Trop J Nat Prod Res, March 2023; 7(3):2574-2579 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Extraction Optimization, Analysis and Biological Activity of Volatile Compounds from *Padina pavonia* Collected from Farasan Island's Coasts, Jazan, Saudi Arabia

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ARTICLE INFO

Article history:
Received 15 January 2023
Revised 01 March 2023
Accepted 03 March 2023
Published online 01 April 2023

ABSTRACT

Marine brown algae are a source of a broad spectrum of active compounds with nutritional and pharmaceutical applications. This study aimed to identify the essential oil (EO) compounds and assay the antimicrobial and antioxidant activity of ethanol and acetone extracts of *Padina pavonia* harvested from Farasan Island's coasts in the Red Sea, Saudi Arabia. Steam distillation was employed to extract the EO and analyze using GC-MS. Forty-one compounds were identified in the volatile fraction of *P. pavonia*, accounting for 89.23% of the total oil. Fatty acids were the primary class of volatile compounds (44%). A freeze-dried algal sample was used for solvent extraction. Optimized extraction with ethanol (50%) and acetone by Soxhlet extractor at 60°C for 2h yielded extracts with high antioxidant activity (IC_{50}: 143 and 222 µg/mL), respectively. Acetone extract showed higher broad-spectrum antimicrobial activity against bacteria and fungi than ethanol 50% extract. The results of this paper present the first report on essential oils from *P. pavonia* in the Red Sea.

Keywords: *Panida pavonia*, Brown algae, Essential oils, Antioxidant activity, Antimicrobial activity

Introduction

In the marine environment, volatile compounds released from marine algae under biotic or abiotic stress play a significant role in communication,[1] attraction, reproduction,[2] and defense against predators.[3-5] It was reported that marine brown algae have been described as an excellent source of different compounds with antioxidant, antimicrobial, antitumor and anticancer activities.[6-8] Phenolic compounds (phenolic acids, flavonoids, and tannins) and terpenes are commonly found in macroalgae.[9] Strong antioxidant properties of phenolic compounds make them ingredients for possible applications as nutraceuticals or pharmaceuticals, as well as in functional food and the cosmetic industries.[10] Marine brown algae are widely distributed around the world. The red sea has been a region of natural history exploration by European scientists for 240 years. In the early 19th century, a British Admiral, Viscount Valentia, made collections of algae from the Red Sea described by Turn in the 17th century.[11] Jazan city lies in the southwest corner of Saudi Arabia. It has a hot desert climate with an average annual temperature above 30°C. The weather is very hot all year round, and the world's hottest average annual temperature, as daily lows average over 20°C and highs over 30°C even in the coldest month of the year. The first study on marine brown algae from Jazan was done in 2005.[12] This is the first study on marine algae collected from Farasan Island. This research aims to identify the compounds of the essential oil of *P. pavonia* and to use *in vitro* assay to determine if the extracts have any antioxidant or antimicrobial activity. This study provides new insights into the biomedical applications of *P. pavonia*.

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Citation: Mohamed SF. Extraction Optimization, Analysis and Biological Activity of Volatile Compounds from *Padina pavonia* Collected from Farasan Island's Coasts, Jazan, Saudi Arabia. Trop J Nat Prod Res. 2023; 7(3):2574-2579 http://www.doi.org/10.26538/tjnpr/v7i3.17

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Material and Methods

Study area

Farasan Island, as shown in Figure 1, is located some 50 Km offshore from Jazan city, the far southwestern part of Saudi Arabia and is among the largest islands in the Red Sea. It is located around 16°42′21″N 41°59′0″E. The Fisheries Research Center supervises the fishing sector through inspection tours to control and regulates fishing to protect the marine environment from pollution. Therefore, Farasan Island was selected as a new pure site for algal sample collection.

