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An In Vitro Usage of *Origanum majorana* Watery Extract as Inhibitory Antibacterial Component against Different Medically Important Bacterial Isolates

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Abstract. *Origanum majorana demonstrates a broad spectrum of effectiveness against various diseases, exerting antimicrobial effects against different pathogenic microorganisms. The goals of this research are to provide light on how Origanum majorana extract inhibits human pathogenic bacteria, how it may prevent biofilm development, and how it affects bacterial adhesion. The antibacterial activity of the water-based Origanum majorana extract was assessed in this research using two different methodologies. "Antibiotics were compared to its effectiveness using an agar-well diffusion assay and disc diffusion method," the first step. Additionally, the extract's ability to inhibit biofilm formation and bacterial adherence was assessed through specialized tests. All bacterial isolates of Gram negative, Gram-positive bacterial types were sensitive to Origanum majorana extract and the range of inhibition zone (30 to 24) mm. Although floxacin was effective against some of these isolates, the majority of them were resistant. Most bacterial Gram negative types were exhibit moderate adherence and biofilm activity to this extracts and some bacterial isolated of bacteria were exhibit high adherence and biofilm activity to the watery extracts of Origanum majorana. This research confirms previous findings that Origanum majorana extracts are very effective against a wide variety of clinical isolates of bacteria, including Gram-negative as well as Gram-positive varieties. Notably, the extracts were found to be more effective than commercially available antibiotics. Furthermore, the extracts displayed significant inhibition of bacterial adherence and biofilm formation. Based on these findings, Origanum majorana extracts have great promise as an effective antibacterial and anti-biofilm agent.*

Keywords *Adherence Inhibition, Antimicrobial Properties, Biofilm Formation, Floxacin and Origanum majorana.*

1. INTRODUCTION

A delicate perennial plant belonging to the Labiatae family, marjoram is officially named Origanum majorana L. Originally from the Eastern Mediterranean, this plant is now more often known as "sweet marjoram" and was once known as Majorana hortensis Moench.

Cultivation of this herb is widespread in countries such as France, India, Hungary, and the United States (Wang et al., 2021). “The phenolic chemicals found in medicinal plants are very promising for use in the creation of new pharmaceuticals.

These compounds offer several advantages, including widespread availability, affordability, and safety. Indeed, plant-derived drugs that are consumed for self-medication and recreational purposes and to alleviate pain and suffering have existed since the dawn of early civilisation(Ghazal et al., 2022). Sweet marjoram has been in use since ancient times and is considered an excellent therapeutic herb and flavouring agent for culinary application. The dried leaf, leaf extract as well as essential oil have been used for the treatment of inflammations of the throat and nose, gastrointestinal and urinary tract issues(Shakya, 2016). Sweet marjoram is recognised for its spasmolytic, diuretic antraheumatic, as well as antiasthma activity. The

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broad pharmacological properties of Marjoram now have a sound scientific evidence base(Tripathy, Satyanarayana, Khan, Raja, & Sciences, 2017). The herb has been shown to possess antioxidant, hepatoprotective, antibacterial, antiulcer, cardioprotective, anticoagulant, antiproliferative, anti-inflammatory, as well as antifungal activity (Ben Salha, Herrera Díaz, Lengliz, Abderrabba, & Labidi, 2019). A few in vitro studies have demonstrated antibacterial and antifungal activity of Marjoram, particularly the active component, terpinen-4-ol, against both Gram-positive (eg, *S. aureus*, E. faecalis) as well as Gram-negative bacteria (E. coli, K pneumoniae, P mirabilis, P multocida) and against a few fungal strains at a wide range of concentrations, including against the drug-resistant clinical isolates (Vasireddy, Bingle, & Davies, 2018). The antioxidant properties of Marjoram have also been demonstrated in the study by (Zeouk, Ouali Lalami, & Bekhti, 2019).

The research objective included an assessment and detection of inhibitory antibacterial effect of Marjoram against different Gram-positive and Gram-negative medically-important pathogenic isolates as well as its inhibitory action against various virulence factors.

