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Microbiological and Pharmacological Investigation of Phytochemicals

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Abstract:

This study investigates the phytochemical composition and biological activities of five ethnomedicinal plants: Zingiber officinale (Ginger), Ocimum basilicum (Basil), Origanum syriacum (Za'atar), Origanum vulgare (Oregano), and Salvia rosmarinus (Rosemary). These plants are traditionally used for their therapeutic properties, yet a comparative evaluation of their bioactivities is limited. Total phenolic and flavonoid contents were quantified using standard colorimetric assays. Antimicrobial activity was assessed against selected bacterial and fungal strains using the agar well diffusion method. Antioxidant capacity was evaluated via the DPPH assay, and cytotoxicity was tested on HCT-116 (colorectal cancer) and HNO-97 (tongue carcinoma) cell lines using MTT assays. Rosemary exhibited the highest phenolic content (189.28 mg/g), while Ginger had the highest flavonoid content (43.13 mg/g). Rosemary demonstrated the strongest antibacterial activity, notably against S. aureus (31 ± 0.5 mm), and Oregano showed significant inhibition against S. enterica (23 ± 0.1 mm). In antifungal assays, Rosemary had the largest inhibition zones against A. flavus (40 ± 0.6 mm) and A. niger (36 ± 0.3 mm). Basil and Za'atar also exhibited notable antifungal activity. Rosemary showed moderate antioxidant activity with an IC50 value of 37.42 µg/mL. Cytotoxicity testing revealed IC50 values of 14.91 µg/mL and 22.3 µg/mL for Rosemary against HCT-116 and HNO-97 cells, respectively. The findings highlight S. rosmarinus as a potent source of antimicrobial, antioxidant, and anticancer compounds. The diverse phytochemical profiles and bioactivities across the studied plants suggest that each offers unique therapeutic potential. These results support further investigation into their clinical applications, particularly concerning bioavailability, mechanisms of action, and potential synergistic effects.

Introduction:

Sinai is a unique example of the geographical distribution of the northern species in the southern areas and vice versa. The fascinating composition of the plant communities growing in this region, Sinai endemics besides the Siberian ones, opens perspectives for practical usage of these herbal resources in different areas such as phytotherapy and agriculture.[1] It contains a detailed phytochemical study on secondary metabolites, ascertaining the active constituents in the selected plant extracts.[2] The antimicrobial and antifungal activities of the chosen plants, along with the explanation of the observed in vitro activities, are also documented.[3]

It is known that due to the emergence of resistant microorganisms, attention has returned to the use of longer plant extracts, and sometimes prophylactic antibiotics are prescribed.[4] Today, the

medical and pharmaceutical benefits of plant extracts are well documented. Nevertheless, the effectiveness of over 80% of the crude drug market is traditional medicine.[5,6] Traditional therapies, as well as the use of herbs in general, have spread to widespread use in many industrialized countries, based on evidence that natural plant products can, in many cases, provide effective therapeutic agents at reduced cost and with few adverse effects.[7]

