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Research Article

Terpenes and Sterols Composition of Marine Brown Algae *Padina pavonica* (Dictyotales) and *Hormophysa triquetra* (Fucales)

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ABSTRACT

In this study the terpenes and sterols composition were identified and estimated qualitatively and quantitatively from the brown algae *Padina pavonica* (Dictyotales) and *Hormophysa triquetra* (Fucales) by using GC/MS (Gas Chromatography-Mass Spectrum). Significant differences were found in the terpenes and sterols composition of the selected algae. The analysis revealed the presence of 19 terpenes in *Padina pavonica* and 20 terpenes in *Hormophysa triquetra*, in addition to 5 sterols recoded in both of them.The total concentration of terpenes in *Hormophysa triquetra* recorded the highest percentage than *Padina pavonica*. In contrast, *Padina pavonica* registered high content of sterols than those in *Hormophysa triquetra*. The main terpene component was the hemiterpene 3-Furoic acid recording in *Hormophysa triquetra* more than in *Padina pavonica*. The diterpene phytol compound occupied the second rank according to their concentration percentage in both of the studied species. *Hormophysa triquetra* characterized by alkylbenzene derivatives more than *Padina pavonica*.Fucosterolwas the major sterol component in both of the selected algae recording a convergent concentration in *Padina pavonica* and *Hormophysa triquetra*. β- Sitosterol was detected only in *Padina pavonica* whereas β–Sitostanol and Stigmasterol were characterized in *Hormophysa triquetra*. Campesterol was found in the two studied species. Some of the high molecular weight compounds of sterols distinguished by binding of a sugar moiety (Steryl glycosides) and sodium adduct (Na^+). Stigmasteryl- glucoside+Na^+ found in *Padina pavonica* only whereas β -sitosteryl- glucoside+ Na^+ detected at the two selected species. Medicinal importance of terpenes and sterols in these brown species was discussed.

Keywords: GC-MS, *Hormophysa triquetra*, *Padina pavonica*,Sterols, Terpenes

INTRODUCTION

Terpenoids are a natural product encompass more than 40 000 structures and form the largest class of all known plant metabolites[1]. Some terpenoids have well-characterized physiological functions that are common to most plant species. In addition, many of the structurally diverse plant terpenoids may function in taxonomically more discrete, specialized interactions with other organisms. Plant terpenoids are widely used as industrially relevant chemicals, including many pharmaceuticals, flavours, fragrances, pesticides and disinfectants, and as large-volume feedstocks for chemical industries[2]. Currently, there is an increased interest in natural substances with valuable medicinal properties, such as terpenoids[3]. Some of the terpenes are most potent drugs against life threatening disease such as cancer[4], malaria[5] and heart disease[6]. Some terpenes also show insecticidal properties[7]. The marine environment is a unique source of bioactive natural products, many of which exhibit structural features not found in terrestrial natural products[8]. Marine seaweeds are rich in bioactive compounds that could potentially be exploited as functional ingredients for both human and animal health applications[9]. Marine macroalgae produce a diverse array of secondary metabolites characterized by a broad spectrum of biological activities, including terpenes, sterols, polyphenols, acetogenins and others[10].

More than 1,140 secondary metabolites have been reported from Phaeophyceae[11]. Species of Dictyotales (brown algae) produce a large array of bioactive secondary metabolites possessing a broad defensive action against herbivores in the marine environment[12]. Almost a third of the reported brown algal chemistry comes from a single genus, *Dictyota*, which has elaborated a wealth of terpenes[13]. Gupta and Abu-Ghannam[9] reported that diterpenoids and sesquiterpenoids have cytotoxic, antiviral and algicidal activities.

