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FT-IR, GC-MS, and HPLC Profiling of the Bioactive Constituents of Ethyl Acetate Fraction of *Eichhornia crassipes* as a Hepatoprotectant

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Abstract: The study was carried out to analyze the phytoconstituents of the leaves of the aquatic weed *E. crassipes.*The plant is being traditionally used as a medicinal plant due to its bioactive constituents, but not many reports are available about *E.crassipes* as a hepatoprotection. The bioactive substances in the ethyl acetate fraction of the hydroethanolic extract of the leaves of *E. crassipes* (Mart.) Solms were analyzed by using Fourier Transform Infrared Spectroscopy (FT-IR), Gas Chromatography-Mass Spectrometer (GC-MS), and High-Pressure Liquid Chromatography (HPLC). The IR spectrum of ethyl acetate fraction of the hydroethanolic extract shows 16 peaks corresponding to peaks ranging from wave number 3624.42 cm^{-1} to 610.40 cm^{-1}. In the GC-MS analysis the existence of 3,5-bis(1,1-dimethylethyl)- ($C_8H_{12}O_3$), 7 oxabicyclo [4.1.0] heptan-1-ol, acetate($C_{14}H_{22}O$), 7,9-di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione, palmitic acid vinyl ester ($C_{18}H_{34}O_2$), phytol ($C_{20}H_{40}O$) as the major constituent of the extract. The extract was further analyzed by HPLC, indicating the presence of phytol as a major constituent that plays a vital role in hepatoprotection. Thus from the results, it is evident that it contains various bioactive compounds and can recommend as a plant of pharmaceutical importance.

Keywords: bioactive compounds; FTIR; GCMS; hepatoprotective; HPLC.

1. Introduction

According to decades of research, plants have a vital role in the safeguarding and therapy of diseases as herbal medicines. This is due to their low cost and simple accessibility. Many plants can even help avoid and mitigate traditional treatments' side effects. Presumably, they can be a potential source of phytochemical compounds, showing important biological and pharmacological activities. According to several studies, plants are also sources of effective medications and will continue to be crucial for screening new lead compounds [1]. Identifying physiologically active chemicals in plants, which leads to additional biological and pharmacological studies, is an important element of plant research [2-4]. Various plants were

investigated to extract the biologically active compounds for their therapeutic activities. However, the leaf extract of *E. crassipes* has gained tremendous attention recently due to its resistance against pathogens [5]. Many physiologically active chemicals have been extracted from *E. crassipes* since it is endowed with many potent phytochemicals like flavonoids, tannins, terpenoids, saponins, cardiac glycosides, quinones, and many others [1]. The *E. crassipes*, were introduced as an ornamental species to beautify bodies of water. Furthermore, the extracts from different parts of this plant, using either organic or water as media, have shown several pharmacological activities, like antitumor, antioxidant, and reduces inflammation, etc. [6-8]. In the current study, the ethyl acetate fraction of *E. crassipes* (leaves) shows the highest antioxidant activities, highest total phenolic, and total Flavonoid content was analyzed for the presence of hepatoprotective compounds.

2. Materials and Methods

2.1. Assemblage of the selected plant.

The aquatic plant *E. crassipes* have been chosen for its medicinal properties and procured in the summer months from Chekhla, Sanand, Ahmedabad district, Gujarat, India. The collected fresh green leaves were cleaned with water, dried, grounded, and stored in airtight containers. The plant was recognized and verified in the Patanjali Research Centre, Haridwar (Authentification no: 2626).

2.2. Preparation of extract.

The powder was macerated with hydroethanolic for 72h. The hydroethanolic crude extract was fractionated with ethyl acetate solvent. The extracts for further use were dried and stored at 4°C.

2.3. Characterizations.

2.3.1. Fourier Transform-Infrared spectrophotometry (FT-IR).

FTIR, a highly precise analytical method, is employed to identify the functional groups present in compounds and specify the structural constituents, providing a quick and non-destructive fingerprinting study of herbal extracts or powders [9-11]. The ethyl acetate extract was applied to the potassium bromide disc (KBr) disc, and the spectrum was recorded in the wave number range of 4000 cm^{-1} to 450 $cm^{-}1$ using FTIR spectroscopy (Bruker optikGmbh, Germany [12-15]. The spectrum was represented in absorption mode.

