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Phytochemical analysis and Antibacterial activity of the 5 different extract from the seagrasses *Posidonia oceanica*

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ABSTRACT
This study was conducted in the faculty of Art and sciences and aimed to determine the chemical composition of the seagrass *Posidonia oceanica* using Ethyl acetate, Chloroform, Ethanol, Cyclohexan and Acetone and to determine the antibacterial activity of the crude extracts against: *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli*. The seagrass was collected, identified, washed, dried, grind and exposed to extraction by soxhlet apparatus, and the crude extracts was subjected to chemical reaction and agar disk diffusion method to detect the presence of the chemical compounds and antimicrobial susceptibility tests. *Posidonia oceanica* possess several chemical compounds including: alkaloids, flavanoides, phenols, tannins, saponins, phlobatannins, sterols, proteins, reducing sugars, polysaccharides, and resins which differ in concentration according to the solvent used and has potent antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with variable activity against other used bacterial strains.

Keywords: Seagrass, *Posidonia oceanica*, Phytochemical analysis, Ethyl acetate, Chloroform, Ethanol, Cyclohexan and Acetone.

1. Introduction
Posidonia oceanica is seagrass able to grow in the clean water with a depth reaching -45 m, and is common plant in the White Mediterranean Sea [1]. *Posidonia oceanica* belongs to the family Posidoniaceae and its genus *Posidonia* has nine species; in which *Posidonia oceanica* is completely restricted to the White Mediterranean Sea [2]. The grasses used as indicator of good seawater quality as it's highly sensitive to marine pollutions [3]. Several researches stated the chemical composition of the species *Posidonia oceanica* and the role of this compound especially the phenolic compounds as biotic and abiotic resistant agents [3-5]. The ability of this compound to kills microbes allow us to look about the chemical activity of different extracts against several bacterial agents.
This study aimed to determine the chemical composition of the seagrass *Posidonia oceanica* using several chemical solvents including (Ethyl acetate, Chloroform, Ethanol, Cyclohexan and Acetone) and to determine the antibacterial activity of the extracts against bacteria including: *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli*.

2. Materials and Methods
2.1 Samples
Posidonia oceanica grasses were collected by scuba diving from 5 meters depth in September 2012, from the White Mediterranean Sea cost at Alkhums City – Libya. The grass was identified and classified in the Faculty of Arts and Sciences – AlMergib University – Libya. The seagrass was washed by tap water (to remove the salts) and then by distilled water and kept away from Sunrays with mixing from time to time for up to 48 hours. Finally, the grasses were dried by oven at 60 ºC for 48 hours. The grasses were grinned with electric grinder till having very fine granules and kept at room temperature in dark bottle until used [6, 7].
The bacterial strains were obtained from the Biotechnology Research Center in Tripoli.

2.2 Method of extraction
Crude extraction of the grasses was done using the soxhlet method by dissolving 10 g of the

powder in 200 ml sterile distilled water and passed through the soxhlet apparatus for 8 hours. The crude extract, then subjected to several solvent including (Acetate, Chloroform, Ethanol, Cyclohexan and ethyl Acetate). Different solvent extracts were reacted with several chemical compounds (Table: 1) to determine the presence of alkaloids, flavanoides, phenols, tannins, saponins, phlobatannins, sterols, proteins, reducing sugars, polysaccharides, and resins. The presence of each compound was reported and the density was scored as 3+, 2+, 1+, ± or 0 according to the intensity of the colour.

Antimicrobial activity of the 5 solvent extracts were done using agar desk diffusion method; in brief 5 mm desks were impregnated in the solvent extract and allowed to dry in desiccant. The desks were placed on Muller and Hinton agar after spreading a swab impregnated in Muller and Hinton broth with 10^8CFU/ml bacterial strains (for each stain single Petri-dish was used). The plate was examined after 24 hours and 48 hours for the presence of inhibition zone and the diameter of the zone was recorded.

Table 1: Different reactions used to determine the presence of some chemical composition of *Posidonia oceanica*

Detected chemicals	Reacted materials/ method	End point of the reaction
Tannins	1% Lead acetate	Appearance of gelatinous precipitate
	1% Ferric chloride	Appearance of bluish – green colour
Phlobotannins	Boiling + 1% HCL	Appearance of Red precipitate
Resins	95% Ethanol + 4% HCL	Turbidity
Saponins	Mercuric chloride	Appearance of white precipitate
Reducing substances (monosaccharide's)	Benedict's solution	Development of yellow – red colour
Flavonoides	Addition of diluted ammonia	Development of yellow colour
Phenolic compound	1% Ferric chloride + Folin's reagent	Appearance of bluish – green colour
Sterols	Chloroform + H2SO4	Development of green, then red colour
Alkaloids	Mayer's	Appearance of white precipitate
Protein	Boiling + H2SO4	Appearance of condensed white precipitate
Polysaccharides	Iodine	Blue colour

3. Results

Several chemical compounds were detected in the *Posidonia oceanica* using five different extraction solutions. The chemical composition of the *Posidonia oceanica* showed high concentration (i.e.: 3+) of phlobatannins obtained by using both acetone and ethanol extraction methods, abundant reducing substances by ethanol and ethyl acetate extraction methods, and sterols using acetone, chloroform and ethanol extracting agents (Table: 2). Alkaloids composition of the grasses was not detected by all extracting agents except the ethanol which showed weak reaction indicating few composition in the grass (Table: 2). Weak saponins concentration was detected by acetone extraction method only (Table: 2).