The presence of sterols in algae was first established by Heilbron *et al*.[14] and later by Tsuda *et al*.[15]. Sterols are known to reduce the blood plasma cholesterol[16]. Most Phaeophyta contained traces of cholesterol.Fucosterol is the main sterol in brown algae[17]. It is biosynthesized through alkylation of 24-methylenecholesterol. It was reported for its antioxidant and hepatoprotective[18], anti-diabetic[19], butyrylcholinesterase inhibitory activities. Sterols may be found either as free sterols, acylated (sterol esters), alkylated (steryl alkyl ethers), sulfated (sterol sulfate), or linked to a glycoside moiety (steryl glycosides) which can be itself acylated (acylated sterol glycosides). Sterol glucosides (SG) were identified in residues that shut down a commercial biodiesel plant. Significant differences in the sterol composition were found within the different

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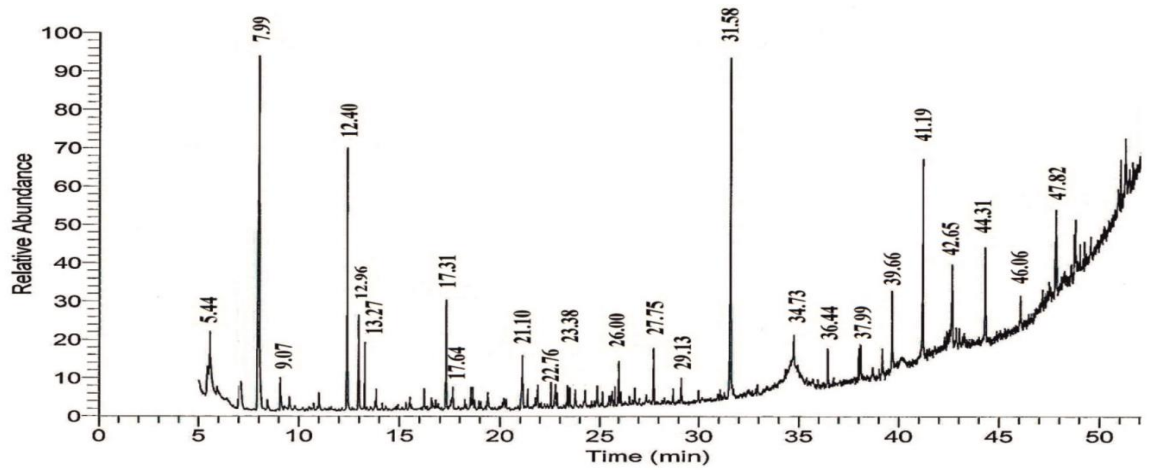

Fig. 1: GC/MS spectra showing terpenes and sterols composition of Padina pavonica

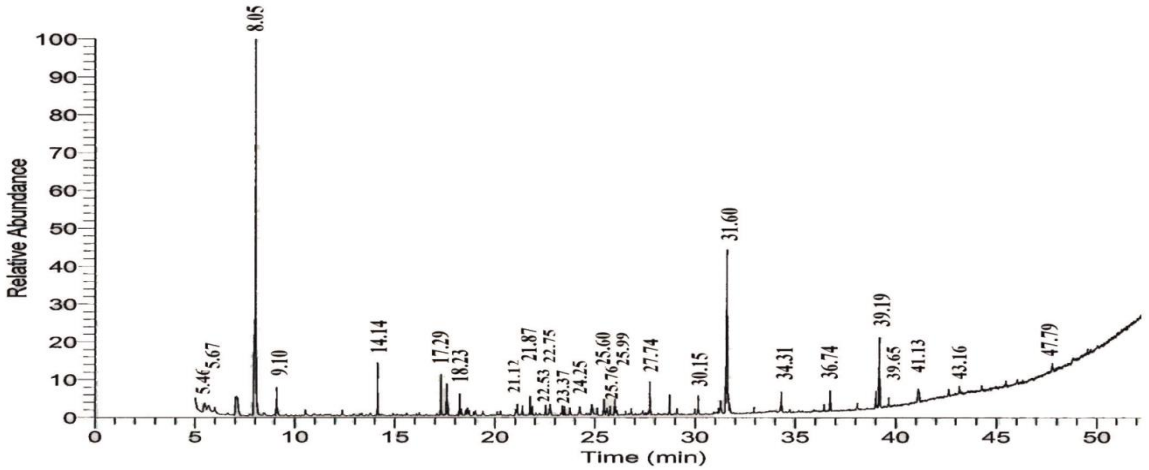

Fig. 2: GC/MS spectra showing terpenes and sterols composition of Hormophysa triquetra

