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Antimicrobial Properties of Lyophilized *Lemna minor* L. Extract Obtained by Microwave Extraction

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Abstract*— Diseases and deaths caused by pathogenic microorganisms are a serious problem worldwide. As a result of the problems caused by the unnecessary and unconscious use of antibiotics and the realization of the side effects of synthetic antimicrobials, there was a need to discover new antimicrobials obtained from natural sources. Lemna minor macrophyte has strong antimicrobial and antioxidant properties due to its secondary metabolites. Because of this feature, it has been used in traditional treatments for years. This study aimed to investigate the antimicrobial activity of L. minor against 11 different microorganisms (E. coli ATCC 25922, S. aureus ATCC 25923, S. aureus MRSA, P. aeruginosa ATCC 27853, B. cereus ATCC 6633, S. epidermidis ATCC 12228, L. monocytogenes, K. pneumoniae, S. infantis, S. typhimurium, Enterococcus faecalis bacteria and C. albicans ATCC 10231). L. minor was extracted by microwave and then lyophilized. The activities of lyophilized extracts were investigated by Minimal Inhibitory Concentration (MIC)(16-1000 µg/ml) and agar diffusion (250-500 µg/ml) methods. In general, it has been determined that concentrations of 250-500 µg/ml cause inhibition of all pathogens. The highest activity was determined K. pneumoniae (62.5 µg/ml) and P. aeruginosa (125 µg/ml).

***Index Terms*—Lemna minor, antimicrobial, microwave extraction.**

I. INTRODUCTION

Due to technological advancements in the 19th and 20th centuries, synthetic and semi-synthetic drugs replaced herbal treatments. Synthetic antioxidants have increasingly supplanted natural antioxidants due to their affordability and accessibility [1], [2]. Similarly, synthetic antimicrobials have been unconsciously used in various sectors, including food, agriculture, and animal husbandry. While antibiotics were originally created for treating infectious diseases, their use in livestock farming has increased in recent years due to their ability to promote animal growth and increase meat production. However, studies have demonstrated that the use of these antimicrobials in agriculture, particularly in animal husbandry, can result in residues in soil and water that may be harmful to both humans and animals [3], [4]. Antimicrobial resistance is a pressing issue in modern society. It has arisen due to various factors and has become one of the most significant current challenges. The incidence of antimicrobial resistance in pathogenic bacteria has become a global problem [5]. Synthetic drugs have many side effects [6] and non-natural antioxidant sources have been found to cause cancer which are serious reasons why research is now focused on natural and harmless antimicrobial and antioxidant sources [7], [8]. The increasing incidence of deaths worldwide due to infectious diseases and cancers highlights the importance of natural antimicrobial agents and anticancer drugs [9].

Approximately 80% of the active compounds obtained from plants used in modern medicine are associated with their traditional use [10]. Medicines derived from natural products are used by 80% of the global population, as per [11]. Nowadays, numerous biologically active metabolites from terrestrial plants have been identified, and modern bioinformatics programs facilitate the design of novel drugs [12]. Research on the medicinal properties of aquatic plants is greatly restricted, with limited existing studies focused mainly on marine plants. Only a few investigations have been carried out on macrophytes distributed in fresh waters [12], [13]. Macrophytes refer to visible, photosynthetic organisms residing in aquatic environments [14], [15]. *Lemna minor* is a floating, perennial aquatic plant that belongs to the Lemanaceae family and inhabits freshwater. *L. minor* has often been utilized in ecotoxicology research due to its fast growth, adaptability to laboratory environments, ease of harvesting and high biomass production. In addition to these studies, *L. minor* has been a traditional medicinal and homeopathic remedy for many years [16]. Its antipyretic, diuretic, and anti-inflammatory properties make it popular for the treatment of upper respiratory tract and chronic rheumatic diseases, as well as for external use in conditions such as eczema, acne, wound healing, and insect bites [17].

