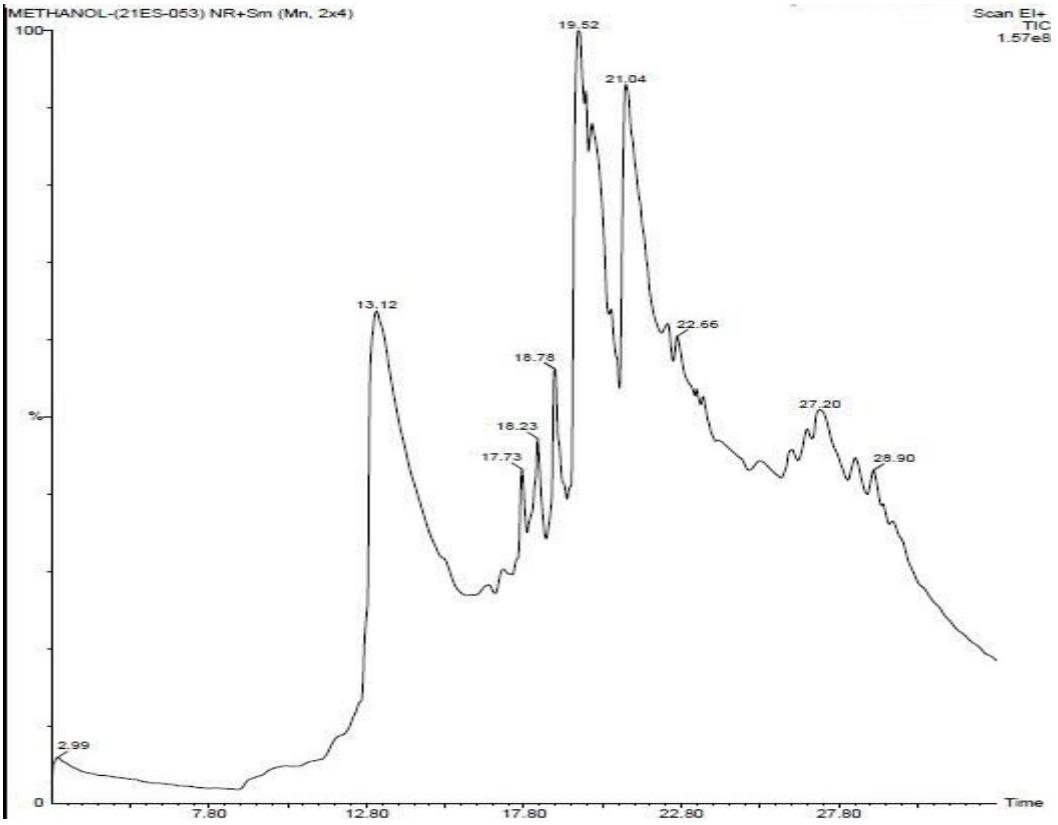


Fig. 1.4: GC-MS Chromatogram of methanol extract of *C. forskaolii* Vahl leaves.

chloroform, acetone, ethanol, and methanol from *C. forskaolii* Vahl leaves identified 35 compounds and some of the bioactive Phyto-compounds have been already reported to possess various biological activities as listed in Table 3 from earlier studies in different species.

Fourier Transform Infrared Spectrophotometer (FTIR):

The FTIR spectrum analysis was used for the identification of major peaks correspondence to the functional groups and bioactive active components were present in the leaves of C. *forskaolii* Vahl as listed in Table 4. In this study, FT-IR analysis observed the peak at 3726.47, 3356.14, 2978.09, 2908.65, 1627.92, 1381.03, 1319.31, 1249.87, 1149.57, 1095.57, 1064.71, 1010.70, 956.69, 894.97, 771.53, 671.23, 601.79, 563.21, 501.49, and 439.77 which correspond to the presence of a hydroxy group, aliphatic secondary amine, alkanes, aromatic tertiary amine, aromatic ethers, secondary amine, alkyl-substituted ether, alkyl-substituted ether, primary alcohol, aromatic, vinylidene, aliphatic chloro compounds, aliphatic bromo compounds, aliphatic iodo compounds, and aryl disulfides (Fig. 2).

