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Bioactivity and Metabolomics Fingerprinting Characterization of Different Organic Solvents Extracts of *Padina pavonica* Collected from Abu Qir Bay, Egypt

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Abstract

In the current study, the extraction of bioactive secondary metabolites from the macroalgae *Padina pavonica* with different organic solvents were examined. The influence of three different solvents, *i.e.* ethyl acetate, acetone, and ethanol on the extraction of *P. pavonica* extracts were analyzed for major phenolic sub-groups, i.e. total phenolics, flavonoids, and tannins as well as total sulfated polysaccharide and carotenoids using spectrometric methods. Meanwhile, the total bioactive secondary metabolites were measured by GC-MS. The antioxidant activities were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the antimicrobial activities were assessed against different pathogenic bacterial and fungal strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans*.). The aim of the study was also to find the correlation between the chemical constituents of the extracts and their biological activities. The current study revealed that these extracts exhibit a good antioxidant activity, especially acetone extract, with high reducing capacity (80.58%). Also, the antimicrobial activities for acetone extract was higher than either the ethanol and ethyl acetate organic solvents due to their higher contents in bioactive compounds as reflected from their phytochemical screening and GC-MC. The GC-MS analysis revealed the presence of different phytochemical compounds resulted in 20 peaks for acetone, 14 for ethanol, and 12 for ethyl acetate. The results of this investigation could be interesting for future studies dealing with the application of *P. pavonica* in marine biotechnology, textile industry, nutrition, and pharmaceuticals for their higher antioxidant as well as antimicrobial activities.

Keywords: Antioxidant; Antimicrobial; *Padina pavonica*; family Dictyotaceae; Egypt.

Introduction

Marine algae are often known as marine macroalgae and are hard substrata or plant-like species found in coastal locations. They usually cling to undersea rocks and sand, as well as snagged on ocean surfaces and sea. Marine algae may be very microscopic or quite large [1]. The three taxonomic groups of marine algae are classified on the basis of pigmentation as red algae (Rhodophyta), green algae (Chlorophyta) and brown algae (Phaeophyta) [2]. Marine algae are rich and possibly renewable resources and its bioactive chemicals are currently being investigated [3-6]. In recent years, the demand for marine species as a prospective and promising source of pharmaceutical compounds has grown. Several studies have shown that chemicals generated by marine algae have a wide range of biological activities such as antifungal, antibacterial, antimalarial, antiviral, anti-inflammatory, antibacterial, etc. [7-12]. Moreover, they provide color pigments and dyes for many purposes such as cosmetics, pharmaceutical products, paints, etc., as these may be utilized to manufacture medical textiles for usage in hospitals due to the antibacterial qualities of the pigments [13].*P. pavonica* is a brown algae belonging to the family Dictyotaceae that can be found in warm temperate to tropical climates all over the world, including North Carolina to Florida in the United States, the Gulf of Mexico, the Caribbean, the tropical and the eastern Atlantic, Mediterranean, and Adriatic Seas [14]. *P. pavonica* can be used for its antioxidants, antimicrobial, insecticidal, antibiotics, hypo-allergic, hepatoprotective anti-inflammatory and antidiabetic activities [15-16]. *P. pavonica* seaweeds' anti-inflammatory and antioxidant properties are well-known for their medical use [17-19]. It is a source of bioactive metabolites that are not found in other species and is used in the pharmaceutical industry. These antioxidant molecules are created in the body's tissues in response to the harsh environment in which they live [20]. Antibacterial chemicals produced by macro algae of

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the *P. Pavonica* are useful in inhibiting the growth of many infections in humans and other animals [21]. Extraction is one of the most important unit operations in industries. Extraction is the typical process of separating the active ingredients and pigments of plants using selected solvents [22-23]. Many factors influence the extraction efficiency of the active agents and colorant components present in natural plant/animal/mineral sources, including:- media type (organic/aqueous solvent or acids/alkali), medium, and extraction conditions such as time, temperature, and particle size substrate, substance-liquid ratio- some physiological elements, such as vibration and the use of ultrasound [24-26]. The aim of the present study is to investigate as well as screening of bioactive compounds inclosing in *P .pavonica* using different organic solvent and determined their activities solvents.

2. Materials and Methods

2.1. Study area and collection of P. pavonica

P. pavonica were collected from the coast of Abu-Qir Bay at Alexandria of Egypt during July 2018 the sample was identified in the national institute of oceanography and fisheries (NIOF). The Bay is considered as one of the most profitable fishing areas, however, it is subjected to major threats which are related to land-based activities including urbanization and coastal development [27-35]. Samples were cleaned with fresh tap water and the epiphytes were removed completely. The cleaned samples were shade-dried at room temperature and well ground [18-19].

2.2. Extraction of bioactive metabolites from P. pavonica

The dry powder of *P. pavonica* was well extracted using three different organic solvents, namely ethyl acetate (EtOAc), acetone, and ethanol (EtOH) (absolute from sigma Aldrich) with the concentration of (70 %), by using an ultrasonic bath for 6 hours at 30°C. The organic solvents were used for extraction in a liquid ratio (algae powder in gram to organic solvents in ml) 1:20 (5gm of powder in 100 ml of organic solvent) and then the extracts were filtered separately and kept for different measurements [36].

2.3. UV-visible spectrophotometry

Screening of the extracted pigment from *P. pavonica* was done by UV-visible spectrophotometric wavelength scan. The absorbance and wavelength of the peaks were determined for the algae extracts by a wavelength scan between 380 and 980 nm. The UV-visible spectra were recorded on a UV-visible spectrophotometer (UNICO).