The Sinai Peninsula is one of the important centers of plant biodiversity.[8] Its flora is the meeting point between the Irano-Turanian and Saharo-Arabian phytogeographic regions and comprises xero-halophytic species. The majority of these plants revealed potential bioactivity either against human pathogenic microbial species or against different pathological conditions. Methanolic extracts of 11 medicinal plants that are traditionally used by Bedouins in the north of Sinai were investigated with a view to evaluate their phytochemical constituents and anti-inflammatory, hypoglycemic, and antimicrobial properties.[9] The findings were tested using various field-medicinal studies that are widely practiced by traditional practitioners in the Sinai Peninsula, which revealed the same claims from the herbal decoctions.[10] The obtained results show certain advantages for more unexplored drug sources as the ethnomedicinal plant claims become highly valued and much more useful. The present study was designed to identify some rich natural resources as probable antiphlogistic, hypoglycemic, and antimicrobial drugs, especially for herpes viral infections of many ethnomedicinal plant extracts that are utilized by traditional practitioners in the Sinai region.[11] In this study, phytochemicals were extracted from five herbs and then tested for their antimicrobial efficiency, as well as their effects on alkaline phosphatase activity in the presence of Salmonella. Both the microbiological and alkaline phosphatase studies clarified that the inhibition efficiency from phytochemicals extracted from the ethnomedicinal herbs is primarily related to their contents of phenolic compounds and not to saponins.[12] Meanwhile, the investigated herbal extracts revealed inhibitory activity in vitro with alkaline phosphatase in the presence of Salmonella.[13] Numerous investigations have examined the antimicrobial and antioxidant qualities of ethnomedicinal plants; however, there are still few thorough comparisons of several phytochemical-rich species. This study aims to investigate the phytochemical composition, antimicrobial, antioxidant, and cytotoxic properties of five ethnomedicinal plants native to the Sinai Peninsula—Z. officinale, O. basilicum, O. syriacum, O. vulgare, and S. rosmarinus. By quantifying the total phenolic and flavonoid content and assessing their bioactivities, the research seeks to evaluate their therapeutic potential, particularly in combating oxidative stress, microbial infections, and cancer, and to highlight their potential as sustainable sources of natural bioactive compounds.

Materials and Methods:

Plant Collection and Identification:

For this study, five plants were selected based on their traditional medicinal use in Egyptian medicine and their availability in the Sinai region 28.5407° N, 33.9761° E (St. Catherine, Sinai Peninsula, Egypt) Fig. 1. The chosen plants included O. basilicum, Z. officinale, S. rosmarinus, O. vulgare, and O. syriacum listed in Table 1. The plants were carefully collected from

natural habitats and cultivated areas within the Sinai Peninsula to ensure their relevance to the local flora. The plants were identified by a local botanist, who examined their distinctive morphological features, such as leaf shape, flower color, and overall growth habit. After confirmation of their identities, voucher specimens of all five species were collected and preserved in the herbarium for future reference. This step was crucial to ensure the accuracy and reliability of the plant species used in the study, and the voucher specimens serve as a permanent record of the plants identified for this research14,15,16. Identification and nomenclature were performed and authenticated by Dr Abdel-Hameed U.K. Professor of flowering plants taxonomy and flora. Voucher specimens were kept at the herbarium of Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt (CAIA) under voucher number UKABS 01- UKABS 02- UKABS 03- UKABS 04- UKABS 05.

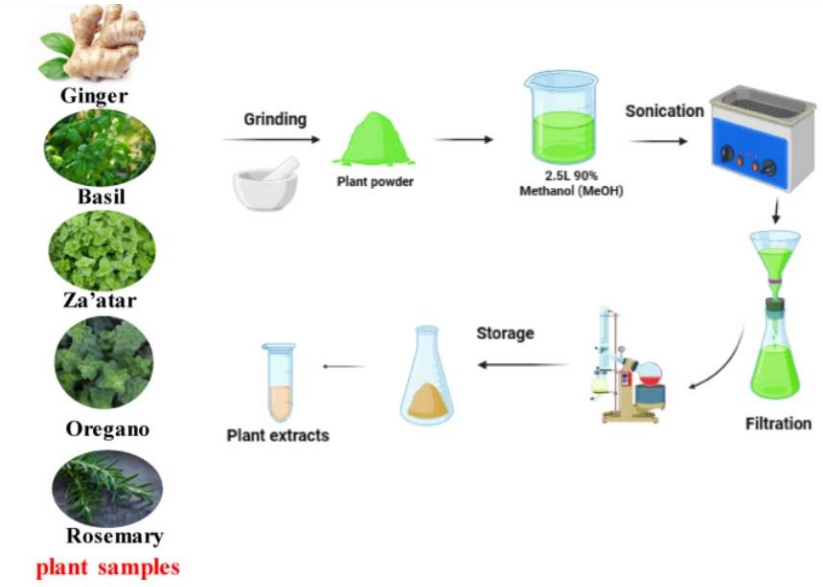
Extraction of Plant Materials:

As illustrated in Fig. 2, 300 g of each provided plant sample as a whole was first ground into a fine powder. The powdered plant material was then subjected to ultrasonic extraction using 2.5 L of 90% methanol (MeOH) as the extraction solvent. The process was carried out using a QSonica Q500 ultrasonic processor equipped with a 13 mm probe, operating at a frequency of 20 kHz and an output power of 500 W.

