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Antimicrobial Profiling of *Origanum majorana* L. and *Marrubium vulgare* L. Extracts Against Selected Pathogenic Isolates

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دراسة الخصائص المضادة للميكروبات لمستخلصات نبات المردقوش البري ونبات الروبيا ضدّ عزلاتٍ ممرضةٍ مختارة

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Abstract:
The diverse secondary phytochemicals found in medicinal and aromatic plants are used as novel antimicrobial agents. This study investigates the biological activity of two distinct species from Lamiaceae family *(Origanum majorana* L. and *Marrubium vulgare* L.) against bacteria and fungi isolates. Our findings showed that most bacterial species tested were resistant to the extracts; however, Gram-positive strains, specifically *Staphylococcus aureus* demonstrated significant growth inhibition when subjected to the ethanolic fraction of *M. vulgare*, suggesting that the architectural nuances of the Gram-positive cell wall may be particularly susceptible to the lipophilic constituents of this extract. Consequently, *M. vulgare* emerges as a viable candidate for targeted therapies against Staphylococcal infections. The study further identifies a clear hierarchy in solvent efficacy, as ethanolic preparations consistently eclipsed their aqueous counterparts in antifungal potency. Inhibition percentage values for *F. oxysporum* were 63 and 89% when using ethanol extracts of *M. vulgare* and *O. majorana.* The lowest inhibition percentage (19%) was obtained by the aqueous extract of *O. majorana* on *A. niger*

Keywords: Antimicrobial, Lamiaceae, *Marrubium vulgare* L., *Origanum majorana* L.

الملخص: تُستخدم المركبات الكيميائية النباتية الثانوية المتنوعة الموجودة في النباتات الطبية والعطرية كعوامل مضادة للميكروبات حديثة. تبحث هذه الدراسة النشاط البيولوجي لنوعين متميزين من الفصيلة الشفوية (نبات المردقوش البري *Origanum majorana* L. ونبات الروبيا *Marrubium vulgare* L) ضد عزلات من البكتيريا والفطريات. أظهرت نتائجنا أن معظم أنواع البكتيريا المختبرة كانت مقاومة للمستخلصات؛ ومع ذلك، أظهرت السلالات موجبة الجرام، وتحديدًا *Staphylococcus aureus* تثبيطًا ملحوظًا للنمو عند تعريضها للجزء الإيثانولي من *Marrubium vulgare*، مما يشير إلى أن الخصائص البنيوية الدقيقة لجدار الخلية موجبة الجرام قد تكون حساسة بشكل خاص للمكونات المحبة للدهون في هذا المستخلص. ونتيجة لذلك، يبرز *Marrubium vulgare* كمرشح واعد للعلاجات الموجهة ضد عدوى المكورات العنقودية. كما تُحدد الدراسة تسلسلًا هرميًا واضحًا في فعالية المذيبات، حيث تفوقت المستحضرات الإيثانولية باستمرار على نظيراتها المائية في الفعالية المضادة للفطريات. بلغت نسب التثبيط *Fusarium oxysporum* 63% و89% عند استخدام مستخلصات الإيثانول لنبات الروبيا ونبات المردقوش البري على التوالي. أما أقل نسبة تثبيط (19%) فقد سُجّلت باستخدام المستخلص المائي لنبات المردقوش البري على فطر *Aspergillus niger*.

الكلمات المفتاحية: مضادات الميكروبات، الفصيلة الشفوية، نبات الروبيا، نبات المردقوش البري.

Introduction

The utilization of botanical agents as robust antimicrobial to a diverse array of pathogens remains an important of pharmacological research (Mashhady *et al.*, 2016). These natural scaffolds possess an inherent ecological advantage; they are characterized by minimal phytotoxicity, and a molecular heterogeneity that far exceeds the structural range of laboratory designed chemicals (Novais *et al.*, 2003; Surendra *et al.*, 2016a). Such secondary metabolites are representing a sophisticated evolutionary defensive strategy, synthesized specifically to impede the invasive progress of bacteria, fungi, parasites, and viruses (Haida *et al.*, 2007).