2.4. Phytochemical profiling. 2.4.1. Determination of total phenolic contents

Total phenolic compounds in the *P. pavonica* extract were determined according to Taga et al. [37], were expressed as mg/g gallic acid equivalent using the standard curve equation: y = 0.110, R2 = 0.998.

2.4.2. Determination of total flavonoid contents.

Total flavonoid contents were determined by a colorimetric method of Zhishen et al. [38], were expressed as mg/g quercetin equivalent using the standard curve equation: y = 0.512, R2 = 0.975

2.4.3. Determination of total tannins.

Tannins (proanthocyanidins) were determined according to Sun et al. [39], were expressed as mg/g catechin equivalent using the standard curve equation: y = 2.3934, R2 = 0.9835.

2.4.4. Determination of total carotenoid.

Total carotenoid contents were measured according to Thaipong et al. [40], were expressed as mg/g β. Carotene equivalent using the standard curve equation: y = 1.891, R2 = 0.997.

.2.4.5. Determination of total Sulfated polysaccharides.

Sulfated polysaccharides was measured according to Dodgson [41], were expressed as mg/g sulfated polysaccharide equivalent using the standard curve equation: y = 0.004, R2 = 0.9971

2.4.6. Total antioxidants capacity

Primary screening assay 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging assay of the total marine extract was performed according to Amarowicz et al.[42].

2.5. Antimicrobial assessment of *P. pavonica*

The microbial activities of different organic solvent extracts of *p. pavonica* against pathogens were determined using the agar-well diffusion method. The pathogens were two Gram-negative strains (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 15442), two Gram-positive strains (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212) and one fungus (*Candida albicans* ATCC 10231) [43].

2.6. FTIR analysis of *P. pavonica.*

FT-IR is a valuable tool for measuring many chemical constituents in marine algae. The algal samples have been studied using Fourier Transform Infrared (FTIR) technique. The composition and structure of molecular functional groups can be determined by analyzing the position, width, and intensity of infrared light absorption [44-46].

2.7. GC-MS analysis of algal extracts

Gas chromatography-mass spectrometry was used to determine the chemical components present in different organic solvent extracts, i.e., EtOAc, Aceton, and EtOH of *P. pavonica*, utilizing a DB5-MS column [30 m × 0.25mm ID J&W Scientific, USA)] with 1 mL/min flow of helium as a carrier gas. The WILEY & NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) libraries were used for recognition of the main peaks [47-51].

3. Results and discussion

The extraction efficiency is greatly influenced by the solvent used and the extraction method [45]. The organic solvents (Concentration 70%) of EtOAc, Acetone and EtOH were used in the present study to extract the bioactive compounds from the collected *P. Pavonica* algae.

3.1. UV-visible spectrophotometry.

UV-visible spectrophotometer readings of organic solvents indicated that acetone extract of *P. pavonica* showed four peaks 502, 533, 609 and 665 nm with absorbance values of 1.379, 1.245, 1.250 and 1.428, while ethanol extract showed four peaks of 504, 529, 613 and 666 nm with absorbance values of 1.245, 1.150, 1.205 and 1.359. The ethyl acetate extract showed four peaks of 502, 533, 609 and 666 nm with absorbance values of 1.151, 1.1006, 0.921 and 1.428, respectively. Based on the results of the present study (Table 1 and Figure 1), the best organic solvent for pigment extraction was acetone, followed by EtOH and finally EtOAc. and that because the higher absorption in the same wavelength range (502 up to 666) in acetone than other organic solvents which indicate the higher pigmentation contents in the acetone extracts.

Table 1. UV-VIS spectrum wavelength and absorption of organic solvents extracts of *P. pavonica*.

Organic solvent	Wavelength	Absorbance
Acetone	502	1.379
	533	1.245
	609	1.250
	665	1.428
EtOH	504	1.245
	529	1.150
	613	1.205
	666	1.425
EtOAc	502	1.151
	533	1.1006
	609	0.921
	666	1.411

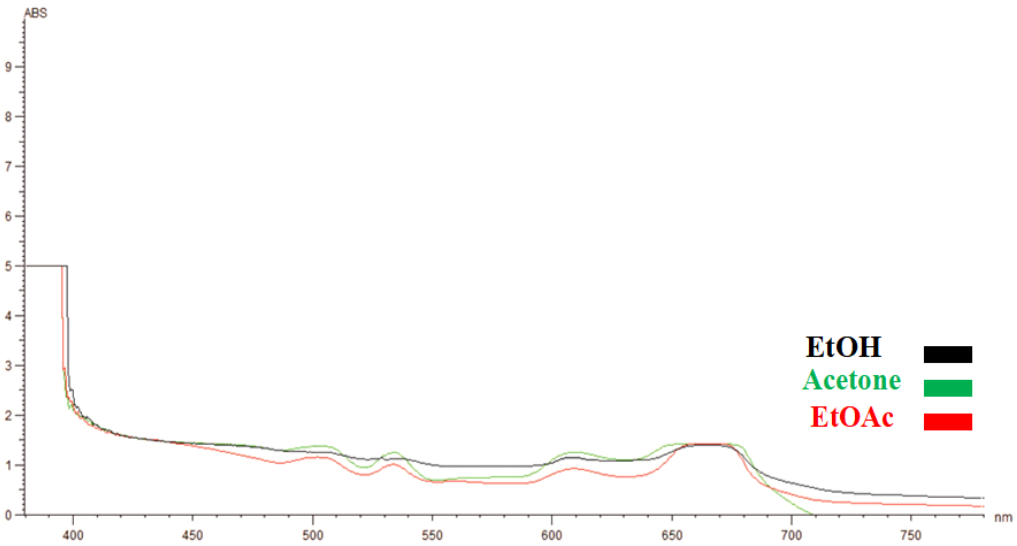

Fig. 1. The UV-visible spectrophotometer of organic solvents extracts of *P. pavonica*.