2.3.2. Gas chromatography-mass spectrometry (GC-MS).

Gas chromatography-mass spectrometry (GC–MS) is used for detecting different types of compounds, including alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters, and amino acids [16,17] isolated from ethyl acetate extract that shows the diverse bioactive therapeutic nature in medicinal plants. The ethyl acetate extract was suspended in acetone to determine the number of chemicals in the ethyl acetate fraction (HPLC grade). Filtration with Varian Bond Elute C18 solid phase extraction at a 1 mg/ml concentration was taken to eliminate impurities. For GC-MS analysis, 1µl of the above solution was used. The GC-MS (GC Clarus 500 Perkin Elmer) analysis was performed using a system

that included an autosampler (AOC-20i). The following conditions were used by the instrument: Column TG 5MS (5% phenyl methyl polysiloxane), operated at 70 eV electron impact mode; helium (99.999%), a split ratio of 10:1, an injection volume of 1µl, and a carrier gas flow rate of 1 ml/min. The injector temperature and the ion source temperature were both 200°C. . The temperature of the oven was set to 110°C (isothermal for 2 min), then increased by 10°C to 200°C/min, then 5°C to 280°C/min, with a 9-min isothermal at 280°C. The mass spectra were collected at 70 eV with a 0.5 s scan interval with fragments ranging from 40 to 650 Da. The temperature of the MS transfer line is 280°C. Using a NIST Ver.2.1MS data library, collated the spectrum acquired using GC-MS and characterized it with the chemicals present in the plant extract.

2.3.3. High-performance liquid chromatography (HPLC).

The HPLC study of *E. crassipes* ethyl acetate extract was carried out on a Shimadzu SCL-10A VP HPLC system with a model LC-10AT VP pump and UV-VIS detector. The C-18 column was Ascentis ES Cyano, 5m, Catalog #: 577307-U. (Dimensions of column: 25cm x 4.6mm.) A variable wavelength programmable photodiode array detector and a gradient HPLC (Shimadzu HPLC class VP series) with LC-10AT VP pumps (Shimadzu). Before usage, the mobile phase HPLC grade methanol was filtered through a 0.21membrane filter and pumped at a flow rate of 1ml/min from the solvent reservoir, resulting in a column backup pressure of 150 kegs/cm 2. The temperature of the column was kept constant at 27°C. Using an injector syringe, 10µl of the sample was injected (Hamilton 750NR).

3. Results and Discussion

Phytochemicals are plant-derived bioactive compounds. They are classified as secondary metabolites, naturally produced in the plant body, including the bark, leaves, stem, root, flower, fruits, and seeds, among other places. The medicinal value of a plant is determined by chemical components that have a physiological effect on the human body. Plants' the most important bioactive constituents, including alkaloids, tannins, polysaccharides, terpenoids, steroids, and flavonoids. It also showed higher total phenolics (372 ± 0.76 mg GAE/g^{-1} dry wt µg/mg of gallic acid equivalent (GAE/µg), and flavonoid content (452.6 ± 0.65 mg RE/g^{-1} dry wt.).The results of the linear regression analysis were calculated as the IC50values. The results showed ethyl acetate extract exhibit strong reducing power and antioxidant activity better than the standards. It contains significant amounts of total phenols (TPC) and total flavonoids (TFC), both of which have potent antioxidant and hepatoprotective properties [18,-20].

3.1. Fourier Transform-Infrared spectrophotometry analysis (FT-IR).

The FTIR spectrum and stretching frequencies corresponding to various functional groups of ethyl acetate extract are shown in Figure 1 and Table 1, respectively. When the extract was passed into FTIR, the functional groups of the compounds were separated based on their peak ratios [- 21]. Molecular structure and vibration of functional groups are obtained information from spectra FTIR at a certain wavenumber [~~21~~ 22]. The FT-IR spectrum shows the stretching frequency at 3624.42 cm-1commensurate to the O-H stretch, indicating the presence of potential -OH groups that can derive from alcohol or phenolic groups. In addition, the stretching frequency at 1724.03 cm^{-1} corresponds to C=O stretching, which reveals the existence of carboxylic acids and related modified esters.

