Biocatalysis and Agricultural Biotechnology 17 (2019) 736–742

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Phytol – A biosurfactant from the aquatic weed *Hydrilla verticillata*

S. Pandi Prabha[a,*], C. Karthik[b], S. Hema Chandrika[a]

[a] *Department of Biotechnology, Sri Venkateswara College of Engineering, Sriperumbudur Taluk, Chennai, Tamil Nadu, India*
[b] *Department of Biotechnology, St. Joseph's College of Engineering, OMR Road, Semmancheri, Chennai, Tamil Nadu, India*

ARTICLE INFO

Keywords:
Phytol
Hydrilla verticillata
High performance Chromatography
Antibacterial activity

ABSTRACT

Phytol, a natural linear diterpene fatty alcohol present in the integral part of the chlorophyll is found in copious amounts in the aquatic weed *Hydrilla verticillata.* It is widely used as a precursor for the manufacture of vitamins E and K and its worldwide demand is 0.1–1.0 metric tons/year. High hydrophobicity of phytol intended its use as a surfactant for various applications. Hence, the main objective of this study is to separate phytol from ethanolic extract of *Hydrilla verticillata* and to use as an anionic surfactant. Phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, saponins and terpenoids. FTIR spectral analysis confirmed the different functional groups such as amides, alcohol, phenol, phosphorus and halogen compounds in the extract. HPLC fingerprint profile of ethanolic extract of hydrilla showed that phytol, a major phytochemical present in the crude extract. Thin layer chromatography is used to determine the proper solvent system for performing separations using column chromatography and effective separation is achieved with mixture of Toluene: Ethyl Acetate in the ratio 7:3. Column chromatography is done for partial purification of phytol and the fractions are analyzed by UV–Visible spectroscopy. The purified phytol is subjected to sulphonation to convert it into phytol sulphate which is found to possess surfactant activity. The concentration of phytol sulphate is assessed by using Methylene Blue Active Substances (MBAS) assay. Phytol sulphate showed potent disinfectant by its antibacterial activity against *E. coli*. Since Phytol is from a natural source and is renewable, its availability in abundance with less risk factor can be a great asset to the humankind.

1. Introduction

Hydrilla verticillata is one of the formidable aquatic weeds abundantly in the invading parts of Asia to almost every continent. It is commonly known as water thyme and termed as Velampasi in Tamil. This is the only species of the genus Hydrilla, an aggressive, opportunistic, nuisance species in the Hydrocharitaceae family. This submersed aquatic macrophyte, pivotal resources for the maintenance of trophic chains and in biogeochemical processes, but they can also be deleterious for several uses if present in excess. These includes providing poor habitat for fish and other wildlife, reduction in the availability of dissolved oxygen in the infested body of water also the stagnant water conditions created by heavy infestations which renders the breeding ground for mosquitoes. It reduces diversity of aquatic nature and fish populations gets affected (Stephen and Sanjeeva Raj, 2013). Moreover, it forms thick mats on the water surface, which can reduce the flow of water in canals, damage dams and interferes with boating and fishing.

Despite these effects, this plant is credited with numerous biological activities. It is a rich source of variable nutrients and chemical constituents like saponins, vitamins, minerals, antioxidants, amino acids, detoxifying agents, etc; and is especially valuable to true vegetarians (Pal and Nimse, 2006). Besides its uses, this plant may be therapeutically used to provide complete nutrition, to improve digestion and gastrointestinal function, circulation, neurological health, blood sugar control, to strengthen immunity and increase endurance (Sutton et al., 2012). The major chemical constituents present in the extract of *Hydrilla verticillata* are Bicyclo heptane, 2,6,6-trimethyl-2-pentadecanone, Hexadecanoic acid, Ethyl ester, Phytol, Linolenic acid ethyl ester and 9,12,15- Octadecatrienoic acid ethyl ester. Of all these chemical constituents, phytol is present in larger amounts (70.29%) (Byju et al., 2013; Pandi Prabha and Rajkumar, 2015). Phytol, a diterpene fatty alcohol, is the integral part of the chlorophyll. It has been widely used as a precursor for the manufacture of synthetic forms of vitamin E and K (Ahmed et al., 2018) while its commercial application includes its usage in cosmetics, shampoos, toilet soaps, household cleaners, and detergents (McGinty et al., 2010). Its worldwide use has been estimated to be approximately 0.1–1.0 metric tons per year. In an attempt to transmute this noxious weed into a beneficial one, the

* Correspondence to: Department of Biotechnology, Sri Venkateswara College of Engineering, Post Bag No: 3, Pennalur, Sriperumbudur Taluk, Chennai 602117, Tamil Nadu, India.

E-mail address: prabha@svce.ac.in (S.P. Prabha).

https://doi.org/10.1016/j.bcab.2019.01.026
Received 2 November 2018; Received in revised form 12 January 2019; Accepted 14 January 2019
Available online 14 January 2019
1878-8181/

compound phytol plays a major role.

Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Many long chain alcohols show some surfactant properties. Noticeable among these are the fatty alcohols, cetyl alcohol, stearyl alcohol, and cetostearyl alcohol (consisting predominantly of cetyl and stearyl alcohols) and oleyl alcohol. Phytol can be directly used as fatty alcohols without the need for any further conversion. Moreover, it is a long chain primary alcohol with high hydrophobicity which marks its utilization as surfactant (Liu et al., 2010). Lauryl alcohol ethoxylate is predominantly used non-ionic surfactant. However, due to the associated risk factors such as skin irritation, inflammation of the mucous membrane and its toxicity to aquatic organisms, a product with less or no risk factor is needed. Since phytol is a compound with less risk factor it could serve the above purpose well. Since its provenance is from a natural source, it is renewable, safe and is available in preponderance amounts. Further natural sources have higher stability when compared to the synthetic one. Hence, the plant *Hydrilla verticillata* and the compound phytol can be a great asset to the humankind and the environment (Diallo et al., 1994)

2. Materials and methods

2.1. Extraction of bioactive molecules

The *Hydrilla verticillata* dry powder (20 g) was extracted in a soxhlet apparatus at 75 °C with 200 ml of 95% ethanol (Schulz et al., 1993). The extract after the ethanol treatment was filtered through Whatman No. 1 filter paper and concentrated by vacuum distillation (Sims and Pettus, 1976). The residue was stored at 4 °C for further investigation (Angajala and Subashini, 2018)

2.1.1. Preliminary phytochemical analysis

The phytochemical analyses of extracts of Hydrilla *verticillata* was performed for the presence alkaloids, phenols, saponins, terpeniods, glycosides, flavonoids and tannins (Harborne, 1988; Sasidharan et al., 2011; Vijayaraghavan et al., 2018; Wani et al., 2018)

2.1.2. FTIR analysis of ethanolic extract of Hydrilla verticillata

Fourier Transform Infrared Spectroscopy (FTIR) perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. Moreover, FTIR spectra of pure compounds are usually unique that they are like a molecular fingerprint. For most common plant compound analysis, the spectrum of an unknown compound can be identified by comparison to a mass of known compounds. The extracts of *Hydrilla verticillata* after the ethanol treatment were taken in 1.5 ml of eppendorf tube. Then the sample was loaded into FTIR Spectroscope. The functional groups of compounds were separated based on its peak ratio and the determination of functional groups was being done by comparing wave number of amide functional groups of the samples to existing standards. The spectra were recorded in the range of 4000–400 cm using FTIR Perkin Elmer Spectrum (Al-Tameme et al., 2015)

2.1.3. Phytol- major phytochemical by HPLC analysis

High Performance Liquid Chromatography is a versatile purification technique widely used for the isolation of natural products. The main principle behind HPLC is the fact that the certain compounds have different migration rates at a particular column and mobile phase. The extent of separation is mostly based on the choice of stationary phase and the mobile phase. The HPLC was performed for the crude extract and the standard phytol. The mobile phase composed of Methanol: Acetonitrile (30:70) was eluted at a flow rate of 1.0 ml/min. The effluent was monitored by a UV detector at 210 nm (Xiao et al., 2013).

2.2. Separation and purification of phytol

2.2.1. Selection of solvent system by TLC

TLC is a simple method to determine the identity of a compound in a mixture when the RF of the compound is compared with the RF of a known compound. A number of 5 TLC plates of 25 × 75 mm size of silica gel was loaded with 50 µl of the crude extract and the standard phytol (bought from Sigma Aldrich). They were allowed to run in 5 different solvent systems of Toluene: Ethyl Acetate (7:3), Chloroform: Methanol (9:1), Hexane: Ethyl Acetate (20:1), Hexane: Ethyl Acetate (15:1), Hexane: Ethyl Acetate (10:1). The TLC plates were air dried and visualized under visible UV lights. RF values (distance travelled by the solute/ distance travelled by the solvent) were then calculated (Thakor et al., 2016).

2.2.2. Separation and purification of phytol by column chromatography

Column Chromatography is basically done to isolate the bioactive compound from the crude extract. The main principle behind column chromatography is the differential adsorption of substance by the solvent. It mainly involves the use of a vertical column, silica gel, cotton plug and the solvent in which the bioactive compound is eluted. The column is packed to obtain a flow rate of 1 ml/min. 0.1 g of the crude extract was dissolved in Toluene: Ethyl acetate (9:1) solvent system. 5 ml of the crude sample was poured to the top of the column. The solvent system Toluene: Ethyl Acetate (9:1) was poured to the column thereby collecting different fractions of the crude extract at regular intervals (Blumer et al., 1969)

2.3. Quantitative analysis of phytol by UV spectroscopy

UV spectroscopy is basically performed for qualitative analysis and for identification of certain classes of compounds. Sometimes UV spectroscopy can also be used for quantitative analysis because some molecules are powerful chromophores in the UV range (Kemp, 1991). This technique does not consume time and much cost effective compared to other techniques (Urbano et al., 2006). The obtained fractions from column chromatography were read at 210 nm and compared with the standard phytol graph to determine the concentration of phytol present in each fraction. Here Toluene: Ethyl Acetate (9:1) is used as a blank (Harborne, 1988)

2.4. Preparation and characterization of anionic surfactant – phytol sulphate

2.4.1. Preparation of phytol sulphate by sulphonation process

The fatty alcohols derived from natural sources usually composes of various linear carbon chain lengths. These are usually converted into alcohol sulphates by treating the alcohols with chlorosulphonic acid.

$$RCH_2OH + ClSO_3H \rightarrow RCH_2OSO_3H + HCl$$

The phytol obtained from the different fraction of column chromatography was subjected to sulphonation. Sulphonation is a process of addition of the sulphonic acid group (SO_3H) directly to carbon in an organic compound. Addition of chlorosulphonic acid to the compound (phytol) yields Phytol sulphate which can be used as an anionic surfactant. The process is an exothermic reaction and should be accompanied by continuous stirring. Replacement of hydrogen group of the fatty alcohol by the sulphonic group is the main mechanism behind this process. (Cripe et al., 1999)

2.4.2. Analysis of anionic surfactant (phytol sulphate) concentration

The surfactant property was estimated using the famous MBAS (Methylene Blue Active Substances) assay. It is a quantitative calorimetric analysis test method that uses methylene blue to detect the presence of anionic surfactants. The principle behind this assay is that the water soluble anionic surfactants forms a 1:1 ion pair with the water soluble cationic dye, methylene blue. The ion pair is effectively neutral and is therefore extractable into water soluble organic solvent such as dichloromethane. The MBAS assay was done by adding 0.1 ml of methylene blue to 2 ml of different concentrations of SDS (standard anionic surfactant) followed by addition of 2 ml of Dichloromethane. They were then centrifuged to remove the upper aqueous layer and the absorbance of the other layer was measured at 650 nm. The same procedure was performed for different fractions of phytol eluted from column chromatography (Jurado et al., 2006).