The Lamiaceae historically designated as the Labiatae stands as within the dicotyledonous, encompassing over 7,000 species and 236 genera of diverse herbaceous and shrub-like forms (Harley *et al.*, 2004). Though its presence is felt globally, the family finds its most concentrated ecological expression within the Mediterranean region. The ubiquity of the Lamiaceae is driven not only by their prized aromatic profiles but also by their remarkable vegetative resilience, often flourishing from simple stem cuttings. From the ornamental of Ajuga, Coleus, and Salvia to the indispensable culinary contributions of thyme (*Thymus*), mint (*Mentha*), sage (*Salvia*), and oregano (*Origanum*) (Khoury *et al.*, 2016).

This family occupies a central role in therapeutic traditions due to its complex volatile profiles, sequestered within specialized external glandular trichomes (Giuliani and Maleci Bini, 2008). These essential oils are the functional engines behind myriad applications in the cosmetic, pharmaceutical, and food industries. The scale of this botanical reliance is staggering; current estimates suggest that 70% to 80% of the world's population still rely on medicinal herbs for primary health care (WHO, 2008). The potent antioxidant and bactericidal efficacy documented in Lamiaceae species is predominantly the result of high phenolic concentrations (Alinezhad *et al*., 2012). Key molecules such as eugenol, carvacrol, and thymol serve as the primary active constituents, fundamentally responsible for the lethal disruption of microbial cell membranes (Tajkarimi *et al.*, 2010).

Marrubium vulgare L., a resilient perennial member of this family, maintains a widespread geographic footprint throughout North Africa, Southern Europe and Central and West Asia (Weel *et al.*, 1999). Traditionally, its decoctions have been employed to mitigate the symptoms of bronchitis, asthma, tuberculosis, and various gastrointestinal distresses (Gruenwald *et al.*, 1998). Scientific validation has since expanded this traditional profile, revealing a staggering biological diversity that includes cytotoxic, anti-hypertensive, and analgesic properties (Bardai *et al.*, 2001; Meyre-Silva *et al.*, 2005; Yamaguchi *et al.*, 2008; Argyropoulou *et al.*, 2012). Furthermore, investigations have highlighted its potential in wound healing and as a formidable agent in managing diabetes and inflammation (Boudjelal *et al.*, 2012; Bokaeian *et al.*, 2014; Amri *et al.*, 2017; Bouterfas *et al.*, 2018).

Origanum majorana, a Mediterranean native, has achieved global distribution through extensive cultivation for its essential oil, popularly termed Marjoram oil (Banchio *et al*., 2008). This essential oil is extracted by steam distillation of the leaves and flower heads, and is a key ingredient in both perfumery and gastronomy. Medicinally, its applications are vast; the plant is characterized as an astringent, stomachic, and anthelmintic agent, utilized to address cardiovascular ailments, hysteria, and respiratory distress (Farooqi and Sreeramu, 2004). Its integration into food preservation is equally significant, predicated on its well-established anti-inflammatory and antimycotic characteristics (López-Cobo *et al*., 2015). Given this background, This study aims to a comparative evaluation of the antibacterial and antifungal of *M. vulgare* and *O. majorana*.

Materials and Methods

The Plant Samples: The leaves of *M. vulgare* and *O. majorana*, were harvested from Al Jabal Al Akhdar, specifically within the Al Bayda region of Libya, during the spring of March 2022.

Preparation of plant extracts

Aqueous extract: To isolate the bioactive fractions, an aqueous extraction was executed in accordance with the foundational principles established by Harborne (1973). This involved macerating 20 g of pulverized dry plant matter in 200 ml of sterile distilled water yielding a 10% w/v concentration under continuous agitation via a mechanical shaker for a duration of 24 hours at a regulated ambient temperature of 25 °C. Subsequent to this period, the mixture filtration using sterilized Whatman No. 1 paper. To isolate the solutes, the filtrate was subjected to concentration through a rotary evaporator at 60 °C, with the resulting extracts sequestered in sterile glass vessels for subsequent microbial screening.