3.2.Quantitative analysis of phytochemical substances and antioxidant activity of *P. pavonica* extracts.

Several studies revealed that when it comes to extracting of bioactive compounds, water is inferior to polar organic solvents [52]. Phenolics, flavonoids carotenoids, tannins, and sulfated polysaccharides contents of *P. pavonica* were varied according to organic solvents used in the extraction process. The highest total phenolic (1.603) and total carotenoids (0.214) was measured in EtOH extract, while the highest total flavonoids (2.279), tannins (0.0396), and sulfated polysaccharides (0.06375) was observed in the acetone extract of P. pavonica. Our results are in agreement with Do et al , who used ethanol (75%) and acetone (75%) to measure total phenolic and total flavonoid. It was concluded that acetone gave a total flavonoid result (0.64) more than ethanol (0.35), but ethanol showed atotal phenolic (1.36) more than acetone (0.87) [53-54].The lowest total carotenoids (0.183), phenolics (0.9), tannins (0.0162), flavonoids (1.875) and sulfated polysaccharides (0.0415) were observed in the EtOAc extract of *p. pavonica* (Figure 2). The DPPH radical scavenging assay is widely used as a prescreening tool for novel antioxidants derived from natural sources due to its reproducibility and stability [36]. It reacts with appropriate reducing agents, to form a hydrogen bond. The number of electrons taken up by the solution loses color [56], resulting in a color change from purple to yellow. The decolonization that results is stoichiometric in terms of the quantity of electrons taken up. It was used to analyze the percentage of free radical scavenging activity presented in organic solvents extracts of *P. pavonica* (Figure 3). The acetone showed the highest percentage as an antioxidant (80.58%), followed by ethanol (74.51%) and then ethyl acetate (69.66%). Because the bioactive compounds in acetone and ethanol extracts (phenols, flavonoids, carotenoids, and tannins), are higher than those in ethyl acetate extract responsible for the antioxidant activity, and that in Agreement With Several published Reports For Examples [57-58] Pigments such as carotenoids and chlorophyll, and vitamin precursors including carotene, ascorbic acid, thiamine, niacin, and phenolic substances such as hydroquinone, polyphenols, and flavonoids may contribute to the antioxidant activity of marine algae. Phospholipids, especially peptides, terpenoids, phosphatidylcholine, and other antioxidant compounds played a role in inhibiting or suppressing oxidation processes, either indirectly or directly [18]. The chemicals are found in a wide variety of plants, including seaweeds, and have been shown to exhibit a variety of biological functions, including antioxidant properties, and polyphenols from brown algae are more powerful free radical scavengers than polyphenols from terrestrial plants [19]. The radical scavenging capacity of the extracts is proportional to their total phenolic content (TPC). Many polyphenols are powerful free-radical scavengers and contribute significantly to antioxidant activity. These are mostly due to their redox characteristics, which can aid in the adsorption of free radicals [59]. Antioxidant activities of *P. Pavonica* seaweeds are well known for their medicinal importance and reported by several workers from all over the world. They are potential sources of biologically active metabolites that are not found in other organisms and are used in the pharma and drug industry. These antioxidant molecules are created in their body tissues in response to the harsh environment in which they inhabit [60]. Alshaikheid et al. [20] investigated the efficiency or efficacy of the polyphenol fraction of *P. pavonica* for in vitro antioxidant activity using DPPH radical scavenging assay method. These findings indicated that the polyphenol fraction of *P. pavonica* is a promising bio source of compounds with antioxidant potential [12]. Many studies have been conducted to determine the antioxidant activity of organic solvents extracts of *P. pavonica* by using the DPPH radical scavenging assay method, which has been shown to be a significant antioxidant activity. The ethanolic extract of *P. pavonica* demonstrated DPPH radical scavenging activity (77.6 %) [61].

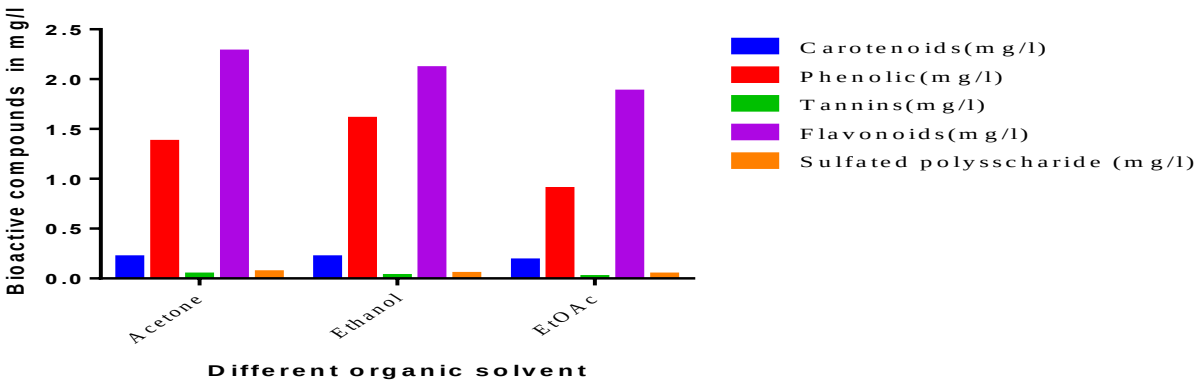

Fig. 2. The phytochemical composition of *P.avonica* extracts in different organic solvents (70%).

