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GC-MS Identification and Antibacterial Activity of *Palmaria Palmata* Algae Extract Against Some Pathogenic Bacteria

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ABSTRACT

The objective of this study was to assess bioactive compounds and antibacterial properties of *Palmaria palmata* algae extract against some pathogenic bacteria. Following gas chromatography (GC-MS), the chemical content of the methanolic extract of the *Palmaria palmata* sample was determined. The antibacterial activity was tested against *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* using the paper disc method. Regardless of the *Palmaria palmata* algae extract, GC-MS analysis enabled the identification of sixteen chemical compounds. The results showed that *Palmaria* extract exhibited significant antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Proteus mirabilis* and *Pseudomonas aeruginosa*, which are common and complex pathogens. Our research contributes to the suggestion that *Palmaria palmata* algae extract have bioactive compounds and antibacterial activity and *Palmaria* extracts have four pharmacological applications.

INTRODUCTION

The ability of marine organisms to produce numerous pharmacologically active compounds, such as antimicrobials, anticancer agents, antivirals, antioxidants, anti-inflammatory agents, and antifungals, sets them apart from other organisms. They are an important source for treating various diseases **(Águila-Ramírez *et al.*, 2012)**. Mortality rates have risen dramatically due to infectious diseases that are difficult to treat due to antimicrobial resistance. Alternative medicine using medicinal plants and marine organisms are one of the available solutions to address this problem **(Mashjoor *et al.*, 2016)**. Since ancient times, humans have relied on plants and marine organisms to treat many diseases due to their therapeutic properties **(Kaushik & Chauhan 2008)**. Based on numerous scientific studies, marine microalgae and medicinal herbs are abundant sources of active biological compounds for the majority of conventional medicines that aid in the treatment of a variety of diseases **(Manlusoc *et al.*, 2019)**. In recent years, a number of researchers have highlighted the discovery and purification of bioactive compounds from marine sources such as algae that have proven their medical effectiveness **(Cotas *et al.*,**

2020). *Palmaria* algae contains many phytochemicals with antimicrobial and antioxidant properties, such as phenols and plant pigments such as r-phycoerythrin and phycocyanin **(Dumay *et al.*, 2013; Savitri *et al.*, 2024)**. The purpose of this study was to examine the *palmata* compounds' antibacterial activity and GC-MS profile.

MATERIALS AND METHODS

1- Preparation of crude extracts

Palmaria sample powder was obtained from the American company Amazon. Then it was soaked in 80% methanol. The solution was then placed in a shaker for 24h before being filtered. Rotary evaporator was used to remove methanol and to obtain the crude material, which was tested at three concentrations 100, 200 and 300mg/ mL against four types of pathogenic bacteria.

2-Antibacterial assay

Staphylococcus aureus, Bacillus cereus, Proteus mirabilis and *Pseudomonas aeruginosa* were used in experiment. The antibacterial assay was conducted on Mueller Hinton agar. *Palmaria palmata* extracts were dissolved in methanolic to obtain a concentration of 100, 200 and 300mg/ mL. According to **Gardea *et al.* (2015)**, the disc diffusion method was used to measure antibacterial activity. Using the same solvent that was used to dissolve the *Palmaria palmata* extract, negative controls were made. The inhibition zone was calculated for all concentrations used in the study on discs in mm.

3-Identification of bioactive compounds using GC MS

The extract of *palmaria palmata* was subjected to GC-MS (Agilent 7890A) testing. Rxi-5 MS capillary columns (Agilent 19091S 433, 30m. 250m internal diameter, 0.25m) were used to analyze the phytochemicals compound of the *Palmaria* extract. Helium was used as the carrier gas at a rate of 1ml/ min, and the GC oven temperature was set at 270-280°C. The separated compounds were identified by comparing the fragmentation pattern of the mass spectra with profiles from GC-MS.

4-Statistical analysis

Using SPSS version 25, a one-way analysis of variance (ANOVA) was performed on the results for statistical analysis. Significant differences (P<0.05) among the bacteria were evaluated by Duncan test.