2.4.3. Analysis of antibacterial activity of phytol sulphate

The use of antimicrobial agents from a natural source is a new non-toxic strategy. The compound phytol from the natural source which is then converted into phytol sulphate was tested for its antibacterial activity. The minimum inhibitory concentration of phytol sulphate for *Escherichia coli* was tested at zeroth hour and 24th hour. The absorbance was read at 650 nm (Lee et al., 2016).

3. Results

3.1. Extraction of bioactive molecules from Hydrilla verticillata

3.1.1. Priliminary phytochemical analysis of ethanolic extract of Hydrilla verticillata

The phytochemical compounds were isolated using ethanol due to its greater ability to solubilize and ease of extraction of the bioactive compounds. Phytochemical screening showed the presence of alkaloids, phenols, terpenoids tannins and saponins, while glycosides and proteins were absent in the extract under the experimental conditions. (Table 1)

3.1.2. FTIR analysis of ethanolic extract of Hydrilla verticillata

The ethanolic extract of Hydrilla sample was subjected to FTIR (Fourier Transform Infrared Spectroscopy) analysis for the identification of different groups and compounds based on the peak value and wavenumber as shown in Fig. 1 and Table 2.

3.1.3. Phytol - major phytochemical by HPLC analysis

High Performance Liquid Chromatography was performed to analyze the different phytochemicals present in the crude extract (Pham et al., 2018). The crude extract showed three peaks at 3.4 min, 3.8 min and 6.6 min as shown in Fig. 2. These peaks corresponds to bergenin, anthraquinone and phytol respectively. The retention time of 6 mins for standard Phytol was seen as shown in Fig. 3.

3.2. Separation and purification of phytol

3.2.1. Selection of solvent system by TLC

TLC was done to analyze mixtures by separating the components in the mixture. Here TLC was used as a confirmatory test for the presence of phytol. The TLC confirmed the presence of phytol in the crude extract comparing with the standard phytol. The RF value of standard phytol was 0.80. Five different solvent systems were used for the elution of plant extract. It was found that the first solvent system (Toluene: Ethyl Acetate (7:3)) gives the best result. Hence it was considered as a desired solvent system for purification of phytol (Table 3).

Table 1
Identification of phytochemical compounds present in *Hydrilla verticillata*.

Compound	Name of the Test	Result
Phenols	Ferric chloride test	+
Saponins	Foam test	+
Alkanoids	Mayer's test	+
Tannins	Braymer's test	+
Proteins	Biuret test	–
Glycosides	Borntrager's test	–
Terpenoids	Salkowski test	+

'+' indicates presence; '-' indicates absence.

3.2.2. Separation and purification of phytol by column chromatography and its analysis

Column chromatography was performed to purify individual compounds from a mixture of compounds. The stationary phase used here is silica gel and the mobile phase is Toluene: Ethyl acetate (7:3). Silica gel is the mainly used adsorbent for particularly nonpolar and medium polar compounds including terpenoids and sterols (Citoglu and Acıkara, 2012). Five different fractions of the compounds are collected from the column and the absorbance values were read at 210 nm (Komiya et al., 1999). The concentration of phytol was determined by comparing with standard graph of phytol (Fig. 4). The concentration of phytol increases gradually from the 1st fraction to 5th fraction.

3.3. Preparation of anionic surfactant – phytol sulphate

3.3.1. Analysis of phytol sulphate concentration

Chlorosulphonation was performed for the five different fractions along with the standard phytol to convert them into phytol sulphate. After the performance of MBAS assay, the absorbance values were read at 650 nm. The OD value of the phytol sulphate obtained from the 5th fraction was in accordance with the OD value of the standard anionic surfactant. It can be concluded that the standard phytol has a concentration of 0.05 μg/ml of surfactant whereas the 5th fraction of phytol from the natural source has a concentration of 0.047 μg/ml of surfactant which is quite desirable (Figs. 5–7).

3.3.2. Analysis of antibacterial activity of phytol sulphate

Phytol Sulphate was found to have antibacterial activity. The minimum inhibitory concentration of phytol sulphate was measured for *Escherichia coli* as similar to the work in Ghaneian et al. (2015). The phytol sulphate incubated with *Escherichia coli* for 24 h was found to inhibit about 64% of the growth whereas there is an 82% growth in the culture without phytol sulphate.

4. Discussion

The *Hydrilla verticillata* possess medicinal values along with other potential values as green manure or fodder. It also contains beta-carotene, which aids in delivering more antioxidants, free radical scavenging, anti-aging and anti-pollution properties. The GC-MS analysis for ethanol extract of *Hydrilla verticillata* revealed the presence of phyto-active compounds such as Bicyclo (3.1.1) heptane 2,6,6-trimethyl- (1.alpha.,2.beta., 5.alpha), 2-penatdecanone, 6,10,14-trimethyl, Hexadecanoic acid ethyl ester, Phytol, Linoleic acid ethyl ester and 9,12,15-Octadecatrienoic acid ethyl ester (z,z,z) exhibiting biological activities (Pandi Prabha and Rajkumar, 2015). In medicinal fields, phytol has shown antinociceptive and antioxidant activities as well as anti- inflammatory and antiallergic effects. phytol is a cholesterol-lowering agent. In the pharma-medico viewpoint, phytol and its derivatives have antimicrobial, cytotoxic, antitumorous, antimutagenic, anti-teratogenic, antibiotic-chemotherapeutic, antidiabetic, lipid lowering, antispasmodic, anticonvulsant, antinociceptive, antioxidant, anti-inflammatory, anxiolytic, antidepressant, immunoadjuvancy, hair growth facilitator, hair fall defense and antidandruff activities (Pal and Nimse, 2006).

