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A comparative study of the effect of extracts extracted from *Ocimum basilicum* leaves using organic extract and essential oil

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Submission Date: January 26th, 2024; **Acceptance Date:** April 29th, 2024; **Publication Date:** June 3rd, 2024

Please cite this article as: Sultan L. J., Fadhil W. G., Hamid M. M., Hadi S. T. A comparative study of the effect of extracts extracted from *Ocimum basilicum* leaves using organic extract and essential oil. *Functional Foods in Health and Disease* 2024; 14(6): 380-387. DOI: https://doi.org/10.31989/ffhd.v14i6.1304

ABSTRACT

Background: Medicinal plants that possess antimicrobial and antioxidant properties have garnered significant attention for their role in maintaining food quality, improving safety, and impeding spoilage. They also can aid in controlling food contamination risks and augmenting the nutritional value of foods.

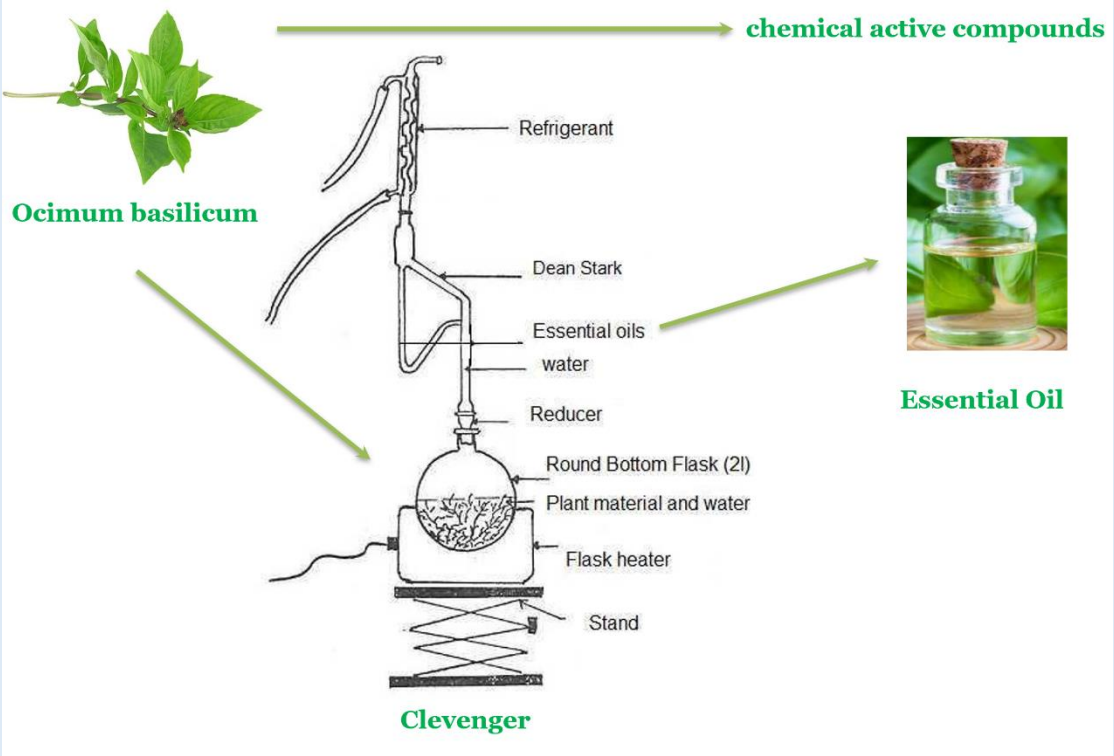
Objective: The study aimed to obtain botanical extracts possessing antimicrobial capabilities and use them to inhibit the growth of molds and yeasts. Additionally, these extracts are aimed at prolonging product shelf life by harnessing their antioxidant attributes.

Methods: Several microorganisms, including *E. coli* and *Pseudomonas*, were subjected to testing. Ethanolic alcohol, chloroform, and essential oil extracts were prepared; the tablets were then immersed in these solutions to test their efficacy.

Results: The leaf extracts displayed significant antimicrobial efficacy against essential oil extracts with *Staphylococcus aureus* and *Serratia marcescens*, while the organic extracts, which included ethyl alcohol and chloroform, did not show any inhibitory effects against bacteria.