Padina species. Terpene and sterol constituent's researches of *Padina pavonica* recognized little information, while it's not available of *Hormophysa triquetra.* The present study aims to analyze and compare the terpenes and sterols composition of *Padina pavonica* and *Hormophysa triquetra* by using GC/MS (Gas Chromatography- Mass Spectrum).

MATERIALAS AND METHODS

Algae material: The brown algae, *Padina pavonica* (Linnaeus) Thivy (Dictyotales) and *Hormopysa triquetra* (C. Agardh) Kützing. (Fucales), were collected from Al Shoaiba area, Saudi Arabia, Red Sea in 2010 (20° 48` - 20° 51` N and 39° 24` - 39° 28` E). These two species were found abundantly in Al Shoaiba coast most of the seasons. They were identified by Papenfuss[21] and Womersley[22]. The brown algae samples were washed with tap water to remove dirt, sand and then dried in air temperature until constant weight. The dried algae were ground in an electric mixer.

Extraction and saponification: The acetone extract of 150g dry weight of algae was evaporated untile dryness and then redissolved in 10% KOH in methanol, and refluxed on water bath for 4 hours (at temperature not more than 50°C) for achieving the saponification process. The reaction mixture should not be allowed to go to dryness, until the salts have been converted to acids[23]. The saponified matter was extracted with ether several times to obtain terpenes and sterols.

Identification and estimation by GC/MS analysis: GC/MC (Gas Chromatography- Mass Spectrum) analysis had been achieved at National Research Centre, El Doki. The GC-MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was employed as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 50°C (hold 3 min) to 300°C as a final temperature at an increasing rate of 5°C /min (hold 5 min). The quantitative estimation of all the identified components was investigated using a percent relative peak area. The identification of the compounds was performed based on the comparison of their relative retention time (Rt) and mass spectra with those of the NIST, WILLY library data of the GC/MS system**.**

Statistical data analysis: The statistical analyses were performed using Minitab (version 16.1). Multivariate descriptive analyses were processed employing to Pearson's correlation of terpenes and sterols concentration percentage between *Padina pavonica* and *Hormophysa*

Table 1: Terpene components identified in *Padina pavonica* and *Hormophysa triquetra* by GC-MS