Interestingly, IR frequency from 11157 cm^{-1} to 1025 cm^{-1} comparable to C-N stretching indicated the presence of related aliphatic amine chain in the extracted compounds primarily responsible for the antibacterial activity. Furthermore, the stretching frequency range 673-622 cm^{-1} attributed to C=C stretching depicted the presence of an unsaturated backbone in the compounds. Overall, FT-IR investigation established the existence of phytocompounds in the extract of the leaves. The spectra showed the existence of functional groups (O–H, C–H,C=O, N–H, N-O, O–H, (=C-H) and -C=C=H: C-H). The phytocompounds such as alkenes, alkynes, aromatic phosphates, carboxylic acid, nitro compounds, aldehydes, alcohols, and phenols were identified by the FT-IR studies are responsible for various medicinal properties of *E. crassipes*. Functional groups of a drug molecule play a critical role in its overall activity, including its interaction with the target and its mechanism of action 19.

Table 1. FTIR peaks of ethyl acetate extract of *E. crassipes.*

Wave number (cm-1)	Frequency ranges (cm-1)	Functional Groups	Phytocompounds Identified
3624.42	>3500	O–H stretch, free hydroxyl	alcohols, phenols
2925.87	3000-2850	C–H stretch	Alkanes
1724.03	1740–1720	C=O stretch	aldehydes, saturated aliphatic
1634.87	1650–1580	N–H bend	1˚ amines
1377.25	1520 –1350	N-O	Nitro compounds
1255.09	1300–1150	C–H wag (–CH2X)	alkyl halides
1157.46	1250–1020	C–N stretch	aliphatic amines
1076.48	1250–1020	C–N stretch	aliphatic amines
1025.50	1250–1020	C–N stretch	aliphatic amines
943.98	950–910	O–H bend	carboxylic acids
885.90	995-850	P-O-C stretch	Aromatic phosphates
673.91	700-650	(=C-H)	Alkenes
659.21	700-650	(=C-H)	Alkenes
642.15	650-600	(-C=C=H:C-H)	Alkynes
622.93	650-600	(-C=C=H:C-H)	Alkynes
610.40	650 – 600	(-C=C=H:C-H)	Alkynes

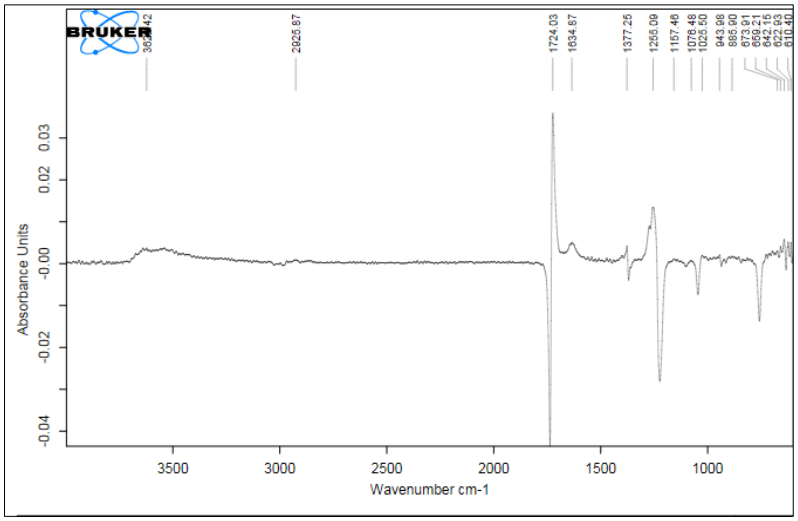


Figure 1. FT-IR spectrum of an ethyl acetate extract of *E. crassipes* leaves.