Extraction was conducted in three consecutive cycles, each lasting 30 min, with sonication applied in a pulse mode (30 s on, 30 s off) to avoid overheating and ensure uniform extraction. This technique enhanced solvent penetration and effectively disrupted plant cell walls, facilitating the release of intracellular bioactive compounds.

After each cycle, the mixture was filtered to remove solid residues, and the resulting filtrate was collected. The combined liquid extracts were concentrated by evaporating the methanol under vacuum at 50 °C using a rotary evaporator (Heidolph Laborota 4000), yielding a concentrated plant extract.

The Plant Materials:



Phytochemical Analysis:

The plant samples were subjected to a detailed phytochemical analysis to evaluate their bioactive potential through the quantification of several key constituents. Total flavonoid content was determined using the aluminum chloride colorimetric method as described by18which involves the formation of a flavonoid-aluminum complex that produces a yellow color, measurable spectrophotometrically at a specific wavelength, thus allowing for the quantification of flavonoids. Additionally, the total phenolic content was assessed using the Folin-Ciocalteu method, following the protocol described by19. This is a widely established assay that detects phenolic compounds by their ability to reduce the Folin-Ciocalteu reagent, resulting in a blue color whose intensity is directly proportional to the phenolic concentration, enabling precise spectrophotometric measurement. These assays collectively provided a comprehensive phytochemical profile, shedding light on the bioactive components present in the plant extracts and laying the groundwork for further investigation into their medicinal properties.

Antimicrobial Screening of Ethnomedicinal Plants Extracts:

The plant extracts were evaluated for their bioactivity against the Gram-positive bacteria, Staphylococcus aureus (ATCC 43300), as well as the Gram-negative bacteria, Escherichia coli (E. coli) (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), and Salmonella enterica (ATCC 14028), in addition to filamentous fungi, such as Aspergillus niger (KF154412), Aspergillus flavus (JF951750), Fusarium oxysporum (LT746253), and yeast, Candida albicans (ATCC 60193). All pathogenic organisms were obtained from the Assiut University Mycological Center (AUMC) in Assiut, Egypt. To assess the antibacterial potential, the agar well diffusion method was employed20. Different nutrient agar media were made and sterilized for the agar well diffusion method: nutritional agar21 for bacteria, Sabouraud agar22 for yeast, and Czapek Dox agar23 for fungal strains. In this procedure, wells were made in an agar plate previously inoculated with the target pathogenic strains. The wells were then filled with the prepared plant extracts at concentration (1 mg/mL), and the plates were incubated under suitable conditions to allow microbial growth. Dimethyl sulfoxide (DMSO) was used as a negative control to assess the potential for contamination and to ensure that no inhibitory effects were present due to the solvents used in the preparation of the extracts. After incubation, the zones of inhibition, defined as the clear areas around the wells where microbial growth was inhibited, were measured. The size of the zone of inhibition is directly correlated with the antimicrobial activity of the extracts, with larger zones indicating stronger antimicrobial effects. This method is widely used for screening antibacterial agents and provides a reliable and effective way to compare the efficacy of different plant extracts24. Upon investigation of the preliminary antimicrobial activity screening, rosemary extract was selected for further analysis due to its strong antimicrobial potential.

Contact Bioautography:

The antimicrobial activity of rosemary extract was examined against S.

aureus (ATCC 43300) using the method of25. Briefly, rosemary extract was dissolved in methanol (1 mg/mL) then 50 µL was applied with a fine bore glass capillary tube, on the entire length of pre-coated glass TLC plates silica gel 60 F254 layer thickness 0.25 mm (Merck, Germany) of size 20 × 20 cm. The mobile phase Hexan: DCM solvent (67:33, V/V) was used for chromatographic separation at room temperature (20 °C). Then, the TLC plates were left to dry for 28 h to remove the solvent completely. The TLC plates were then transferred to the surface of tryptone soya agar plates that had already been inoculated with S. aureus (ATCC 43300) as standard strain (1% v/v; 1.0 × 106 CFU/mL) using sterile swabs. After pre-diffusion for 90 min at 4 °C, The TLC plates were removed from the surface by using sterile forceps, and the plates were incubated in an inverted position for 24 h at 37 °C. Finally, areas of inhibition on the agar surface, corresponding to the spots in chromatographic plates, are indicative of the antimicrobial substances of the extract26.