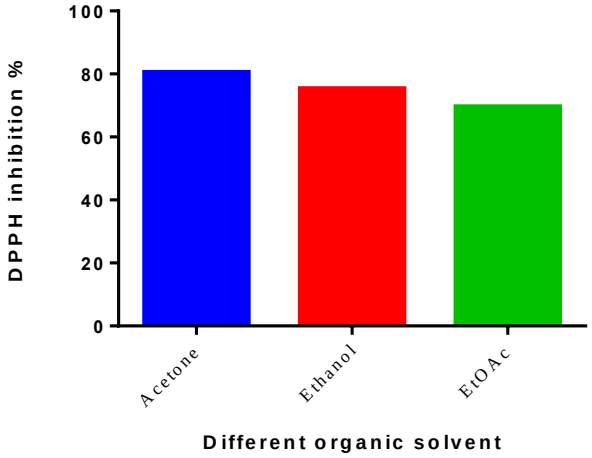

Fig. 3. The total antioxidant capacity using DPPH assay

3.3. Antibacterial and antifungal activity.

Organic solvents are often more efficient in extracting molecules with antibacterial action. Many studies have shown that organic solvents extracts of *P. pavonica are* effective antibacterial. Antifungal and antioxidant, such as using ethanol [61], methanol [62], Ethyl acetate [63] etc. The antibacterial activity of *P.pavonica* organic solvent extracts were evaluated according to their inhibition zone. The present study results (Figure 4) showed that acetone extract recorded higher activity against all Gram positive and Gram-negative bacteria followed by ethanol then ethyl Acetate. Acetone extract revealed significant antibacterial activity against *Staphylococcus aureus* (25 mm), *Escherichia coli* (23 mm), *Enterococcus faecalis* (18mm) and *Pseudomonas aeruginosa* (20 mm) .Much research has been carried out to investigate the antibacterial activity of organic solvent extracts of *P.pavonica* utilising the agar well-diffusion method, which has been demonstrated to have significant antibacterial activity. In the study of Al-Enazi et al. The ethanol extract of *P. pavonica* revealed significant antibacterial activity against *Staphylococcus aureus* (21.7 mm), *Pseudomonas aeruginosa* (19.60mm) and *Escherichia coli* (18.20) [61]. In another study of Madkour et al.(year), the effectiveness of organic solvent extracts of *P. pavonica* against different types of bacteria was measured. *Staphylococcus aureus* was the most sensitive bacteria for *P. pavonica* extracts, especially acetone extract (inhibition zone: 15 mm) [64]. On the other hand, the antifungal activity of the three organic solvent extracts revealed that the ethyl Acetate solvent had the maximum activity (25mm), followed by ethanol and acetone (23mm). In the study of Saidani *et al*., the methanolic extract of *P. pavonica* revealed significant antifungal activity against *Candida albicans* (25 mm) [65], another study of *Al-Enazi et al*., The ethanol extract of *P. pavonica* revealed significant antifungal activity against *Candida albicans* (23.7 mm) [61], another study of *Saleh* & *Al-Mariri* The ethanol extract of *P. pavonica* revealed significant antifungal activity against *Candida albicans* (16 mm) and acetone extract showed antifungal activity against *Candida albicans* (18 mm) [66].

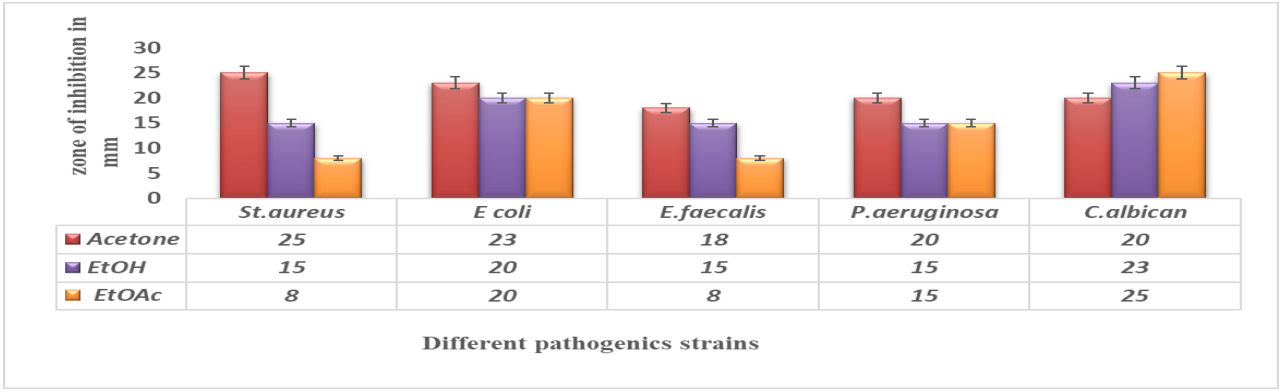

Fig.4. The antimicrobial activity of different solvents of P. pavonica (A), and Heat map with correlation analysis of effect of different organic solvent on different pathogenic Bacteria(B)

3.4. FTIR

The current study on *P. pavonica* extracts using three different organic solvents, 70% of (Acetone, EtOAc, and EtOH) showed that: The Acetone. Extract of *P. pavonica* demonstrated the presence of 13 peaks in FTIR from 13 peaks we identified 12 peaks using the standard FTIR library.The EtOH extract of *P. pavonica* shows the presence of 13 peaks in FTIR from 13 peaks we identified 10 peaks using the standard FT-IR library and The EtOAc. Extract of *P. pavonica* shows the presence of 11 peaks in FTIR from 11 peaks we identified 9 peaks using the standard FTIR library (Figure 5 & Table 2).