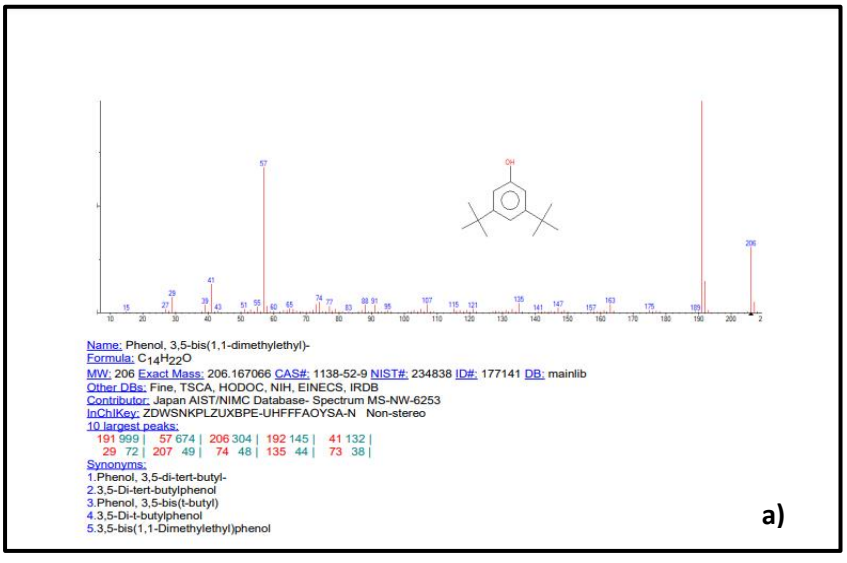
3.2. Gas chromatography-mass spectrometry (GC-MS) investigation.

In the phytoconstituent evaluation and chemotaxonomic research of medicinal plants containing biologically active components, GCMS is significant [18]. The ethyl acetate extract was studied by GC-MS technique to identify the presence of possible chemicals in the leaf extract. The GC-MS chromatograms and the possible interpretation are shown in Figure 2a-e and Table 2. The ethyl acetate fraction of *E. crassipes* leaves showed various peaks, indicating

the existence of various phytocompounds. The phytocompounds characterized were further recognized by their chemical properties (Table 2). The most prevailing components contributing to medicinal activities identified in the ethyl acetate extract of *E. crassipes* having different functional groups are Phenol,3,5-bis(1,1-dimethylethyl) antioxidants (Figure 2a); 7-Oxabicyclo [4.1.0] heptan-1-ol, acetate (Figure 2b),9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione (Figure2c); Palmitic acid vinyl ester (figure 2d), and Phytol (Figure 2e). These compounds showed pharmacological activities and were found to be antioxidant, antimicrobial, hepatoprotective, anti-inflammatory, antiulcer, antidiuretic, and ant-asthmatics, against neuro-degenerative disorders and antiarthritic [23,-25]. Among these components, phytol having a probability of 41.59, is found to be an interesting finding because of its hepatoprotective role [21]. These phytochemical constituents can be isolated and potentially novel drugs against various hepatoprotective disorders.

Table 2. GC-MS investigation of ethyl acetate extract of *E. crassipes leaves.*

Phytochemical constituents	**M.Wt**	**Retention time**	**Probability**	**Biological Activity**
Phenol, 3,5-bis(1,1-dimethylethyl)- ($C_{14}H_{22}O$)	206.167	23.56	23.00	Anti-asthmatics Bronchodilators, dermatological disorders, for treating wounds, ulcers, burns, scars, keloids, or the like, Antipsoriatics
7 Oxabicyclo [4.1.0]heptan-1-ol, acetate $C_8H_{12}O_3$	156.078	29.31	55.80	treating neurodegenerative disorders
7,9-Di-tert-butyl-1-oxaspiro(4.5)deca-6,9-diene-2 ,8-dione	276.172	37.90	62.88	Manufacture or treatment of nanostructures
Palmitic acid vinyl ester $C_{18}H_{34}O_2$	282.255	40.34	58.29	Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor Antimicrobial, Antifungal, Antitumour, Antibacterial
Phytol $C_{20}H_{40}O$	296.307	43.95	41.59	Cancer-Preventive Antimicrobial anti-inflammatory antidiuretic Antioxidant, Hepatoprotective. Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal Antimalaria



