Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 5 (2025)

Complete Analysis And Extraction Of Antimicrobial Compounds From Oregano Plant

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Article Details

Keywords: Oregano, Antimicrobial Properties, Antibiotic Resistance

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ABSTRACT

This comprehensive study investigates the antimicrobial potential of Origanum vulgare, focusing on compounds such as thymol and carvacrol known for their efficacy against diverse pathogens. Employing advanced extraction techniques, the research integrates botany, chemistry, and microbiology to position oregano as a sustainable alternative to synthetic antimicrobials. The investigation reveals clear zones of inhibition in agar plates, showcasing the dose-dependent antimicrobial effect of oregano extracts. The comprehensive study outlined in the abstract delves deeply into the antimicrobial potential of Origanum vulgare, commonly known as oregano, shedding light on its efficacy against a broad spectrum of pathogens. At the heart of the investigation lie two key compounds, thymol and carvacrol, which have garnered attention for their potent antimicrobial properties. These compounds, found abundantly in oregano, serve as the focal point of the study due to their ability to combat diverse pathogens effectively. To unravel the antimicrobial potential of oregano, the research employs advanced extraction techniques that blend insights from botany, chemistry, and microbiology.

INTRODUCTION

The natural world is an abundant source of compounds with potential antimicrobial properties, and researchers have long sought to unlock the secrets hidden within plants to combat the relentless evolution of drug-resistant microbes. The exploration of natural resources for antimicrobial compounds has become an imperative quest in our battle against the ever-adaptive world of drug-resistant microbes. *Origanum vulgare*, a herb celebrated in kitchens worldwide, has garnered attention for its suspected antimicrobial properties (Muriel-Galet *et al.*, 2015). Recent years have witnessed a significant surge in scientific enthusiasm for the thorough investigation and extraction of these compounds from the oregano plant. This surge is driven by the urgent need to develop novel therapeutic agents that can stand as a formidable defense against the burgeoning crisis of antibiotic resistance (Karakaya *et al.*, 2011).

Oregano's medicinal use can be traced back to ancient civilizations, including the Greeks and Romans. It was employed for its antibacterial and antifungal properties. Historically, oregano was used to treat various ailments, such as respiratory infections, digestive issues, and skin conditions. Its essential oils, rich in carvacrol and thymol, played a central role in these traditional medicinal practices (Oleynikov, 2020). Over time, oregano's antimicrobial potential has garnered scientific interest and validation. In this literature review, we will explore the methods employed in the extraction and analysis of antimicrobial compounds from oregano, with a particular focus on Response Surface Methodology, incubation, autoclaving, vortex mixing, pH monitoring, agar-based culturing, bacterial strains, and microbiological techniques. By delving into these aspects, we aim to better understand the potential of oregano as a source of natural antimicrobials and its applications in medicine and food preservation (Castilho *et al.*, 2012)

In a world where microbial infections and antibiotic resistance pose ongoing challenges, the multifaceted world of oregano's antimicrobial compounds offers hope and possibility. It reminds us that nature, combined with scientific precision, has the power to provide answers to some of our most pressing challenges (Kocic-Tanackov *et a*l., 2014).

Oregano contains an array of phytochemicals, with the most notable being thymol and carvacrol. These compounds are categorized as phenolic monoterpenes and have demonstrated significant antimicrobial activity against a broad spectrum of microorganisms (Sharifi-Rad *et al.*, 2021).

The extraction of antimicrobial compounds from oregano involves various methods, including steam distillation, solvent extraction, and supercritical fluid extraction. Steam distillation is a traditional and widely used method, but newer techniques like supercritical fluid extraction offer higher yields and selectivity (Cavero *et al.*, 2006).

Oregano's antimicrobial efficacy is well-documented. Thymol and carvacrol have exhibited potent antibacterial properties against both Gram-positive and Gram-negative bacteria. Additionally, they have antifungal effects against various fungal strains, making oregano a valuable natural antimicrobial source (Chun et al., 2005).