2. METHODS

The leaves of *Origanum majorana* were collected, washed with distilled water and dried by air. The aquatic extract was prepared according to (N. K. J. A. j. o. p. Hindi & Therapeutics, 2013). by weighting 25 gram of the powder of the plant, put it in pyrex beaker and add 100 ml of boiling water. Left for 30 min, and put the beaker on the magnetic stirrer overnight. Later on the extract was centrifuged with 2500 rotary/min for 10min. Then used Whatman filter paper and left overnight, put the extract in the oven at 40-45. Weight the extract and dissolve it in a suitable amount of water to get the final stock concentration as 25%; Floxacin was used as a positive control drug.

3. BACTERIAL ISOLATES:

The research made use of twenty-two different bacterial isolates that were obtained from patient samples. Agar slants containing these isolates were cultivated and "activated in three consecutive sets" before being kept at 4ºC. Multiple biochemical analyses verified the taxonomic assignments of the isolates (Forbes, Sahm, & Weissfeld, 2007). In accordance with what is shown in Table 1, fourteen of the isolates were Gram-negative bacteria, whereas eight were Gram-positive bacteria as (*Aggregatibacter actinomycetemcomitans, Acinetobacter baumannii, Enterobacter aeruginosa, Klebsiaella pneumoniae, Prevotella intermedia, Porphyromonas gingivalis, Proteus spp. as mirabilis* and *vulgaris, Serratia spp., Salmonella*

spp. as typhi and *typhimurium, in addition to the Pseudomonas as aeruginosa* and *flourscences*), whereas eight were Gram-positive bacteria (different Staphylococci spp. as aureus, epidermidis, and saprophyticus; different streptococci spp. as agalactiae, faecalis, mutans, pneumoniae and pyogenes).

Antibiotic susceptibility test and antibacterial effect of the extract:

Disk-diffusion test was used to detect susceptibility to Floxacin and the antibacterial inhibitory effect of the extract; as 4-5 colonies were picked from pure culture of each isolate and put it to a test tube containing 5 ml of normal saline, then turbidity was adjusted to about 1.5 x 108 CFU/ml. Few drops of suspension were taken by a sterile cotton swab and spread evenly on the surface of Muller-Hinton agar medium, left to dry and add one disk of Floxacin on the surface of each plate. Then after wells were made in each plate by cork-borer to put 200 micro-milliliter of the extract in each well; the test being run in triplicate to assure consistency and reliability (N. K. J. A. j. o. p. Hindi & Therapeutics, 2013). Later, plates allowed to dry and incubated at 37°C for 24 hr. Inhibition zones around the wells and disks were measured by millimeter (mm) unit by using a metric ruler. The results were interpreted as according to CLSI (2020).

Adherence test:

One of the most important virulence factors shown by bacteria is their ability to cling to the cell membranes of humans. The technique employed to assess bacterial adherence, specifically for Gram-negative bacteria, is described in references (N. K. K. Hindi, Al-Mahdi, & Chabuck, 2014).

Biofilm Formation Assay:

The gold standard for identifying biofilm development was the semi-quantitative microtiter plate test, which was devised by (Ávila-Campos et al., 2000). This test is also known as the Tissue Culture Plate (TCP) technique assay. Table 2 provides an overview of the results obtained using this method, specifically for Gram-negative bacteria.

Table 1: displays the results of bacterial adherence and biofilm formation using the TCP method (N. Hindi, Yasir, Al-Mahdi, & Jebur, 2016).

Formation of Biofilm	Adhesion activity	Mean of O.D. values at 630nm
No formation (Negative)	Negative	Less than 0.120
Moderate formation (Moderate positive)	Moderate adhesion	0.240 – 0.120
High formation (Highly positive)	Strong adhesion	More than 0.240

4. RESULTS

The antibacterial effect of *Origanum majorana* at 25% concentration against bacteria by agar well method was studied (Figure 1 and Figure 2), all bacterial isolated of Gram negative as well as Gram-positive bacteria were sensitive to this extract and the range of inhibition zone were (30 to 24) mm.