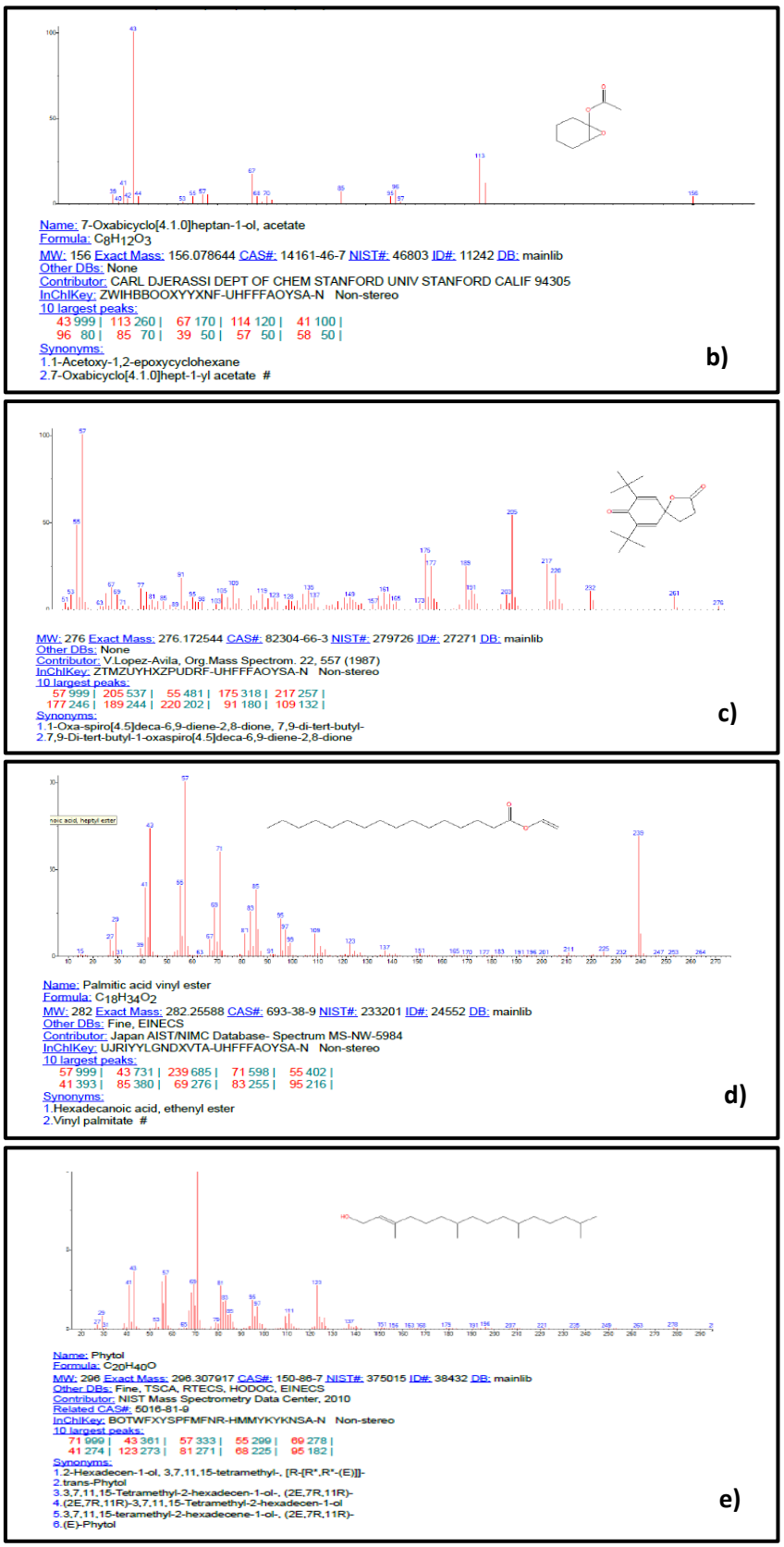


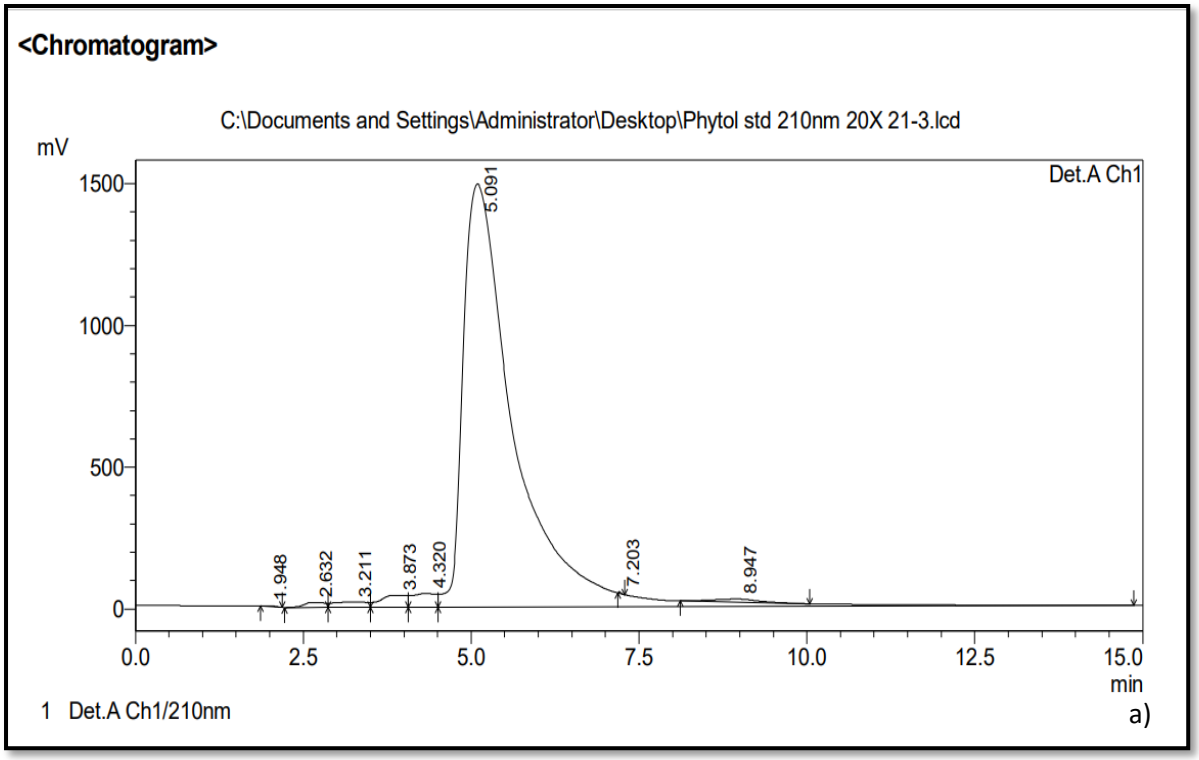
Figure 2. Phytocompounds identified from ethyl acetate extract of *E. crassipes* leaves by GC-MS analysischromatogram (a) Phenol, 3,5-bis(1,1-dimethylethyl) antioxidants, (b) 7-Oxabicyclo [4.1.0] heptan-1-ol,acetate, (c)7, 9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-diene-2,8-dione, (d) Palmitic acid vinyl ester and (e) Phytol.

3.3. High-performance liquid chromatography (HPLC) analysis.

It's the most extensively utilized liquid chromatographic technique in the analysis of drugs. It's essential for identifying and evaluating chemicals in pharmaceutical development, and it has been employed worldwide [26-30]. It was subjected to HPLC analysis to assess the presence of phytol exclusively and isolate the constituent of the extract. The HPLC profile of the ethyl acetate is represented in Figure 3. From the elution profile, it is observed that the extract contains one major phytochemical compound corresponding to retention times at 5.091. The height of each peak relates to the abundance of that compound present in the extract. The retention times at 5.091 corresponds to two solvent phases used in HPLC. In order to assign these HPLC peaks and asses GC-MS analysis, phytol was run in HPLC as the standards separately, and run profiles were shown in Figure 3, respectively. In the standard HPLC profiles, phytol runs at a retention time of 5.091 (Figure 3a). On correlating the retention times of phytol in extract and standard runs, it is observed that the phytol in the extract run was observed at a retention time of 5.070 (Figure 3b). Furthermore, based on the area under the peak of phytol from the HPLC profile of extract, it indicated that the presence of phytol has 2.3589 µg/mg of sample. Several researchers explained the use of HPLC for the quantification and characterization of secondary metabolites of plant extracts [31, 32].