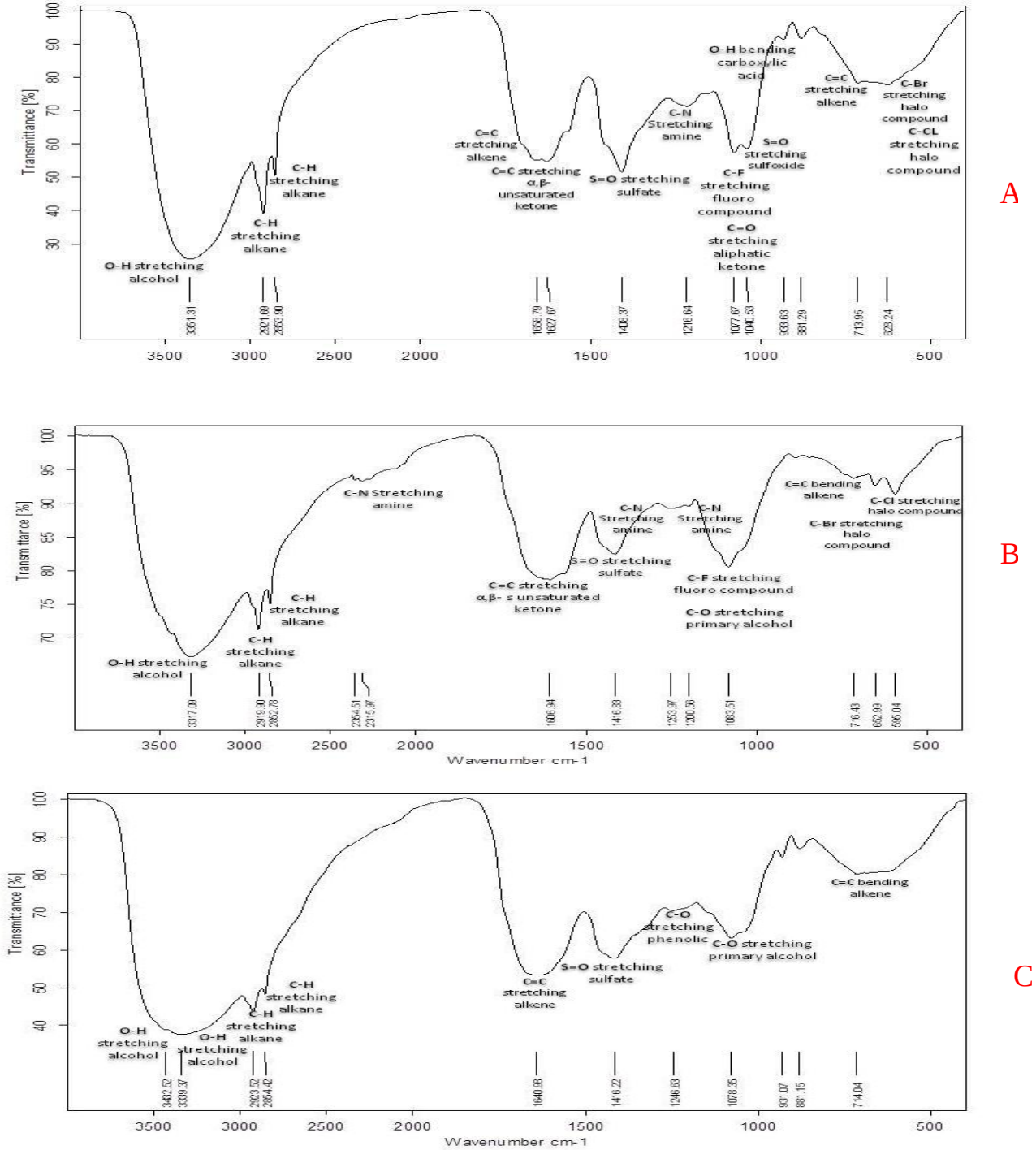

Fig. 5. FT-IR Spectrum of organic solvents extract of *P. pavonica*: (a) Acetone extract, (b) EtOH extract and (c) EtOAc.

Table 2. FTIR peak values and functional groups of different extracts of *P. pavonica*.

Functional groups	Acetone	EtOAc	EtOH	Related bioactive compounds
(O-H stretching)	3351.3071	3432.5230	3317.0932	alcohol
		3339.3692		alcohol
(C-H stretching)	2921.6887	2923.5146	2919.9004	alkane
	2853.8979	2854.4219	2852.7831	alkane
(C=C stretching)	1627.6649	___	1606.9385	α,β- s unsaturated ketone
(S=O stretching)	1408.3723	1416.2234	1416.8268	Sulfate
(C-N stretching)	1216.6382	___	1253.9649	amine
			1200.5546	amine
(C-F stretching)	1077.6732	___	1083.5109	Fluoro compound
(C-O group)	___	1246.6313	___	phenolic
(C-O stretching)	1077.6732	1078.3522	1083.5109	primary alcohol
(S=O stretching)	1040.5303	___	___	sulfoxide
(O-H bending)	933.6250	931.0739	___	carboxylic acid
(C=C bending)	713.9454	714.0408	716.4252	alkene
(C=C stretching)	1658.7943	1640.9769	___	alkene
(C-I stretching)	___	___	595.0372	halo compound
(C-Br stretching)	628.2369	___	652.9850	halo compound
(C-Cl stretching)	713.9454	___	___	halo compound
	628.2369		___	halo compound