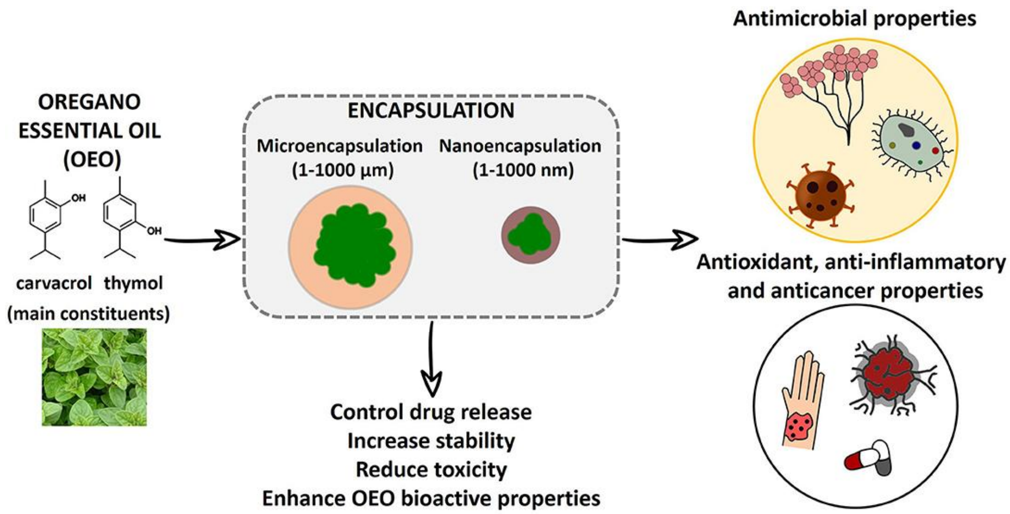

FIGURE:1 ANTIMICROBIAL COMPOUND ANALYSIS (SKANDAMIS, 2011)

MATERIALS AND METHODS

INITIAL PHASE

Objective: Prepare oregano leaves for subsequent processing. Careful Drying: Oregano leaves were subjected to meticulous drying to remove moisture. Facilitating Processing: Moisture removal aimed to facilitate and enhance further processing steps.

GRINDING PROCESS

Follow-up Step: After the drying phase, the dried leaves were taken through an additional step. Coarse Powder Formation: The leaves were ground meticulously to achieve a coarse powder texture. Surface Area Enhancement: Grinding aimed to increase the surface area, aiding in subsequent extraction processes.

SOLVENT MIXTURE COMPOSITION

Ground oregano powder used in the extraction. Solvent mixture included ethanol, methanol, and acetone. Strategic choice of solvents aimed to extract a diverse range of compounds with varying polarities.

EXTRACTION PROCEDURE

Ground oregano powder subjected to the solvent mixture. Mixture underwent a shaking process to facilitate thorough interaction between the solvents and the plant material. Extended steeping period of precisely a week chosen to allow sufficient time for comprehensive extraction.

SHAKING PROCESS

Mechanical agitation applied to the mixture to enhance the interaction between the solvents and the oregano powder. Shaking served to promote the dissolution of a wide range of compounds into the solvent mixture.

INITIATION OF EVAPORATION

The primary objective was to kick start the evaporation process on the petri plates. Evaporation aimed to eliminate the liquid component, leaving behind a dry, concentrated residue.

CAREFUL EXTRACTION AND REFINEMENT

Extraction of compounds from oregano leaves involved a strategic mixture of solvents—ethanol, methanol, and acetone. The extraction process aimed to obtain a diverse range of compounds, considering their varying polarities. Extracted material underwent a meticulous refinement process to enhance its purity and concentration.

BASIS FOR SAMPLE PREPARATION

The refined extracts served as the fundamental building blocks for subsequent sample preparation. These extracts were enriched with compounds obtained from oregano, ensuring a representative composition for further analyses. To understand the impact of oregano extracts comprehensively, samples with various concentrations were prepared. Different concentrations allowed researchers to explore a spectrum of dilutions, providing insights into the concentration-dependent effects of the extracts.

BACTERIAL STRAIN CULTIVATION:

The study concurrently focused on two primary aspects: plant material preparation/extraction and bacterial strain cultivation. Specific bacterial strains, namely *Staphylococcus aureus* and *E. coli*, were selected for cultivation. The inclusion of *Staphylococcus aureus* and *E. coli* added a microbial dimension to the study.

STRATEGIC SELECTION

Staphylococcus aureus and *E. coli* were strategically chosen for their relevance in microbial studies and their prevalence in various environments.

RESPONSE SURFACE METHODOLOGY

Despite the lack of statistics, Mini-tab empowers all parts of an organization to communicate better results, create better products, and improve efficiencies to maximize costs and minimize costs. Only Mini-tab offers a unique, integrated approach, delivering software and services that drive business success anywhere with the cloud.

RESULTS AND DISCUSSION

OBSERVATION OF INHIBITION ZONES

Upon the incubation of agar plates containing oregano extracts, a notable phenomenon was observed—clear zones of inhibition. This observation holds significance in understanding the antimicrobial properties of oregano extracts.