The crude extract of *Hydrilla verticillata* can be obtained by soxhlet apparatus using ethanol. Ethanol was used as the extraction solvent since it has a better solvent polarity and was able to penetrate the cellular membrane to extract the non-polar compound phytol from the leaves (Adham, 2015; Yadav et al., 2009). Moreover, ethanol was the most effective one, producing the highest yield in extracting the bioactive compounds. The yield of 1.19 g of crude extract was obtained

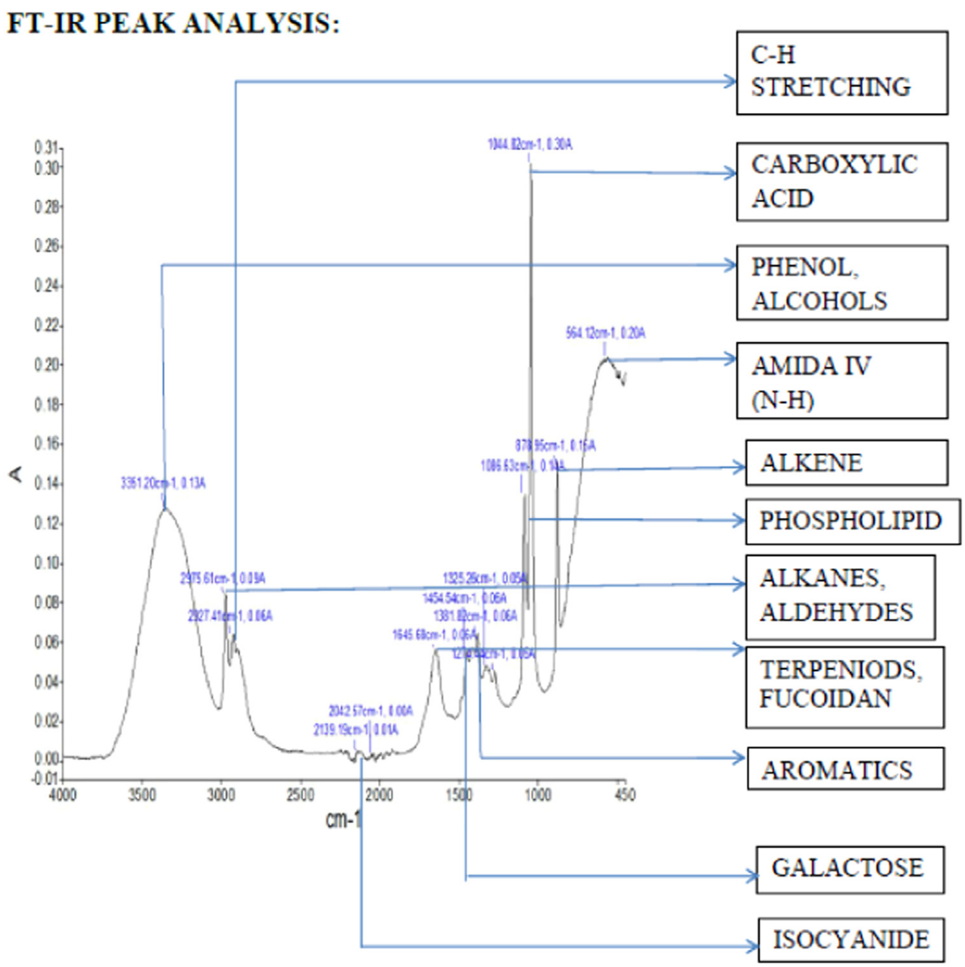

Fig. 1. FTIR Spectroscopy analysis of ethanolic extract of *Hydrilla verticillata*.

Table 2
FTIR Spectroscopy analysis of Ethanolic extract of *Hydrilla verticillata*.

S. No.	Wave Number (cm^{-1})	Responsible Phytochemical
1	564.12	Amide IV (N-H)
2	878.95	Alkene
3	1044.82	Carboxylic acid
4	1086.63	Phospholipids
5	1325.26	Galactose
6	1454.54	Aromatics
7	1645.68	Terpenoids, Fucoidan
8	2139.19	Isocyanides
9	2927.41	C-Hstretching, Aldehydes
10	2975.20	Alkanes
11	3351.20	Phenols, Alcohols

from 20 g of the dry leaf powder. Pal and Nimse (2006) reported the extraction of Hydrilla plant leaves using ethanol and the yield was found to be 2.4% (w/w). The crude extract was found to be dissolved in water, toluene, acetone, ethyl acetate, ethanol, benzene and methanol. The preliminary confirmation for the presence of phytol was carried out by Salkowski test. The appearance of reddish brown ring indicated the presence of diterpenoid compound (phytol) in the extract. The protonation of the hydroxyl group of the phytol when treated with strong acids like sulphuric acid leads to the formation of charge transfer complexes in the resulting conjugated dienes which was responsible for the colour change (Alhakmani et al., 2013).