Conclusion: The basil leaf extract demonstrated significant antimicrobial activity against microorganisms, particularly in the case of essential oil extracts compared with other methods of extraction. However, Gram-positive bacteria showed a better response for the essential oils method than that of Gram-negative bacteria.

Keywords: *Ocimum basilicum;* basil leaf; Clevenger; chemical active compounds; essential oil; organic extracts



Graphical Abstract

INTRODUCTION

Since ancient times, humans have turned to natural remedies as a form of pharmacy; they often resorted to using herbs and their parts for therapeutic purposes due to observing or believing these could improve ailments individuals experienced. Basil, recognized as an aromatic medicinal plant, has been utilized for various purposes throughout history [1]. Basil leaves, whether dried or fresh, are commonly used as flavoring agents or spices in the production of sauces, vinegars, green sweetened syrups, and confectioneries [2]. Warm water infusions of basil leaves and flowers are traditionally employed to treat diseases of the stomach and digestive system, acting as an antispasmodic and relieving gas [3-4]. Externally, basil can be applied to treat acne, skin injuries, and loss of smell. Internally, it can be used to manage fever, nausea, intestinal infections, and fatigue [5]. Moreover, alcohol extract of basil leaves can have antimicrobial effects [6].

Given concerns regarding potential food poisoning associated with the use of chemical and industrial compounds, such as antimicrobials, there has been a surge in demand for natural sources of food preservation. Consequently, scientists have turned their attention to

exploring the efficacy of inhibitory compounds, such as essential oils. Essential oils are a combination of monoterpenes, particularly sesquiterpenes, and their oxidized derivatives, including alcohol, aldehydes, phenols, ethers, ketones, and oxides. Due to containing organic compounds, essential oils are known for their unique aroma and flavor profiles [7-9]. Essential oils, also known as volatile oils, also easily dissolve in ether; are present in cosmetics, perfumes, soap, and detergents [5]; and exhibit properties that are anti-mold, anti-yeast, antibacterial, anti-mycotoxin, and antioxidant [12]. In particular, basil oil, a type of essential oil, has medicinal benefits [6].

METHODS

Preparation of basil leaf extract using organic solvents (ethyl alcohol and chloroform): Fresh basil (*Ocimum basilicum*) leaves were locally sourced, collected, and air-dried by the Herbarium at the College of Science, University of Baghdad. To prepare alcohol extract from basil plants, a sample was processed following a method described in references [13-14], which involved using 20 g dry basil powder and extracting the substances with a saxholate extraction device. The extraction was carried out using 400 ml of 95% ethyl alcohol over a period of 24 hours. After extraction, the material was dried in an electric oven at 40°C.

Essential oil extraction: It was performed using a Clevenger apparatus, as described in the British Pharmacopoeia for Herbs [15].

Detection of active compounds [16-18] and precipitation reagents for secondary chemical compounds in basil leaf extracts:

Alkaloids' reagents: (a) Mayer's reagents and (b) tannic acid reagent.

Phenols' reagents: (a) lead acetate reagents (1%) and (b) potassium hydroxide (KOH) reagent.

Terpenoids' reagents: (a) foam reagent and (b) $HgCl_2$ reagent.

Preparation of plant extract concentrations: To prepare a stock solution for the alcoholic extract, 2 g of the stock solution was dissolved in 3 mL of ethyl alcohol, and the volume was then adjusted to 10 mL with distilled water, yielding a final concentration of 200 mg/mL. From this solution, further dilutions were made to achieve the desired concentration of 50 mg/mL.

Bacterial samples: Bacterial samples isolated and identified included *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Salmonella Typhi* (*S. typhi*), *and Serratia marcescens* (*S. marcescens*). The samples were obtained from the microbiology laboratories at the College of Science, University of Baghdad. Bacterial isolates were calibrated using a McFarland standard and then spread onto Muller-Hinton medium to study the effects of the extracts on them.