Antioxidant Assay:

Antioxidant activity evaluation of S. rosmarinus extracts using the DPPH radical scavenging assay25. The antioxidant potential of S. rosmarinus extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. This assay utilizes the DPPH radical, a stable free radical, to evaluate the ability of the rosemary extract to neutralize free radicals.

Sample Preparation and Reaction:

A stock solution of 20 µg/mL concentration of trolox was prepared in methanol from which 5 concentration were prepared including 6.25, 5, 3.75, 2.5 and 1.25 µg/mL. These concentrations were then incubated with a freshly prepared DPPH solution (0.1% in methanol). In each reaction well, 100 µL of the DPPH reagent was combined with 100 µL of the rosemary extract solution. The reaction mixture was incubated at room temperature for 30 min in the dark to allow for radical scavenging25.

Measurement of Antioxidant Activity:

The DPPH radical reacts with antioxidants, resulting in a reduction of its characteristic purple color. The extent of this color change is directly proportional to the scavenging activity of the extract. After incubation, the absorbance of the reaction mixture was measured at 517 nm using a FluoStar Omega microplate reader.

Control and Data Calculation:

Ascorbic acid, a well-known antioxidant, was used as a positive control to benchmark the radical scavenging ability of the rosemary extract. The percentage of inhibition of the DPPH radical was calculated using the following formula.

Cell Culture:

The two cancer cell line (HCT-116: Colorectal Cancer and HNO-97: Tongue carcinoma) was acquired from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). The cells were cultured in RPMI medium supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% heat-inactivated fetal bovine serum. Cultures were maintained in a humidified incubator at 37 °C with a 5% (v/v) CO2 atmosphere.

Cytotoxicity Testing:

The cytotoxicity of S. rosmarinus extract was evaluated using both the MTT and SRB assays on HCT-116 colorectal cancer cells and HNO-97: Tongue carcinoma. For the MTT assay, cells were seeded in 96-well plates at a density of 5 × 103 cells per well in 100 µL of complete RPMI medium and

incubated for 24 h. After incubation, the cells were treated with varying concentrations of the rosemary extract for a specified period (typically 72 h)27.

Following treatment, 20 µL of MTT reagent (5 mg/mL) was added to each well. Viable cells reduced MTT to formazan, resulting in a color change that could be quantified spectrophotometrically. The optical density (OD) was measured at 570 nm using a BMGLABTECH® FLUOstar Omega microplate reader. The number of viable cells was determined by comparing the absorbance in treated wells to control wells. To further confirm and quantify the cytotoxic effects, the SRB assay was performed. After the MTT procedure, the medium was replaced with 150 µL of 10% trichloroacetic acid (TCA) to fix the cells. The plates were incubated at 4 °C for 1 h, followed by washing five times with distilled water to remove excess TCA. The fixed cells were stained with 70 µL of 0.4% SRB solution and incubated in the dark at room temperature for 10 min. After washing three times with 1% acetic acid to remove unbound SRB, the cells were air-dried overnight. Protein-bound SRB was solubilized by adding 150 µL of 10 mM TRIS buffer, and the absorbance was measured at 540 nm28. The concentration of rosemary extract required to inhibit 50% of cell growth (IC50) was calculated using the dose-response curve derived from both MTT and SRB assay results. The IC50 values indicate the potency of the rosemary extract in inhibiting cell proliferation, with lower IC50 values corresponding to higher cytotoxicity. This dual assay approach provided comprehensive insight into the selective cytotoxic effects of S. rosmarinus on HCT-116 colorectal cancer cells, highlighting its potential as a therapeutic agent for cancer treatment29.