FTIR spectrum was used to identify the functional group of the active compounds based on the peak value in the region of infrared radiation. The result of FTIR analysis showed different peaks at 564.12, 878.95, 1044.82, 1086.63, 1325.26, 1454.54, 1645.68, 2139.19, 2927.41, 2975.20 and 3351.20 cm^{-1} and confirmed the presence of amide IV (N-H), alkene, carboxylic acid, phospholipids, galactose, aromatics, terpenoids, fucoidan, isocyanides, C-H stretching, Aldehydes, alkanes, phenols and alcohols respectively (Arulkumar et al., 2018). The FTIR study is more sensitive as it gives the precise and exact determination over a specific concentration range without time consumption. (Janakiraman et al., 2011)

The selection of two phase solvent system was the most crucial step and was selected according to Xiao et al. (2013). The HPLC profile of the crude extract indicated the presence of not only the desired compound phytol but also other important phytochemicals like bergenin and anthraquinone (Wouters, 1985; Mello da Silva et al., 2014). Several peaks were formed, and the height of each peak corresponds to the abundance of that phytochemical. Thus, it was found that the compound anthraquinone was present in higher amounts in the crude extract (Phatangare et al., 2017). However the slight variation in the retention times of standard phytol and phytol in the crude extract may be due to the mobile phase composition where some of the organic solvent may have lost through evaporation or when there is a change in the fixed flow rate of the pump (Dolan John, 2005).

In this study, five different solvent systems were used for the elution of bioactive molecules from plant extract. Different spots were noted on the TLC plates and each spot corresponds to different phytochemicals (Zhang et al., 2003). The spot and RF value of phytol in the extract was in accordance with the standard phytol were noted. The solvent system Toluene: Ethyl Acetate (7:3) yields the RF value of 0.81 which was so similar with the RF value of the standard phytol (Sharma and Paliwal,

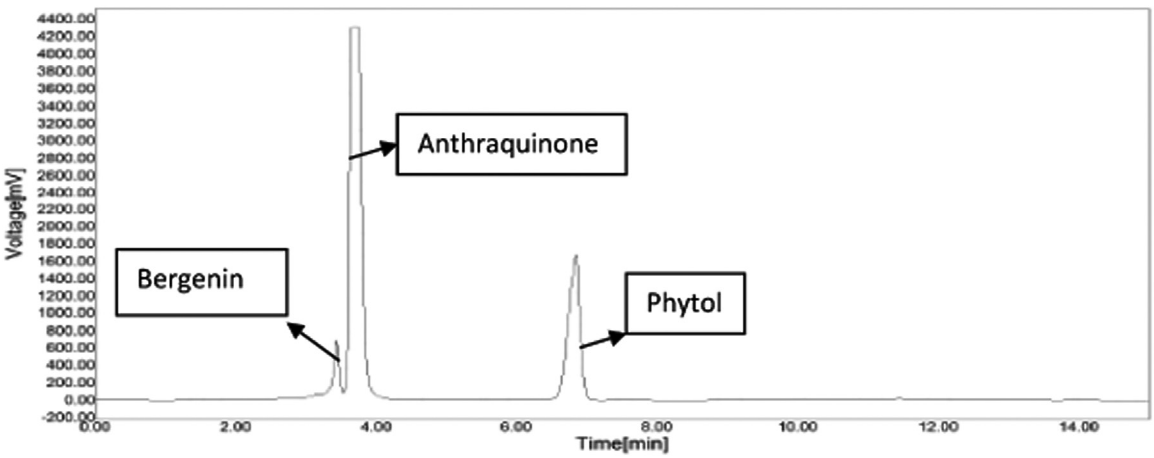

Fig. 2. Chromatogram of the crude extract of *Hydrilla verticillata*.

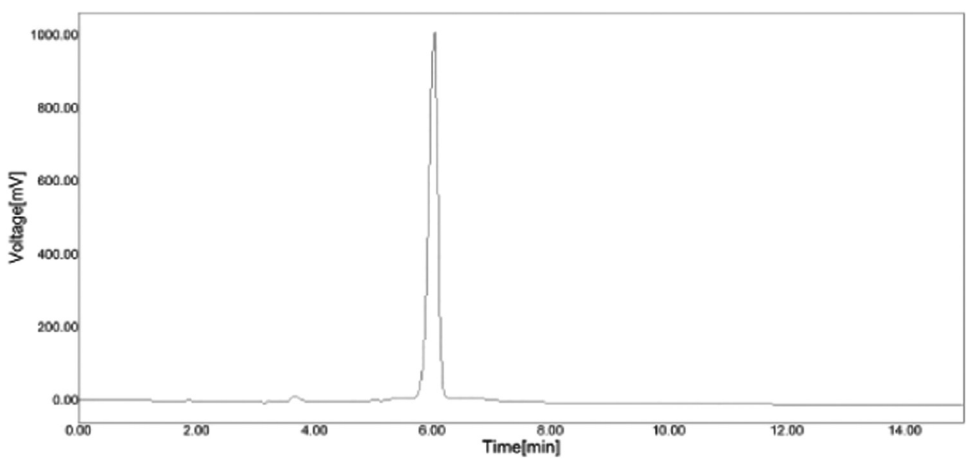

Fig. 3. Chromatogram of standard phytol (Retention time-6 min).

Table 3
Different solvent systems and their RF values.