Algal sample

The algal sample (*P. pavonia*) used for this study was harvested off the island of Farasan in the Red sea in November 2021. The sea temperature was 21°C. It was identified by Ibraheem B. Mohammad of the Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Egypt, 2014.[13] *P. Pavonia* belong to Phylum (Ochrophyta), Class (Phaeophyceae), Order (Dictyotales), Family (Dictyotaceae) Genus (Padina), Species (Pavonia). It grows in a depth of 0.5-5m on Farasan Island coasts. The algal sample was washed with running tap water to clean it from epiphytes, freeze-dried for 2 days using a freeze-dryer (FreeZone 2.5, Labconco, Kansas City, MO, USA) and ground (for 1 min in a high-speed grinder).[14]

Materials

The solvents and reagents used for this study include ethanol (Merck), acetone (Merck), and DPPH (Sigma Aldrich, P.A).

Extraction of essential oil

The essential oil was extracted by steam distillation at 100°C for 4 h using Clevenger apparatus. The freeze-dried algal material (300 g) was loaded into a round bottom flask and filled with water (two-thirds). The produced oil:water mixture was separated by a rotary vacuum evaporator (BUCHI Rotavapor R-200). The oil obtained was dried over anhydrous sodium sulfate and kept at 4°C until further analysis.

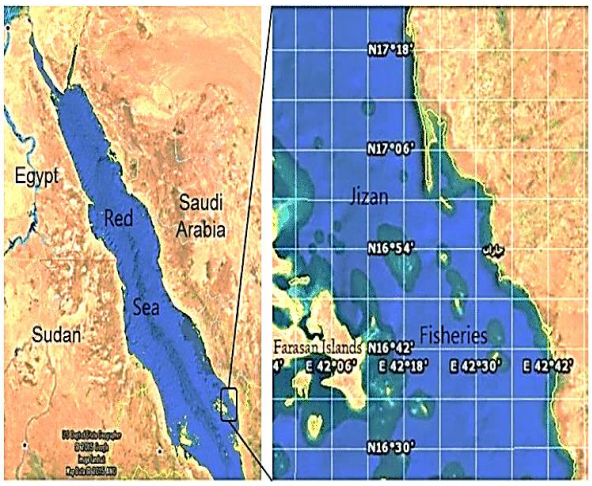

Figure 1: The map for the study area showing the location of Farasan Island and its distance from Jazan city

Extraction with organic solvents
Extraction with ethanol (50%)
The freeze-dried and ground algal sample (50 g) was extracted with 500 mL of ethanol (50%). The extraction was done by shaking in an incubator at 60ºC for 2h. After the extraction, samples were centrifuged at 5000 rpm for 8 min at room temperature.[14] The supernatant was dried by exposure to air. The dried viscous extract was dissolved in dimethylsulfoxide (DMSO) for biological activity assay.

Extraction with acetone
About 50 g of the freeze-dried and ground algal sample was extracted with acetone using a Soxhlet extractor at 60ºC for 2h. The mixture was collected and filtered through Whatmann No 1. filter paper. The filtrate was dried in a rotary evaporator (BUCHI Rotavapor R-200). The resulting material was kept in a refrigerator until used.[15]

Gas chromatography-mass spectrometry GC-MS analysis
The essential oil (10 µL) sample was used for GC-MS analysis (Chromatograph Hewlett Packard Agilent 6890, mass spectrometer Hewlett Packard Agilent 5973 instrument). The sample was injected into capillary column HP-5MS (30 m x 0.25 mm, 0.25 µm of film thickness, Agilent Technologies, Wilmington, DE, CA, USA) using helium as a carrier gas at a flow rate of 0.3 ml /min. The programmed temperature mode was started at 40ºC for 10 minutes and then increased to 250ºC with a rate of 6ºC/min.[16,17] The structural identification was done using the software library NIST 2002 (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, USA).