3.5. GC-MS analysis of algal extracts

The components of acetone extract of *P. pavonica* were identified with the aid of GC-MS technique, The GC chromatogram showed twenty peaks (Figure 6). Corresponding to 35 compounds were characterized by comparing their mass spectra with those reported by NIST library. Most of the compounds had antioxidant, antimicrobial, antifungal, anti-insect anti-inflammatory, and cancer preventive properties (Table 3). The components of EtOAc extract of *P. pavonica* were identified by the aid of GC-MS technique, The GC chromatogram showed 12 peaks (Figure 6) corresponding to 17 compounds were characterized by comparing their mass spectra with those reported by NIST library. All compounds had antioxidant, antimicrobial, Anti fungi, Antiulcer, anti-inflammatory, and anti-cancer (Table 4). The components of EtOH extract of *P. pavonica* were identified with the aid of the GC-MS technique, The GC chromatogram showed 14 peaks as shown in fig 6 corresponding to 21 compounds were characterized by comparing their mass spectra with those reported by NIST library. All compounds had antioxidant, antimicrobial, Anti fungi, antiviruses, Antiulcer, anti-inflammatory, and anti-cancer (Table 5). In similar study of *Sudha* and *Balasundaram*, a total of twenty bioactive chemicals have been reported from ethanolic extract of *P. pavoica* by (GC-MS) analysis [54] In another study of *Usha* and *Rani* GC-MS analysis revealed eighteen components in the methanol extract of *P. pavonica* [67].

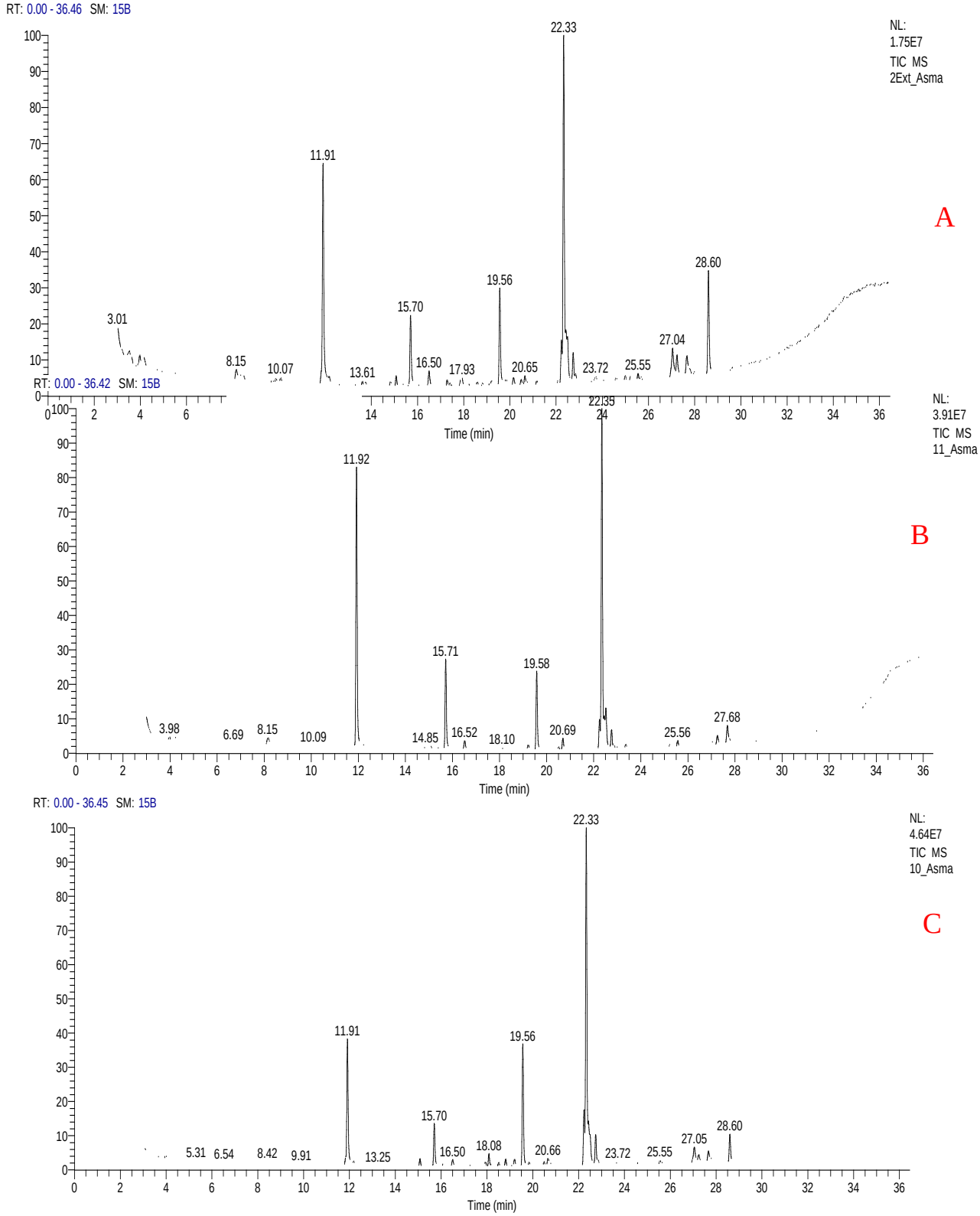

Fig. 6. GC-MS chromatogram of of organic solvents extract of *P. pavonica*: (a) Acetone extract, (b) EtOAc extract and (c) EtOH extract.