Solvent Systems	Distance travelled by the solute (phytol)	Distance travelled by the solvent	RF value	Desired solvent system
Toluene: Ethyl Acetate (7:3)	4.5	5.5	0.81	Yes
Chloroform: Methanol (9:1)	3.8	5.5	0.69	No
Hexane: Ethyl Acetate (20:1)	2.8	5.5	0.50	No
Hexane: Ethyl Acetate (15:1)	2.2	5.5	0.40	No
Hexane: Ethyl Acetate (10:1)	2.5	5.5	0.46	No

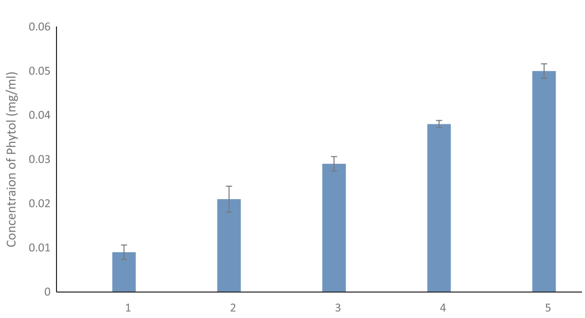

Fig. 4. Concentration of Phytol in different fractions of column chromatography.

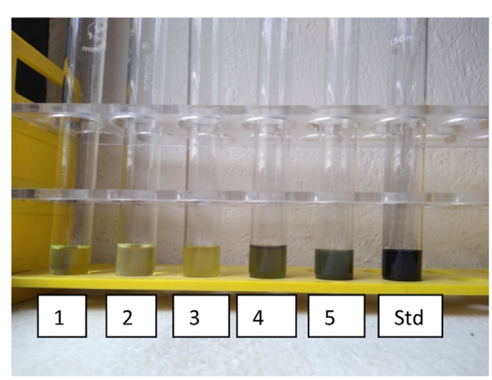

Fig. 5. Fractions 1, 2, 3, 4, 5 and Std. Phytol after MBAS assay.

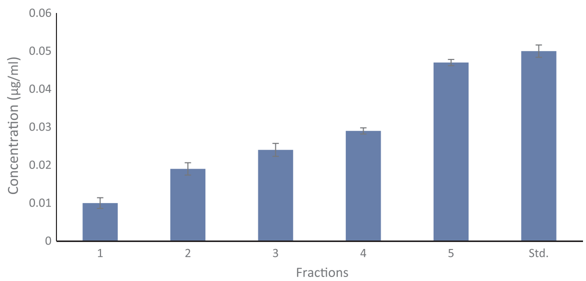

Fig. 6. Concentration of Phytol sulphate in different fractions of column chromatography.

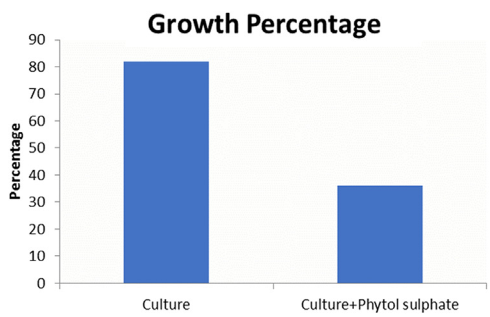

Fig. 7. Antibacterial activity of phytol sulphate.

2013; Thakor et al., 2016). Toluene: Ethyl acetate was found to be a desired one because both toluene and ethyl acetate are more polar and hence can easily elute the non-polar compound phytol when compared to other solvent systems since the other solvents do not have more polarity combination (Brent Friesen and Pauli, 2005).

The bioactive molecule was purified by column chromatography mainly due to its convenience, economy and availability of stationary phase (Zhang et al., 2005; Citoglu and Acıkara, 2012). The non-polar compound phytol gets eluted easily with polar solvents like toluene, ethyl acetate, methanol, ethanol, etc. Hence, in the present study silica gel and Toluene: Ethyl Acetate (7:3) was used as the adsorbent and solvent system respectively. Five different fractions were collected at time interval of 10 min. The increasing concentration of phytol from the first fraction was mainly due to the presence of phytol in the least quantity in the 1st fraction and in the maximum quantity in the 5th fraction. As the elution time increased, the colour intensity in the fraction also increased and hence the absorbance value (Tiselius et al., 1956). The increasing concentration was mainly due to the solvent polarity of phytol (non-polar) which was eluted well in Toluene: Ethyl Acetate solvent system (Brent Friesen and Pauli, 2005).

The purified phytol is then converted into phytol sulphate by sulphonation process which has significant surfactant activity. The increasing concentration of phytol sulphate in the five fractions was mainly due to the increase in concentration of phytol. Performance of chlorosulphonation directly converts the phytol into Phytol sulphate and hence the reason for the increasing concentration (George and White, 1999). The main mechanism behind this conversion is that the SO_3H group of the chlorosulphonic acid replaces the hydrogen atom of the alcoholic group of the phytol to form phytol sulphate and HCl gas is liberated (Gilbert, 1962).

Albeit different disinfectants, (e.g. Deconex, Microten, Cidex, and Alprocide) have been in use for the past two decades ago, the vast majority of them are lethal and flimsy (Ghaneian et al., 2015). In this study, phytol extracted from *H. verticillata* was used as a new disinfectant (Pejin et al., 2014). However, the mechanism of its antimicrobial activity is not fully described. It is suggested that protein and enzyme inactivation is one of the important mechanisms for inactivation of microbes. The main mode of actions are damage of cell membrane by the induction of oxidative stress (Lee et al., 2016) and denaturation of *Escherichia coli* dehydrogenases (Sykes, 1939). The phytol causes membrane damage and rapid denaturation of proteins by subsequent interference with metabolism and cell lysis (Larson and Morton, 1991).

From the present examination, it is significant to make reference that phytol is the major phytochemical present in the aquatic weed *Hydrilla verticillata* which has shown strong antibacterial action. Numerous engineered anionic surfactants have shown toxic and carcinogenesis, which has incited more noteworthy consideration towards hunting down normally happening surfactants. The natural phytol sulphate surfactant prepared from the *Hydrilla verticillata* was observed to be a good source to replace synthetic compounds.