INCUBATION PROCESS: The incubation process played a crucial role in the development and visualization of inhibition zones. The plates were allowed to incubate under specific conditions to promote the growth and interaction of microorganisms with the oregano extracts.

CLEAR ZONES OF INHIBITION

The clear zones of inhibition refer to areas surrounding the wells containing oregano extracts where the growth of microorganisms is visibly hindered or inhibited.

ZONE SIZE VARIATION

The observed zones of inhibition exhibit a spectrum of sizes, reflecting the diverse degrees of bacterial growth inhibition. This variation prompts further investigation into the factors influencing these differences.

CONCENTRATION-DEPENDENT EFFECT

Analyzing the relationship between the concentration of the oregano extract and the resulting size of the inhibition zones. Higher concentrations tend to correlate with larger zones, suggesting a concentration-dependent antimicrobial effect.

DOSE-RESPONSE RELATIONSHIP

A more in-depth exploration of the dose-response relationship reveals the specific nature of the concentration-dependent effect. Understanding how the oregano extract influences bacterial susceptibility at different concentrations is crucial for drawing meaningful conclusions.

ACTIVITY AGAINST SPECIFIC STRAINS

STAPHYLOCOCCUS AUREUS STRAINS SUSCEPTIBILITY

The oregano extracts demonstrated effectiveness against *Staphylococcus aureus* strains. Distinct zones of inhibition were observed, indicating the ability to hinder *Staphylococcus aureus* growth.

COLI STRAINS SUSCEPTIBILITY

The oregano extracts exhibited susceptibility against *E. coli* strains. Clear zones of inhibition were noted, signifying the extract's ability to impede the growth of *E. coli*. Discuss the importance of inhibiting E. coli and its relevance in different applications or environments.

This detailed exploration provides a comprehensive understanding of the activity of oregano extracts against specific strains, emphasizing both *Staphylococcus aureus* and *E. coli*, and delving into various aspects such as comparative analysis and potential mechanisms of action.

TABLE: RSM DESIGN OF METHANOL EXTRACT OF OREGANO *(E.COLI)*

Sr	**Temperature ℃**	**pH**	**Concentration µg/l**	**Zone of inhibition**
1	35	6.5	750	12
2	40	5	250	7
3	37.5	6.5	750	19
4	37.5	8	750	13
5	35	8	250	5
6	35	8	250	5
7	35	5	500	11
8	37.5	5	750	12
9	37.5	6.5	500	11
10	37.5	6.5	750	19
11	40	6.5	750	6
12	37.5	6.5	750	19
13	40	8	500	5
14	40	5	250	4
15	40	5	500	5
16	40	5	500	5
17	40	6.5	750	5

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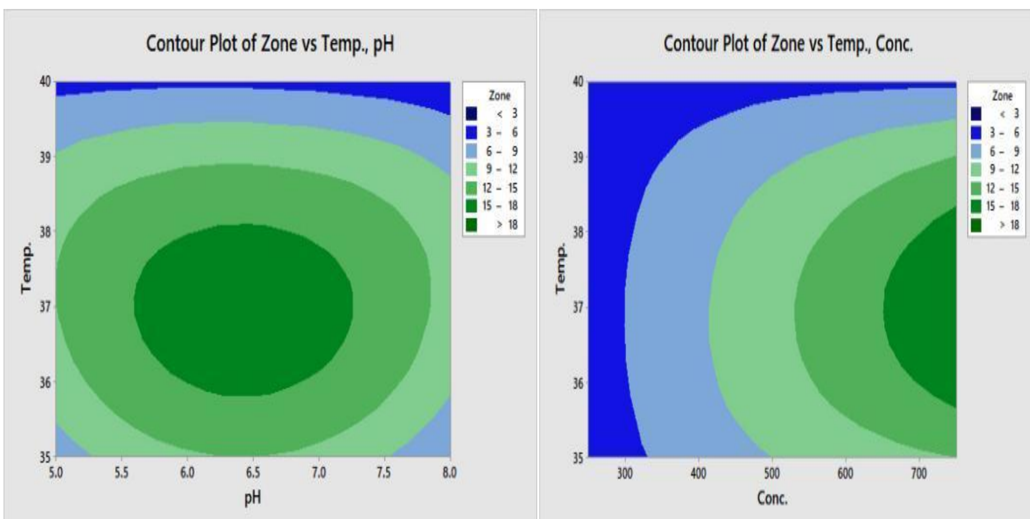
18	35	5	250	4
19	37.5	8	750	10
20	35	5	250	4
21	35	6.5	750	12
22	37.5	6.5	250	5
23	40	8	250	3
24	35	5	250	3
25	37.5	6.5	750	19
26	40	8	250	2
27	37.5	6.5	750	19
28	37.5	6.5	250	4
29	37.5	6.5	750	19
30	37.5	6.5	750	19
31	40	5	500	5
32	37.5	5	750	12
33	37.5	8	750	10
34	40	8	500	5
35	37.5	6.5	750	19
36	40	6.5	750	5
37	27.5	6.5	750	19
38	40	8	250	3
39	35	6.5	750	12
40	37.5	6.5	750	19
41	35	5	500	11
42	40	8	500	5
43	37.5	5	750	12
44	37.5	6.5	750	19
45	37.5	6.5	750	19
46	35	8	500	7
47	35	8	500	7
48	37.5	6.5	500	11