Excellent antibacterial activity of the *Posidonia oceanica* was noted in the cyclohexane extract. The extract showed good inhibition against all tested organisms except *P. mirabilis*. Interestingly, the highly resistant P. aeruginosa had the biggest zone of inhibition (figure: 1). Ethyl acetate extract showed no antibacterial activity, while Proteus mirabilis on the other hands showed high resistant mechanisms (figure: 1). The most sensitive strain was *Staphylococcus aureus* which showed sensitivity zone to all but not the ethyl acetate extract (figure: 1).

Table 2: Phytochemical constituent of the *Posidonia oceanica* using different extracts

Chemical compound	Chemical extracts				
	Acetone	Chloroform	Cyclohexan	Ethanol	Ethyl acetate
Alkaloids	0	0	0	1+	0
Flavanoides	1+	2+	2+	1+	±
Phenols	0	0	0	1+	0
Phlobatannins	3+	2+	1+	3+	2+
Polysaccharides	1+	1+	1+	1+	1+
Proteins	1+	1+	1+	0	1+
Reducing sugars	1+	1+	1+	3+	3+
Resins	1+	2+	0	0	1+
Saponins	1+	0	0	0	0
Sterols	3+	3+	1+	3+	2+
Tannins	1+	1+	±	2+	1+

0= No reaction, ± = unclear results, += weak positive, 2+ = good reaction, 3+= strong reactions

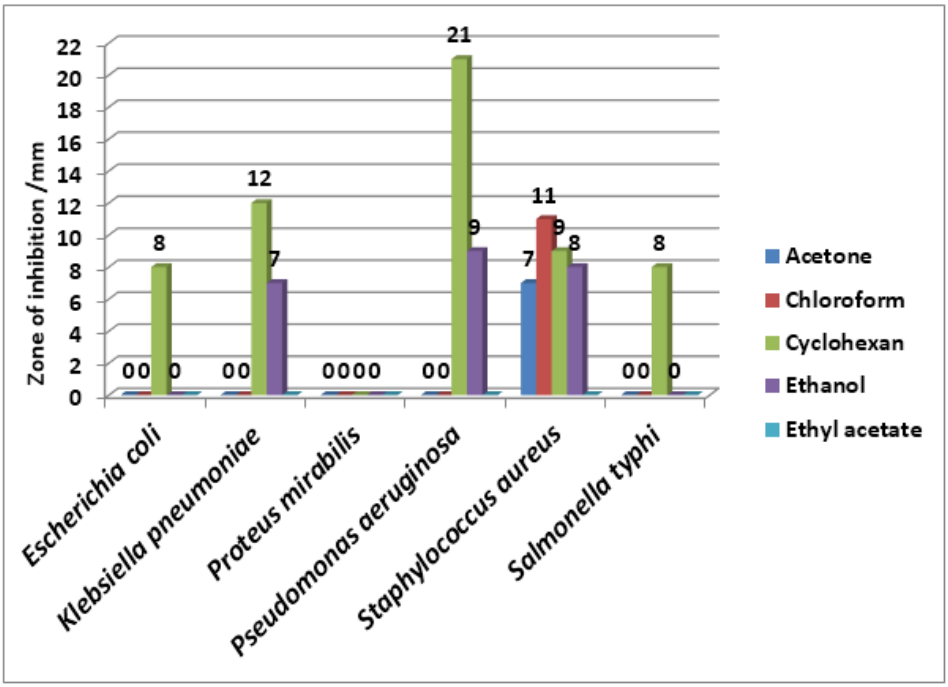

Fig 1: Antimicrobial susceptibility of the *Posidonia oceanica* different extracts

4. Discussion

Posidonia oceanica is an important White Mediterranean seagrass, as these grasses attenuate the wave streams, protect the hinterland from wave attack, and stabilize the seabed [8]. They also use as indication of marine contamination as they are highly sensitive to marine pollutions [3].

Several chemical compounds including (alkaloids, flavanoides, phenols, tannins, saponins, phlobatannins, sterols, proteins, monosaccharides, polysaccharides, and resins) have been detected in the seagrass *Posidonia oceanica*. Some of these have also been detected in similar seagrasses or other plants such as *Piper umbellatum* and *Piper pellucid* [9], *Syringodium isoetifolium* [10, 11], *Raphanus sativus* niger [12], *Cymodocea serrulata* [11, 13], *Halodule pinifolia* [11], and *Halophila ovalis* [11].

Acetone considered one of the best extracting agents able to extract 9/11 chemical compounds, while cyclohexan failed to extract 4/11 chemical compounds, although the uses of multiple extracting agents is better for good extraction of different chemical components. Several researcher describe different fatty acids, phenolic compounds and tannins content of the seagrass *Posidonia oceanica* [3, 5, 14, 15], although our study detected small concentration of alkaloids, phenols and saponins, this might be due to differences in the extraction and/or detection methods.

Posidonia oceanica seagrass extracts showed good antimicrobial activity against several bacterial strains. This finding contradicts with the finding of Orhan I. *et al* [16] and Ballesteros E. *et al* [17] who showed no activity of *Posidonia oceanica* extracts against bacterial strains. This difference might be due to differences in the extraction methods or in the tested bacterial strains. Best antimicrobial activity was obtained by cyclohexan followed by ethanol extracts, while poor activity was noted from ethyl acetate. This difference may be contributed to ability of different chemical extraction method to extract chemical compounds with different concentration and may contributes to its antimicrobial activity against different bacterial strains by masking them or inhibiting their activity.

5. Conclusion

This study concluded that the seagrass *Posidonia oceanica* possess several chemical compounds including: alkaloids, flavanoides, phenols, tannins, saponins, phlobatannins, sterols, proteins, reducing sugars, polysaccharides, and resins which differ in concentration according to the solvent used and has potent antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with variable activity against other used bacterial strains.

6. Conflict of interest

We, the authors of this manuscript certify that there was no conflict of interest to any private or Governmental institutes.

7. Acknowledgements

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