5. Conclusion

The compound phytol was isolated from the leaves of *Hydrilla verticillata*. It was found that the ethanol was the most suitable solvent for extraction of bioactive molecules. The chromatogram of the crude extract revealed not only the presence of phytol but also other compounds like bergenin and anthraquinone. Toluene: Ethyl Acetate was found to be the most desired solvent system for the elution of phytol. The partially purified phytol was then converted to phytol sulphate by the process of sulphonation. The fatty alcohol chain of phytol is responsible for the surfactant activity. Moreover, phytol sulphate was found to possess antibacterial activity and can be used as a novel disinfectant. Since phytol is from a natural source and is renewable and available in abundance with less risk factor, it can be a great asset to the humankind.

References

Adham, A.N., 2015. Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content *in Mentha piperita, Mentha longifolia and Osimum basilicum*. J. Pharmacogn. Phytochem. 3 (6), 130–139.

Ahmed, B.E., Badawi, M.H., Mostafa, S.S., Higazy, A.M., 2018. Human anticancers and antidiabetic activities of the *cyanobacterium Fischerella sp*. BS1-EG isolated from River Nile, Egypt. Int. J. Curr. Microbiol. Appl. Sci. 7 (1), 3473–3485.

Alhakmani, F., Kumar, S., Khan, S.A., 2013. Estimation of total phenolic content, in–vitro antioxidant and anti–inflammatory activity of flowers of *Moringa oleifera*. Asian Pac. J. Trop. Biomed. 3 (8), 623–627.

Al-Tameme, H.J., Hameed, I.H., Idan, S.A., Hadi, M.Y., 2015. Biochemical analysis of *Origanumvulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). J. Pharmacogn. Phytother. 7 (9), 221–237.

Angajala, G., Subashini, R., 2018. Evaluation of larvicidal potential of β-sitosterol isolated from indigenous *Aegle marmelos Correa* crude leaf extracts against blood feeding parasites and its binding affinity studies towards sterol carrier protein. Biocatal. Agric. Biotechnol. 16, 586–593.

Arulkumar, A., Rosemary, T., Paramasivam, S., Rajendran, R.B., 2018. Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (*Gracilaria corticata and Gracilaria edulis*) from Palk Bay, India. Biocatal. Agric. Biotechnol. 15, 63–71.

Blumer, M., Robertson, J.C., Gordon, J.E., Sass, J., 1969. Phytol-derived C19 di-and triolefinic hydrocarbons in marine zooplankton and fishes. Biochemistry 8 (10), 4067–4074.

Brent Friesen, J., Pauli, G.F., 2005. Guess - A generally useful estimate of solvent systems for CCC. J. Liq. Chromatogr. Relat. Technol. 28 (17), 2777–2806.

Byju, K., Anuradha, V., Rosmine, E., Kumar, N.C., Nair, S.M., 2013. Chemical characterization of the lipophilic extract of Hydrilla verticillata: a widely spread aquatic weed. J. Plant Biochem. Biotechnol. 22 (3), 304–311.

Citoglu, G.S., Acıkara, O.B., 2012. Column Chromatography for Terpenoids and Flavonoids. In: Chromatography and Its Applications, pp. 761–768.

Cripe, T.A., Connor, D.S., Vinson, P.K., Burckett-St, J.C.T.R., Willman, K.W., 1999. U.S. Patent No. 6,008,181. Washington, DC: U.S. Patent and Trademark Office.

Diallo, M.S., Abriola, L.M., Weber, W.J., 1994. Solubilization of nonaqueous phase liquid hydrocarbons in micellar solutions of dodecyl alcohol ethoxylates. Environ. Sci. Technol. 28 (11), 1829–1837.

Dolan John, W., 2005. LC troubleshooting, retention time changes. LC• GC Eur. 18 (1), 12–16.

George, A.L., White, G.F., 1999. Optimization of the methylene blue assay for anionic surfactants added to estuarine and marine water. Environ. Toxicol. Chem. 18 (10), 2232–2236.

Ghaneian, M.T., Ehrampoush, M.H., Jebali, A., Hekmatimoghaddam, S., Mahmoudi, M., 2015. Antimicrobial activity, toxicity and stability of phytol as a novel surface