Compound Name	M.W.	M.f.	*Padina pavonica* Conc. %	Rt	*Hormophysa triquetra* Conc. %	Rt
1. 4-Cyclopentene-1,2,3-trione	115	$C_5H_2O_3$	1.20	5.44	-	-
2. D-limonene	136	$C_{10}H_{16}$	-	-	1.33	5.46
3. 1,7,7-Trimethyl-camphor	152	$C_{10}H_{16}O$	-	-	0.67	5.67
4. 3-Furoic acid	112	$C_5H_4O_3$	13.39	7.99	25.64	8.05
5. 2-ethylbutanoic acid	132	$C_6H_{12}O_2$	1.08	9.07	-	-
6. 1,3,4 Trimethoxy-butanol	164	$C_7H_{16}O_4$	-	-	2.04	9.10
7. Menthol	156	$C_{10}H_{20}O$	6.70	12.40	-	-
8. Geraniol	159	$C_{10}H_{18}O$	2.22	12.96	-	-
9. 2-ethylhexyl acrylate	184	$C_{11}H_{20}O_2$	1.33	13.27	-	-
10. 2,4-bis(1,1-dimethylethyl)phenol	206	$C_{14}H_{22}O$	-	-	2.60	14.14
11.2,6-di(t-butyl)-4-hydroxy- 4-methyl-2,5-cyclohexadien-1-one	236	$C_{15}H_{24}O_2$	2.64	17.31	2.60	17.29
12. Palmitoyl	239	$C_{16}H_{31}O$	0.79	17.64	-	-
13. Butylhydroxy Toluene	220	$C_{15}H_{24}O$	-	-	1.49	18.23
14. Benzene, (1-ethylnonyl)	232	$C_{17}H_{28}$	2.16	21.10	0.67	21.87
15.Benzene, (1-butylheptyl)	232	$C_{17}H_{28}$	-	-	0.82	21.12
16. Benzene, (1-methyldecyl)	232	$C_{17}H_{28}$	0.93	22.76	1.06	22.75
17. Heptadecane	240	$C_{17}H_{36}$	-	-	0.63	22.53
18 Benzene, (1-pentylheptyl)	246	$C_{18}H_{30}$	0.68	23.38	0.67	23.37
19. Benzene, (1-ethyldecyl)	246	$C_{18}H_{30}$	-	-	0.75	24.25
20. Benzene, (1-pentyloctyl)	260	$C_{19}H_{32}$	-	-	0.67	25.60
21. Benzene, (1-butylnonyl)	260	$C_{19}H_{32}$	-	-	0.63	25.76
22. 2-pentadecanone,6,10,14-trimethyl	268	$C_{18}H_{36}O$	1.24	26.00	1.43	25.99
23. Hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$	1.49	27.75	2.17	27.74
24. Hexadecanoic acid ethyl ester	284	$C_{18}H_{36}O_2$	0.71	29.13	-	-
25 9-Ecosene	280	$C_{20}H_{40}$	-	-	1.30	30.15
26. Phytol	296	$C_{20}H_{40}O$	10.70	31.58	15.45	31.60
27. Ambrein	428	$C_{30}H_{52}O$	1.34	34.73	1.48	34.31
28. n-Dotriacontane	450	$C_{32}H_{66}$	0.96	36.44	-	-
29. Sorbitan	446	$C_{24}H_{46}O_7$	0.78	37.99	-	-
30. 3', 4'-Didehydro-β,ψ-carotene	539	$C_{40}H_{54}$	2.33	39.66	1.42	36.74

triquetra ($P \leq 0.05$), in addition to analysis one way ANOVA was tested for Mean±standard deviation (M±StDev).

RESULTS AND DISCUSSION

Figures 1 and 2 showed terpenes and sterols composition of *Padina pavonica* and *Hormophysa triquetra* respectively as detected by using GC-MS spectra technique. Retention time and the relative abundance of each compound were recognized.The GC-mass analysis revealed the presence of 19 peaks of terpenes in *Padina pavonica* compatible with their fragmentation patterns and 20 terpenes in *Hormophysa triquetra*, in addition to 5 sterols recoded in both of them. The total concentration of terpenes in *Hormophysa triquetra* recorded the highest percentage (64.85%) than those of *Padina pavonica* (52.67%). In contrast, *Padina pavonica* registered high content of sterols (15.3%) more than in *Hormophysa triquetra* (8.93%). The terpene components in *Padina pavonica* were separated at retention time interval from 5.22 to 36.44min, while sterols interval from 39.19 to 47.82min as shown in Figure 1. Terpenes of *Hormophysa triquetra* detected at retention time interval ranging from 5.46 to 36.76min and from 39.65 to 47.79min for sterol components (Figure 2).

Table 1 summarized the terpene components, retention time (Rt), molecular weight (M.W.), molecular formula (M.f) and concentration percentage in *Padina pavonica* and *Hormophysa triquetra*. Thirty one compounds of the terpene constituents were recognized at the two selected algal species. *Padina pavonica* contained 19 terpene compounds whereas *Hormophysa triquetra* registered 21 compounds. There was a significant difference in terpenes content between the studied species. The Pearson correlation of terpenes between *Padina pavonica*and *Hormophysa triquetra* of terpenes concentration % was found highly significance (ρ-Value = 0.000). *Padina pavonica* recorded M±StDev 1.756 ± 3.119 whereas *Hormophysa triquetra* registered 2.184 ± 5.227. It was noted that some of the Shared same compounds in the studied species showed slightly differences in their retention time. This is may be attributed to the sequence of increasing polarity of the separated compounds that detected in GC- mass detector of each species.