Ethanolic extract: Prepare the ethanolic extract in the same way as the aqueous extract, but by using ethyl alcohol 70% to replace water (Xian-gou and Ursula, 1994).

Microbial Cultivation

The pathogenic battery employed for this assessment included the bacterial isolates *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, all of which were sourced from the Botany Department's at Omar Mukhtar University. These strains were revitalized on nutrient agar at 37 °C for an 18-hour incubation window. Fungal isolates of *Aspergillus niger* and *Fusarium oxysporum* were procured from the same institution. These mycological cultures were established on Potato Dextrose Agar (PDA), maintained at a stable 30 °C for a developmental period spanning 3 to 5 days.

Antimicrobial Bioassays

The evaluation of antibacterial potency was conducted via the agar well diffusion technique (Reeves, 1989). Within nutrient agar matrices pre-seeded with the target organisms, four distinct wells (0.6 cm diameter) were bored to accommodate extract concentrations of 25, 50, 75, and 100 mg/ml. Following a 24-to-48-hour incubation period at 37 °C, the diameters of the resulting zones of inhibition were quantified in millimeters. To ensure statistical rigor, each assay was performed in triplicate.

Antifungal efficacy was scrutinized by inoculating 5 mm mycelial disks of *A. niger* and *F. oxysporum* onto solidified PDA media. These were incubated alongside negative controls at 25 °C. After a 7-day maturation phase, the radial growth of the mycelium was recorded. The relative suppressive capacity of the extracts was then calculated using the mathematical expression formalized by Deans and Soboda (1990):

$$\text{Growth Inhibition (\%)} = [Dc\text{-}De/Dc]\times 100$$

In this equation, Dc refers to the diameter of the colony in the control group, while De refers to the diameter in the presence of extracts.

Results and Discussion

The growth modulation of *Aspergillus niger* and *Fusarium oxysporum* under the influence of *Marrubium vulgare* and *Origanum majorana* extracts reveals a starkly divergent efficacy dictated by the polarity of the extraction medium (Figure 1). Most salient was the performance of the ethanolic *O. majorana* fraction, which achieved formidable inhibition peaks of 78% against A. niger and 89% against *F. oxysporum*. In contrast, the aqueous counterpart of *O. majorana* exhibited negligible fungistatic impact, suggesting that the essential bioactive metabolites are poorly sequestered in hydrous environments. Furthermore, while the ethanolic and aqueous preparations of *M. vulgare* performed with comparable mediocrity against *A. niger*, the ethanolic variant demonstrated a robust antagonistic capacity specifically against the mycelial proliferation of *F. oxysporum* (Figures 2 and 3).

The screening of these Lamiaceae plant against a battery of pathogenic strains including *Escherichia coli, Salmonella enterica, Staphylococcus aureus,* and *Streptococcus pyogenes* uncovered a localized rather than universal potency. The ethanolic *M. vulgare* extract emerged as a selective inhibitor of *S. aureus*, manifesting clear zones of inhibition measuring 10, 5, and 2 mm at descending concentrations of 100, 75, and 25 mg/ml, respectively (Table 1, Figures 4 and 5).

As for the extract of *O. majorana*, it had no significant effect on the four types of bacteria used in this study. The resistance of Gram-negative species (e.g., *E. coli* and *S. enterica*) is due to the outer membrane's lipopolysaccharide layer, which functions as a robust permeability barrier against the diffusion of the hydrophobic antimicrobial molecules present in *M. vulgare* and *O. majorana* extracts. The limited antibacterial activity of O. majorana in this study, compared to previous reports, is likely due to the use of ethanolic and aqueous extraction rather than hydro-distillation. Solvent extraction prioritizes polar constituents, whereas essential oils are rich in volatile terpenes like thymol, which possess superior antimicrobial efficacy (Tajkarimi *et al*., 2010).