disinfectant. Environ. Health Eng. Manag. J. 2 (1), 13–16.
Gilbert, E.E., 1962. The reactions of sulfur trioxide, and its adducts, with organic compounds. Chem. Rev. 62 (6), 549–589.
Harborne, J.B., 1988. Phytochemical Methods, A Guide to Modern Techniques of Plananalysis, 3rd edn. Chapman and Hall, New York, pp. 1–150.
Janakiraman, N., Sahaya Sathish, S., Johnson, M., 2011. UV–vis and FTIR spectroscopic studies on Peristrophe bicalyculata (Retz.) Nees. Asian J. Pharm. Clin. Res. 4 (4), 125–129.
Jurado, E., Fernandez-Serrano, M., Nunez-Olea, J., Luzon, G., Lechuga, M., 2006. Simplified spectrophotometric method using methylene blue for determining anionic surfactants: applications to the study of primary biodegradation in aerobic screening tests. Chemosphere 65 (2), 278–285.
Kemp, W., 1991. Energy and electromagnetic spectrum. In: Kemp, W. (Ed.), Organic Spectroscopy. Macmillan Press, London, UK, pp. 1–7.
Komiya, T., Kyohkon, M., Ohwaki, S., Eto, J., Katsuzaki, H., Imai, K., Hibasami, H., 1999. Phytol induces programmed cell death in human lymphoid leukemia Molt 4B cells'. Int. J. Mol. Med. 4 (4), 377–457.
Larson, E.L., Morton, H.E., 1991. Disinfection, Sterilization and Preservation, 4th edition. pp. 11–15.
Lee, W., Woo, E.R., Lee, D.G., 2016. Phytol has antibacterial property by inducing oxidative stress response in *pseudomonas aeruginosa*. Free Radic. Res. 50 (12), 1309–1318.
Liu, Xiadong, Tracy, Mark, Pohl, Christopher, 2010. The strategy of surfactant analysis by HPLC. J. Anal. Biochem. 35 (3), 93–98.
McGinty, D., Letizia, C.S., Api, A.M., 2010. Fragrance material review on phytol. Food Chem. Toxicol. 48, S59–S63.
Mello da Silva, J., Antinarelli, L.M.R., Pinto, N.D.C.C., Coimbra, E.S., de Souza-Fagundes, E.M., Ribeiro, A., Scio, E., 2014. HPLC-DAD analysis, antileishmanial, antiproliferative, and antibacterial activities of *Lacistema pubescens*: an Amazonian medicinal plant. BioMe. Res. Int. 1–7.
Pal, D.K., Nimse, S.B., 2006. Little known uses of common aquatic plant *Hydrilla verticillata* (Linn. f.) Royle. Nat. Prod. Resour. 5, 108–111.
Pandi Prabha, S., Rajkumar, J., 2015. J. Chem. Pharm. Res. 7 (3), 1809–1815.
Pejin, B., Savic, A., Sokovic, M., Glamoclija, J., Ciric, A., Nikolic, M., Mojovic, M., 2014. Further in vitro evaluation of antiradical and antimicrobial activities of phytol. Nat. Product. Res. 28 (6), 372–376.
Pham, H.N.T., Sakoff, J.A., Van Vuong, Q., Bowyer, M.C., Scarlett, C.J., 2018. Screening phytochemical content, antioxidant, antimicrobial and cytotoxic activities of *Catharanthus roseus (L.) G. Don* stem extract and its fractions. Biocatal. Agric. Biotechnol. 16, 405–411.
Phatangare, N.D., Deshmukh, K.K., Murade, V.D., Hase, G.J., Gaje, T.R., 2017. Isolation and characterization of phytol from *Justicia gendarussa Burm. f.* - An anti-inflammatory compound. Int. J. Pharmacogn. Phytochem. Res. 9 (6), 64–872.
Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Latha, L.Y., 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr. J. Tradit. Complement. Altern. Med. 8 (1), 1–10.
Schulz, B., Wanke, U., Draeger, S., Aust, H.J., 1993. Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycol. Res. 97 (12), 1447–1450.
Sharma, V., Paliwal, R., 2013. Preliminary phytochemical investigation and thin layer chromatography profiling of sequential extracts of Moringa *oleifera* pods. Int. J. Green Pharm. 7 (1), 41–45.
Sims, J.J., Pettus Jr, J.A., 1976. Isolation of free cis and trans-phytol from the red alga *Gracilariaandersoniana*. Phytochemistry 15 (6), 1076–1077.
Stephen, S., Sanjeeva Raj, P.J., 2013. Keystone functions of *Hydrilla verticillata*. Resonance 18 (2), 156–162.
Sutton, D.L., Vandiver Jr, V.V., Hill, J.E., 2012. Grass Carp: A Fish for Biological Management of *Hydrilla* and other Aquatic Weeds in Florida. University of florida IFAS Extension: Bulletin, pp. 867.
Sykes, G., 1939. The influence of germicides on the dehydrogenases of Bacteria coli: i. The succinic acid dehydrogenase of Bact Coli. J. Hyg. 39 (4), 463–467.
Thakor, P., Mehta, J.B., Patel, R.R., Patel, D.D., Subramanian, R.B., Thakkar, V.R., 2016. Extraction and purification of phytol from Abutilon indicum: cytotoxic and apoptotic activity. RSC Adv. 6 (54), 48336–48345.
Tiselius, A., Hjerten, S., Levin, O., 1956. Protein chromatography on calcium phosphate columns. Arch. Biochem. Biophys. 65 (1), 132–155.
Urbano, M., Luque de Castro, M.D., Perez, P.M., García-Olmo, J., Gomez-Nieto, M.A., 2006. Ultraviolet–visible spectroscopy and pattern recognition methods for differentiation and classification of wines. Food Chem. 97, 166–175.
Vijayaraghavan, K., Rajkumar, J., Seyed, M.A., 2018. Phytochemical screening, free radical scavenging and antimicrobial potential of Chromolaena odorata leaf extracts against pathogenic bacterium in wound infections–a multispectrum perspective. Biocatal. Agric. Biotechnol. 15, 103–112.
Wani, P.A., Tolu, A.M., Wahid, S., 2018. Antioxidant, antimicrobial and antibiotic resistance modifying effect of Heliotropium indicum. Biocatal. Agric. Biotechnol. 15, 113–118.
Wouters, J., 1985. High performance liquid chromatography of anthraquinones: analysis of plant and insect extracts and dyed textiles'. Stud. Conserv. 30 (3), 119–128.
Xiao, X.H., Yuan, Z.Q., Li, G.K., 2013. Preparation of phytosterols and phytol from edible marine algae by microwave-assisted extraction and high-speed counter-current chromatography. Sep. Purif. Technol. 104, 284–289.
Yadav, P.P., Maurya, R., Sarkar, J., Arora, A., Kanojiya, S., Sinha, S., Raghubir, R., 2009. Cassane diterpenes from *Caesalpinia bonduc*. Phytochemistry 70 (2), 256–261.
Zhang, Z., Pang, X., Xuewu, D., Ji, Z., Jiang, Y., 2005. Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. Food Chem. 90, 47–52.