Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2024, 37(3)

Benefits of Marjoram (*Origanum majorana*) and Chamomile (*Matricaria chamomilla*) in Preserving some Foods from Spoilage

Ibtisam Fareed Ali Karm[1*]
[1]Market Research and Consumer Protection Center, University of Baghdad, ,Baghdad, Iraq.
*Corresponding Author.

Received:20 May 2023 Accepted: 25 June 2023 Published: 20 July 2024
doi.org/10.30526/37.3.3504

Abstract

A global scientific study found that reducing the excessive and indiscriminate use of antimicrobial medicinal drugs in all their forms significantly improved food safety. We made aqueous and organic extracts (Ex1, Ex2, and Ex3) from two plants (*Origanum majorana* and *Matricaria chamomilla*) to create the best alternative form for antimicrobial preventive agents that cause food spoilage. Traditional methods determined the most essential compounds in plants, and we used the FTIR analysis technique for all treatments to observe impurities such as glycosides, alkaloids, flavonoids, carbohydrates, and phenolic substances. This study points to synergistic activity for plant extracts (*Origanum majorana* and *Matricaria chamomilla*). The safe use of antimicrobials that are of natural origin, as much as possible, is necessary to manage the risks in order to reduce the emergence of microorganisms resistant to traditional antibiotics.

Keywords: Preventive agents, FTIR technique, food safety, pharmacological herbs.

1. Introduction

In harsh environments, microorganisms can spread, and their impact on human life can be positive and negative. Not only do they contribute to human sickness, but they also cause food to spoil. Food rotting is an unwanted process that poses a significant challenge for people. Food deterioration is accelerated by a variety of causes [1], including antimicrobial peptides (AMPs) generated from plants that have been researched for potential bioactivities against a variety of human, plant, and food infections [2]. Many beneficial substances, including phenolic compounds, which may scavenge free radicals and thus lessen oxidative stress, are found in plant material. Flavonoids, phenolic acids, lignans, and stilbenes are examples of phenolic compounds that have antioxidant characteristics. Plants use the chemicals mentioned above' ' characteristics as a defensive strategy against UV radiation, temperature changes, and mechanical harm [3]. They also serve as a significant chemical barrier against herbivores because they operate physiologically on insects [4, 5]. One of the Lamiaceae family's well-researched species is *Origanum majorana*, it has been identified as a plant with valuable pharmacological properties due to its rich chemical profile, which may be applied to both the plant's extracts and essential oil fraction and it was found that their leaves extract hold magnificent composites such as rosmarinic acid, thymol, tannins, flavonoids, triterpenes, and phenol. Numerous researches have specifically looked at the biological

activity of the essential oil extracted from the plant's aerial section [6, 7, 8]. The Asteraceae family includes the well-known medicinal plant species chamomile (*Matricaria chamomilla L.*), which is frequently referred to as the "star among therapeutic species." It is now a very popular and widely utilized therapeutic herb in both folk and traditional medicine. Years of conventional and scientific use and research have confirmed its multitherapeutic, aesthetic, and nutritional properties **Figure 1**. Numerous chemical and biological interactions could result in food deterioration. To prevent food from deteriorating due to bacteria and chemicals, in order to keep food quality at the required level and to maximize nutritional benefits, food preservation comprises a variety of food processing techniques. Food preservation techniques include growing, harvesting, processing, packaging, and distribution of food are all examples of food preservation techniques. The main goals of food preservation are to provide value-added goods, provide dietary variety, and combat improper agricultural planning. [9]. Plants are well-known for their historical use as medical herbs to treat disorders of the neurological and gastrointestinal systems. Since consumers worldwide wish to explore alternatives to antimicrobial treatments based on natural components, the use of synthetic antimicrobial agents for food safety has come under scrutiny recently [10].

Figure 1. *Origanum majorana* and its powder on the left *Matricaria chamomilla* and its powder on the wright.

2. Materials and Methods

2.1. Plant Materials:

The herb was obtained from the local market in Baghdad and was in the form of leaves and flowers. Air-dried plant samples were spread out on a paper sheet in a shadowy part of the room, kept from direct contact with the sun until their weight became constant, and then ground with a French-made electric mill and kept in a polyethylene bag at a laboratory temperature of 25±3 ◦C until use.

2.2. Plants Extractions:

- Cold aqueous extract: At cold extract, 25 grams of fresh leaves powders of each plant were weighed out and dipped into 300 ml cold distilled water for 24 hour with occasional shaking at room temperature then filtered and stored into a clean conical flask and marked Ex1.
- Hot aqueous Extract: Adding 50 grams of studied plants to 500 ml hot boiling water using shaking incubator, for six hours, after gradually cooling it filtered then stored for farther use and named Ex2.
- Organic maceration in methanol (metha), ethanol (etha) and water was used to extract some chemicals, especially phenolic compounds. In a 500 mL conical flask, 100 mL of each type of

solvents were added to 25 grams of each powdered plant, the flask's mouth was covered, and it was put in shaker incubator for six hours, then it has been cooled, filtered then stored for farther use and named Ex3.