Biological activities investigation
Antimicrobial study
A set of human pathogenic bacteria and fungi were selected as test organisms. Gram-positive bacteria used were *Bacillus subtilis NRRL B-94*, *Staphylococcus aureus* NRRL, while the Gram-negative bacteria include *Psedumonas aeruginosa* NRRL and *Escherichia coli* NRRL B-3703. Fungi were *Aspergillus niger* NRRL313 and *Candida albicans* NRRL477. All the test organisms were obtained from the culture collection of Cairo Microbiological Resource Centre - Cairo Mircen, Faculty of Agriculture, Cairo, Egypt

Antibacterial test
The agar well diffusion method was used to test the antibacterial activity of the extracts.[18] The different bacterial suspensions (0.2 mL) in nutrient broth (1.0 x 10^5cfu/mL) were spread homogeneously on the surface of nutrient agar plates. In each petri dish, 3 wells with a diameter of 10 mm were prepared. The algal extracts (ethanol and acetone 50%) were dissolved in 5% dimethylsulfoxide (DMSO) at a 5 mg/mL concentration. Extract (0.1 mL) was dropped separately in two wells, and DMSO alone was used as blank for pre-diffusion, whereas the standard ciprofloxacin disc (5 mg/disc) was placed on the culture surface as a positive control agent. The petri dishes were kept at 4ºC for 30 min and then transferred to an incubator at 37ºC for 24h. The inhibition activity was measured as the diameter of the inhibitory zone in the agar layer.

Antifungal test
The fungi were cultivated on Sabouraud glucose agar medium.[18,19] The same method of agar well diffusion stated above was applied. 200 µL of fungal and yeast suspensions (1.0x10^7 cell/mL) were spread homogeneously on the surface of the agar plates. The incubation period was 72h at 37ºC.

Antioxidant activity
Both ethanol (50%) and acetone extract were subjected to the 2, 2-diphenyl-1-picryl-hydrazine-hydrate (DPPH) free radical scavenging assay based on hydrogen atom transfer mechanism. Different concentrations of both extracts (50, 100, 150, 200 and 250 µg/mL) were prepared using DMSO (10%) in absolute ethanol as solvent. Extracts were added at an equal volume to the methanol solution of DPPH (0.3 mM). The mixture was shaken and left to stand at room temperature in the dark for 30 minutes. Ascorbic acid prepared in methanol was used as the standard antioxidant agent. A triplicate determination was carried out for each sample. The changes in the absorbance of the test samples were measured at 517 nm using a spectrophotometer.[20] The percentage of DPPH scavenging activity was calculated using the following equation:

DPPH radical scavenging (%) = [(A control –A sample) / A control] × 100

Where: $A_{control}$ is the absorbance value of DPPH. A_{sample} is the absorbance value of the test sample and DPPH.
The extract concentration giving 50% inhibition (IC_{50}) was calculated from the plotted graph of inhibition percentage against extract concentration.

Results and Discussion

Essential oil, characterized by greenish yellow, represented 0.09 % of the algal material dry weight. The yield was comparable with that reported from other brown alga *Cystoseira sediodes* (0.1 %).[21]

GC-MS analysis of essential oil
The essential oil was subjected to chemical analysis by GC-MS. The major constituents were identified by combining library spectra software and literature data. 41 compounds were identified, representing 89.23% of the total compounds in the extract. Table 1 reports the chemical profile of volatile fractions and the relative contents of detected components obtained from the steam distillation of *P. pavonia.* As presented in Figure 2, essential oils from *P. pavonia* are characterized by a high content of fatty acids representing 44% of total compounds. The major fatty acid is a saturated fatty acid, palmitic acid ($C_{16}H_{32}O_2$) (26.17%). This compound was also reported as a significant component of essential oils from *Cystoseira* species.[22,23] Other fatty acids were detected in low amounts, including Palmitoleic acid (7.01%), Myristic acid (6.06%), Eicosanoic acid (2.25%), (*Z*)-Dodec-5-enoic acid (2.13%). A previous study on *Cystoseira barbata*[24] confirmed that hydrocarbons were predominant in essential oils, but in this study, hydrocarbons represent (5%). It could be due to degradation at high temperatures during extraction. Alcohols represent the second major class of compounds accounting for 23% of the total content. This result is comparable with that reported previously in a study on *C. compressa*. Where the alcohol content was between 13-48% depending on the season of algal collection.[25] Ketones were detected (5%), followed by esters (3%). In contrast, aldehydes, an important odour compound, were detected in the lowest percentage (1%). Tridecanal was the major of this class, with (0.54%). Terpenes were detected, which play an essential role in defence mechanism and reproduction in the marine environment. Also, it is responsible for the ocean smell of algae, particularly non-isoprenoid C11-hydrocarbons.[26]