Antibacterial study:

The room dried and grained leaves of *C. forskaolii* Vahl were extracted with different solvents including aqueous, ethanol, methanol, acetone, and chloroform, and preliminary screened for their antimicrobial activities against fish pathogens. They have shown antibacterial activities on gram-positive bacteria (*Streptococcus agalactiae* and *Streptococcus iniae*) and gram negative bacteria (*A.hydrophilia, Pseudomonas aeruginosa,* and *Vibrio cholerae*) as well as antifungal activity on *Aspergillus niger* and *Asperigillus flavus*. Antibacterial activities of all the five different extracts of *C. forskaolii* Vahl leaves have been evaluated by measuring the diameters of zones of inhibition on bacterial strain and the results are presented in Table 5.

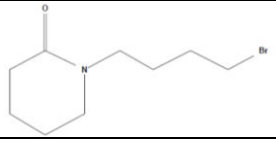
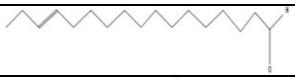
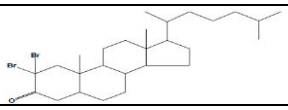
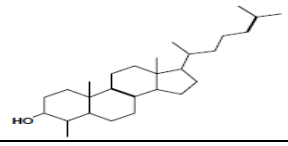
According to the zone of inhibition produced by 75 µl of aqueous extract of *C. forskaolii* Vahl leaves for *S. agalactiae, S. iniae, A. hydrophilia, P. aeruginosa and V. cholerae* were 18±0.2, 12±0.1, 13±0.1, 14±0.1 and 13±0.2, respectively, and for 50 µl concentration was 15±0.1, 11±0.1, 12±0.26, 12±0.26 and no activity, respectively. At the minimum concentration of 25 µl, the clear zone was 13±0.1, 10±0.1, 10±0.2, 11±0.1 and no activity, respectively. The clear zone of inhibition produced by amoxyclav was 21±0.1, 13±0.2, 15±0.17, 17±0.3 and 16±0.26, respectively.

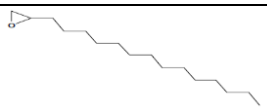
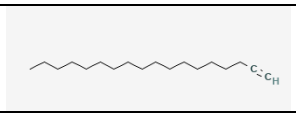
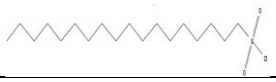
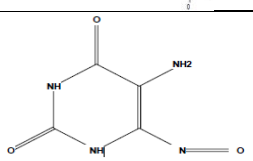
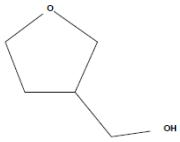
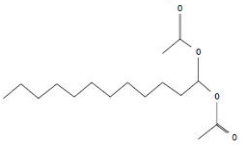
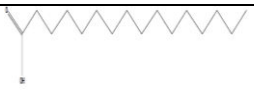
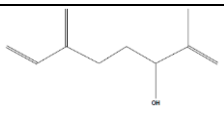
Synergetic antibacterial activities of ethanolic extract of *C. forskaolii* Vahl leaves were tested against bacterial pathogen and marked their highest growth inhibition at 75 µl recorded 12±0.26 and 12±0.4 against *S. agalactiae* and *A. hydrophilia* followed by 11±0.1 and 11±0.17 at 50 µl concentration. All three concentrations with the zone of inhibition at 08±0.35, 09±0.2, and 10±0.3 were observed against *S. iniae* and no results were found in *P. aeruginosa* and *V. cholerae.* On the other hand, amoxyclav showed zone of inhibition with 20±0.2 (*S. agalactiae*), 15±0.17 (*S. iniae*), 15±0.26 (*A. hydrophilia*) and 17±0.26 (*V. cholerae*).

