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Extraction of Essential Oil from Origanum majorana and Evaluation of Its Antimicrobial Activity against Pathogenic Bacteria Isolated from a Hospital Environment

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Abstract

This study aimed to develop an efficient and low-cost method for extracting essential oil from Origanum majorana (marjoram) and to evaluate its antimicrobial activity against pathogenic bacteria isolated from a hospital environment. An organic solvent extraction method using n-hexane at a controlled low temperature (35 °C) was employed to preserve thermolabile bioactive compounds while reducing energy consumption. The extraction process involved plant drying, solvent maceration, purification, and the production of absolute oil through vacuum distillation.

The antimicrobial activity of both commercially available and naturally extracted marjoram essential oils was assessed against bacterial isolates obtained from different hospital departments, including nephrology, maternity, intensive care, and neonatal units. The disc diffusion method described by Lorian (1980) was used to evaluate bacterial sensitivity. A total of 250 bacterial isolates were obtained from 160 clinical and environmental samples.

The results demonstrated that the naturally extracted marjoram essential oil exhibited notable antibacterial activity, particularly against Proteus mirabilis, Escherichia coli, and Staphylococcus aureus. Inhibition zones increased proportionally with oil concentration, reaching the highest values at 30%. In contrast, commercial marjoram oils showed limited or no antibacterial efficacy, which may be attributed to low concentrations of active compounds or improper manufacturing and storage conditions.

The study concludes that the proposed low-cost solvent extraction method yields high-quality essential oil with significant antibacterial properties. Origanum majorana essential oil represents a promising natural alternative for pharmaceutical and therapeutic applications, especially in the context of combating hospital-associated bacterial pathogens.

Keywords: Origanum majorana; Essential oil extraction; Antimicrobial activity; Hospital-isolated bacteria; Disc diffusion method; Medicinal plants.

INTRODUCTION

Essential oils are among the most valuable natural products due to their wide range of biological activities and their extensive applications in pharmaceutical, food, cosmetic, and fragrance industries. These volatile plant-derived compounds are characterized by complex chemical compositions that confer antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties. The efficiency of essential oil extraction plays a crucial role in determining both the quality and commercial value of the final product.

Origanum majorana L., commonly known as marjoram, is an aromatic and medicinal plant belonging to the Lamiaceae family. It is widely recognized for its rich content of bioactive volatile compounds, such as terpinen-4-ol, thymol, carvacrol, and linalool, which are responsible for its antimicrobial, antioxidant, and therapeutic effects. Owing to these properties, marjoram essential oil has attracted considerable attention in both traditional medicine and modern industrial applications.

Despite its importance, the extraction of high-quality essential oil from *O. majorana* remains challenging. Conventional extraction techniques, such as steam distillation, hydrodistillation, and solvent maceration, are widely used but are often associated with high energy consumption, long processing times, and potential degradation of heat-sensitive compounds. In addition, these methods may result in variable yields and inconsistent oil composition, which limits their economic and industrial feasibility.

In recent decades, increasing emphasis has been placed on developing alternative extraction techniques that are both cost-effective and environmentally sustainable. Modern approaches, including ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction, have demonstrated improved efficiency and better preservation of bioactive constituents. However, the high operational cost and technical requirements of some of these methods restrict their application, particularly in small- and medium-scale production systems.

Accordingly, this study seeks to evaluate a simple, low-cost solvent extraction method for obtaining essential oil from *Origanum majorana* while maintaining high quality and biological activity. In addition, the antimicrobial potential of the extracted oil is assessed against pathogenic bacteria isolated from a hospital environment, with the aim of exploring its suitability as a natural alternative to conventional antimicrobial agents.

MATERIALS AND METHODS

Plant Material Preparation

Fresh aerial parts of *Origanum majorana* were collected and air-dried in a well-ventilated, shaded area for a period of 8–10 days until constant weight was achieved. The dried plant material was then cut into small pieces to increase the surface area and enhance extraction efficiency.