Table 1: The chemical composition of the essential oil of *P. pavonia*. The data are expressed as relative percentages of each single peak area with respect to the total peak area.

No	Compound	Percentage%	Chemical formula	Retention time	MW
1	Tetrahydro-2-5-dimethyl-furan	0.2	$C_6H_{12}O$	7.32	100.15
2	Hexanal	0.09	$C_6H_{12}O$	12.13	100.16
3	Oct-1-en-3-one	0.03	$C_8H_{14}O$	20.78	126.19
4	2,6,6,-trimethyl-2-cyclohexene-1,4-dione	0.04	$C_9H_{18}O$	30.24	142.24
5	3,5,5,-trimethyl-hex-1-ene	0.039	C_9H_{18}	36.1	126.24
6	Decan-1-ol	0.42	$C_{10}H_{22}O$	36.72	158.28
7	Dec-1-en-3-one	0.21	$C_{10}H_{18}O$	37.10	154.22
8	Undecan-2-one	0.13	$C_{11}H_{22}O$	37.76	170.29
9	Undecanal	0.097	$C_{11}H_{22}O$	37.88	170.29
10	Ethyl cyclohexane carboxylate	0.41	$C_9H_{16}O_2$	38.35	156.22
11	Bicyclo(3.3.1)nonane-2,6-dione	0.23	$C_9H_{12}O_2$	38.64	152.19
12	(R)-5,7-dimethyl-1,6-octadiene	0.76	$C_{10}H_{18}$	39.12	138.25
13	Eugenol	0.18	$C_{10}H_{12}O_2$	40.12	164.20
14	Geranylacetone	0.13	$C_{13}H_{22}O$	45.12	194.31
15	3,7,11, trimethyl-dodecan-1-ol	0.35	$C_{15}H_{32}O$	45.13	228.31
16	Dodecan-1-ol	2.12	$C_{12}H_{26}O$	45.48	186.33
17	(*E*)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	2.28	$C_{13}H_{20}O$	46.17	192.30
18	Tridecan-2-one	0.82	$C_{13}H_{26}O$	46.59	198.34
19	Hexadecane	1.8	$C_{16}H_{34}$	46.99	226.44
20	Dimethyl 1,4-benzenedicarboxylate	0.39	$C_{10}H_{10}O_4$	47.18	194.18
21	5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	0.37	$C_{11}H_{16}O_2$	47.89	180.24
22	(*Z*)-dodec-5-enoic acid	2.13	$C_{11}H_{22}O_2$	48.89	198.30
23	Dodecanoic acid (lauric acid)	0.25	$C_{12}H_{24}O_2$	49.28	200.13
24	alpha-Cadinol	1.23	$C_{15}H_{26}O$	53.11	222.36
25	Hexadecan-1-ol	5.20	$C_{16}H_{34}O$	53.46	242.44
26	Pentadec-1-ene	2.04	$C_{15}H_{30}$	53.80	210.40
27	11-pentan-3-ylhenicosane	1.12	$C_{26}H_{54}$	53.96	366.70
28	Tridecanal	0.54	$C_{13}H_{26}O$	54.12	198.34
29	2-octadecoxyethanol	0.40	$C_{20}H_{42}O_2$	54.67	314.54
30	Myristic acid	6.06	$C_{14}H_{28}O_2$	55.17	228.37
31	n-pentadecanol	1.27	$C_{15}H_{32}O$	55.64	288.41
32	Eicosanoic acid	2.25	$C_{20}H_{40}O_2$	55.85	312.53
33	**6,10,14-Trimethyl-2-pentadecanone**	4.27	$C_{18}H_{36}O$	56.87	268.5
34	Oleyl alcohol	2.48	$C_{18}H_{36}O$	57.54	268.5
35	Farnesyl acetone	1.05	$C_{18}H_{30}O$	58.45	266.43
36	**Palmitoleic acid**	7.01	$C_{16}H_{30}O_2$	58.96	254.40
37	**Palmitic acid**	26.17	$C_{16}H_{32}O_2$	59.15	256.42
38	**Methyl Arachidonate**	2.51	$C_{21}H_{34}O_2$	60.58	318.49
39	**n-Nonadecan-1-ol**	3.02	$C_{19}H_{40}O$	60.97	284.52
40	**Phytol**	7.56	$C_{20}H_{40}O$	61.49	296.53
41	2-(Octadecyloxy)ethanol	1.23	$C_{20}H_{42}O_2$	65.22	314.55