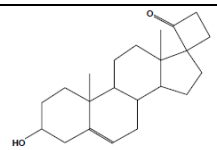
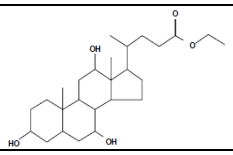
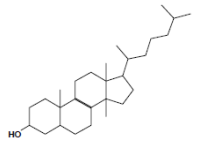
Among the gram-negative bacteria, *P. aeruginosa and V. cholerae* were the most sensitive strains at 75 µl methanolic extract with a zone of growth inhibition of 23±0.17 and 16±0.3, respectively followed by 11±0.17 and 11±0.26 at medium concentration 50 µl for both the strains and also 10±0.17 at 25 µl for *V. cholerae.* In addition, no results were shown in all three concentrations of the methanolic extract on *S. agalactiae, S. iniae,* and *A. hydrophilia.* The strong zone of inhibition produced by amoxyclav was 23±0.36 (*S. agalactiae*), 21±0.17 (*A. hydrophilia*), 12±0.2 (*P. aeruginosa*), 20±0.1 (*V. cholerae*) and no inhibition for *S. iniae*.

No inhibitory effect was found in *S. agalactiae, A. hydrophilia,* and *P. aeruginosa* using 25 µl, 50 µl, and 75 µl concentrations of acetone extract-treated group. Amongst gram-positive bacteria *S. iniae* and gram-negative bacteria *V. cholerae* show diameters of inhibition of 12±0.2, 13±0.17, 15±0.3 and 12±0.3, 13±0.3, 16±0.3 at 25 µl, 50 µl, and 75

Table 3: Biological activity of different compounds of *C. forskaolii* Vahl leaves

S. No.	Name of the compounds	Nature of the compound	Chemical structure	Biological Activity	References
1	Phytol	Diterpene alcohol		Antimicrobial, Anticancer, Antioxidant, Diuretic, Anti-inflammatory, Antinociceptive, Chemopreventive properties	(Camila *et al*., 2013)
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol		Antimicrobial, Anti-diuretic, Anticancer, Anti-inflammatory, Antioxidant	(Sudha *et al.*, 2013; Ismail *et al*., 2020)
3	Pentadecanoic acid	Saturated fatty acid		Lubricants and Adhesive agents	(Sunita *et al*., 2017)
4	2-Piperidinone, N-[4-bromo-n-butyl]-	Alkaloid		Antimicrobial, Antioxidant, Anti-inflammatory	(Dukes 1992-2016)
5	Octadecanal	Fatty aldehyde		Alkane-lyase activity, Sex pheromone	(Arora and Meena, 2017)
6	1-Octadecyne	-		No activity reported	-
7	Octadecanal, 2-bromo	-		Nontoxic and Anti-microbial agents; Anti-inflammatory, Anti-apoptotic effects	(Kumar *et al*., 2018)
8	Cis-9,10-Epoxyoctadecan-1-ol	Alcoholic compound		Antimicrobial	(Subavathy and Thilaga, 2016)
9	17-Pentatriacontene	Alkene		Anticancer, Antibacterial, Antiarthritic, Antioxidant, Antimicrobial activity, Anti-inflammatory	(Yogeswari *et al*., 2012)
10	14-Heptadecenal	-		No activity reported	-
11	Hexatriacontane	-		Analgesic activity, Radical scavenger, Anti-inflammatory and Antioxidant activity	(Ashwathanarayana and Raja Naika, 2018)
12	2,2-Dibromocholestanone	-		Inhibitor of alpha-amylase enzyme	(Ramesh and Ravi, 2020)
13	1-Heptatriacotanol	-		Antibacterial activity, Anti-hypercholesterolemic effects	(Olufunmiso *et al*., 2018)
14	Tetradecane, 1-chloro	-		No activity reported	-
15	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	-		No activity reported	-

16	Oxirane, tetradecyl	Oxirane		No activity reported	-
17	1-Octadecyne	-		No activity reported	-
18	1-Eicosanol	Aliphatic alcohol		Antimalarial, Antifungal, Antioxidant, Emollient for cosmetics	(Chatterjee *et al*., 2018)
19	n-Nonadecanol-1	Long chain alcohol		Antimicrobial and Cytotoxic properties	Kuppuswamy *et al*., (2013)
20	9-Octadecenal	-		No activity reported	-
21	Hexadecanal	-		No activity reported	-
22	Octadecane, 1-(ethenyloxy)-	Ether		Antisepsis	(Nor Qhairul Izzreen, and Vijaya Ratnam, 2014)
23	1-Octadecanesulphonyl chloride	-		No activity reported	-
24	Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitroso	-		No activity reported	-
25	Tetrahydro-3-furanmethanol	-		No activity reported	-
26	1,1-Dodecanediol, diacetate	-		No activity reported	-
27	n-hexadecanoic acid	Palmitic acid		Anti-cancer, Antioxidant, Anti androgenic, Alpha reductase inhibitor, Antifungal, Anti-inflammatory, Antifibrinolytic, Antimalarial, Antimicrobial Agent, Hypocholesterolemic Nematicide, Hemolytic, 5- Potent mosquito larvicide, Flavour, Hemolytic, Antialopecic, and Potent prostaglandin-E2 9-reductase) inhibitor	(Aparna *et al*., 2012; Korbecki and Bajdak-Rusinek, 2019)
28	2-Methyl-6-methylene-octa-1,7-dien-3-ol	-		No activity reported	-
29	Dodecanal	Aldehyde		Analgesic effect, Antimicrobial, Anti-inflammatory and Cytotoxic Activities	(Bae *et al*., 2019)