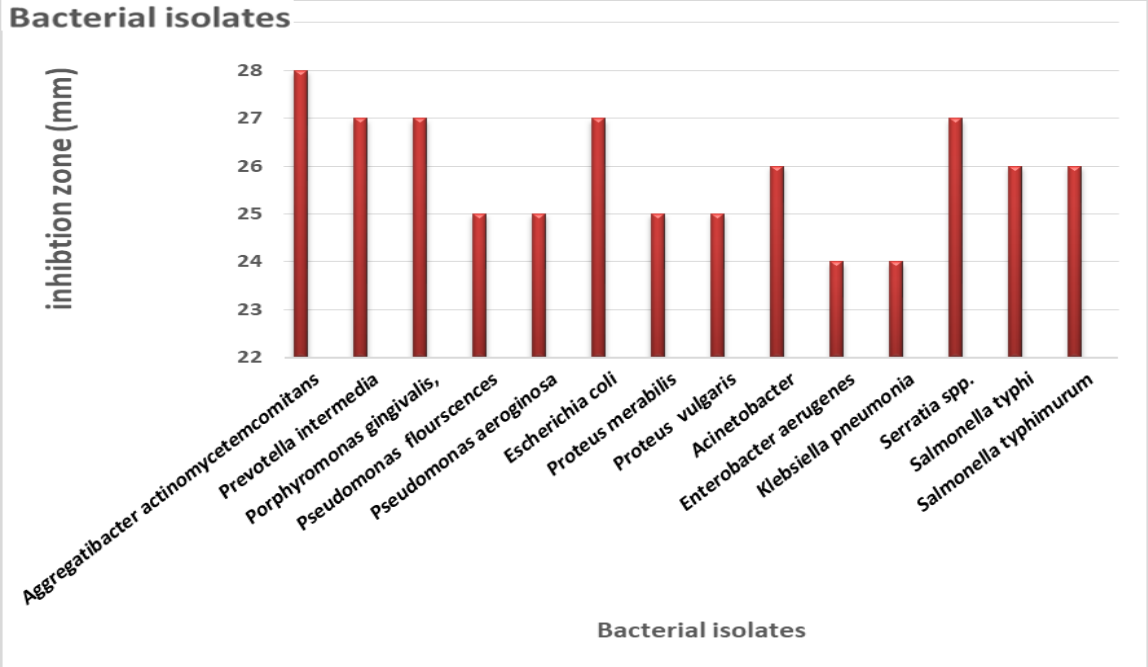

Figure 1: Inhibitory Antibacterial Effect of 25% *Origanum majorana* Aqueous Extract against Different Clinical Gram-negative Bacterial Isolates by Agar Well-Diffusion Assay.

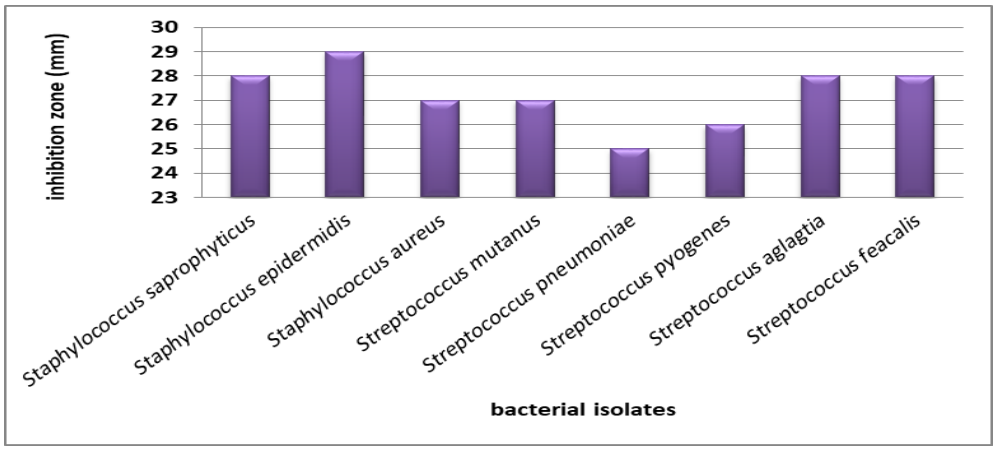

Figure 2: Inhibitory Antibacterial Effect of 25% *Origanum majorana* Aqueous Extract against Different Clinical Gram-positive Bacterial Isolates by Agar Well-Diffusion Assay.

