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Determination of Phytochemical and Antibacterial Properties of *Momordica charantia* Seed Extracts

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Abstract

In recent years, it has been essential to discover safe and effective antibacterial drugs because of rising antibiotic-resistant bacteria. In traditional medicine, plant extracts including biological active components have been used for therapeutic purposes. We aimed to evaluate the antimicrobial properties of the aqueous seed extract of *Momordica charantia* (*M. charantia*) on 9 pathogenic bacteria. The antibacterial activity of the extract was assessed against strains using disc diffusion and broth microdilution tests. A total of 21 constituents were identified from *M.charantia* seeds. Alcohols, esters, aldehydes, monoterpenes, and monoterpenoids were found as the prevalent groups. The seed extract showed the greatest antimicrobial activity on *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) with an inhibition zone diameter (IZD) value of 15.75±0.50 mm and 15.25±0.957 mm, respectively. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) test results ranged from 12.5 to 100 (mg/mL). The seed extract of *M.charantia* could be used for the cure of bacterial infections as a new natural drug.

Keywords: Antibacterial activity, *Momordica charantia*, Phytochemical components

1. Introduction

Several classes of antibiotics are used for the treatment of bacterial diseases and reduced morbidity and mortality [1]. Bacteria have acquired resistance to antibiotics via biochemical and genetic mechanisms [2]. According to the World Health Organization (WHO), the failure of antibiotic treatment is due to multi-drug-resistant bacteria and their toxic effects [3]. Researchers have been focused to discover new plant-derived antimicrobials against resistant bacteria [4,5].

Since ancient times, botanical drugs have been used for the cure of bacterial diseases [6]. Any plant that has therapeutic properties or can be used as a precursor of drugs is named a medicinal plant by the WHO [7]. Plant extracts containing various phytochemicals (tannins, alkaloids, flavonoids, and phenolic compounds) are responsible for their therapeutic properties such as antimicrobial, antioxidant, and anticancer [8]. *M. charantia* (*Cucurbitaceae* family) is considered a medicinal plant [9] and has been used for the cure of toothache, diarrhea, cancer, pneumonia, and bacterial infections in traditional medicine [10-12] due to containing bioactive components like phenolic compounds, triterpenes, and carotenoids [13,14]. The purpose of our study was to determine the phytochemical components and antibacterial properties of an aqueous extract of *M. charantia* against human pathogens.

2. Materials and Methods

2.1. Preparation of Seed Extracts

Seeds of *M. charantia* were purchased from the local market in Turkey and identified by a taxonomist in Canakkale Onsekiz Mart University, Herbarium of the Department of Biology, and the voucher specimen was 003081.

Seeds were washed, dried, and finally powdered. Seed extracts were obtained using the maceration method. Briefly, 10 gr *M. charantia* seeds were milled into powder and dissolved in distilled water (100 mL) in a sterile Erlenmeyer flask using a magnetic stirrer at 70°C for 1 hour. The solution was filtered (Whatman-No 1) and evaporated in an incubator at 50 °C for 3 days. Then, the extract was stored at 4 °C in the dark until antibacterial assays. Aqueous extract was sterilized using a syringe filter (0.45 µm).

2.2. GCMS

The sample was prepared by SPME technique. The components of the seed of *M. charantia* were isolated using divinylbenzene/carboxen on polydimethylsiloxane (DVB/CAR on PDMS) 50-/30-mm fibers. After each fiber was exposed for 45 min at 50 °C, they were injected to the port of the GC-MS. Shimadzu GCMS QP 2010 ULTRA (USA)) equipped with an RXI-5MS capillary column (30 m; 0.25 mm; 0.25 μm) was used with helium as the carrier gas (flow rate: 1.0 ml/min). The inlet temperature was set at 250°C. The split ratio was 1:30. Column oven temperature was 50 C. The oven temperature was programmed from 50 to 270 °C at 5 °C/min. All components were identified by comparison of their retention indices with Wiley 9 (Wiley, New York, NY, USA) and NIST 11 (National Institute of Standards and Technology) (Gaithersburg, MD, USA) libraries.