Table 3. HPLC ANALYSIS of the standard and the hydroethanolic fraction of Ethyl acetate leaves of *E. Crassipes.*

Peak	Retention Time		Area		Area %	
	Std Phytol	Sample	Std Phytol	Sample	Std Phytol	Sample
1	-	3.758	-	23299036	-	43.011
2	5.091	5.070	68425614	645638	100.000	56.989



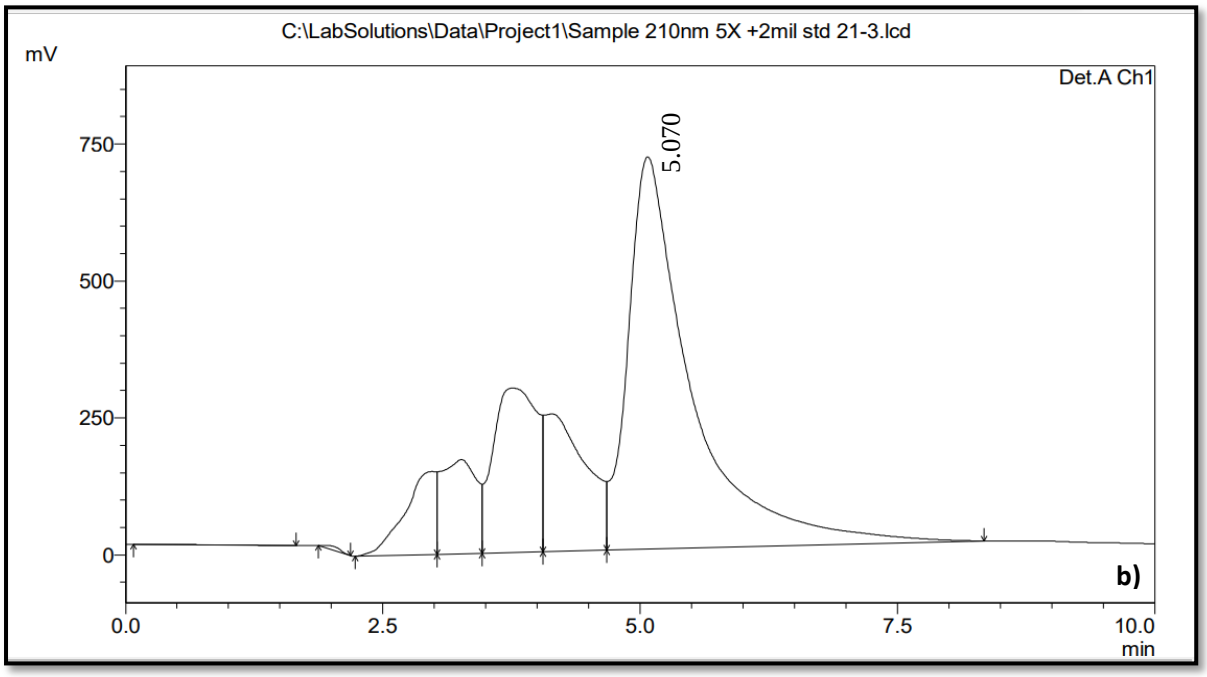


Figure 3. HPLC chromatogram of the Ethyl acetate fraction of the Hydro ethanolic extract of *E. crassipes* (a) Phytol Standard (b)Ethyl acetate fraction.

4. Conclusions

In conclusion, the phytol was isolated as the majority of components among other phytochemical compounds from the leaves of *E. crassipes* and also indicates that ethyl acetate was the best solvent for this extraction. The GC-MS chromatogram of the crude extract identified not limited to phytol but infers the other possible phytochemical compounds which have potential activity against various diseases. Phytol is a phytochemical phytoconstituents found in plants. It can be found in all types of plants in nature. The antioxidant activity, free radical scavenging activity, and additive action of phytol could be the reason behind its hepatoprotective effects. The amount of phytol is significantly more than any other constituents in the ethyl acetate extract, which indicates that the leaf of *E. crassipes* could be a great herbal asset for future drug discovery.

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Conflicts of Interest

The authors declare no conflict of interest.

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