30	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3.beta.,17.beta.)-	-		Antimicrobial Antiarthritic, Anticancer, Anti-inflammatory, Antiasthma, and Hepatoprotective	(Sathiyabalan *et al.*, 2014)
31	Ethyl iso-allocholate,	Steroid derivative		Antimicrobial activity	(Malathi *et al.*, 2016)
32	Cholest-8-en-3-ol, 14-methyl-, (3.beta.,5.alpha.)-	-		No activity reported	-
33	2-Butanone, 4-(acetyloxy)-	-		No activity reported	-
34	3-Acetoxydodecane	-		No activity reported	-
35	Decanal	-		No activity reported	-

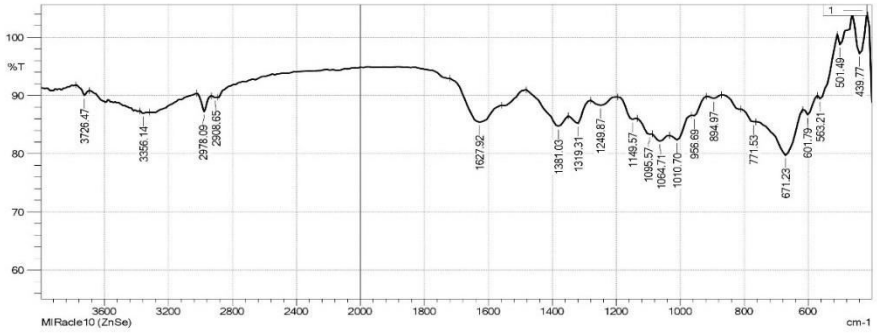


Fig. 2: FTIR spectrum analysis of *C. forskaolii* Vahl leaves.

µl concentrations, respectively. A clear zone of growth inhibition was produced by amoxyclav as the disc was 35±0.26 (*S. agalactiae*), 21±0.2 (*S. iniae*), 16±0.1 (*A. hydrophilia*), 21±0.3 (*V. cholerae*) and no inhibition for *P. aeruginosa*.

The results showed that 75 µl of chloroform extract had inhibition zone on all five test bacteria *S. agalactiae, S. iniae, A. hydrophilia, P. aeruginosa* and *V. cholerae* with inhibition zone diameters as 23±0, 16±0.17, 14±0.3, 13±0.1, and 24±0.3, respectively. Additionally, 50 µl concentration showed inhibition as 22±0.26, 13±0.17, 12±0.2, and 14±0.2 followed by 25 µl concentration with 19±0.2, 12±0.36, 11±0.17, and 11±0.1 against *S. agalactiae, A. hydrophilia, P. aeruginosa,* and *V. cholerae*, respectively. *S. iniae* did not show any inhibitory activity against chloroform extract at 50 µl and 25 µl while amoxyclav as the disc with inhibition zones of 28±0.1, 23±0.1, 14±0.17 and 32±0.1 against *S. agalactiae, A.hydrophilia, P. aeruginosa* and *V. cholerae*, respectively.