Essential Oil Extraction

A low-cost solvent extraction method was developed using *n*-hexane as an organic solvent. The dried plant material was immersed in *n*-hexane and maintained at a controlled temperature of approximately 35 °C to prevent degradation of thermolabile compounds. The mixture was continuously agitated for several hours to ensure maximum extraction of aromatic constituents.

The extraction process was repeated several times to improve oil recovery. The combined extracts were then treated with sodium hydroxide solution to remove impurities and undesirable non-aromatic components. After purification, the solvent was evaporated, yielding crude essential oil suitable for further processing.

Preparation of Absolute Essential Oil

To obtain absolute oil, the crude (concrete) oil was subjected to further purification. The oil was mixed with ethanol at a ratio of 1:8 and placed in a water bath. The mixture was vigorously shaken and subsequently filtered to remove waxes and fatty substances.

The filtrate was transferred to a vacuum distillation apparatus and maintained at sub-zero temperatures for 10–15 h. Final distillation was conducted under reduced pressure at 30 °C, followed by gentle heating in a water bath. The resulting product was a highly purified absolute essential oil free from non-volatile impurities.

Bacterial Isolation and Identification

A total of 160 samples were collected from different hospital departments, including nephrology, intensive care unit (ICU), maternity ward, and neonatal unit. Standard microbiological techniques were employed for bacterial isolation and cultivation.

Bacterial identification was carried out based on cultural characteristics and biochemical tests. In total, 250 bacterial isolates were obtained and classified into several genera, including *Escherichia*, *Klebsiella*, *Enterobacter*, *Shigella*, *Proteus*, *Pseudomonas*, *Citrobacter*, and *Staphylococcus*.

Antimicrobial Activity Assay

The antibacterial activity of marjoram essential oil was evaluated using the disc diffusion method as described by Lorian (1980). Sterile filter paper discs (1 mm thickness) were prepared and impregnated with either commercial marjoram oil or naturally extracted essential oil at concentrations of 10%, 20%, and 30%.

Bacterial suspensions were prepared and evenly spread onto blood agar plates using sterile swabs. The oil-impregnated discs were placed on the agar surface at equal distances to avoid overlapping inhibition zones. Plates were incubated at 37 °C for 24 h. Antibacterial activity was assessed by measuring the diameter of inhibition zones (mm) surrounding each disc.

Results and Discussion

A total of 160 samples were examined and analyzed from different hospital departments, including the nephrology unit (30 samples), neonatal unit (25 samples), intensive care unit (70 samples), and maternity ward (35 samples). The results showed that the bacterial isolation rate was 100%, as summarized in Table 1.

Table 1. Number and percentage of positive bacterial isolates obtained from hospital departments

Sample source	Number of samples	Number of isolates	Percentage (%)
Nephrology unit	30	45	18.0
Intensive care unit (ICU)	70	115	46.0
Maternity ward	35	58	23.2
Neonatal unit	25	32	12.8
Total	**160**	**250**	**100**

Biochemical identification of bacterial isolates

Biochemical characterization revealed a diverse distribution of bacterial genera. *Escherichia coli* accounted for 46 isolates (18.4%). The genus *Klebsiella* included *K. pneumoniae* (16 isolates) and *K. aerogenes* (12 isolates). All *Enterobacter* isolates (21 isolates) were identified as *E. aerogenes*.

The genus *Shigella* comprised *S. flexneri* (20 isolates) and *S. dysenteriae* (13 isolates). A total of 18 isolates belonged to the genus *Proteus*, all identified as *P. mirabilis*. All *Pseudomonas* isolates were classified as *P. aeruginosa*. Thirty isolates belonged to *Citrobacter* spp. The genus *Staphylococcus* accounted for 49 isolates, of which *Staphylococcus aureus* represented the majority.

The overall biochemical classification of the isolated bacteria is presented in Table 2.