2.3. *In Vitro* Antibacterial Activity Assays

2.3.1. Microorganisms and Cultures

Escherichia coli ATCC 25922, *Streptococcus agalactiae* ATCC 12386, *Proteus vulgaris* ATCC 13315, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 10145, *Streptococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* ATCC 19615, and *Staphylococcus aureus* ATCC 25923 bacterial strains were used as test microorganisms and obtained from American Type Culture Collection (ATCC). All strains were stored at -20 °C in brain heart infusion broth (BHI) with 20 % glycerol. The turbidity of the bacterial suspensions was equalized to 0.5 Mc Farland standard.

2.3.2. Agar Disc Diffusion Method

The method was used to detect the antibacterial properties of *M. charantia* seed extracts against 9 human pathogens according to the Clinical & Laboratory Standards Institute (CLSI). 100 µL of each inoculum was spread on brain heart agar (BHA) (Biokar, France) plates. 25 µL of 100 mg/mL concentration of the seed extract were pipetted onto steril blank paper discs (Oxoid, Bioanalyse) under aseptic conditions. After incubation at 37 °C for 18-24 h, the IZDs were recorded. The negative control was distilled water; positive controls were gentamicin (10 µg/disc, Bioanalyse), ampicillin (10 µg/disc, Bioanalyse), and vancomycin (30 µg/disc, Bioanalyse). Experiments were repeated three times.

2.3.3. Determination of MIC and MBC

The microdilution broth method was used to detect MIC using sterile 96 well plates. Firstly, 100 µl of BHI was pipetted into each well. Two-fold serial dilutions (200-12.5 mg/mL) of seed extract were prepared in BHI. Then, 20 µl of inoculum was put into each well except for sterility control. Growth control was put into 12th wells. After incubation, 10 µL of suspension from four negative wells was transferred to BHA plates. After the same incubation as MIC, the lowest extract concentration at which bacterial strains did not grow, was determined MBC.

2.3.4. Statistical Analysis

IZD results of seed extract were compared with antibiotics using one-way ANOVA and post-hoc-Turkey tests (SPSS 19 version).

3. Results

SPME-GC/MS identified 21 compounds in *M. charantia* seed as presented in Table 1 and the GC chromatogram is seen in Figure 1. The seed extract exhibited an antimicrobial effect on 9 pathogens. The IZD and MIC results are displayed in Table 2. The extract exhibited the greatest antibacterial effect on *B. subtilis* and *S. aureus* with the IZDs being 15.75±0.50 mm and 15.25±0.957 mm, respectively. No significant antibacterial activity was detected against *S. pyogenes* (IZD: 9.00 mm) compared to the antibiotics ($p<0.05$). The MIC/MBC values of the seed extract were between 12.5-100 (mg/mL).

Table 1. Compounds of *M. charantia* seeds using SPME/GC/MS

Peak#	Retention Time	Area %	Name
1	1.942	1.35	Acetic acid
2	2.333	1.40	1-Butanol
3	2.728	14.49	Pentanal (CAS)
4	4.194	36.28	1-Pentanol
5	4.915	0.94	Hexanal (CAS)
6	6.553	1.30	Hex-2(E)-enal
7	7.094	2.66	p-Xylene
8	7.181	2.26	Hexanol <n->
9	7.926	1.35	o-Xylene
10	9.017	7.06	Oxime-, methoxy-phenyl-_
11	10.403	4.63	Hept-2(E)-enal
12	10.969	3.34	2-Hepten-1-ol, (E)-
13	11.894	1.34	2-Furanmethanol, tetrahydro- (CAS)
14	13.072	1.54	o-Cymene
15	13.238	4.47	D-Limonene
16	13.332	3.11	Eucalyptol (1,8-CINEOLE)
17	15.066	2.75	3-Heptanol, 2-methyl- (CAS)
18	19.993	2.48	Dodecane
19	20.456	4.67	Acetic acid, octyl ester
20	23.645	1.47	Tridecane (CAS)
21	32.354	1.11	Nerolidol
		100.00	