The extracts were then filtered by Whatman No. 1 filter paper, and then a vacuum pressure pump (AP-9925 Auto Science) was used. Using the Rotary Vacuum Evaporator RE52 with a 50°C water reservoir, the solvent from the extract was evaporated. The residues were eventually gathered and stored at 4 °C for biological activity tests [11-13].

2.3. Treatments preparing

From the investigated plants, three remedies were created. To investigate the synergistic activity of both plants against microbes, the plant extract was prepared separately, as well as an extract from a combination of the two plant extracts see **Table 1**.

Table 1. Treatments of plants extracts and ciprofloxacin (control)

No.	Treat.	Plant Extract
1	Ex1.	(*O. majorana*) 25 gm. +(*M. chamomilla*) 25 gm. + cold water 300 ml
2	Ex2.	(*O. majorana*) 25 gm.+*(M. chamomilla*)25 gm.+ boiling water 300 ml
3	Ex3.	*(O. majorana)* 25gm.+ *(M. chamomilla)*25gm. + metha 100 ml + etha 100 ml+ water 100 ml
4	Con.	Aqueous solution of the antibiotic ciprofloxacin

2.4. Micro-organisms (harmful bacteria and fungi) isolates:

Some bacterial species that cause food spoilage were determined using visual and biochemical testing on samples of ruined foods, selected canned seafood including tuna and sardines, and certain pathogenic microorganisms including some fungi and bacteria were isolated from symptomatic parts of infected food, then transferring some parts of food to peptone Water (PW) for 6 hours then specific volume transfer to plates of Nutrient agar (NA) and incubated under 37ºC for 24 hr then culture stored, observed colonies of bacteria shifted to solid culture media to acquire clear and pure isolates of bacteria, these bacterial strains were purified by subculture on Mueller-Hinton agar and incubated for 24h in at 37°C which were used to test the interactions of successfully isolated bacteria towards treatments. [14, 15].

2.5. Phytochemical analysis and the active Groups:

In phytochemical analysis, the Fourier transform infrared spectrophotometer (FTIR) is one of the most effective instruments for determining the kinds of chemical bonds (functional groups) that are present in compounds. With a scan range of 400 to 4000 cm-1 and a resolution of 4 cm-1, various extracts were employed for the FTIR analysis using the Shimadzu FTIR Spectroscope (Japan) [16].

2.6. Bioactivity for extracts:

2.6.1. Zone Inhibition Measurement

Nearly 100 ml of the supplement agar was spread with a specialized culture of hazardous microscopic organisms. Next, the disc diffusion method was employed to validate the antibacterial effects. Leaf extracts and the antibacterial medication ciprofloxacin were applied to particular areas. On each plate, the inhibition zone was finally evaluated after incubation for 24 hrs. [17, 18].

2.6.2. The MIC Evaluate

The MIC of each concentration against several pathogenic bacteria has been determined through research. In response, stocks were also created for it, and the tested concentrations for Ex1, Ex2, and Ex3 were 25, 50, 100, 200, 400, and 800 µg/mL. Researchers have also conducted research to

determine the minimum inhibitory concentration (MIC) of the anti-infection, aiming to evaluate the efficacy of the plant extract as an alternative to chemical antibiotics. [18, 19].

3. Results and Discussion

Three pathogenic microorganisms were noticed, and three species of genera of bacteria were chosen to be tested due to their connection with human poisoning and food spoilage. The bacteria were diagnosed according to morphology and biochemical tests (**Table 2**).

Table 2. Pathogenic tested bacteria that isolated from some food products

No.	Pathogenic bacteria	Gram stain	Food product
1	*Pseudomonas* spp.	Negative	Milk products
2	*Streptococcus* spp.	Positive	Meat products
3	*Bacillus subtilis*	Positive	Vegetable products

3.1. Phytochemical analysis and the active Groups

Tanning, alkaloids, flavonoids, glycosides, carbohydrates, sterols, and phenolic substances were found during phytochemical screening for plant extracts (treatments). This result is in line with research by [20, 21]. Using the FT-IR analytical technique indicated in **Table 3 and Figure 2**, the absorbance peaks of the treatment extracts revealed the presence of many functional groups with varying absorbance. Because of the existence of hydrogen bonds in flavonoids, the broad peak at wave number 3350 cm^{-1} in FT-IR **Figure 2**. reveals the presence of a phenolic hydroxyl group (OH), which reflects the stretching vibration. The stretching vibration of the aromatic (C-H) bond is shown by the two medium bands at 2990 cm^{-1} and 2800 cm^{-1}. A faint band that appears at 1688 cm^{-1} also originates from the stretching vibration of the ketone (C=O) group. The stretching vibration of the (C=N) group is responsible for the sharp band's appearance at 1600 cm^{-1}, whereas the stretching vibration of the (N-H) group is responsible for that at 1473 cm^{-1}. Finally, the cm^{-1} and 1320 cm^{-1} waves are dedicated to C-H bending alkane and O-H bending carboxylic acids. (**Table 3 and Figure 2**).