Figure 3 shows the disk diffusion technique demonstrating the antibacterial activity of floxacin against Gram-negative bacteria. Using the same method, Figure 4 shows that floxacin is effective against Gram-positive bacteria. It is noteworthy that a significant number of

bacterial isolates, both Gram-negative and Gram-positive, exhibited resistance to this antibiotic. However, there were also instances where certain bacterial isolates demonstrated sensitivity to floxacin.

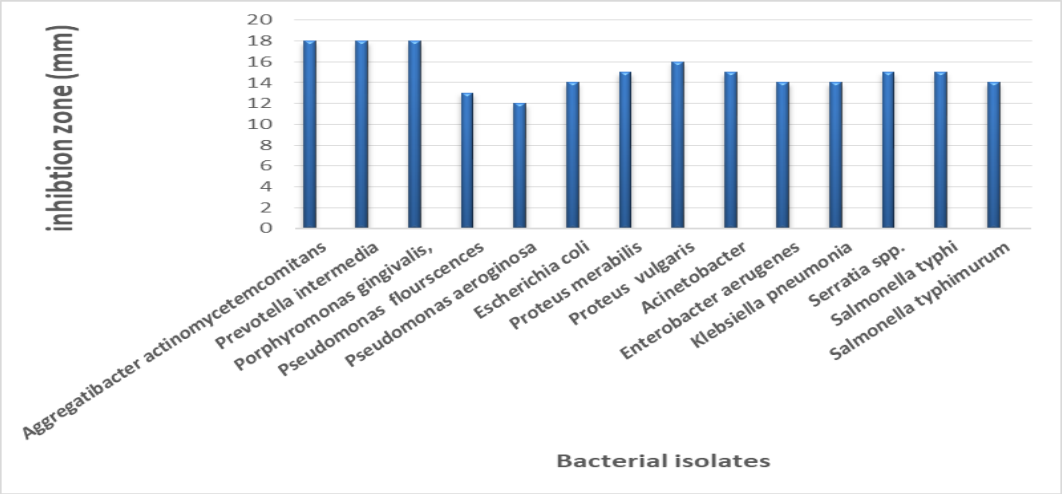

Figure 3: Antibiotic Susceptibility Test of Floxacin against Different Clinical Gram-negative Bacterial Isolates by Disk-Diffusion Test.

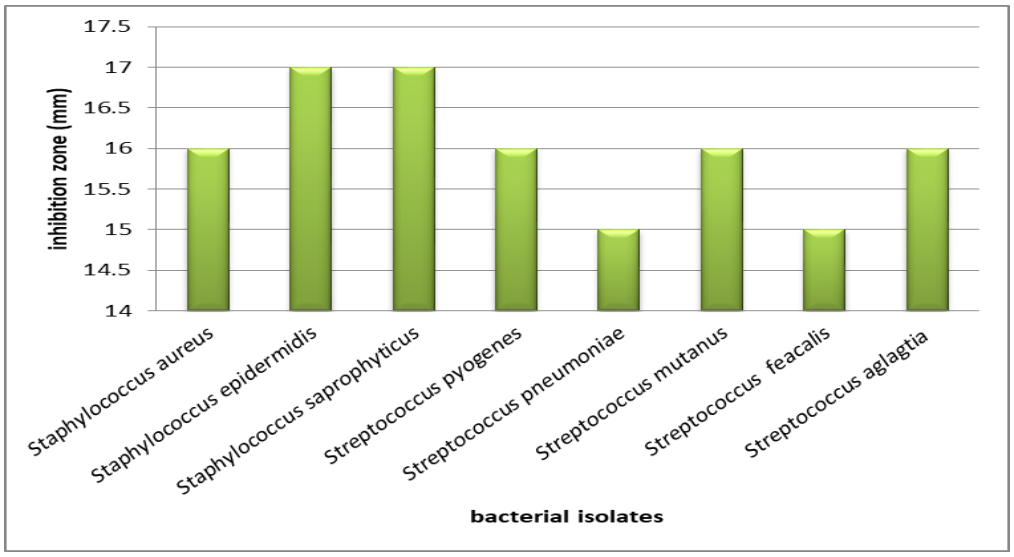

Figure 4: Antibiotic Susceptibility Test of Floxacin against Different Clinical Gram-positive Bacterial Isolates by Disk-Diffusion Test.