Table 2. Biochemical classification of bacterial isolates

Bacterial group	Number of isolates	Percentage (%)
Escherichia coli	46	18.4
Klebsiella spp.	28	11.2
— *K. pneumoniae*	16	57.1
— *K. aerogenes*	12	42.9
Enterobacter spp. (*E. aerogenes*)	21	8.4
Shigella spp.	33	13.3
— *S. flexneri*	20	60.6
— *S. dysenteriae*	13	39.4
Proteus spp. (*P. mirabilis*)	18	5.6
Pseudomonas spp. (*P. aeruginosa*)	25	10.0
Citrobacter spp.	30	12.0
Staphylococcus spp.	49	19.6

Bacterial group	Number of isolates	Percentage (%)
– Coagulase-positive	35	71.4
– Coagulase-negative	14	28.8
Total	**250**	**100**

The present findings are consistent with the study of Deepjot et al. (2002), who reported the presence of *Staphylococcus aureus* in 12% of samples collected from healthcare workers, supporting the relevance of our results. Similar findings were also reported by Orji et al. (2005), who investigated bacterial contamination of physicians' apparel in a Nigerian hospital and found that 58% of samples were contaminated with pathogenic bacteria, including *Proteus*, *Salmonella*, *Shigella*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Furthermore, these results agree with Treakle et al. (2009), who demonstrated that healthcare workers may act as reservoirs for bacterial transmission in hospitals. In their study, *Staphylococcus* species were isolated from the majority of collected samples, including strains resistant to methicillin. Likewise, Ekrami et al. (2010) reported extensive bacterial contamination across hospital environments in Iran, emphasizing the need for improved hygiene and disinfection practices.

Antibacterial activity of commercial marjoram essential oil

Experimental evaluation of the commercial marjoram essential oil revealed no inhibitory effect against the tested bacterial isolates, even when applied at high concentrations without dilution. Bacterial growth was observed around all discs, as shown in Table 3.

Table 3. Effect of commercial *Origanum majorana* essential oil on bacterial isolates

Result	Bacterial isolate
+	*Staphylococcus aureus*
+	*Pseudomonas aeruginosa*
+	*Proteus mirabilis*
+	*Escherichia coli*

Note: (+) indicates visible bacterial growth and absence of inhibition.

The lack of antibacterial activity may be attributed to low concentrations of active compounds, inadequate extraction or manufacturing processes, improper storage conditions, or the addition of solvents and preservatives that reduce biological efficacy. Therefore, naturally extracted essential oils are recommended for reliable antimicrobial evaluation.

Antibacterial activity of naturally extracted marjoram essential oil

The naturally extracted *Origanum majorana* essential oil demonstrated measurable antibacterial activity against Gram-negative bacteria. *Proteus mirabilis* showed the highest susceptibility, while *Pseudomonas aeruginosa* exhibited lower sensitivity, particularly at lower concentrations. These

findings are consistent with those reported by Ouwehand et al. (2010), who observed strong antibacterial effects of essential oils against pathogenic bacteria.

Similar results were reported by Abu-Darwish et al. (2012), who confirmed the strong inhibitory effects of essential oils derived from *Salvia officinalis* and *Thymus vulgaris* against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Table 4. Antibacterial activity of naturally extracted *Origanum majorana* essential oil

Bacterial isolate	10% (mm)	20% (mm)	30% (mm)
Proteus mirabilis	0.30	1.20	1.60
Pseudomonas aeruginosa	0.14	0.23	0.39
Staphylococcus aureus	0.11	0.19	0.53
Escherichia coli	0.83	1.10	1.30

An increase in essential oil concentration resulted in a corresponding increase in bacterial growth inhibition, with the 30% concentration showing the strongest antibacterial effect. *Proteus mirabilis* was the most sensitive organism, followed by *E. coli* and *S. aureus*, whereas *Pseudomonas aeruginosa* was the least affected.