The study's objective was to assess the antimicrobial efficacy of *L. minor* against 11 pathogens using the Minimal Inhibitory Concentration (MIC) and Agar diffusion techniques.

II. MATERIALS- METHODS

A. Plant growth

Lemna minor used in the study was purchased from a local aquarium products store and grown in aquariums for 4 weeks before starting the study to adapt to laboratory conditions. The plants were identified by comparing the morphological characteristics of fresh plant samples [18]. The plants used in the study were grown in aquaria in the Hydrobiology Laboratory of the Biology Department of Ankara University. Until the experiments, the plants were kept in 100-liter

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aquariums containing 40% Hoagland medium, without any heater or filter system, under a 12:12 light:dark photoperiod, with a water temperature of 19.3±2.1 °C. The water in the aquariums was replenished every two days by drawing 1/5 from the bottom [19].

The microwave-assisted extraction method was utilised to extract plant extracts. This technique has gained popularity in recent years due to its benefits, including reduced solvent consumption compared to traditional Saxlet methods, quicker processing times, typically expressed in minutes, and the absence of a volatilisation stage afterwards [20].

B. Preparation of Plant Extracts

One gram of plant sample was transferred into a flask containing 20 millilitres of 60% ethanol (99.5% Merck, Germany) and subjected to microwave-assisted extraction at 850 watts for 30-30-30 in 90 seconds. The obtained extracts were subsequently centrifuged at 2500 rpm for 20 minutes, and the resulting supernatant was filtered through Whatman No:1 filter paper [21]. The extracts were frozen and dried using a lyophilizer (Christ, Alpha 1-2). They were then stored at a temperature of +4°C until analysis [22], [23].

C. Investigation of Antimicrobial Activity

The study assessed the antimicrobial activity of *L. minor* extracts against 11 pathogenic microorganisms: *Esherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Salmonella infantis*, *S. typhimurium*, *Enterococcus faecalis* bacteria, and *Candida albicans* ATCC 10231 yeast. Bacteria were cultured in Nutrient Broth (NB) (Merck, Germany) and *C. albicans* was cultured in Sabouraud (2%) Dextrose Broth (SDB) (Merck, Germany) at 37°C for 18 hours [24], [25]. Two distinct approaches were adopted to evaluate antimicrobial activity. These methods are described in detail under separate headings.

Minimum Inhibitory Concentration (MIC) Method

One hundred milligrams of plant extract were dissolved in 10 millilitres of DMSO. The resulting stock solution was sterilised using a 0.22 µm filter. Serial dilutions were made in Nutrient Broth medium to prepare samples with concentrations ranging from 1000-16 µg/ml. McFarland 0.5 density pathogens were inoculated into these media containing the plant extracts and incubated at 37°C for 24 hours. After the incubation period concluded, the minimum inhibitory concentration (MIC) was determined by establishing the concentration that exhibited no visible turbidity [26], [27].

Agar Diffusion Method

The pathogens, which were adjusted to a density of McFarland 0.5, were seeded on Muller Hinton Agar (MHA) medium. Extract concentrations (250 and 500 µg/ml), which were determined to be effective in the MIC study, were added to drilled wells with a 5 mm diameter on the agars. These were then incubated at 37ºC for 24 hours. Antimicrobial activity was determined by measuring the diameter (mm) of the inhibition zones around the wells towards the end of the incubation period [26], [27].