Effect of plant extracts on bacterial growth: The agar well diffusion method [19] was used to assess bacterial sensitivity to the plant extract. For each type of microorganism, 0.1 mL of bacterial suspension was spread onto Mueller-Hinton agar with a Cork borer. Wells, each with a 6 mm diameter, were drilled into the agar; into each well, 0.1 mL of the plant extract was added at various concentrations, with distilled water serving as a negative control. The dishes were left for 15 minutes, then incubated at 37°C for 24 hours. After

incubation, the diameter of the inhibition zones was measured.

RESULTS AND DISCUSSION

Table 1 presents the results of chemical detection, revealing numerous active ingredients in basil plants. Notably, alkaloids were identified, with spanning being particularly significant due to its role in antimicrobial activity. The extracts are characterized by their sticky texture, dark green color, and aromatic odor. The green color was attributed to chlorophyll and xanthine, while the distinctive aromatic smell of basil was due to its volatile oils. Additionally, basil contains mucous materials, vegetable gel, and gums. The analysis confirmed that basil leaves contain saponins, tannins, glycosides, phenols, and flavonoids in varying proportions [19].

Table 1. Chemical detection of active compounds in basil leaves.

Active compounds	Detector type	Detection result
Alkaloids	Dragendorf	+
	Mayer	+
	Wachner	+
	Brick	+
Saponins	Mercuric chloride	+
	Shake the extract	+
Tannins	Ferric chloride (1%)	+
	Lead acetate (1%)	+
Glycosides	Kashif Fahlenk	+
	Benedict's detector	+
Phenols	Ferric chloride (1%)	+
Flavonoids	Ethyl alcohol + KOH	+

Effect of organic extracts of basil leaves on microorganisms: Different polar solvents were used to identify the main components with inhibitory effects on microorganisms. The effectiveness of the extracts is summarized in Table 2, where the inhibition zones for *Salmonella Typhi* and *Staphylococcus aureus* were 6 mm and 9.5 mm, respectively. The alcoholic extract contained effective compounds, including flavonoids, alkaloids, and tannins, which possess inhibitory effects on certain microorganisms [20]. These phenolic compounds inhibit enzymes responsible for basic metabolic reactions by protein denaturation and preventing bacterial growth. These findings are consistent with a 2008 study that evaluated antioxidant and antimicrobial activity of essential oil and corroborated with a 2015 literature review that found compelling evidence for the ability of basil oil to inhibit bacterial growth [20-21].

The chloroform extract had a weak effect on *S. typhi* (1.5 mm), *K. pneumonia*e (7 mm), and *E. coli* (5 mm). This is because the chloroform extract contained

flavonoids and terpenoids, which can rupture cell membranes by forming complexes with proteins [22].

It is evident from Table 2 that Gram-positive bacteria are more affected by essential oils. The average diameter of the inhibition zone for *S. aureus* was 24 mm due to the peptidoglycan layer that directly interacts with the oils. The Gram-positive bacteria lack additional protective substances outside the peptidoglycan layer, making them more susceptible [22]. Conversely, the Gram-negative bacteria exhibit less susceptibility, as evidenced by *S. typhi* showing an inhibition zone of 14 mm, *E. coli* 8 mm, *S. marcescens* 7 mm, and *K. pneumoniae* 20 mm. This difference may be attributed to the nature, composition, and sensitivity level of the bacterial cell walls. Variations may also arise from the type of plant extract and preparation method [23]. Table 1 shows that the different extraction methods and compounds used played a significant role in the efficacy of the extracts in inhibiting microorganisms. The results closely aligned with expectations due to the presence of eugenol, which is known to be effective against lipophilic microorganisms. The abundance of eugenol in volatile oils enhances their solubility in cell membranes, rendering them toxic to microorganisms [24]. The findings are consistent with previous research which indicated a higher antimicrobial effect against Gram-positive organisms compared to their Gram-negative counterparts [25]. The higher the concentration of the extract, the greater the area of inhibition observed. Basil extract is beneficial for treating synergistic wounds and infections [27]. Its antibiotic activity is attributed to the formation of complexes in the cell wall by incorporating cellular and soluble proteins, leading to microbial membrane disruption [28].