Total identified compounds (from peak total area) 89.23

Compounds detected in an amount higher than 1% are written in bold

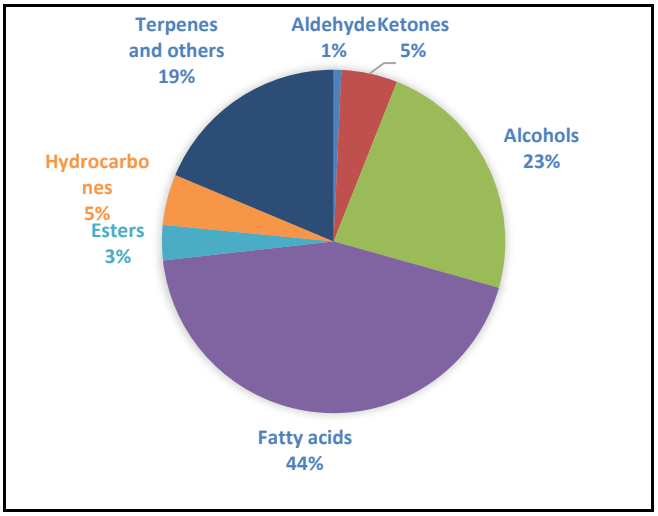

Figure 2: The relative content of each chemical class of compounds detected in essential oil from *P. pavonia*

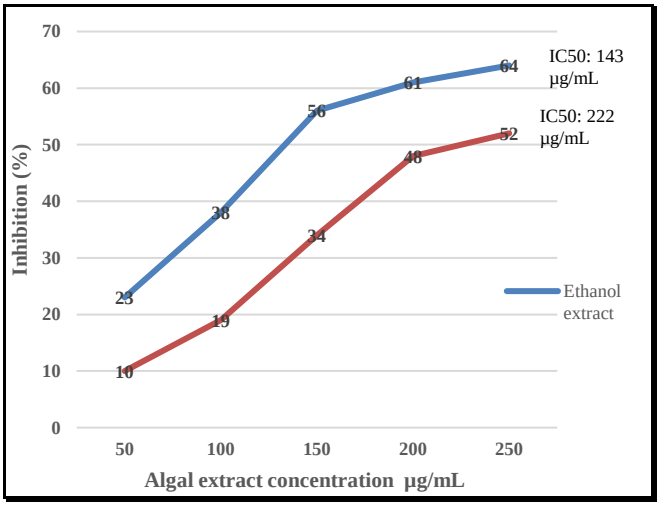

Figure 3: DPPH Scavenger activity of ethanol 50% and acetone extracts from *P. pavonia*
*Ascorbic acid 250 µg/mL gave inhibition% 95 ± 23

Table 2: The yield and characterization of solvent extracts of *P. pavonia*

Solvent extract	Appearance	Colour	Yield %
Ethanol 50%	Viscus liquid	Greenish brown	4.95
Acetone	Viscus liquid	Dark greenish brown	3.87