Antifungal activity:

The agar well diffusion method for antifungal activity against *Aspergillus niger* and *Aspergillus flavus* showed a significantly reduced zone of inhibition by concentration-dependent manner (Table 6). The maximum zone of inhibition was displayed in 75 µl concentration of all solvent extracts than 25 µl and 50 µl for *Aspergillus niger* and *Aspergillus flavus*. The aqueous and methanol extract of *C. forskaolii* Vahl leaves with the highest zone of inhibition of 17±0.25 and

Table 4: FTIR Interpretation of compounds of *C. forskaolii* Vahl Leaves

S. No.	Peak value [Wave number cm^{-1}]	Wave number cm^{-1} [Reference article]	Stretching	Functional group
1	3726.47	>3500	Non bonded, O-H	Hydroxy group
2	3356.14	3360–3310	>N-H	Aliphatic secondary amine
3	2978.09	3000–2850	C–H	Alkanes
4	2908.65	3000–2850	C–H	Alkanes
5	1627.92	1662-1626	N-H	Secondary amide
6	1381.03	1385-1380	C-H	Alkane
7	1319.31	1360–1310	C-N	Aromatic tertiary amine
8	1249.87	1270–1230	aryl -O	Aromatic ethers
9	1149.57	1190–1130	C-N	Secondary amine
10	1095.57	1150–1050	C-O	Alkyl-substituted ether
11	1064.71	1150–1050	C-O	Alkyl-substituted ether
12	1010.70	1000-1050	C-F	Aliphatic fluoro compounds
13	956.69	1225–950	C-H	Aromatic
14	894.97	895–885	C-H	Vinylidene
15	771.53	800–700	C-Cl	Aliphatic chloro compounds
16	671.23	700–600	C-Br	Aliphatic bromo compounds
17	601.79	700–600	C-Br	Aliphatic bromo compounds
18	563.21	600–500	C-I	Aliphatic iodo compounds
19	501.49	600–500	C-I	Aliphatic iodo compounds
20	439.77	500–430	S-S	Aryl disulfides

17±0.5 while ethanol, acetone, and chloroform extracts showed 13±0.05, 14±0, and 13±0.6, respectively against *Aspergillus flavus.* Chloroform and acetone extract of *C. forskaolii* Vahl leaves were found to have an uppermost inhibition zone at 75 µl concentration which showed inhibition with a diameter of 16±0.5 and 15±0.5 against *Aspergillus niger.* In addition, the maximum inhibitory concentration of 75 µl aqueous, ethanol, and methanol extract showed 13±0.5, 13±0.6, and 14±0.4. The relatively low inhibitory activity was noted at 25 µl and 50 µl for all five extracts against test fungal pathogens.

Discussion

Previous, literature documented various plant extracts with their bioactive phytoconstituents which have different susceptibility against pathogenic bacteria and fungal strains. Kavitha (2021) reported that the phytochemical profiling, ethanolic extracts of leaf and fruits of *Trichosanthes dioica* Roxb. revealed the

Table 5: Antibacterial activity of *C. forskaolii* Vahl leaves

S. No.	Solvent extracts	Concentration µl	Zone of inhibition (mm) SD ± Mean				
			Gram positive bacteria		Gram negative bacteria		
			Sa	Si	Ah	Pa	Vc
1	Aqueous	25 µl	13±0.1**	10±0.1**	10±0.2**	11±0.1**	-
		50 µl	15±0.1**	11±0.1**	12±0.26**	12±0.26**	-
		75 µl	18±0.2**	12±0.1**	13±0.1**	14±0.1**	13±0.2**
		Disc	21±0.1**	13±0.2**	15±0.17**	17±0.3**	16±0.26**
2	Ethanol	25 µl	-	08±0.35**	-	-	-
		50 µl	11±0.1**	09±0.2**	11±0.17**	-	-
		75 µl	12±0.26**	10±0.3**	12±0.4**	-	-
		Disc	20±0.2**	15±0.17**	15±0.26**	-	17±0.26**
3	Methanol	25 µl	-	-	-	-	10±0.17**
		50 µl	-	-	-	11±0.17**	11±0.26**
		75 µl	-	-	-	23±0.17**	16±0.3**
		Disc	23±0.36**	-	21±0.17**	12±0.2**	20±0.1**
4	Acetone	25 µl	-	12±0.2**	-	-	12±0.3**
		50 µl	-	13±0.17**	-	-	13±0.3**
		75 µl	-	15±0.3**	-	-	16±0.3**
		Disc	35±0.26**	21±0.2**	16±0.1**	-	21±0.3**
5	Chloroform	25 µl	19±0.2**	-	12±0.36**	11±0.17**	11±0.1**
		50 µl	22±0.26**	-	13±0.17**	12±0.2**	14±0.2**
		75 µl	23±0.3**	16±0.17**	14±0.3**	13±0.1**	24±0.3**
		Disc	28±0.1**	-	23±0.1**	14±0.17**	32±0.1**