Results and Discussion:

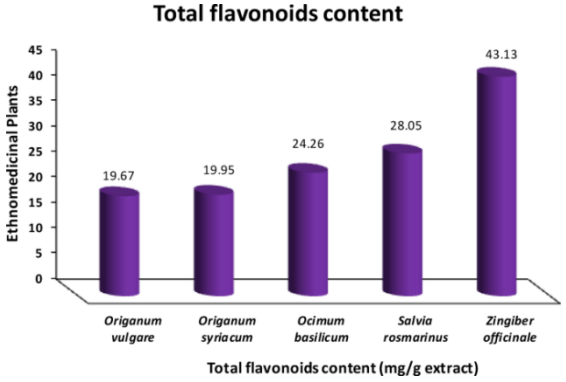
Taxonomical features, morphological comparison, and medicinal insights of five medicinal plants from the zingiberaceae and lamiaceae families

This study examines the taxonomical features and morphological characteristics of five medicinal plants: Z. officinale, O. basilicum, O. syriacum, O. vulgare, and S. rosmarinus30. These plants were selected from two primary families, Zingiberaceae and Lamiaceae, known for their rich medicinal properties. The analysis revealed significant distinctions between plants of the Zingiberaceae family and those within the Lamiaceae family31. Z. officinale, with its rhizomatous root system, erect stem, and large, yellow, fragrant flowers, is distinct in both its structure and medicinal use compared to the other plants, which share several common features32. In contrast, the Lamiaceae family members exhibited similar morphological traits such as square stems, aromatic glands, and opposite leaf arrangements, with slight variations in flower and leaf shape between species. O. basilicum, O. syriacum, O. vulgare, and S. rosmarinus all shared these features, though Rosemary stood out for its needle-like, aromatic leaves suited to dry climates. These findings underscore the importance of plant morphology for identification and classification, highlighting shared characteristics within the Lamiaceae family, and offer insights into their potential medicinal uses, particularly related to their aromatic oils and therapeutic properties.

Phytochemical Content:

The Total Flavonoid Content:

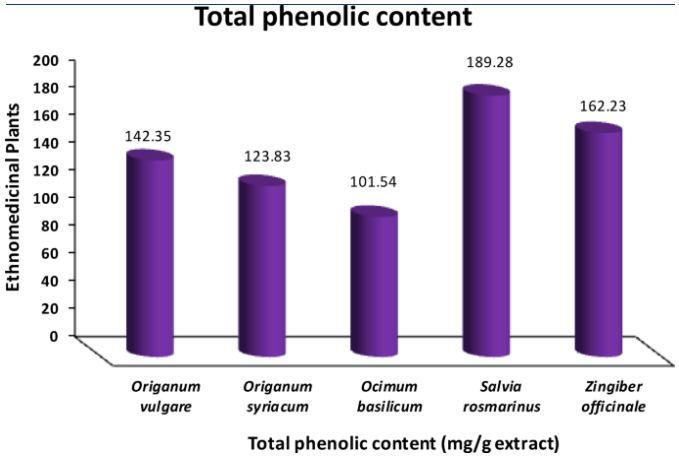
As demonstrated in Fig. 3 the total flavonoid content (mg/g extract) of five ethnomedicinal plants was evaluated, revealing significant variation across the species. Z. officinale exhibited the highest flavonoid content at 43.13 mg/g, followed by S. rosmarinus at 28.05 mg/g, O. basilicum at 24.26 mg/g, and O. syriacum and O. vulgare, which both had relatively similar values of 19.95 mg/g and 19.67 mg/g, respectively. The higher flavonoid content in Z. officinale is consistent with its well-established antioxidant and anti-inflammatory properties, which are largely attributed to its polyphenolic compounds33. Similarly, S. rosmarinus is known for its rich polyphenolic content, including flavonoids like rosmarinic acid, which contribute to its anti-inflammatory and antioxidant effects34. O. basilicum also demonstrated a moderate flavonoid content, reinforcing its traditional use as an adaptogen and immune booster, while O. vulgare and O. syriacum, though lower in flavonoid concentration, are recognized for their antimicrobial and antioxidant properties35. The variation in flavonoid content across these plants could be due to genetic factors, environmental conditions, and cultivation practices, as these factors have been shown to influence secondary metabolite production in plants36. The high flavonoid content in Z. officinale suggests it may be particularly beneficial for managing oxidative stress-related conditions such as cardiovascular diseases and certain cancers, aligning with its traditional use in treating nausea, digestive issues, and inflammation37. Likewise, the flavonoids in S. rosmarinus and O. basilicum may contribute to their therapeutic applications in managing stress, digestive disorders, and inflammatory diseases. Although O. vulgare and O. syriacum show lower flavonoid concentrations, their significant antioxidant and antimicrobial activities still make them valuable in ethnomedicinal practices. These findings emphasize the medicinal potential of these plants, particularly their flavonoid-rich profiles, and underscore the importance of further studies to isolate specific flavonoid compounds and explore their bioavailability and synergistic effects in humans. The diversity of flavonoid content among plants in the Zingiberaceae and Lamiaceae families also highlights the varied therapeutic potentials of different plant species, warranting additional research into how environmental factors and harvesting methods might influence flavonoid production in these ethnomedicinal plants. In brief, the plants evaluated, particularly Z. officinale and S. rosmarinus, with their high flavonoid content, demonstrate considerable potential as sources of natural antioxidants, which could support their traditional uses in improving health and preventing disease, with further research needed to substantiate these findings through clinical trials and bioavailability studies.