III. RESULTS

The extract of *L. minor* demonstrated antimicrobial activity against all microorganisms studied. The greatest activity was observed against *K. pneumoniae* (62.5 µg/ml). The bacterium with the highest resistance was S. epidermidis for which a dose of 1000 µg/ml was necessary (Table 1). *Lemna* species contain phenolic compounds such as gallic acid, tannins, flavonoids, anthocyanins, quercetin and other compounds including thiol, terpenes and steroids. These compounds possess antimicrobial activity and can be effective against *B. subtilis*, *B. cereus*, *S. aureus*, *S. saprophyticus*, *S. warneri*, *Proteus vulgaris*, *Citrobacter freundii*, *C. koseri*, *Neisseria lactamica*, *Micrococcus luteus*, and *Streptococcus pneumoniae* bacteria as well as various *Candida* sp. fungi have been documented in previous studies [28], [29]. *Pseudomonas fluorescens* is identified as an opportunistic pathogen that affects fish, birds, and humans, causing illnesses in numerous fish in brackish, fresh and marine waters across the globe. In their study, Gonzalez-Renteria [28] examined the antimicrobial effects of extracts from *Lemna minor* on the *P. fluorescens* pathogen. Three different solvents (methanol, hexane, and chloroform) were used at three different incubation periods (24, 48, and 72 hours) and six different concentrations (5000, 500, 50, 5, 0.5, and 0.05 µg/ml) to determine their effect. The results showed that the highest effect was observed with hexane and chloroform solvents during a 24-48 hour incubation period. The minimum inhibitory concentration (MIC) of the 24-hour methanol extract was found to be 0.05 µg/ml. In their study using methanol extract of *L. minor* and 11 different pathogens, Tan [30], reported that the MIC values ranged from 1.8-2.0 mg/ml. In a similar study, discovered that the MIC values of methanolic *L. minor* extract were 12, 40, 60, 90 and 170 µg/ml for *Shigella flexneri*, *B. subtilis*, *M. luteus*, *P. aeruginosa and S. aureus* pathogens, respectively. Further research on the topic indicates that the MIC value of *L. minor* may fluctuate, but in general, it has promising antimicrobial activity [17].

Table I. Antimicrobial activities (µg/ml)

	Microorganism	**MIC (µg/ml)**	**Agar Diffusion (mm)**	
			250 µg	**500 µg**
1	***E. coli***	250	-	-
2	***S. aureus***	250	-	-
3	***S. aureus* – MRSA**	500	10	13

	Microorganism	MIC (µg/ml)	Agar Diffusion (mm)	
			250 µg	500 µg
4	***E. faecalis***	500	-	7
5	***S. epidermidis***	1000	13	14
6	***L. monocytogenes***	125	8	10
7	***K. pneumoniae***	62.5	12	15
8	***S. infantis***	250	10	13
9	***S. typhimurium***	250	-	-
10	***P. aeruginosa***	250	8	11
11	***C. albicans***	250	-	-

The agar diffusion analysis results were found to be generally consistent with the MIC study, with parallel zones observed around the well. Similar to the MIC study, *K. pneumoniae* was identified as the most sensitive pathogen, with a diameter of 15 mm. However, extracts that displayed activity in the MIC study did not produce any zones in the agar diffusion method for *E. coli*, *S. aureus*, *S. typhimurium*, and *C. albicans* microorganisms. Negative results can occur with this method due to polarity and diffusion problems [29]. In a study, *L. minor* macrophyte was extracted from Hamam Creek (Erzurum) using ethanol and methanol. The antimicrobial activity of the extract was investigated on reference and clinical strains comprising of 21 bacteria and 4 fungi using the disc diffusion method. The study resulted in the extraction of plants in both solvents, which were tested against various bacterial strains including *S. epidermidis*, *S. saprophyticus*, *S. warneri*, *C. freundii*, *C. koseri*, *Neisseria lactamica*, *N. sicca*, *M. luteus*, *B. cereus*, *B. subtilis*, and *S. pneumoniae*. The researchers reported that *L. minor* exhibits anticandidal activity against *C. parapsilosis* and *C. glabrata* yeasts. Therefore, it is considered a promising natural food preservative [28].

IV. CONCLUSION

Our study suggests that the lyophilised extract of L. minor we analysed displays potential antimicrobial activity on pathogens responsible for diseases such as meningitis, diarrhoea, colitis, endocarditis and pneumonia. Further research can evaluate the viability of this extract as a raw material for drugs and a preserving agent for food.

The studies were carried out in Gazi University Life Sciences Application and Research Center Laboratories.

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