Sa: *S.agalactiae,* Si: *S.iniae;* Ah: *A.hydrophilia;* Pa: *P.aeruginosa;* Vc: *V.cholerae,* Disc-amoxiclav; - Indicates nil activity; SD – Standard Deviation; **Significance p< 0.01.

Table 6: Antifungal activity of *C. forskaolii* Vahl leaves

S. No.	Fungal strain	Solvent extracts	Zone of inhibition (mm) SD ± Mean			
			25 µl	50 µl	75 µl	Control
1	*Aspergillus niger*	Aqueous	10±0.5**	12±0.5**	13±0.5**	13±0.5
		Ethanol	11±0.5**	13±0.6**	13±0.6**	14±0.8
		Methanol	10±0.5**	11±0.35**	14±0.4**	13±0.95
		Acetone	12±0.6**	14±0.5**	15±0.5**	18±0.5
		Chloroform	12±0.5**	14±0.5**	16±0.5**	15±0.4
2	*Aspergillus flavus*	Aqueous	12±0.5**	15±0.3**	17±0.25**	12±0.5
		Ethanol	9±0.5**	10±0.6**	13±0.05**	11±0.8
		Methanol	11±0.5**	14±0.5**	17±0.5**	10±0.5
		Acetone	9±0.8**	12±0.5**	14±0.7**	11±0.4
		Chloroform	10±0.5**	11±0.7**	13±0.6**	11±0.5

SD – Standard Deviation, **Significance p<0.01

Presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids, reducing sugar, tannins, and terpenoids. Biologically active components like tannins, flavonoids, cardiac glycosides, anthocyanins, terpenoids, carotenoids, ascorbic acid, and reducing compounds were present in all solvent extracts including *n*-hexane, petroleum ether, chloroform, ethyl acetate, ethanol, acetone, and water of *Phaseolus vulgaris* seeds (Nawaz *et al*., 2020). Preliminary screening of phytochemicals showed the presence of alkaloids, flavonoids, saponins, steroids, and tannins were present in distilled water, methanol, acetone, chloroform, ethyl acetate, and hexane from the leaves of *Datura metel* (Dhawan and Gupta, 2017). Madhankumar and Murugesan (2019) showed the presence of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, tannins, amino acids, oils, and resins while carbohydrates were absent in the methanolic extracts in the methanolic leaf extract of *Andrographis serpyllifolia* (*A. serpyllifolia*). Similar results were also noted by Malarvizhi *et al.* (2019) as higher amount of the different phytoconstituents present in the methanolic extract followed by aqueous and petroleum ether of *Commelina diffusa* Burm. f. (*C. diffusa*) shoots. Olivia *et al*. (2021) screened phytochemical characterization and GC-MS analysis of *Hibiscus asper* leaves using aqueous and methanol fraction. Earlier phytochemical and GC-MS studies have shown the presence of various secondary metabolites and bioactive compounds from different plant species (Kalaimagal, 2019; Padma *et al*., 2019).

Rizwana *et al*. (2019) observed acetone, methanol, ethanol, and ethyl acetate extract from *Passiflora edulis* f. edulis fruit for GC-MS, FTIR and also tested against gram-positive and gram-negative bacteria namely *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa,* and *Klebsiella pneumonia*. The results indicated that gram-positive bacteria especially *B. subtilis* exhibited strong inhibition while gram-negative bacteria showed weak inhibition against

all extracts and antifungal activity reported against *Candida albicans*. This is similar to present studt. Sangeetha *et al*. (2020) evaluated the phytochemical, GC-MS and antibacterial activity of *Calotropis gigantea* which showed maximum inhibition in chloroform and acetone extracts than aqueous and no inhibition in petroleum ether against tested organisms *Escherichia coli, Klebsiella* sp*., Streptococcus* sp*.* and *Pseudomonas* sp. In addition, chloroform and petroleum ether extracts from *Calotropis gigantea* indicated more effectiveness when compared with aqueous and acetone extract against *Aspergillus* sp. than yeast. Further, hexane, ethyl acetate, and methanol extracts of *Muniria angustisepala* leaves showed presence of phytochemical constituents and antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhii, Klebsiella pneumonae, Staphylococcus aureus, Bacillus subtilis,* and antifungal activity against *Aspergillus niger, Penicillium notatum, Rhizopus stolon* and *Candida albicans* (Shehu *et al*., 2019).