Table 3. Bioactive compounds identified by using GC-MS analysis in acetone extract of *P. pavonica*

Compound Name	RT	Area %	Molecular formula	M.W	Activity	Reference
2-Hydroxy-3-[(9E)-9-Octadecenoyloxy] Propyl (9E)-9-Octadecenoate #	3.52	1.26	C39H72O5	620	Antimicrobial	[68]
9-Octadecenoic Acid (Z)-	3.52	1.26	C18H34O2	282	Anti fungi	[69]
Oxiraneoctanoic acid, 3-octyl-, cis-	3.52	1.26	C18H34O3	298	Antifungal, Insecticidal, Antibacterial	[70]
5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-Z)-	3.97	0.72	C27H38O2	394	Cardio protective	[71]
6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	3.97	0.72	C25H36O2	368	Anticancer, Anti-inflammatory, Insectifuge	[72]
3,5-Heptadienal, 2-ethylidene-6-methyl-	8.15	1.03	C10H14O	150	Anti insect	[73-74]
1-Dodecanamine, N,N-dimethyl	11.91	18.87	C14H31N	213	Anti insect	[75]
Benzoic acid, 2,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	15.07	0.81	C16H30O4Si3	370	No activity reported	
1-Tetradecanamine, N,N-dimethyl	15.70	5.92	C16H35N	241	Anti cancer	[76]
2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S-[3à,6à(R*)]]-	16.50	1.51	C15H26O2	238	Antimicrobial	[68]
1-Nonadecene	17.28	0.60	C19H38	266	Antifungal, Anticancer	[77]
Hexadecanoic acid, methyl ester	19.56	8.10	C17H34O2	270	Antioxidant	[78]
1-Dodecanol, 3,7,11-trimethyl-	20.64	0.76	C15H32O	228	Antimicrobial	[79]
1-Hexadecanol, 2-methy -	20.64	0.76	C17H36O	256	Antimicrobial	[80]
8,11-Octadecadienoic Acid, Methyl Ester	22.23	3.12	C19H34O2	294	Antifungal	[81]
Methyl 9-cis,11-trans-octadecadienoate	22.23	3.12	C19H34O2	294	Anti bacterial	[82]
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	22.23	3.12	C19H34O2	294	Antioxidant	[83]
9-Octadecenoic acid (Z)-, methyl ester	22.33	30.50	C19H36O2	296	Anti-inflammatory, Anticancer	[84-85]
11-Octadecenoic acid, methyl ester	22.33	30.50	C19H36O2	296	Antimicrobial	[86]
6-Octadecenoic acid, methyl ester(Z)	22.42	3.93	C19H36O2	296	Antimicrobial Antioxidant	[87]

2-Methylenebrexane	22.49	2.99	C10H14	134	Antimycotoxigenic	[87]
N-Methyl-N-benzyltetradecanamine	22.49	2.99	C22H39N	317	Anti ulcer agents	[88]
Octadecanoic Acid, Methyl Ester	22.74	2.31	C19H38O2	298	Anti-tumor, cytotoxic and anti-microbial Antioxidant, Anti-inflammatory	[89-91]
Methyl stearate	22.74	2.31	C19H38O2	298	Antifungal and antioxidant	[92]
1,25-Dihydroxyvitamin D3, TMS derivative	25.55	0.59	C30H52O3Si	488	Anti-inflammatory	[93]
9,10-Secocholesta-5,7,10(19)-Triene-1,3-Diol, 25-[(Trimethylsilyl)Oxy]-, (3á,5Z,7E)-	25.55	0.59	C30H52O3Si	488	Anticancer	[94]
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	27.04	3.00	C21H40O4	356	Antimicrobial	[69]
9-Octadecenoic acid (Z)-, phenylmethyl ester	27.23	1.71	C25H40O2	372	Anti inflammatory, anti androgenic cancer	[97]
4-Hexyl-1-(7-methoxycarbonylheptyl)bicyclo [4.4.0]deca-2,5,7-triene	27.23	1.71	C25H40O2	372	Antimicrobial	[98]
Glycidyl oleate	27.66	6967886.30	C21H38O3	816	Antimicrobial, Antioxidant and Anticancer	[99]
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	27.66	3.22	C19H34O2	294	Antioxidant	[100]
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	27.66	3.22	C21H38O4	354	Anti insect	[78]
Diisooctyl phalate	28.60	9.06	C24H38O4	131-20-4	Antifungal activity Antimicrobial, Cytotoxic activity	[101-102]
1,2- Benzenedicarboxylic acid	28.60	9.06	C24H38O4	390	Antioxidant, anti microbial	[103]
Bis(2-ethylhexyl) phthalate	28.60	9.06	C24H38O4	390	Antimicrobial	[102]

Table 4. Bioactive compounds identified by using GC-MS analysis in EtOAc extract of *P. pavonica* by using GC-MS