The historical reliance on aromatic flora for biocidal applications finds its modern justification in the complex richness of secondary metabolites, namely phenols, terpenoids, and alkaloids. The volatile organic compounds (VOCs) characteristic of the Lamiaceae family are hypothesized to be the primary drivers of this biological interference (Edris, 2007; Bakkali *et al.*, 2008). However, the therapeutic yield of these extracts is not a static property; it is an emergent outcome of the interplay between the solvent's chemical nature, the culture media, and the specific physiological traits of the microbial target (Angioni *et al.*, 2006; Dai *et al*., 2010).

Historical data underscores this trend: while methanolic extracts of *T. vulgaris* and *S. officinalis* have shown significant suppression of *S. aureus* (Shan *et al*., 2007), ethanolic S. officinalis has demonstrated broader efficacy against *B. cereus* and *E. coli* (Kokoska *et al*., 2002). This supports the consensus that Gram-positive organisms are generally more susceptible to herbal interventions due to the absence of the complex Gram-negative outer envelope (Ceylan *et al*., 2004). This sensitivity confirms the potential of traditional pharmacopeia as a viable source for modern bactericidal development (Rakholiya and Chanda, 2012; Tekwu *et al.*, 2012).

Previous inquiries into the essential oils of *O. majorana* suggest a potent efficacy against diverse pathogens, including Bacillus anthracis and Proteus vulgaris (Ben *et al.*, 2001). Methanolic extracts have even been proposed as eco-friendly alternatives to commercial pesticides due to their non-toxic profile (Leeja and Thoppil, 2007). Our results align with the observations of Mahmood *et al.* (2008), which posit that the failure of aqueous extracts is a direct consequence of the incomplete recovery of non-polar active ingredients. The thermodynamic limitations of water as a solvent preclude the solubility of hydrophobic phenolics, which remain the primary executioners of bactericidal action (Pinelo *et al.*, 2004).

The necessity for such natural alternatives is emphasized by the public health risks associated with E. coli food poisoning and the persistence of *S. aureus* as a primary agent of hospital-acquired infections (Vogt *et al.*, 2005; Brodsky *et al*., 2007). Moreover, the agricultural crisis posed by *Fusarium oxysporum* (tomato wilt), *Phytophthora infestans* (late blight), and *Rhizoctonia solani* (root rot) demands a shift away from synthetic fungicides (Gautam *et al*., 2003; Rai *et al*., 2011; Marcin *et al*., 2012). While chemical fungicides offer a temporary shield, their toxic residues and environmental persistence necessitate the urgent discovery of biological strategies (Copping and Menn, 2000; Carvalho, 2017; Shuping and Eloff, 2017; Abbey *et al*., 2019).

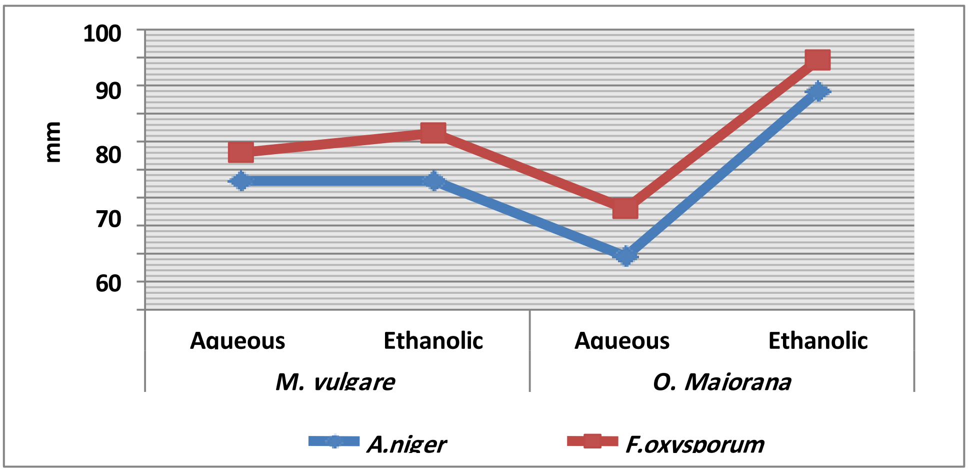

Fig. (1): Antifungal activity of *M. vulgare* and *O. majorana* extracts.