49	37.5	6.5	750	19
50	37.5	6.5	750	19
51	37.5	6.5	500	11
52	40	5	250	4
53	37.5	6.5	750	19
54	37.5	6.5	750	19
55	35	8	250	5
56	37.5	6.5	750	19
57	37.5	6.5	750	19
58	35	8	500	7
59	37.5	6.5	250	5
60	35	5	500	11

EFFECTS OF TEMPERATURE AND PH ON ANTIMICROBIAL ACTIVITY OF *ORIGANUM VULGARE* AGAINST (*E. COLI)*



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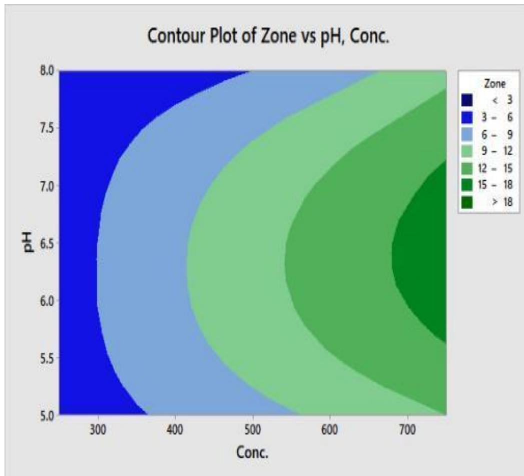

FIGURE 2.1: SHOWS THAT BY KEEPING CONCENTRATION ON X-AXIS AND TEMPERATURE ON Y-AXIS AND BY ALTERING THE MAXIMUM ZONE OF INHIBITION IS ON DARK GREEN AREA AND MINIMUM IS ON DARK BLUE AREA AT PH OF (6-7) AND CONCENTRATION OF 750MG/L SO IT'S ALSO PROVES THAT IT WORK MORE EFFICIENTLY IN ACIDIC ENVIRONMENT.

TABLE:1 RSM DESIGN OF METHANOL EXTRACT OF OREGANO *(STAPHYLOCOCCUS AUREUS)*

Sr No	Temperature ℃	pH	Concentration µg/l	Zone of inhibition
1	35	6.5	750	19
2	40	5	250	15
3	37.5	6.5	750	27
4	37.5	8	750	21
5	35	8	250	16
6	35	8	250	16
7	35	5	500	17
8	37.5	5	750	22
9	37.5	6.5	500	19
10	37.5	6.5	750	27
11	40	6.5	750	21
12	37.5	6.5	750	27
13	40	8	500	21

14	40	5	250	14
15	40	5	500	16
16	40	5	500	16
17	40	6.5	750	14
18	35	5	250	13
19	37.5	8	750	21
20	35	5	250	13
21	35	6.5	750	18
22	37.5	6.5	250	12
23	40	8	250	14
24	35	5	250	12
25	37.5	6.5	750	27
26	40	8	250	17
27	37.5	6.5	750	27
28	37.5	6.5	250	12
29	37.5	6.5	750	27
30	37.5	6.5	750	27
31	40	5	500	15
32	37.5	5	750	18
33	37.5	8	750	21
34	40	8	500	19
35	37.5	6.5	750	27
36	40	6.5	750	22
37	27.5	6.5	750	27
38	40	8	250	12
39	35	6.5	750	15
40	37.5	6.5	750	27
41	35	5	500	19
42	40	8	500	20
43	37.5	5	750	22
44	37.5	6.5	750	27