In past several investigators assessed the antibacterial and antifungal activity of different plant extracts against various bacterial and fungal pathogens (Yemata *et al*., 2019; Yusof *et al*., 2020). Nandagopalan and Kavitha (2021) evaluated the antimicrobial activity of methanol, petroleum ether and aqueous extracts of *Calanthe masuca* against bacterial (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli*) and fungal pathogens (*Candida albicans* and *Aspergillus niger*). Furthermore, they observed that methanol extract showed highest antibacterial and antifungal activity than other solvents. Habtom and Gebrehiwot (2019) demonstrated the antibacterial and antifungal activity of ethanol, methanol, and aqueous extracts from leaves of two plant species- *Vernonia amygdalina* and *Croton macrostachyus.* They reported that all methanol and ethanol extracts showed the highest growth inhibitory effects activity against *Staphylococcus aureus, Streptococcus agalactiae, Salmonella typhi, Escherichia coli* and ethanol extract of *Croton macrostachyus* showed antifungal activity against *A. niger* and *A. flavus.* Methanol crude extract from the root of *Barringtonia asiatica, Barringtonia racemose* and *Leptadenia hastata* were tested against *Aspergillus niger, Aspergillus flavin, Fasarium oxysporium*, and *Candida tropicalis* at different concentration (Reference). Moreover, their results revealed that the antifungal activity of all plant extract. The flower of *Acacia auriculiformis* was assayed against *Aspergillus niger* and *Candida tropicallis* and its antifungal activity was proven at 1000 ppm concentration (Samling *et al*., 2018). Zahid (2019) examined *Aspergillus niger, Rhizopus oryzae* and *Alternaria solani* using extract from different plant species including *Euphorbia helioscopia*, *Phyllanthus emblica*, *Ricinus communis*, *Putranjiva roxburghii*, *Croton tiglium*, *Euphorbia hirta*, *Euphorbia splendens*, *Jatropha integerrima* and *Euphorbia prostrata*. They observed maximum inhibitory action of all plant extracts depending on their concentration. Listyorini *et al*. (2021) have investigated and tested antifungal activity against *A. flavus* due to the presence of fatty acid and glycoside from aqueous extract of *P. edule* seed.

Conclusion

In the present investigation, preliminary phytochemical screening, GC-MS analysis, FTIR, and antimicrobial activities of *C. forskaolii* Vahl leaves extract was studied for the first time using five different solvent extracts such as chloroform, acetone, ethanol, methanol, and aqueous. This is the first available information about the qualitative preliminary phytochemical screening of *C. forskaolii* Vahl leaves which revealed the presence of several secondary metabolites including proteins, carbohydrates, alkaloids, phenols, flavonoids, tannins, saponins, triterpenoids, and steroids. The 35 major bioactive compounds present in *C. forskaolii* Vahl leaves using different solvent extracts were identified by GC-MS. FTIR which showed the available functional groups present in the bioactive molecules of *C. forskaolii* Vahl leaves.

The results of the present study revealed that all solvent extracts showed variable antimicrobial activity against aquatic pathogenic bacterial strains and fungal strains. Among the tested extracts results of aqueous and chloroform solvent extracts of leaves showed the most potent antibacterial activity followed by ethanol, acetone, and methanol extracts. In addition, *A. niger* was most potent resistant fungal strain against chloroform extract, and *A. flavus* was effective against aqueous extract followed by all other solvent extracts. It is concluded from the present study that the leaves of *C. forskaolii* Vahl have shown significant antimicrobial activities against pathogens due to the presence of different bioactive phytoconstituents and various functional groups. The present investigation suggests that the contribution of these biologically active compounds to the aquaculture and fisheries industries should be evaluated.

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