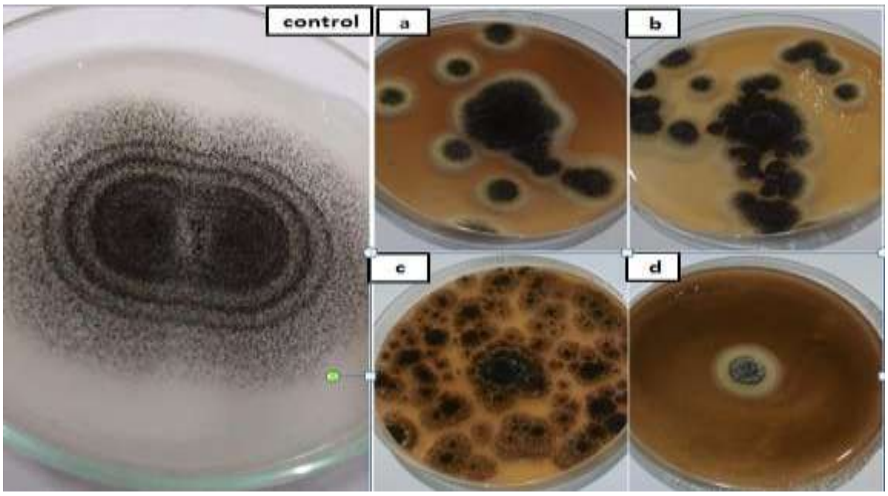

Fig.(2): Growth inhibition of *A. niger* by aqueous (a, c) and ethanolic (b, d) extracts of *M. vulgare* and *O. majorana*.

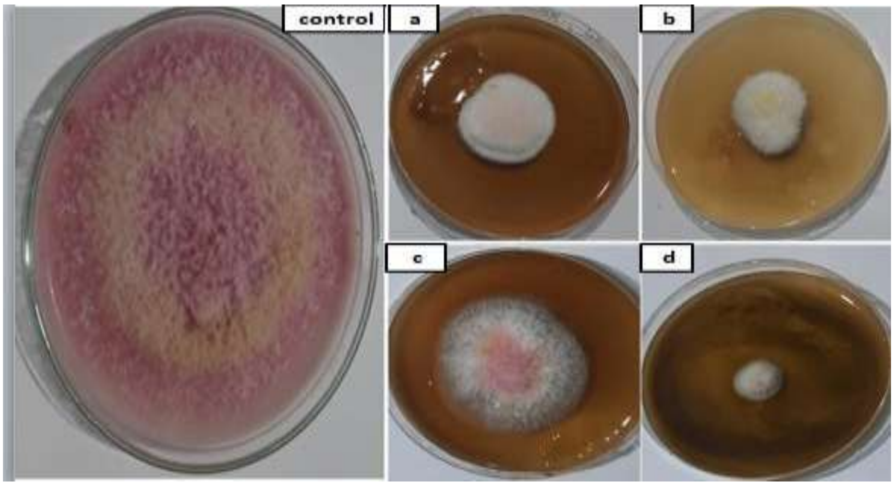

Fig.(3): Growth inhibition of *F. oxysporum* by aqueous (a, c) and ethanolic (b, d) extracts of *M. vulgare* and *O. majorana*.