RESULTS AND DISSCUSION

1- Antibacterial activity

Anti-infective agents may come primarily from marine organisms. In the current study, the antibacterial activity of Palmaria extract on pathogenic strains of *Staphylococcus aureus, Bacillus cereus, Proteus mirabilis,* and *Pseudomonas aeruginosa* bacteria was evaluated (Table 1). Depending on the bacterial species used, the Palmaria extract's antibacterial activity varies. *Proteus mirabilis* and *Pseudomonas aeruginosa* were the bacteria with the highest sensitivity because they were the most resistant to other types of bacteria and to all concentrations. The inhibition zone varied in diameter between 14.00 and 25.00mm for 100mg/ mL and between 14.00 and 25.00mm for

300mg/ mL (Table 1). *Pseudomonas aeruginosa* was found to have the highest antimicrobial activity in Palmaria extract, while *Staphylococcus aureus* had the lowest. Additionally, the antibacterial activity of *Palmaria* extract might also be related with many bioactive compounds (Table 2) as antimicrobials for example 1,4-Methanonaphthalen-9-ol, 1,2,3,4-tetrahydro-, stereoisomer, 2H-Benzopyrane-3-carbotriazanyde, 2-oxo-, 11-Eicosenoic acid, Linoleic acid, Pentadecanoic acid, 2-Pentadecanone, Tetradecanoic acid, Fumaric acid, 1-Methyl-5-fluorouracil, Phenacylidene diacetate, Dimethyl Sulfoxide, Dodecane, Tetradecane and n-Hexadecanoic acid. This finding is consistent with several other studies that have demonstrated that natural product extracts' bioactive compounds have a stronger inhibitory effect on both Gram-positive and Gram-negative bacteria **(Saeed *et al.*, 2020; Addai *et al.*, 2022; Prasetyaningsih *et al.*, 2025)**. Biologically active compounds negatively affect bacterial cell membranes, growth and reproduction, as well as protein and cell wall, while disrupting enzymes and DNA (**Addai *et al.*, 2022; Evika *et al.*, 2024)**

2- GC-MS profiling

The *Palmaria* extract was analyzed using gas chromatography-mass spectrometry (GC-MS) and revealed the presence of several groups of bioactive compounds. Table (2) shows the bioactive compounds along with their biological activity, retention time (RT), molecular formula, area, and molecular weight. A total of 16 compounds were identified in *Palmaria* extract, 1,4-Methanonaphthalen-9-ol, 1,2,3,4-tetrahydro-, stereoisomer, 2H-Benzopyrane-3-carbotriazanyde, 2-oxo-, 11-Eicosenoic acid, Linoleic acid, Pentadecanoic acid, 2-Pentadecanone, 6,10,14-trimethyl-, Tetradecanoic acid, Fumaric acid, 1-Methyl-5-fluorouracil, Phenacylidene diacetate, Dimethyl Sulfoxide, Dodecane, Tetradecane, n-Hexadecanoic acid, Hydroxyurea, N, N', O-trim ethyl- and Dimethyl Sulfoxide; antimicrobial, hypocholesterolemic, anticancer, antifungal antioxidants , antidiabetic, antibiofilm, antimicrobial, anti-inflammatory anti-nociceptive and antineoplastic **(Erasto & Viljoen 2008; Liu *et al.*, 2020; Chirumamilla *et al.*, 2022)**.

Table 3. Antibacterial activity of *Palmaria Palmata* extracts

Types of bacteria	**Concentration mg/mL**		
	100	**200**	**300**
Staphylococcus aureus	14.00 ± 1.28 c	16.00 ± 0.73 d	17.00 ± 1.38 c
Bacillus cereus	15.00 ± 1.67 b	18.00 ± 1.50 c	23.00 ± 2.79 b
Proteus mirabilis	18.00 ± 2.53 a	24.00 ± 2.67 a	25.00 ± 2.58 a
Pseudomonas aeruginosa	18.00 ± 2.10 a	23.00 ± 2.52 b	25.00 ± 1.06 a

a–d Different letters within each column indicate significant difference ($P < 0.05$).