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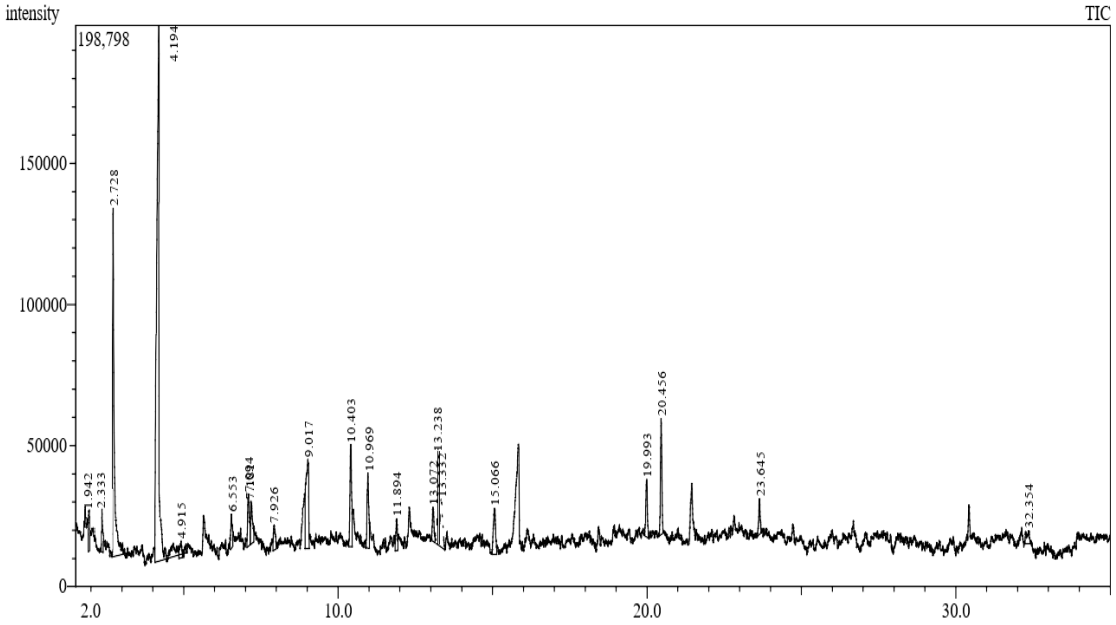

Figure 1. GC chromatograph for the *M. charantia* seeds

Table 2. IZD and MIC values of *M. charantia* seed extract on 9 standard strains

Bacterial strains	IZD (mm)				MIC (mg/mL)	MBC (mg/mL)
	Extract (100 mg/mL)	G	A	V		
E. faecalis **ATCC 29212**	13.00±0.816	14.75±0.50 (p:0.008)	11.75±0.50 (p:0.058)	11.50±0.577 (p<0.05)	25	50
E. coli **ATCC 25922**	11.50±0.577	16.75±0.957 (p<0.05)	16.00±0.816 (p<0.05)	-	50	100
P. vulgaris **ATCC 13315**	13.00±0.816	12.25±0.957 (p:0.432)	9.75±0.50 (p<0.05)	-	25	50
B. subtilis **ATCC 6633**	15.75±0.50	15.25±0.50 (p:0.515)	8.75±0.50 (p<0.05)	8.25±0.50 (p<0.05)	12.5	25
S. agalactiae **ATCC 12386**	9.75±0.50	21.75±0.50 (p<0.05)	15.50±0.577 (p<0.05)	14.25±0.50 (p<0.05)	100	100
P. aeruginosa **ATCC 10145**	12.00±0.816	14.25±0.50 (p<0.05)	9.75±0.50 (p<0.05)	-	50	50
S. epidermidis **ATCC 12228**	9.75±0.50	25.50±0.577 (p<0.05)	19.25±0.957 (p<0.05)	13.75±0.50 (p<0.05)	100	100
S. pyogenes **ATCC 19615**	9.00±0.816	20.25±0.50 (p<0.05)	25.50±1.290 (p<0.05)	15.75±0.50 (p<0.05)	100	100
S. aureus **ATCC 25923**	15.25±0.957	-	-	-	12.5	12.5

IZD in mm (Mean±SD: Standard Deviation) G: Gentamicin, A: Ampicillin, V: Vancomycin, -: ≤6 (disc zone diameter 6mm)

4. Discussion

M. charantia has been used in medicine as an herbal drug for the cure of disorders like hemorrhoids, gastrointestinal systems, skin, bones, and blood cancer [15]. Pharmacologically active components such as flavonoids, phenols, terpenes, and triterpenoids of *M. charantia* extracts are responsible for their antibacterial activity [16,17].

