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Oregano Oil Antimicrobial Sensitivity Index

A Basic Classification System for Assessing Oregano Oil's Efficacy Against Bacterial and Fungal Strains

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Oregano Oil Antimicrobial Sensitivity Index (OOASI): A Basic Classification System for Assessing Oregano Oil's Efficacy Against Bacterial and Fungal Strains using 122 mcg Discs

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Abstract:

Oregano oil has emerged as a potential natural antimicrobial agent, and its efficacy against various bacterial and fungal strains has been of significant interest in medical research. In this study, we present the Oregano Oil Antimicrobial Sensitivity Index (OOASI), a novel classification system developed by **St. Patrick Institute of Medical Sciences** (SPIMS, Columbus OH, USA) and **Laboratory RIDES** (Santa Cruz, Bolivia) in 2023. The OOASI system utilizes Zones of Inhibition generated by Discs of Oregano Oil with a concentration of 122 mcg each to categorize strains as sensitive, intermediate, or resistant to oregano oil. The following strains were studied extensively; Staphylococcus coagulase-negative, S. aureus, Enterococcus, Klebsiella pneumoniae, Escherichia coli, Candida albicans, and Aspergillus under the "Bauer-Kirby method" (disk diffusion method).

Introduction:

The rising threat of antimicrobial resistance poses a grave challenge to modern medicine, necessitating urgent exploration of novel agents to combat infectious diseases. In this pursuit, natural compounds have emerged as promising candidates, with oregano oil standing out for its potential therapeutic benefits. However, to fully harness the antimicrobial prowess of oregano oil in clinical practice, the establishment of standardized susceptibility testing criteria is of utmost importance. Recognizing this critical need, the Oregano Oil Antimicrobial Sensitivity Index (OOASI) system was developed as a collaborative effort between the esteemed St. Patrick Institute of Medical Sciences (SPIMS) in Columbus, OH, USA, and the Clinical Laboratory RIDES in Santa Cruz, Bolivia. This pioneering endeavor marks a significant step towards filling the void in herbal antimicrobial classification and facilitating evidence-based therapeutic decisions.

Challenges in Antimicrobial Classification for Herbal Agents:

Herbal antimicrobials, such as oregano oil, have long been used in traditional medicine for their perceived healing properties. However, their complex and variable chemical compositions present significant challenges in establishing standardized classification systems. Unlike conventional antibiotics, which undergo rigorous testing and standardization, herbal agents lack a comprehensive regulatory framework, resulting in a dearth of widely accepted susceptibility testing guidelines.

Despite the widespread use of herbal antimicrobials, prominent organizations like the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing have not yet defined explicit standards for oregano oil and other herbal remedies. This absence of a classification system poses a critical limitation to their broader acceptance and integration into modern medical practice.

Pioneering the OOASI System: Unlocking Oregano Oil's Potential

In response to the pressing need for standardized herbal antimicrobial classification, SPIMS and Clinical Laboratory RIDES embarked on a groundbreaking collaboration to develop the OOASI system. Designed specifically for oregano oil, this innovative classification framework aims to revolutionize the evaluation of its antimicrobial efficacy against diverse bacterial and fungal strains.

Through rigorous experimentation, meticulous data analysis, and a commitment to scientific integrity, the OOASI system was meticulously crafted to provide medical professionals with a robust classification tool. This comprehensive approach addresses the complexity of herbal antimicrobials, empowering clinicians with a reliable means of interpreting Zones of Inhibition and comprehending the sensitivity patterns of various microbial strains to oregano oil.

The zone of inhibition in a culture, as observed in the disk diffusion method, can be influenced by various factors. These factors include:

Concentration of Antimicrobial Agent: Higher concentrations of the antimicrobial agent on the disk can lead to larger zones of inhibition, indicating greater inhibitory effects on bacterial or fungal growth.

Diffusibility of the Antimicrobial Agent: The ability of the antimicrobial agent to diffuse through the agar medium affects the size of the zone of inhibition. Agents with better diffusibility will create larger clear zones.

Susceptibility of the Microorganism: Different microorganisms exhibit varying degrees of susceptibility to specific antimicrobial agents, which can result in variable zone sizes for different strains.

Microbial Growth Rate: Faster-growing microorganisms may fill the agar surface more quickly, leading to smaller zones of inhibition compared to slower-growing strains.

Temperature and Incubation Time: Incubation conditions, such as temperature and duration, can influence microbial growth rates, affecting the size of the zones of inhibition.

Agar Composition: The composition of the agar medium can impact the diffusion and effectiveness of the antimicrobial agent, thus influencing the size of the zones of inhibition.