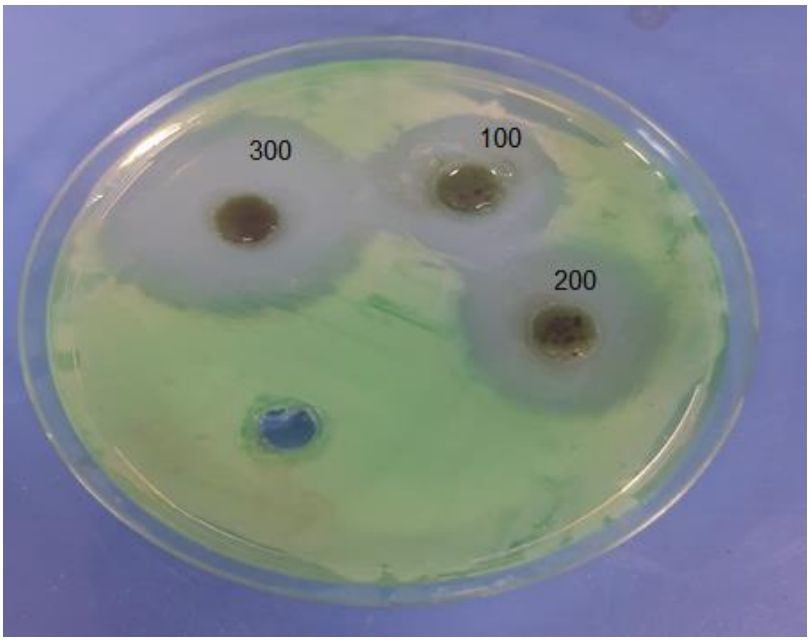

Fig. 1. Effect of *Palmaria palmate* algae on *Psedomonas aeruginosa*

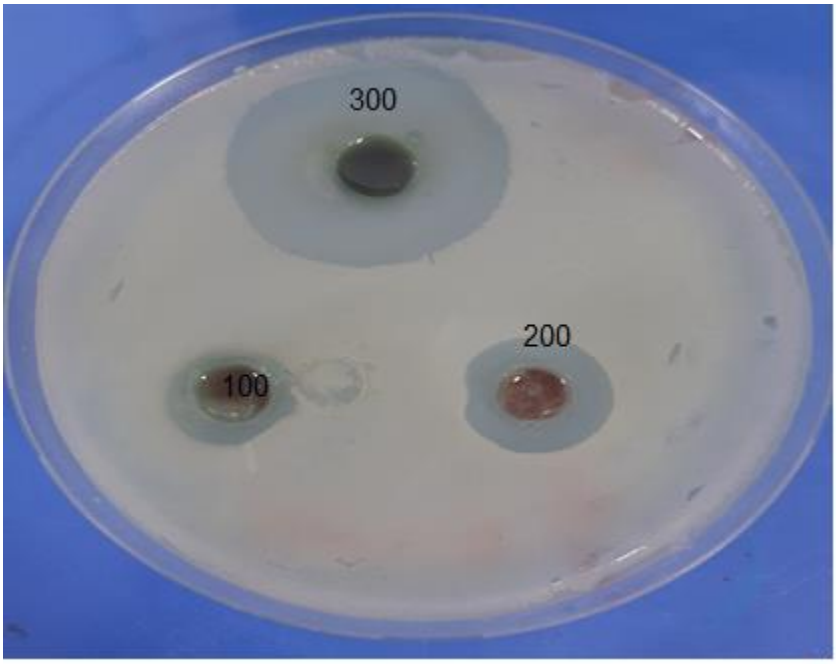

Fig. 2. Effect of *Palmaria palmate* algae on *Bacillus cereus*

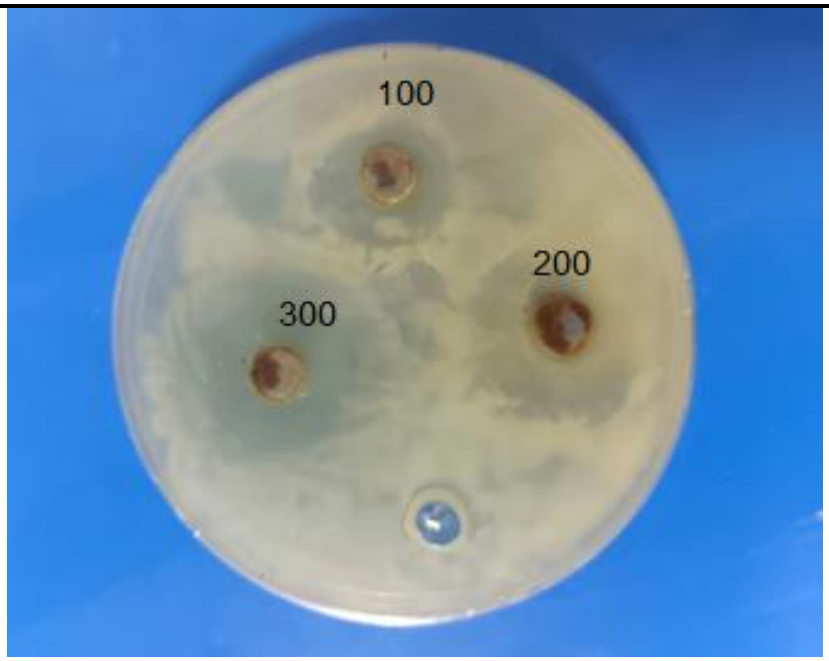

Fig. 3. Effect of *Palmaria palmate* algae on *Staphylococcus aureus*

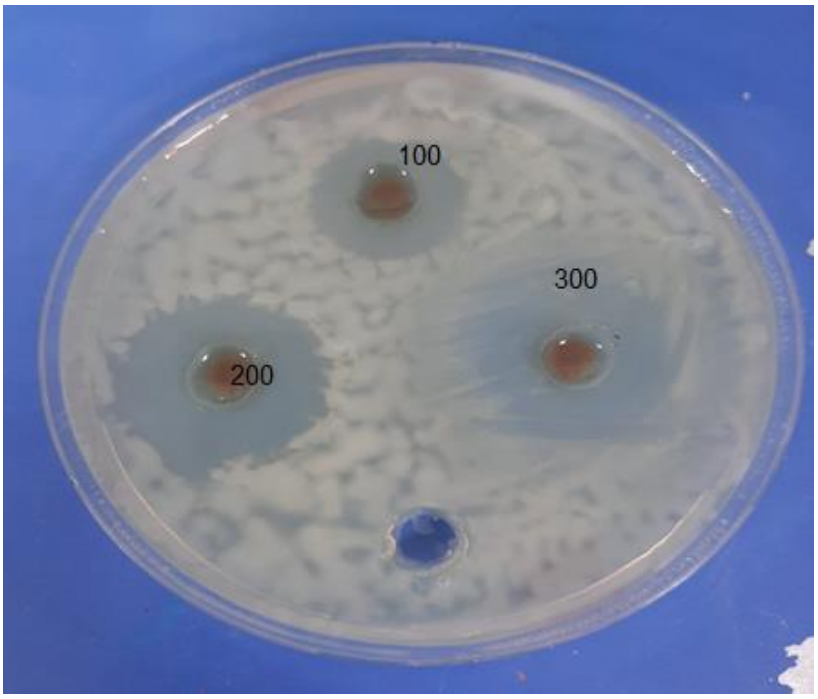

Fig. 4. Effect of *Palmaria palmate* algae on *Proteus* sp.

Table 2. Compounds identified of *Palmaria* extracts

No.	Compound name	Formula	M.W	RT (min)	M.W	Biological activity
1	**1,4-Methanonaphthalen-9-ol, 1,2,3,4-tetrahydro-, stereoisomer**	$C_{11}H_{12}O$	202.25	5.07	202.25	Antimicrobial Anti-inflammatory
2	**2H-Benzopyrane-3-carbotriazanyde, 2-oxo-**	$C_{10}H_9N_3O_3$	207.20	7.23	207.20	Antimicrobial
3	**11-Eicosenoic acid**	$C_{20}H_{38}O_2$	310.51	8.55	310.51	Antimicrobial Anti-inflammatory
4	**Linoleic acid**	$C_{20}H_{36}O_2$	308.50	9.10	308.50	Anticancer Antioxidant Antimicrobial
5	**Pentadecanoic acid**	$C_{15}H_{28}O_2$	240.38	10.63	240.38	Anti-inflammatory Antioxidant Antimicrobial,
6	**2-Pentadecanone, 6,10,14-trimethyl-**	C18H36O	268.48	12.44	268.48	Hypocholesterolemic, Anti-inflammatory Antibacterial Anti-nociceptive Antioxidant