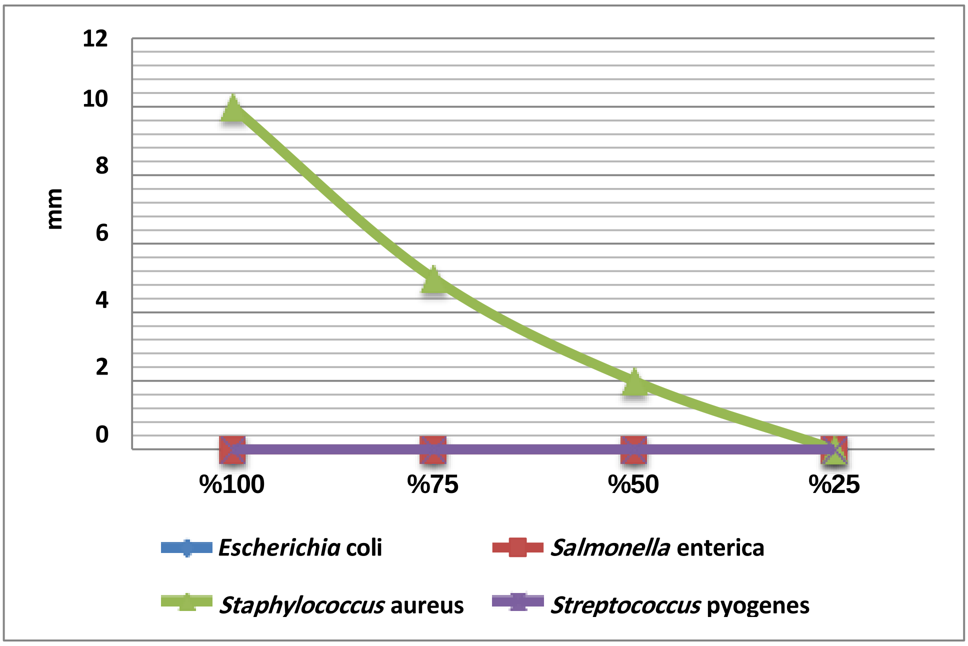

Fig. (4): Antibacterial activity of ethanolic *M. vulgare* extracts against selected pathogens.

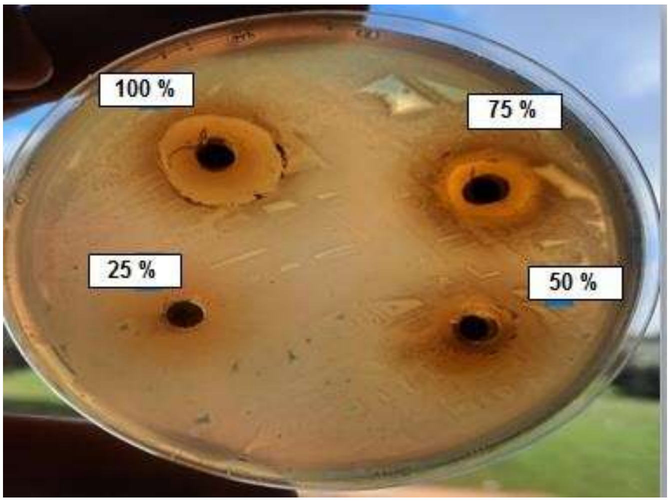

Fig. (5): Inhibitory efficacy of *M. vulgare* ethanolic extracts against *Staphylococcus aureus.*

Conclusion

The synthesis of this investigation underscores a compelling biocidal potential inherent in *Marrubium vulgare* and *Origanum majorana*, specifically manifesting as high-potency antagonism toward Gram-positive bacterial lineages and select mycological isolates. The study utilized aqueous and ethanolic solvent systems media characterized by low ecological toxicity to effectively sequester a rich payload of phenolic constituents. These plant extracts fortified by the presence of volatile essential oils, function as complex bioactive delivery systems that disrupt microbial homeostasis. Such empirical evidence robustly supports the current academic trajectory toward integrating plant-derived extracts as natural antimicrobial agents. Beyond their pharmacological promise, these extracts offer a viable pathway for the biorational management of recalcitrant phytopathogens in agricultural ecosystems. However, the transition from laboratory efficacy to clinical utility necessitates further granular investigation to calibrate standardized therapeutic dosages and elucidate the full pharmacodynamic spectrum of these Lamiaceae derived compounds.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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