Solvent Extracts Yield
The yield of both ethanol 50% and acetone extracts is shown in Table 2. The ethanol 50% extract was higher (4.95%). This yield was lower than that obtained for *P. pavonica* (6.56%).[27]

Antimicrobial activity
Seaweed extracts in different solvents exhibited different antimicrobial activities.[28] In the present study, ethanol (50%) and acetone extracts showed good antibacterial and antifungal activity (Table 3). The maximum inhibition activities were shown for acetone extract (22 and 23 mm) on *B. subtilis and S. aureus*, respectively. In contrast, ethanol extract exhibited a 19 mm zone of inhibition for both bacterial strains. Fungi showed less sensitivity towards both two extracts. *C. albicans* was moderately sensitive towards ethanol (50%) extract (13mm), and *A. niger* showed moderate sensitivity towards acetone extract (12 mm). These results are comparable with that reported in the previous study,[29] where extracts from brown algae C*ystoseira mediterranea* and C*ystoseira usneoides* showed potent activity against *S. aureus.* In contrast to our results, some previous studies reported the non-efficacy of algal methanol extract of *Sargassum vulgare* against *E. coli and S. aureus.*[30] Other studies showed that brown algae collected in spring and autumn showed good antimicrobial activity.[31] The biological activity of marine brown algae is thought to be influenced by environmental factors, reproductive state and seasonality.[32] The extraction protocol and the harvest period are other vital factors.[33] The higher antimicrobial activity of the acetone extract could be related to the high flavonoid content of *P. pavonia.* A previous study, [34] reported that acetone extract of air-dried *P. pavonica* showed a high total flavonoid content. Also, the potential antimicrobial activity of the flavonoid-rich extract (70.08 mg/g) of *P. pavonia* was reported in another previous study.[35]

Antioxidant activity
The ability of *P. pavonia* extracts to scavenge the DPPH radical at a concentration of 250 µg/mL was higher in the ethanol (50%) extract (64 ± 0.4) (with IC_{50} = 143 µg/mL) than in acetone extract (52 ± 9) (IC_{50} = 222 µg/mL), as shown in Figure 3. The modified freeze-drying and the extraction method positively affected the DPPH scavenger activity compared with other traditional drying and extraction methods, such as maceration.[36] As previously reported, the total phenolic content (TPC) and antioxidant activity correlate. Extraction with ethanol 50% at 60ºC could preserve the phenolic compounds susceptible to thermal degradation.[14] At the same time, in previous studies, when the extraction was performed at two different temperatures (24 and 60ºC), it was reported that a higher temperature, 60ºC, increased the intermolecular interaction within the solvent, which increased the solubility of phenolic compound and showed higher antioxidant activity.[37]The mechanism of phenolic compounds as antioxidant depends on its hydroxyl group which is directly attached to the carbon atoms of the aromatic ring and the hydrogen atom donated to the free radicals, thereby preventing the oxidation of other compounds.[38]

Conclusion

The results of this study contribute to the characterization of the brown alga, *P. pavonia*, as a matrix that can yield different components with antimicrobial and antioxidant activities. The analysis of essential oil showed various chemical compounds reflecting the high value of *P. pavonia*, which can be considered a good candidate for different industrial and pharmaceutical applications. Using the freeze-dried algal sample improved the extract yield and quality. Also, the modified extraction protocol with ethanol (50%) improved the total phenolic compounds, which showed high antioxidant activity. Similarly, the extraction with acetone using a freeze-dried algal sample showed an extract with broad-spectrum antimicrobial activity. The promising results obtained in this study will open a broad spectrum of possibilities.

Table 3: Antibacterial and antifungal activity of solvent extract from *P. pavonia*

Algal extract	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	B. subtilis	***S. aureus***	***P. aeruginosa***	***E. coli***	***A. niger***	***C.albicans***
	Diameter of inhibitory zone (mm)					
Ethanol 50%	19	19	17	15	10	13
Acetone	22	23	21	17	12	11

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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