Total Flavonoids in the Extracts of the Five Ethnomedicinal Plants:

The Total Phenolic Content:

As presented in Fig. 4 the total phenolic content (mg/g extract) of five ethnomedicinal plants was evaluated, revealing significant variation across the species. S. rosmarinus exhibited the highest phenolic content at 189.28 mg/g, followed by Z. officinale (Ginger) with 162.23 mg/g, O. vulgare (Oregano) at 142.35 mg/g, O. syriacum (Za'atar) at 123.83 mg/g, and O. basilicum (Basil) with 101.54 mg/g. The high phenolic content in S. rosmarinus is consistent with its well-documented antioxidant and anti-inflammatory properties, primarily attributed to compounds like rosmarinic acid, which have been shown to neutralize free radicals and reduce inflammation38. Similarly, Z. officinale contains substantial phenolic compounds, contributing to its therapeutic effects, including antioxidant, anti-inflammatory, and anticancer activities39. O. vulgare (Oregano) also contains significant phenolic compounds, supporting its antimicrobial and antioxidant uses40,41while O. syriacum and O. basilicum, though lower in phenolic content, are still recognized for their health-promoting properties, particularly in immune function and stress reduction. The variation in phenolic content can be attributed to factors such as genetic differences, environmental conditions, and cultivation practices, all of which influence the biosynthesis of secondary metabolites in plants42,43. The high phenolic content in S. rosmarinus and Z. officinale supports their traditional uses in treating a variety of ailments, from digestive issues to inflammation, while O. vulgare and O. syriacum continue to be valuable for their antimicrobial and antioxidant properties. The synergistic effects of phenolics and flavonoids in these plants enhance their medicinal potential, as both groups of compounds have complementary effects on human health, with phenolics mainly offering antioxidant and anti-inflammatory benefits, and flavonoids supporting cardiovascular, immune, and neuroprotective health. These findings underline the importance of these ethnomedicinal plants as natural sources of bioactive compounds with therapeutic value, emphasizing the need for further research to explore their specific phenolic compounds, bioavailability, and potential clinical applications. Future studies should also examine how environmental factors and cultivation methods influence the phenolic content in these plants to optimize their medicinal use.