Origanum majorana aquatic extracts were tested for their anti-adherence and anti-biofilm activities against Gram-negative bacteria. The findings are shown in Table 3 and Table 4, respectively. It was observed that the majority of the Gram-negative bacterial isolates displayed moderate adherence and biofilm activity in response to these extracts. However, there were some isolates that exhibited high adherence and biofilm activity when exposed to the aquatic extracts of *Origanum majorana.*

Table 2: Anti-Adhesion Activity of aquatic extracts of *Origanum majorana* against Different Clinical Gram-negative Bacterial Isolates.

Bacterial type	**Extract effect**
Aggregatibacter actinomycetemcomitans	High inhibition
Prevotella intermedia	
Porphyromonas gingivalis	
Acinetobacter baumannii	
Enterobacter aeruginosa	Moderate inhibition
Klebsiaella pneumoniae	
E. coli	
Pseudomonas (aeruginosa and *flourscences)*	
Serratia spp.	
Proteus spp. (mirabilis and *vulgaris)*	
Salmonella spp. (typhi and *typhimurium)*	

Table 3: Active anti-biofilm properties of aquatic *Origanum marjorana* extracts against Different Clinical Gram-negative Bacterial Isolates

Bacterial type	**Extract effect**
Aggregatibacter actinomycetemcomitans	High inhibition
Prevotella intermedia	
Porphyromonas gingivalis	
Acinetobacter baumannii	
Enterobacter aeruginosa	Moderate inhibition
Klebsiaella pneumoniae	
Pseudomonas (aeruginosa and flourscences)	
Proteus spp. (mirabilis and vulgaris)	
Salmonella spp. (typhi and typhimurium)	
Serratia spp.	No effect
E. coli	

5. DISCUSSION

The utilization of natural substances is currently being promoted as a result of the growing resistance of bacteria to various natural and synthetic antibiotics, as well as increased recognition of the adverse effects associated with these chemicals. Past attempts to manage antibiotic resistance and reduce the predominance of harmful microbes failed, alternative approaches involving natural compounds are gaining importance (Pernin, Guillier, & Dubois-Brissonnet, 2019).

“A major public health concern globally is the proliferation of microorganisms that are resistant to more than one antibiotic. It is generally believed that the main cause of the rise of antibiotic-resistant strains is the inappropriate and overuse of antibiotics in their treatment of microbiological diseases (Szemerédi et al., 2020).

The results of this study showed that most of bacterial isolates were resistant to the antibiotic floxacin, in comparison to good response to the *Origanum majorana* extract which showed a notable inhibitory effect against most of the studied isolates; because it inhibited the growth of all tested isolates, regardless of their diameters of inhibition. In addition to its role to inhibit adherence and biofilm formation.

Plant extracts contain secondary metabolites, including alkaloids, phenolics, flavonoids, quinines, polyphenols, flavones, tannins, flavonols, terpenoids, polypeptides, lectins, proanthocyanidins, as well as quercetin, which contribute to their antimicrobial activity (Szemerédi et al., 2020). These compounds are responsible for the immune-stimulating properties of plant extracts, providing protection against microorganisms. It is worth noting that plant extracts are generally considered safe and do not have known side effects.

O. majorana (marjoram) is particularly rich in terpenoids, including monoterpene essential oil constituents, diterpenes, and triterpenes. Additionally, "flavonoid aglycons and glycosides as well as hydroxyquinone derivatives such as arbutin, hydroxyquinone, and methylarbutin, as well as tannins and phenolic acids for example caffeic acid, rosmarinic acid, chlorogenic acid, caftaric acid, in addition to protocatechuic acid, have been identified in ethyl acetate, hydroalcoholic, and aquatic extracts of the plant" (Tripathy et al., 2017).

Certain study applied by (Tripathy et al., 2017). that carried on 3 plants' extracts including majorana, shown that these extracts have powerful antibiotic and antifungal properties, opening the door to the development of new treatments for a variety of ailments.