Salinas-Sánchez et al. (2021) reported that linalool oxide and limonene oxide were found the major components of the hexane extract of *M. charantia* seeds [18]. In this study, GC-MS revealed five main compounds*:* alcohols, aldehydes, esters, monoterpenes, and monoterpenoids. The antibacterial properties of *M. charantia* is related to its bioactive components like seed oil, tannins, proteins, terpenoids, alkaloids, and steroids [19-27]. Furthermore, research suggests that polysaccharides are the primary bioactive agents in *M. charantia* responsible for its antimicrobial effects [28]. Polysaccharides extracted from *M. charantia* have demonstrated significant bactericidal activity against various bacterial strains, including *B. subtilis*, *S. aureus*, *S. typhimurium*, and *E. coli* [29].

Previous studies have reported that *M. charantia* had antibacterial potential. In a study, IZD values of aqueous seed extract of *M. charantia* on *S. aureus*, *E. coli*, and *P. aeruginosa* were 20 mm±0.51, 13 mm±0.51, and 16±0.51 mm, respectively [30]. A study by Ibisanmi et al. (2022) demonstrated that the IZD of water extracts of *M. charantia* was 16±0.29 mm against *E. coli*, 20±0.29 mm against *B. cereus*; 18±0.29 mm against *S. aureus*, 14±0.17 mm against *P. aeruginosa* at a concentration of 100 mg/mL [31]. In another study reported by Top et al. (2018), the antibacterial activity of *M. charantia* plant extract on *E. coli, S. aureus, E. faecalis, K. pneumoniae,* and *P. aeruginosa* was investigated and the highest IZD at 100 mg/mL concentration was *E. faecalis* (8.00 mm) [32]. In our study, the antibacterial activities of the aqueous seed extract of *M. charantia* were assessed with IZD. The significant antibacterial effect was detected against *S. aureus* (IZD: 15.25±0.957 mm), and *B. subtilis* (15.75±0.50mm).

A study conducted by Khalid et al. in 2021 found that the aqueous methanolic extract obtained from the leaves exhibited the highest effectiveness with a 30 mm diameter of the IZD against *P. multocida*. In contrast, the absolute alcoholic leaf extracts showed no discernible impact on *S. aureus*. The aqueous methanolic seed extract demonstrated significant antibacterial activity, displaying a IZD of 22 mm against *E. coli* [9].

The ethanolic leaf extract of *M. charantia* exhibited low antimicrobial activity against *Proteus mirabilis* and *Klebsiella pneumoniae* with MIC values of 312.5 and 625 µg/mL, respectively [33]. The MIC/MBC value of the acetone extract of *M. charantia* was 0.31/0.62 mg/mL against *Acinetobacter baumannii* [34]. In our experiment data, the seed extract was the most effective on the *S. aureus* strain (MIC/MBC:12.5 mg/mL).

According to the studies, the chemical composition of the *M. charantia* seed, IZD, and MIC/MBC results of its extract against bacteria are varied. The differences between the results are related to the type of solvent, extraction method, and harvest season [35].

5. Conclusion

Alcohols, aldehydes, esters, monoterpenes, and monoterpenoids have found as the principal components of *M. charantia* seeds. Based on our results, the aqueous seed extract of *M. charantia* has antibacterial activity against human pathogens. After toxicity assays, the seed extracts of *M. charantia* alone or combined with antibiotics could be utilized to cure bacterial infections in the pharmacological industry.

Author's Contributions

Mehzat Altun: Conducted the experiment, performed statistical analysis, evaluated the results, and wrote the manuscript.
Zerife Orhan: Contributed to the experiment's advancement, manuscript preparation interpreted findings.

Ethics

There are no ethical issues after the publication of this manuscript.

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