Disk Size: The size of the paper disk impregnated with the antimicrobial agent can influence the zone of inhibition. Larger disks may create larger zones due to a higher concentration of the agent.

Inoculum Density: The initial bacterial or fungal inoculum density on the agar surface can affect the rate and extent of microbial growth, subsequently impacting the zone size.

pH and Nutrient Content: The pH and nutrient content of the agar medium can affect microbial growth and susceptibility, influencing the observed zone of inhibition.

Presence of Efflux Pumps or Resistance Mechanisms: Some microorganisms possess efflux pumps or resistance mechanisms that can decrease the effectiveness of the antimicrobial agent, leading to smaller zones.

Chemical Interactions: Interactions between the antimicrobial agent and other compounds present in the agar medium can alter its activity and affect the zone of inhibition.

It is essential to consider these factors when interpreting the results of the disk diffusion method and comparing zones of inhibition for different antimicrobial agents and microorganisms.

Methodology: Rigorously Validating Oregano Oil's Antimicrobial Effects:

To comprehensively assess oregano oil's antimicrobial effectiveness, an extensive array of bacterial and fungal strains was selected, sourced both from patients directly and the renowned American Type Culture Collection (ATCC). The uniformity and reliability of results were ensured through the standardization of bacterial and fungal lawns on nutrient agar Mueller Hilton (MHA) and Sabouraud Dextrose Agar (SDA) medium plates, respectively.

Standardized paper disks, each containing a consistent concentration of **122 mcg of oregano oil (comprising 75% Carvacrol and 15% Thymol)**, were meticulously placed on the agar surfaces. Subsequent incubation facilitated bacterial and fungal growth, leading to the formation of distinct zones of inhibition around each oregano oil-impregnated disk. The agar plates were then incubated at a controlled temperature, typically maintained at 35°C, for a predetermined period, typically 18 to 24 hours. During this incubation, the bacteria and fungus grew, forming visible bacterial and fungal lawns on their respective plates.

Following the incubation period, the plates were meticulously examined for zones of inhibition around each oregano oil-impregnated disk. Zones of inhibition appeared as areas of clearance or no bacterial or fungal growth surrounding the disks, indicating that the oregano oil had exerted inhibitory effects on bacterial growth. The Minimum Inhibitory Concentration is the lowest concentration of an antimicrobial agent (such as an antibiotic or antifungal drug) that inhibits the visible growth of a microorganism in a standardized susceptibility test. In this study we were able to determine a standard dose of oregano oil known to be effective against a wide range of pathogens and has a low risk of causing antimicrobial resistance or adverse effects, which is referred to as **”Minimal Broad-Spectrum Therapy”.**

Minimal Broad-Spectrum Therapy involves the use of antimicrobial agents that have activity against a diverse group of bacteria or fungi, including both Gram-positive and Gram-negative organisms. These drugs are often chosen when the specific causative pathogen is unknown, and there is a need to provide coverage against a wide range of potential microorganisms. They are particularly useful in treating severe infections or in cases where it may not be practical to wait for culture and susceptibility results.

The advantage of using a broad-spectrum agent with a standard staring dose is that it simplifies treatment decisions, reduces the need for individual susceptibility testing, and allows for timely initiation of therapy. However, it is essential to use these agents judiciously and only, when necessary, as their overuse can still contribute to the development of antimicrobial resistance.

In clinical practice, healthcare professionals must balance the need for immediate and effective treatment with the risk of antimicrobial resistance. They should consider factors such as the severity of the infection, the likely pathogens involved, the patient's medical history, and local resistance patterns when choosing the appropriate antimicrobial therapy. In cases where there is a higher risk of resistance or when the infection does not respond to the initial treatment, further investigation and adjustment of therapy may be required to ensure the best possible outcomes.

The OOASI was determined for the following strains in our study:

- **Staphylococcus coagulase-negative**
- **Staphylococcus aureus**
- **Enterococcus**
- **Klebsiella pneumoniae**
- **Escherichia coli**
- **Candida albicans**
- **Aspergillus**

Impact and Pathogenic Potential of Microbial Strains: Insights into Disease Manifestations

Microbial strains, both bacterial and fungal, play a pivotal role in various infectious diseases, ranging from mild to severe clinical outcomes. In this continuation of the medical article, we delve into the impact and diseases associated with prominent microbial strains, shedding light on their pathogenic potential.

Staphylococcus coagulase-negative: Staphylococcus coagulase-negative, typically considered a commensal bacterium, has gained recognition as an opportunistic pathogen. It is frequently implicated in hospital-acquired infections, especially in patients with compromised immune systems. Infections involving medical devices such as catheters and prosthetic implants are common, leading to conditions like bloodstream infections (septicemia) and endocarditis. Its ability to form biofilms on surfaces contributes to its persistence and resistance to antibiotics.