The main component in *Padina pavonica* and *Hormophysa triquetra* was the hemiterpene (C_5) (3-Furoic acid) of the

Table 2: Sterol components identified of *Padina pavonica* and *Hormophysa triquetra* by GC-MS

Compound Name	M.W.	M.f.	*Padina pavonica* Conc. %	Rt	*Hormophysa triquetra* Conc. %	Rt
1.Fucosterol 24-ethylidene cholest-5-en-3β-ol	412.69	$C_{29}H_{48}O$	5.16	41.19	5.28	39.19
2.β - Sitosterol (3β)-Stigmasta-5-en-3-ol	414.71	$C_{35}H_{50}O$	2.68	42.65	-	-
3.β - Sitostanol 5α-stigmastan-3β-ol	416.73	$C_{29}H_{52}O$	-	-	0.63	39.65
4.Stigmasterol 3β-Hydroxy-24-ethyl-5,22-cholestadiene	412.69	$C_{29}H_{48}O$	-	-	1.54	41.13
5.Campesterol (24R)-Methylcholes	400.68	$C_{28}H_{48}O$	3.14	44.31	0.70	43.16
6.Stigmasteryl- glucoside+Na^+	597.41	$C_{35}H_{58}O_6$+Na^+	1.20	46.06	-	-
7.β -sitosteryl- glucoside+Na^+	599.43	$C_{35}H_{60}O_6$+Na^+	3.12	47.82	0.78	47.79

molecular weight (112) and detected approximately at the same retention time 8 minutes. The 3-Furoic acid of *Hormophysa triquetra* recorded high concentration (25.64%) than that of *Padina pavonica* (13.39%). 3-Furoic acid exhibits hypolipidemic activity in rodents and lowers serum cholesterol and serum triglyceride levels in mice and rats[24].

The diterpene phytol (C_{20}) occupied the second rank according their concentration percentage in the studied species. The concentration of phytol in *Hormophysa triquetra* was (15.45%), while (10.70%) in *Padina pavonica*. Claude[25] mentioned that the diterpenes have exceptionally open chain, as found in phytol which forms a part of chlorophyll. Ghazala and Shameel[26] identified diterpene phytol from brown algae while Gupta and Abu-Ghannam[9] confirmed that several types of diterpenoids and sesquiterpenoids have been found to be the main secondary metabolites of the species belonging to Dictyotales. Bianco *et al*.[27] mentioned that if geographical diterpene distribution patterns may be considered within an evolutionary perspective, rather than as a purely ecological topic[28], the defensive action of diterpene in *Dictyota* species adds a new component to enable us to understand the complex world of the evolution of defensive chemistry and chemotaxonomy in seaweeds. The bio-activities of diterpenes especially Dictyotaceae attributed to some of their metabolites include ichthyotoxicity, phyotoxicity, antibiosis, cytotoxicity, antifungal, antiviral, insecticidal antifeedant properties in addition to activity against the Eatumorin mice[29].

The volatile organic compounds (Alkylbenzene) groups were found in the selected species mainly in *Hormophysa triquetra*. Alkylbenzenes were represented by 7 compounds in *Hormophysa triquetra* [Benzene, (1-butylheptyl); Benzene, (1-ethylnonyl); Benzene, (1-methyldecyl); Benzene, (1-pentylheptyl); Benzene, (1-ethyldecyl); Benzene, (1-pentyloctyl) and Benzene, (1-butylnonyl)] whereas 3 compounds [Benzene, (1-ethylnonyl); Benzene, (1-methyldecyl) and Benzene, (1-pentylheptyl)] were only detected in *Padina pavonica*. Alkylbenzenes play the role of pheromone in marine brown algae [30] as well, enhancing the sweet aroma of a ripening mango. Alkylbenzenes are important constituents of vehicular fuels and exhausts[31].