7	**Tetradecanoic acid**	C14H28O2	228.37	13.18	228.37	Antibacterial Antibiofilm Antioxidant
8	**Fumaric acid**	$C_{10}H_{14}O_4$	198.21	14.61	198.21	Antibacterial
9	**1-Methyl-5-fluorouracil**	$C_5H_5FN_2O_2$	144.10	16.08	144.10	Anti-cancer
10	**Phenacylidene diacetate**	$C_{12}H_{12}O^5$	236.22	18.10	236.22	Antibacterial Antioxidant
11	**Dimethyl Sulfoxide**	C2H6OS	78.13	19.32	78.13	Anti-inflammatory Antioxidant Antimicrobial
12	**Dodecane**	C12H26	170.33	19.82	170.33	Anti-diabetic Anti-bacterial
13	**Tetradecane**	C14H30	198.39	23.24	198.39	Antibacterial Antifungal
14	**n-Hexadecanoic acid**	$C_{16}H_{32}O_2$	256.42	26.40	256.42	Antioxidants Hypocholesterolemic
15	**Hydroxyurea, N, N', O-trim ethyl-**	C4H10N2O2	118.13	27.03	118.13	Antineoplastic
16	**Dimethyl Sulfoxide**	C_2H_6OS	78.13	27.95	78.13	Antimicrobial

CONCLUSION

From the current results, it can be concluded that the Palmaria algae extract contains countless bioactive compounds with high antibacterial efficacy. Natural antibiotics against a variety of drug-resistant pathogens can be made with these bioactive compounds.

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