Both Table 3 and Figure 1 demonstrate the effectiveness of the extract against molds. The diameter of inhibition zones for *A. niger* and *Penicillium* was 32 mm and 53 mm, respectively, when using chloroform. However, ethyl alcohol did not exhibit any inhibitory activity against yeasts, including *C. albicans* [29]. The superior inhibitory activity of the oil can be attributed to its terpene compounds, specifically α-pinene, which interferes with the cytoplasmic membranes and affects the transport mechanisms [30].

Table 2. Effect of extracts on inhibiting bacterial growth.

Microorganisms	Ethyl alcohol extract	Chloroform extract	Essential oil
Escherichia coli	10	5	8
Staphylococcus aureus	9.5	0	24
Klebsiella pneumoniae	9	7	20
Salmonella typhi	6	1.5	14
Serratia marcescens	0	0	7

Table 3. Effect of extracts on inhibiting yeast and mold growth.

Yeasts and molds	Ethyl alcohol extract	Chloroform extract	Essential oil
Candida albicans	ND	ND	16
Aspergillus niger	ND	32	40
Penicillium	ND	53	55

ND = Not detected.

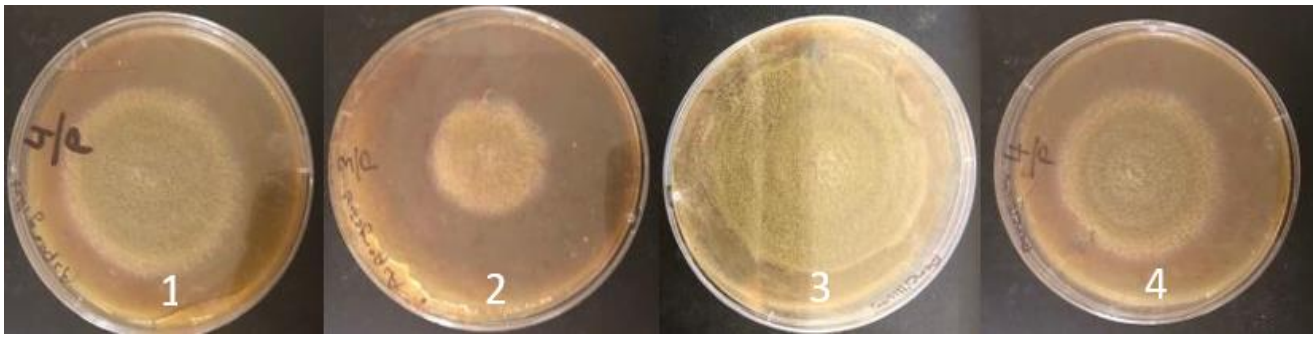


Figure 1. Effect of extracts on inhibiting growth of yeasts and molds. 1 = *Aspergillus niger*; 2 = *Candida albicans*; 3 = *Penicillium*; 4 – *Chloroform.*

CONCLUSION

Essential oils extraction methods proved more effective than organic extraction methods for basil leaves in terms of exhibiting inhibitory activity against microorganisms. This increased efficacy may be attributed to the presence of the terpene compound α-pinene, which disrupts cytoplasmic membranes and impacts transport mechanisms. Essential oil extraction appears to be a promising alternative method for obtaining compounds from basil plants, with potential applications in the food, pharmaceutical, and cosmetic industries. Chemical detection results indicated that alcoholic extracts contain effective compounds with inhibitory effects on certain microorganisms, whereas chloroform extracts demonstrate a weaker effect on the same organisms. Additionally, the results showed that Gram-positive bacteria are more affected by essential oils than Gram-negative bacteria.

List of Abbreviations: mm: measuring unit, gm: gram, mL: milliliter, °C: Celsius degree

Authors' Contributions: Linh Jaafar Sultan: formal analysis; methodology; project administration; funding acquisition; validation; writing original draft. Wafaa Ghazi Fadhil: data duration; formal analysis; methodology. Mohammed Majed Hamid: project administration; supervision; resources; validation. Sara Thamer Hadi: writing review and editing.

Competing Interests: The authors have no conflict of interest.

Acknowledgement and Funding: The authors would like to acknowledge the contribution of the University of Anbar (www.uoanbar.edu.iq) via their prestigious academic staff, who supported this research with all required technical and academic support.

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