Compound Name	**RT**	**Area %**	**Molecular formula**	**M.W**	**Activity**	**Reference**
1-Dodecanamine, N,N-dimethyl-	11.92	28.95	C14H31N	213	Anti bacterial	[101]
1-Tetradecanamine, N,N-dimethyl-	15.71	9.28	C16H35N	241	Anti bacterial	[102]
2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S-[3à,6à(R*)]]-	16.51	1.02	C15H26O2	238	Antimicrobial	[57]
Hexadecanoic acid, methyl ester	19.58	8.26	C17H34O2	270	Anti bacterial - Antifungal	[103-104]
Hexadecanoic acid, ethyl ester	20.69	1.37	C18H36O2	284	Anti-oxidant- Anti-inflammatory-	[84]
Methyl 9-cis,11-trans-octadecadienoate	22.25	2.80	C19H34O2	294	Anti bacterial	[82]
11,14-Octadecadienoic acid, methyl ester	22.25	2.80	C19H34O2	294	Antibacterial	[105]
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	22.25	2.80	C19H34O2	294	Anti inflammatory, Anti-oxidant, Anti cancer	[106-107]
9-Octadecenoic acid (Z)-, methyl ester	22.35	35.67	C19H36O2	296	Anti-inflammatory Anti cancer	[73]
10-Octadecenoic acid, methyl ester	22.44	2.98	C19H36O2	296	Antibacterial, Antifungal, Antioxidant, Decrease blood cholesterol	[108]
N-Methyl-N-benzyltetradecanamine	22.52	3.86	C22H39N	317	Anti ulcer agents	[88]
Octadecanoic Acid, Methyl Eter	22.76	1.88	C19H38O2	298	Anti-oxidant, Antiinflammatory	[84]
Methyl stearate	22.76	1.88	C19H38O2	298	Antifungal and antioxidant	[92]
2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	27.25	1.20	C22H20OS	332	Antimicrobial	[109]
Glycidyl oleate	27.67	2.72	C21H38O3	338	Antimicrobial, Antioxidant and Anticancer	[99]
9-Octadecenoic Acid (Z)-	27.67	2.72	C18H34O2	282	Anti fungi	[69]
2-hydroxy-3-[(9E)-9-Octadecenoyloxy]Propyl (9E)-9-Octadecenoate #	27.67	2.72	C39H72O5	620	Antimicrobial	[68]

Table 5. Bioactive compounds identified by using GC-MS analysis of EtOH extract of *P. pavonica*.

Compound Name	RT	Area %	Molecular formula	M.W	Activity	Reference
1-Dodecanamine, N,N-dimethyl-	11.91	14.73	C14H31N	213	Anti bacterial	[110]
Cyclooctasiloxane, hexadecamethyl-	15.08	0.81	C16H48O8Si8	592	Anti bacterial	[111-112]
1-Tetradecanamine, N,N-dimethyl-	15.7	4.95	C16H35N	241	Anti bacterial	[113]
Neophytadiene	18.08	1.43	C20H38	278	anti bacteria, anti viruses, anti fungi,	[102 & 109& 111-112]
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	18.08	1.43	C20H40O	296	Antimicrobial, anti-inflammatory	[72]
2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	18.08	1.43	C20H38	278	anticancer	[57]
17-Octadecynoic acid	18.81	0.88	C18H32O2	280	antimicrobial,	[48]
Hexadecanoic acid, methyl ester	19.56	13.92	C17H34O2	270	Anti bacterial - antifungal	[87 & 94]
Methyl 9-cis,11-trans-octadecadienoate	22.23	5.8	C19H34O2	294	Anti bacterial	[82]
Methyl 10-trans,12-cis-octadecadienoate	22.23	5.8	C19H34O2	294	antimicrobial, antioxidant and anticancer	[49]
9-Octadecenoic acid (Z)-, methyl ester	22.33	39.6	C19H36O2	296	Anti-inflammatory anti cancer	[84-85]
11-Octadecenoic acid, methyl ester	22.33	39.6	C19H36O2	296	antimicrobial	[86]
N-Methyl-N-benzyltetradecanamine	22.5	2.28	C22H39N	317	Anti ulcer agents	[88]
Methyl stearate	22.74	3.31	C19H38O2	298	Antifungal and antioxidant	[92]
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	27.04	2.43	C21H40O4	356	Antimicrobial	[69]
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	27.04	2.43	C21H40O4	356	Antimicrobial, Anticancer	[115]
Glycidyl oleate	27.66	1.85	C21H38O3	338	Antimicrobial, Antioxidant and Anticancer	[99]
2-Hy-3-[(9E)-9-Octadecenoyloxy]Propyl (9E)-9-Octadecenoate #	27.66	1.85	C39H72O5	620	Antimicrobial	[68]
9-Octadecenoic Acid (Z)-	27.66	1.85	C18H34O2	282	Anti fungi	[69]
Diisooctyl phthalate	28.6	3.42	C24H38O4	390	Antifungal activity Antimicrobial and Cytotoxic Activity	[101-102]
Bis(2-ethylhexyl) phthalate	28.6	3.42	C24H38O4	390	Antimicrobial	[102]

Conclusion

The present study revealed that *P. pavonica* are an excellent source of bioactive compounds such as polyphenolic contents (phenolic, flavonoids and tannic acids) which can be used as a potential agent in the textile, cosmetic, pharmaceutical and nutrition industries. The results of UV-visible spectrophotometry, GC-MS analysis, phytochemical profiling, antioxidant and antimicrobial activities revealed that the acetone extract is the best solvent for extracting bioactive compounds from *P. pavonica*, followed by ethanol and then ethyl acetate. Further research studies are recommended for investigation of other extraction parameters as will as other biological properties of *P.povonica* extracts.

Data Availability Statement

The data presented in this study are available within the article

Conflicts of Interest

The authors declare no conflict of interest.

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