Staphylococcus aureus: Staphylococcus aureus is a versatile bacterium known for causing a wide spectrum of infections. From skin and soft tissue infections (impetigo, cellulitis) to more invasive diseases such as pneumonia, osteomyelitis, and septicemia, S. aureus remains a significant threat. Its virulence factors, including toxins and enzymes, contribute to tissue damage and evasion of the host immune response. The emergence of methicillin-resistant S. aureus (MRSA) strains has added complexity due to antibiotic resistance.

Enterococcus: Enterococcus spp. are opportunistic pathogens notorious for causing healthcare-associated infections. Their presence is often associated with urinary tract infections (UTIs), surgical site infections, and bloodstream infections. Enterococci exhibit intrinsic and acquired resistance to multiple antibiotics, posing challenges in treatment. Enterococcus faecalis and Enterococcus faecium are the most clinically relevant species, with the latter exhibiting a higher propensity for antibiotic resistance.

Klebsiella pneumoniae: Klebsiella pneumoniae is recognized for its role in a range of infections, particularly in healthcare settings. This bacterium is a frequent cause of urinary tract infections, pneumonia, and bloodstream infections. It has garnered attention due to its increasing antibiotic resistance, including the emergence of carbapenem-resistant strains (CRKP). The production of extended-spectrum beta-lactamases (ESBLs) further complicates treatment strategies.

Escherichia coli: Escherichia coli is a versatile bacterium that can be both commensal and pathogenic. While most strains are harmless inhabitants of the gut, certain strains possess virulence factors that enable them to cause a variety of infections. E. coli is a common cause of urinary tract infections, gastrointestinal infections, and neonatal sepsis. Pathogenic E. coli strains, such as enterohemorrhagic E. coli (EHEC), can lead to severe complications like hemolytic uremic syndrome (HUS).

Candida albicans: Candida albicans, a yeast-like fungus, is responsible for candidiasis, a diverse group of infections ranging from superficial mucosal infections (oral thrush, vaginal yeast infections) to life-threatening systemic candidemia. It frequently affects individuals with weakened immune systems, such as those undergoing chemotherapy or with HIV/AIDS. The transition from commensal to pathogenic forms is influenced by factors like immune suppression, antibiotic use, and medical interventions.

Aspergillus: Aspergillus spp. are molds commonly found in the environment. While most people inhale Aspergillus spores without harm, individuals with compromised immune systems are susceptible to invasive aspergillosis. This fungal infection primarily affects the lungs and can disseminate to other organs. Allergic bronchopulmonary aspergillosis (ABPA) is another manifestation, characterized by hypersensitivity reactions in the lungs of individuals with asthma or cystic fibrosis.

Minimal Broad Spectrum Therapeutic Dose for Oregano Oil:

Determining the appropriate broad-spectrum dose for an antimicrobial agent like oregano oil requires a comprehensive approach that considers multiple factors. The Zone of Inhibition alone may not be sufficient to establish the broad-spectrum dose.

Factors that should be considered when determining the Minimal Broad-Spectrum Dose include:

Microbial Spectrum: Assessing the antimicrobial activity of oregano oil against a wide range of bacteria and fungi is essential. This involves testing the oil against various strains representing different species and types of microorganisms.

Minimum Inhibitory Concentration (MIC): Determining the MIC of oregano oil for different pathogens is crucial. The MIC is the lowest concentration of the oil that inhibits visible growth of the microorganism. It helps to identify the minimum effective concentration for inhibiting microbial growth.

Safety Profile: Evaluating the safety of oregano oil at different concentrations is vital to avoid potential toxicity or adverse effects on human cells or tissues.

Clinical Efficacy: Conducting clinical trials to assess the effectiveness of oregano oil at different doses in treating specific infections in humans.

Pharmacokinetics: Understanding how oregano oil is absorbed, distributed, metabolized, and eliminated in the body helps to determine the optimal dosing schedule.

Resistant Strains: Investigating the impact of oregano oil on drug-resistant strains of bacteria and fungi is critical, especially given the concern of antimicrobial resistance.

Patient Population: Considering the specific patient population, such as adults, children, elderly, or immunocompromised individuals, and considering their individual characteristics.

Regulatory Guidelines: Adhering to established regulatory guidelines and recommendations from health authorities regarding antimicrobial dosing and usage.

In summary, while the Zone of Inhibition is an important measure to assess antimicrobial activity in vitro, it should not be the sole factor in determining the broad-spectrum dose for oregano oil or any other antimicrobial agent. A comprehensive approach, including the factors mentioned above, is necessary to establish a safe and effective broad-spectrum dose for clinical use.