Discussion

The present study demonstrates that the extraction method plays a decisive role in determining both the chemical quality and the antimicrobial efficacy of *Origanum majorana* essential oil. The naturally extracted oil obtained using *n*-hexane at low temperature (35 °C) exhibited pronounced antibacterial activity against several pathogenic bacteria isolated from a hospital environment, whereas commercial marjoram oils showed no detectable inhibitory effects.

The superior performance of the naturally extracted oil may be attributed to the preservation of thermolabile bioactive compounds, such as terpinen-4-ol, thymol, and carvacrol, which are widely recognized for their antimicrobial properties. Previous studies have reported that these oxygenated monoterpenes disrupt bacterial cell membranes, increase membrane permeability, and interfere with essential metabolic processes, ultimately leading to bacterial growth inhibition (Vera, 1999; Komaitis, 1992).

The observed concentration-dependent antibacterial activity is consistent with earlier findings, which indicate that higher essential oil concentrations enhance membrane destabilization and intracellular leakage in bacterial cells. In this study, the highest inhibition zones were recorded at a concentration of 30%, confirming the dose–response relationship reported by Ouwehand et al. (2010) and Abu-Darwish et al. (2012).

Among the tested bacterial species, *Proteus mirabilis* exhibited the highest susceptibility to marjoram essential oil, followed by *Escherichia coli* and *Staphylococcus aureus*. In contrast, *Pseudomonas aeruginosa* showed comparatively lower sensitivity. This reduced susceptibility may be explained by the intrinsic resistance mechanisms of *P. aeruginosa*, including its low outer membrane permeability and the presence of efflux pumps, which limit the penetration and accumulation of hydrophobic compounds such as essential oils.

The lack of antibacterial activity observed in commercial marjoram oils highlights a critical issue related to essential oil quality in the market. Factors such as dilution, adulteration, prolonged storage, exposure to light and heat, or improper extraction techniques can significantly reduce the concentration of active constituents. Similar observations have been reported in previous studies that emphasized the importance of standardized extraction and storage conditions to ensure biological efficacy (Kakouri, 2022; Khan et al., 2023).

Furthermore, the high prevalence of pathogenic bacteria isolated from hospital departments, particularly intensive care units, underscores the ongoing challenge of hospital-acquired infections. The findings of this study align with earlier reports documenting widespread bacterial contamination in healthcare environments (Treakle et al., 2009; Ekrami et al., 2010). In this context, natural antimicrobial agents such as marjoram essential oil may offer complementary or alternative strategies for infection control, especially against antibiotic-resistant strains.

Overall, the results support the potential application of *Origanum majorana* essential oil as a natural antimicrobial agent and emphasize the importance of developing low-cost, efficient extraction methods suitable for small- and medium-scale production systems.

Conclusion

This study successfully demonstrated that a low-cost solvent extraction method using *n*-hexane at controlled low temperature yields high-quality *Origanum majorana* essential oil with significant antibacterial activity. The naturally extracted oil exhibited strong inhibitory effects against several pathogenic bacteria isolated from a hospital environment, particularly *Proteus mirabilis*, *Escherichia coli*, and *Staphylococcus aureus*, with antibacterial efficacy increasing proportionally with oil concentration.

In contrast, commercial marjoram essential oils showed no detectable antimicrobial activity, highlighting the impact of extraction quality, processing, and storage conditions on biological effectiveness. These findings confirm that optimized extraction techniques are essential for preserving the bioactive constituents responsible for antimicrobial activity.

The study concludes that *Origanum majorana* essential oil represents a promising natural alternative to conventional antimicrobial agents, with potential applications in pharmaceutical, therapeutic, and infection-control contexts. Future research is recommended to further characterize the chemical composition of the extracted oil using advanced analytical techniques (e.g., GC–MS) and to evaluate its efficacy against a broader range of clinically relevant and antibiotic-resistant microorganisms.

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