The triterpenes (C_{30}) Ambrein concentration percentage in *Padina pavonica* was 1.34% and 1.48% in *Hormophysa triquetra*. Ambrein was used as an analgesic[32]. 3', 4'-Didehydro-β,ψ-carotene as a tetraterpene (C_{40}) was detected in *Padina pavonica* (2.33%) more than in *Hormophysa triquetra* (1.42%). Among the important group (Carotenoids) β-carotene perform three major functions in plants: accessory pigments for light harvesting, prevention of photooxidative damage and pigmentation attracting insects[25]. The sesquiterpene (C_{15}) 2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5cyclohexadien-1-one found in *Padina pavonica* and *Hormophysa triquetra* recording 2.64 and 2.60% respectively. Sesquiterpenes have generally potent antibacterial and antifungal activities, and they are toxic to several invertebrates and also in fish[25]. The monoterpene (C_{10}) menthol characterized only in *Padina pavonica* (6.70%). Monoterpenes are an important constituent of essential oils in plants.

There was no significance of sterol concentration % between *Padina pavonica* and *Hormophysa triquetra*. The Pearson correlation was ρ-Value=0.1. M±StDev of *Padina pavonica* registered 2.186 ± 1.889 and *Hormophysa triquetra* was 1.276 ± 1.842. Table 2 showed the sterol compounds in both of *Padina pavonica*and *Hormophysa triquetra*. Fucosterol was the major sterol in the studied species recording approximately similar concentration percentage 5.16% in *Padina pavonica* and 5.28% in *Hormophysa triquetra*. This was agreement with Kamenarska *et al*.[33] who mentioned that Fucosterol predominated in *Padina gymnospora*, *Padina crassa*,*Padina vickersiae*. In contrast, they found that the main sterol in *Padina pavonia* from the Aegean Sea was Cholesterol (34.0%) then Fucosterol (24.3%). Kamenarska*et al*.[33] compared the sterol composition of *Cystoseira crinita* from two different seas: the Black Sea and the Mediterranean Sea, which differ strongly in salinity and water temperature. He found the fucosterol concentrations in the two samples were very close and the concentrations of cholesterol and 24-methylenecholesterol were significantly increased. Furthermore in the Black

Sea, Kamenarska *et al*.[34] present low concentrations of fucosterol in the brown algae *Stilophora rhizodes*, *Punctaria latifolia* and *Punctaria plantaginea* and the main sterol composition was cholesterol and 24-methylenecholesterol. Ikekawa[35] mentioned that sterol content in Chlorophyta is more similar to higher plant, and it contains large amount of cholesterol while in Phaeophyta, fucosterol is the main sterol and small amounts of cholesterol, 24-methylene-cholesterol and saringosterol. In a sample of *Padina pavonia* from the Aegean Sea, Kanias *et al*.[36] was found only four sterols (cholesterol, fucosterol, stigmasterol and campesterol), comprising 98% of the total sterol mixture. The differences found in the sterol composition could be due to differences in the life cycle of the algae[37] and to differences in the ecological conditions[38]. El Gamal[39] also created Fucosterol from brown algae whereas Kumar*et al*.[40] isolatedFucosterol from the brown alga *Turbinaria conoides* and he found that Fucosterol shows significant antihistaminic and anticholinergic activities.