Moreover, Abu Ghazal and his coworkers (Ghazal et al., 2022). studied the effect of *Origanum majorana* extracts as an antibacterial inhibitory with the biofilm formation inhibitory effect, where they showed that it had a "strong antibacterial activity especially against *S. epidermidis*, *S. aureus*, MRSA, E. coli AG-100, E. coli, as well as *K. pneumoniae* bacteria. In addition to its effect as inhibitor of efflux pump", thus can be applied as a resistance-reversing agent against antibiotic-resistant strains.

6. CONCLUSION

Origanum majorana extracts showed outstanding effectiveness against a variety of clinical isolates, including gram-negative and gram-positive bacteria, according to the research. These extracts demonstrated superior effectiveness compared to commercially available antibiotics. Furthermore, they showed significant inhibition of bacterial adhesion and biofilm formation.

REFERENCES

Ávila-Campos, M. J., Simionato, M. R. L., Cai, S., Mayer, M. P. A., De Lorenzo, J. L., & Zelante, F. J. P. O. B. (2000). Virulence factors of Actinobacillus actinomycetemcomitans: other putative factors. 14, 05-11.

Ben Salha, G., Herrera Díaz, R., Lengliz, O., Abderrabba, M., & Labidi, J. J. M. (2019). Effect of the chemical composition of free-terpene hydrocarbons essential oils on antifungal activity. 24(19), 3532.

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). Diagnostic microbiology: Mosby St Louis.

Ghazal, T. S. A., Schelz, Z., Vidács, L., Szemerédi, N., Veres, K., Spengler, G., & Hohmann, J. J. P. (2022). Antimicrobial, multidrug resistance reversal and biofilm formation inhibitory effect of Origanum majorana extracts, essential oil and monoterpenes. 11(11), 1432.

Hindi, N., Yasir, A., Al-Mahdi, Z., & Jebur, M. H. J. I. J. P. R. (2016). Evaluation of antibacterial activity: anti adherence, anti biofilm and anti swarming of the aquatic extract of black raisins and vinegar of black raisins in Hilla City, Iraq. 9(9), 271-280.

Hindi, N. K. J. A. j. o. p., & Therapeutics, C. (2013). In vitro antibacterial activity of aquatic garlic extract, apple vinegar and apple vinegar-garlic extract combination. 1(1), 42-51.

Hindi, N. K. K., Al-Mahdi, Z. K. A., & Chabuck, Z. A. G. J. P. (2014). Antibacterial activity of the aquatic extractof fresh, dry powder ginger, apple vinegar extract of fresh ginger and crud oil of ginger (zingiberofficinale) against different types of bacteria in Hilla City, Iraq. 3(6).

Pernin, A., Guillier, L., & Dubois-Brissonnet, F. J. F. m. (2019). Inhibitory activity of phenolic acids against Listeria monocytogenes: deciphering the mechanisms of action using three different models. 80, 18-24.

Shakya, A. K. J. I. j. o. h. m. (2016). Medicinal plants: Future source of new drugs. 4(4), 59-64.

Szemerédi, N., Kincses, A., Rehorova, K., Hoang, L., Salardón-Jiménez, N., Sevilla-Hernández, C., . . . Spengler, G. J. A. (2020). Ketone-and cyano-selenoesters to overcome efflux pump, quorum-sensing, and biofilm-mediated resistance. 9(12), 896.

Tripathy, B., Satyanarayana, S., Khan, K. A., Raja, K. J. I. J. o. P. R., & Sciences, H. (2017). An updated review on traditional uses, taxonomy, phytochemistry, pharmacology and toxicology of Origanum majorana. 5(4), 1717-1723.

Vasireddy, L., Bingle, L. E., & Davies, M. S. J. P. O. (2018). Antimicrobial activity of essential oils against multidrug-resistant clinical isolates of the Burkholderia cepacia complex. 13(8), e0201835.

Wang, S., Zhou, L., Attia, F. A.-Z. K., Tang, Q., Wang, M., Liu, Z., . . . Kang, W. J. F. i. N. (2021). Origanum majorana L.: a nutritional supplement with immunomodulatory effects. 8, 748031.

Zeouk, I., Ouali Lalami, A., & Bekhti, K. J. A. J. P. C. R. (2019). In vitro antibacterial activity of medicinal plants in the central north of Morocco: a possible source of alternative drugs against methicillin-resistant Staphylococcus aureus. 12, 285-292.