Total Phenolic in the Extracts of the Five Ethnomedicinal Plants:

The results of the antimicrobial screening of ethnomedicinal plant extracts reveal significant variability in the antimicrobial activity of the plants tested, indicating their potential therapeutic value against both bacterial, fungal and yeast pathogens (Fig. 5; Table 2). Ginger, despite its common use in traditional medicine, showed no inhibition against E. coli, S. aureus, and P. aeruginosa, which may be

attributed to the relatively lower concentrations of bioactive compounds in the extract used44. However, it exhibited moderate antibacterial inhibition against S. enterica (20 ± 0.3 mm), suggesting that its antibacterial potential may be more specific to certain pathogens, particularly Gram-negative bacteria. This result is consistent with some studies that suggest Ginger contains bioactive compounds such as gingerols, which have been shown to have antimicrobial properties, though their activity may be more effective against certain types of bacteria45. On the other hand, Basil demonstrated moderate antibacterial activity across all tested bacteria, with significant inhibition zones observed against E. coli (10 ± 0.1 mm), S. enterica (21 ± 0.3 mm), S. aureus (15 ± 0.01 mm), and P. aeruginosa (24 ± 0.6 mm), showing broader activity across different pathogens. The presence of compounds such as eugenol, a key constituent in Basil's essential oil, may explain this activity, as eugenol has been shown to disrupt bacterial cell membranes and inhibit microbial growth46. Similarly, Za'atar displayed broad-spectrum antibacterial effects, particularly effective against S. enterica and S. aureus (with inhibition zones of 21 ± 0.3 mm and 20 ± 0.01 mm, respectively), but it did not show any inhibitory effects against P. aeruginosa. This suggests that while Za'atar's antibacterial properties are robust, they may be more specific to certain bacteria, and the lack of activity against P. aeruginosa might indicate the need for different extraction methods or higher concentrations to capture all bioactive components47. Oregano was another plant extract that demonstrated significant antibacterial activity, especially against S. enterica (23 ± 0.1 mm) and S. aureus (17 ± 0.6 mm), suggesting it is an effective agent against both Gram-negative and Gram-positive bacteria. This could be attributed to the high levels of carvacrol and thymol in oregano, compounds known for their strong antimicrobial properties48,49. Finally, Rosemary showed the strongest antibacterial activity across all tested bacteria, with the highest inhibition zones of 31 ± 0.5 mm against S. aureus and 22 ± 0.4 mm against S. enterica, suggesting its broad-spectrum and potent antibacterial potential.

When assessing the antifungal activity, Ginger exhibited moderate inhibition against A. niger (20 ± 0.11 mm), A. flavus (30 ± 0.3 mm), and C. albicans (24 ± 0.3 mm), suggesting it may be useful for treating fungal infections, though its antifungal effects were less pronounced compared to other plants in the study50. This could be due to the presence of bioactive compounds such as gingerols and shogaols, which are known to exhibit antifungal properties, albeit at higher concentrations51. Basil demonstrated particularly strong antifungal activity, with inhibition zones of 30 ± 0.11 mm against A. niger, 32 ± 0.3 mm against A. flavus, and 28 ± 0.3 mm against C. albicans, indicating its potent ability to combat fungal pathogens. Basil's antifungal properties are likely linked to compounds such as eugenol and other phenolic compounds, which have been reported to disrupt fungal cell membranes and inhibit spore germination52. Za'atar also exhibited high antifungal activity, particularly against A. niger (30 ± 0.11 mm) and A. flavus (29 ± 0.3 mm), suggesting that it may be effective in preventing fungal growth and could be valuable in treating fungal infections. The antifungal potential of Za'atar could be attributed to its essential oil composition, which contains several

phenolic compounds with proven antifungal activity53. Oregano showed significant antifungal effects, particularly against A. niger (35 ± 0.3 mm), highlighting its potential as a natural remedy for fungal infections, especially those caused by Aspergillus species. The high levels of carvacrol and thymol in oregano are well-known for their ability to inhibit fungal growth by interfering with membrane integrity and cell wall synthesis54. Rosemary exhibited the strongest antifungal activity among all the plant extracts tested, with inhibition zones of 36 ± 0.3 mm against A. niger, 40 ± 0.6 mm against A. flavus, and 21 ± 0.3 mm against C. albicans, underscoring its potent antifungal properties. The high levels of rosmarinic acid and essential oils in Rosemary contribute significantly to its broad-spectrum antifungal effects, making it an effective natural agent against common fungal pathogens55.