β- Sitosterol [(3β)-Stigmasta-5-en-3-ol] was recorded only in *Padina pavonica* (2.68%) whereas β–Sitostanol [5α-stigmastan-3β-ol] and Stigmasterol [3β-Hydroxy-24-ethyl-5,22-cholestadiene] was characterized in *Hormophysa triquetra* recording 0.63 and 1.54% respectively. β-Sitosterol is present in all plant lipids and is used for steroid synthesis. Stigmasterol is used for the synthesis of progesterone and vitamin D3, act as a potential anti-inflammatory compound[41]. Zelazny *et al*.[42] showed that free sterols in the plasma membrane of the marine alga *Dunaliella* are absolutely required for sensing osmotic changes.In our study, Campesterol [(24R)-Methylcholest-5-en-3β-ol] was registered in the two studied species. *Padina pavonica* (3.14%) contain concentration of Campesterol more than *Hormophysa triquetra* (0.70%). Campesterol was widespread occurrence in plants reduced the permeability of biomembranes.

Some compounds of sterols in the selected species distinguished by binding of a sugar moiety (Steryl glycosides) and sodium adduct (Na^+). Steryl glycosides (SG) are generically called 'phytosterol conjugates.Moreau *et al*.[43] mentioned that most of the SG is localized in the plasma membrane and the involvement of SG in a biosynthetic process that occurs adjacent to the plasma membrane which is a reasonable hypothesis. In nature there is a distribution of isotopes for all elements as sodium adduct (Na^+). The monoisotopic peak corresponds to the exact mass of a particular formula. The masses will increase by M^{+1}, M^{+2}, and so on where additional neutrons are incorporated somewhere into the formula. These isotopic distributions are very predictable in natural systems without enrichment. The observed isotopic distribution patterns match closely with the theoretical isotopic distribution patterns. The use of isotopic patterns was found to be extremely useful for quickly assessing other unknown peaks and ascertaining if there were large organic compounds present in the sample rather than noise/contamination peaks[44].

Our data concerning revealed that the presence of sugar moiety and Na^+ adduct at high molecular weight 597.41 and 599.43 as shown in Table 2. *Padina pavonica* recorded 3.12% of β-sitosteryl-glucoside+Na more than *Hormophysa triquetra* (0.78%). Stigmasteryl-glucoside+Na found in *Padina pavonica* (1.20%) only. The sterol glucoside sodium adducts observed in this study as a characteristic isotopic abundance patterns. The attachment of a sugar moiety to the 3-hydroxy group of a sterol drastically increases the size of the hydrophilic part of the lipid. It is obvious that the glycosylation of a considerable fraction of membrane bound free sterols alters the biophysical properties of the membrane[45]. Steroid glycosides are derivatives of a typical membrane-bound sterol molecule and carry one or more sugar residues at the 3-hydroxy group at other hydroxy groups in the rings, or at the side chain of a modified sterol backbone. Khatun *et al*.[46] showed the biological activity of stigmasterol and stigmasterol glucoside. They domenstrated that these sterol glucoside contain more hydrophilic part (glucose moiety) and these glucosides is being thought to create effective hindrance to the esterification of cholesterol and thus resulting in the inhibition of entry of cholesterol into the blood vessel.

CONCLUSION

The brown algae *Padina pavonica* (Dictyotales) and *Hormophysa triquetra* (Fucales) are rich of terpenes and sterols composition. Significant differences were found in the amounts and types of the terpenes and sterols in the two algae. The analysis by GC/MS revealed that *Hormophysa triquetra* contain 20 compounds of terpenes more than in *Padina pavonica* which included 19 terpene compounds in addition to 7 compounds of sterols recording 5 for each. The total concentration of terpenes in *Hormophysa triquetra* recorded the highest percentage than those of *Padina pavonica*. In contrast, *Padina pavonica* registered high content of sterols more than in *Hormophysa triquetra*. The main component of terpenes in the studied specieswas hemiterpene 3-Furoic acid Followed by diterpene phytol.*Hormophysa triquetra* recorded high concentration of the previous two terpenes than *Padina pavonica*. The selected species contained approximately similar concentration percentage of the main sterol Fucosterol. Some compounds of sterols in the selected species distinguished by binding of a sugar moiety (Steryl glycosides) and sodium adduct (Na^+) at high molecular weight 597.41 and 599.43. Pharmaceutical significances of terpenes and sterols components in these species were discussed.

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