45	37.5	6.5	750	27
46	35	8	500	17
47	35	8	500	15
48	37.5	6.5	500	22
49	37.5	6.5	750	27
50	37.5	6.5	750	27
51	37.5	6.5	500	26
52	40	5	250	12
53	37.5	6.5	750	27
54	37.5	6.5	750	27
55	35	8	250	13
56	37.5	6.5	750	27
57	37.5	6.5	750	27
58	35	8	500	17
59	37.5	6.5	250	12
60	35	5	500	17

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EFFECTS OF TEMPERATURE AND PH ON ANTIMICROBIAL ACTIVITY OF ORIGANUM VULGARE AGAINST STAPHYLOCOCCUS AUREUS

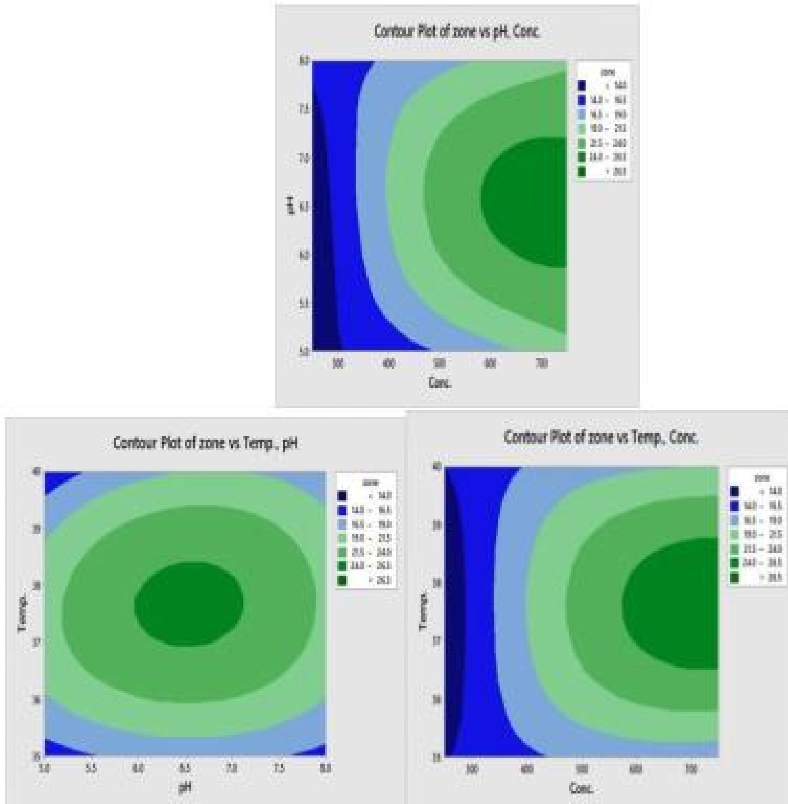

FIGURE 2.2: SHOWS THAT BY KEEPING CONCENTRATION, PH AND TEMPERATURE ON X-AXIS AND Y-AXIS AT RESPECTIVELYTHE MAXIMUM ZONE OF INHIBITION IS ON DARK GREEN AREA AND MINIMUM IS ON DARK BLUE AREA AT PH 6.5 AND CONCENTRATION 750 MG/L.

DISCUSSION

The introductory section sets the context for the discussion on the herbal potential of oregano. It outlines the primary focus on how oregano, a herb with historical significance in traditional medicine and cuisine, is being re-evaluated through contemporary scientific exploration. This chapter serves as a bridge between the historical uses of oregano and its modern application in addressing microbial threats (Padulosi, 1997). The exploration of its rich history serves to underscore its traditional uses and sets the stage for a more comprehensive understanding of its potential in the scientific realm (Adame-Gallegos *et al.*, 2016). The versatility of oregano, serving both medicinal and culinary purposes, underscores its historical significance and the reverence with which it was held in various traditions (Fleisher & Sneer, 1982) The fact that oregano has persisted as a culinary and medicinal staple speaks to its resilience and the enduring appreciation of its unique qualities (Węglarz *et al.*, 2020) This shift represents a pivotal moment in the understanding of oregano's potential, moving beyond anecdotal evidence to a more rigorous and evidence-based examination (P. N. Skandamis & Nychas, 2001). Carvacrol, another prominent constituent, exhibits similar attributes, contributing to oregano's overall antimicrobial potency. Rosmarinic acid, with its antioxidant and antimicrobial properties, adds a multifaceted dimension to the herb's bioactive profile (Voidarou *et al.*, 2021).

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