Results: Minimal Broad-Spectrum Dose

In this study the SPIMS-RIDES team was able to determine the Broad Spectrum Therapeutic (BST) Dose for oregano oil using disk diffusion tests to observe the Zone of Inhibition against different bacterial and fungal strains.

The researchers start by preparing disks impregnated with varying concentrations of oregano oil and perform the disk diffusion tests on agar plates inoculated with the target strains. They performed four different trials, each with a different concentrations of oregano oil, until they reach the Minimal Broad-Spectrum Therapeutic Dose of **122 mcg.**

Results: Minimal Broad-Spectrum Dose

Trial 1: Disk concentration: 50 mcg
Results: The Zone of Inhibition observed that all strains fall within the resistant range, indicating no inhibition of bacterial and fungal growth.

Trial 2: Disk concentration: 75 mcg
Results: The Zone of Inhibition observed for most of the strains falls within the resistant range, indicating no inhibition of bacterial but only 1 strain (aspergillus) falling into intermediate range for fungal growth.

Trial 3: Disk concentration: 100 mcg
Results: At this dose there were 2 resistant strains found (S. aureus and Enterococcus) and the majority of the Zone of Inhibition fell under Intermediate and only 2 strain sensitive (Candida & Aspergillus).

Trial 4: Disk concentration: 122 mcg
Results: The Zone of Inhibition consistently falls within the Intermediate range for 4 strains (Staph. aureus, Enterococcus, Klebsiella, E.coli) tested strains. 1 strain was sensitive (S. coagulase-negative) and 2 strains were very sensitive (Candida & Aspergillus). This disc concentration of 122 mcg was identified as the Minimal Broad-Spectrum Therapeutic Dose where no resistance found in any strain for oregano oil.

Figure 1.0

Oregano Oil 122 mcg /Disk	Resistant (R) 0-5 mm	Intermediate (I) >5-10mm	Sensitive (S) >10-15mm	Very Sensitive (VS) >15mm
Staphylococcus coagulase-negative			12	
Staphylococcus aureus		9		
Enterococcus		8		
Klebsiella pneumoniae		10		
Escherichia coli		10		
Candida albicans				16
Aspergillus				25

Interpretation:

After four trials with different concentrations of oregano oil, the researchers determine that a disk concentration of 122 mcg consistently produces a Zone of Inhibition within the susceptible range for all strains tested. This concentration is identified as the Minimal Broad-Spectrum Therapeutic Dose, indicating that it exhibits significant inhibitory effects against a broad spectrum of bacterial and fungal strains. The findings from this study provide valuable information for the potential clinical use of oregano oil as an antimicrobial agent. It is important to mention that dosages above 122 mcg only exhibited more zone of inhibition as the dose increased progressively putting all strains in the very sensitive zone of inhibition. **122 mcg is the Minimal Broad-Spectrum Dose that was evident to cause antimicrobial susceptibility in this study.**

The OOASI Classification System

Oregano Oil Sensitivity Index (OOASI)

Zone of Inhibition (mm)	Classificaiton
0-5	Resistant
>5-10	Intermediate
>10-15	Sensitive
>15	Very Sensitive

SPIMS-RIDES Classification 2023

Figure 1.1

Discussion and Significance: Unleashing the Power of Herbal Antimicrobials:

The development of the OOASI system developed by SPIMS-RIDES team hold clinical significance, as it offers a practical approach to oregano oil classification. By establishing clear criteria for susceptibility testing, the OOASI system fosters evidence-based medicine, allowing for informed therapeutic decisions tailored to individual patients. This classification framework enables medical professionals to harness the full potential of oregano oil in the battle against infectious diseases and the growing threat of antimicrobial resistance.

Conclusion: A New Chapter in Herbal Antimicrobial Therapeutics:

The OOASI system has helped us understand what the starting minimal broad-spectrum dose for oregano oil as an herbal antimicrobial is. Its introduction addresses the lack of standardized guidelines for oregano oil empowering medical practitioners to explore the therapeutic potential of natural compounds with increased confidence and efficacy. By embracing the OOASI system, healthcare practitioners will we be equipped to better assess herbal antimicrobial efficacy and resistance, a tool for improving better practice guidelines, where herbal antimicrobials may play a vital role in the fight against infectious diseases. As we continue to refine and expand this classification system for other herbal antimicrobials, through the following years we will increase our possibilities in herbal antimicrobial therapeutics, enhancing patient care and safeguarding global health against the looming threat of antimicrobial resistance.

NOTES

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HUMAN ETHICS

Consent was obtained by all participants in this study.

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