Mechanisms of Antimicrobial Activity in Ethnomedicinal Plant Extracts:

The antimicrobial activity of the ethnomedicinal plant extracts is primarily attributed to several mechanisms, including the disruption of microbial cell membranes, inhibition of enzyme activity, and interference with nucleic acid synthesis. Compounds such as eugenol (from Basil), carvacrol and thymol (from Oregano), and rosmarinic acid (from Rosemary) disrupt microbial cell membranes, increasing permeability and causing leakage of cellular contents, leading to cell death. These compounds also inhibit bacterial and fungal cell wall synthesis, impair spore formation, and interfere with DNA replication, preventing microbial growth. Additionally, phenolic compounds in these plants may chelate essential metal ions, disrupting key enzymatic processes. The antioxidant properties of some extracts, particularly Rosmarinic acid in Rosemary, neutralize reactive oxygen species (ROS), further damaging microbial cells. Furthermore, some compounds inhibit biofilm formation, which is crucial for microbial resistance, by disrupting quorum sensing. Together, these mechanisms provide a broad spectrum of antimicrobial action, suggesting that these plants can be potent natural agents against both bacterial and fungal pathogens.

Antimicrobial Activity of Rosemary Extract against S. Aureus Using Bioautography:

The antimicrobial activity of rosemary extract against S. aureus (ATCC 43300) was evaluated using bioautography as illustrated in Fig. 6. After applying 50 µL of rosemary extract to the TLC plate and performing chromatographic separation with the mobile phase (hexane: DCM, 67:33, V/V), zones of inhibition were observed after incubation of the TLC plate on the inoculated tryptone soya agar. The TLC bioautographic assay revealed the presence of several clear inhibition zones, which corresponded to specific spots on the TLC plate. These inhibition zones varied in size and intensity, indicating that different components of the rosemary extract exhibited antimicrobial activity. The most prominent zone of inhibition was observed at a spot located approximately halfway along the TLC plate, suggesting that the compound(s) at this location had the strongest antimicrobial activity against S. aureus. Smaller inhibition zones were also observed at other spots, indicating the presence of additional antimicrobial compounds with varying degrees of potency. No inhibition was observed in the areas where the rosemary extract did not contain active compounds, further confirming the

specificity of the antimicrobial action to certain components of the extract. The bioautography results from this study provide clear evidence that the rosemary extract contains antimicrobial compounds capable of inhibiting the growth of S. aureus (ATCC 43300), a Gram-positive pathogen that is a major cause of skin, respiratory, and wound infections. The formation of inhibition zones on the agar plate correlates directly with the spots on the TLC plate, indicating that the rosemary extract's bioactive components were successfully separated during chromatography and that some of these components have antimicrobial properties. The varying sizes of the inhibition zones observed suggest that rosemary contains multiple antimicrobial compounds with different levels of efficacy. The largest inhibition zone, found at one of the major spots, indicates the presence of a potent antimicrobial compound, while the smaller zones suggest weaker or less potent compounds. This is consistent with previous research indicating that rosemary contains several bioactive constituents, such as rosmarinic acid, carnosic acid, and essential oils, all known for their antimicrobial properties. Among these, rosmarinic acid is particularly well-documented for its activity against S. aureus and other pathogenic bacteria, which may explain some of the inhibition observed in this study. In addition, the bioautography method effectively identified and localized antimicrobial compounds without the need for sophisticated analytical equipment, such as mass spectrometry or NMR, which would be necessary to identify the specific chemical structures of the active compounds. The presence of multiple inhibition zones suggests that rosemary's antimicrobial activity is not due to a single compound but rather a combination of bioactive substances that together contribute to the overall antibacterial effect. The findings from this study are consistent with previous reports that highlight the antimicrobial potential of rosemary, particularly in fighting bacterial infections. The results are particularly relevant in light of the increasing global concern regarding antibiotic resistance, as natural products like rosemary offer a promising alternative to conventional antibiotics. While the rosemary extract demonstrated antimicrobial activity in vitro, further studies are needed to explore the specific compounds responsible, as well as their efficacy in vivo, toxicity, and potential for clinical application.

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