Table 3. Common active groups at treatments (Ex1, Ex2 and Ex3).

n.	Wave number (cm^{-1})	The dynamic groups
1	3350	O-H, N-H Over lapping amide, carboxylic acid
2	2990	C-H (-CH3) stretching
3	2800	C-H (-CH2) stretching
4	1688	C=O carboxylic acid
5	1600	C=O amide stretching
6	1473	N-H bending,
7	1400	C-O (COO-), O-H bending,
8	1320	C-H bending alkanes,
9	1100	O-H bending carboxylic acids

During the metabolic profiling of aerial sections using Fourier-transform infrared spectroscopy (FTIR), we found the existence of primary amides, secondary amides, polymer alcohols such as polyvinyl alcohol (PVA), and phenols, indicating the presence of several metabolites and several functional groupings attributed to certain peaks. These results agree with studies by [21-23].

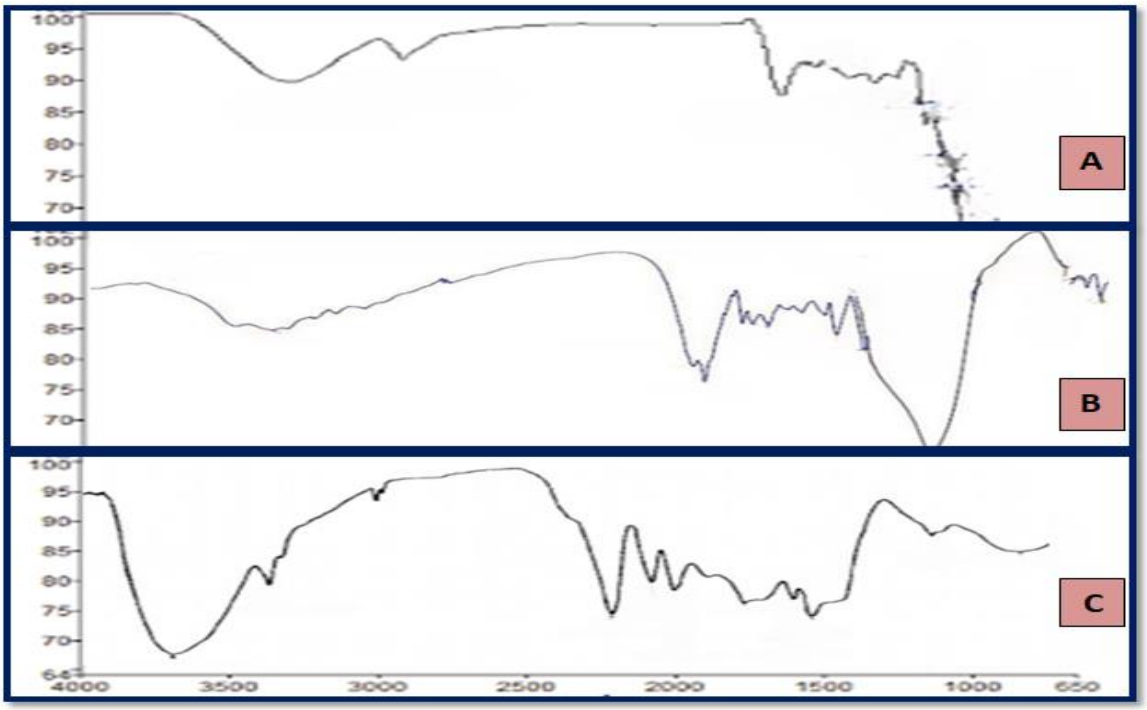

Figure 2. FTIR for treatments (A:Ex1, B:Ex2 and C: Ex3).

3.2. Biological activity

The study's findings demonstrated that the response to specific extracts could vary based on the type of bacteria and how they interacted with the extract's active ingredients in one of two ways.

3.2.1. Inhibition zone test

The antibacterial activity of Ex1, Ex2, and Ex3 treatments showed a clear effect that was seen in all treatments of plant extracts. In addition to the control treatment, Ex1 worked on *Pseudomonas* spp., *Streptococcus* spp., and *Bacillus subtilis*, with inhibition zones of 8, 10, and 11 mm, respectively, while Ex2 inhibition results were 9.8, 11, and 10 mm, and Ex3 recorded 15.5, 13, and 14 mm. Compared with ciprofloxacin as the control treatment, it was clear that there are various data points pointing that the best treatment is Ex3 with clear inhibition zones, as shown in **Table 4**.

Table 4. Inhibition zone (mm) for bacteria growth treated with treatments

Tests	*Pseudomonas* spp.	*Streptococcus* spp	*Bacillus subtilis*
Ex1	8 mm	10 mm	11 mm
Ex2	.9.8	11 mm	10 mm
Ex3	15.5 mm	13 mm	14 mm
Con.	13 mm	10. 5mm	12 mm

3.2.2. Regulating the (MIC) for treatments

The (MIC) findings for treatments and the control treatment against the investigated microorganisms were 400 µg \ml at Ex1. However, when compared to the control and other treatments, Ex3 was clearly the best treatment for all tested bacteria, including *Pseudomonas* spp., *Streptococcus* spp., *and Bacillus subtilis*, at 100, 100, and 200 µg \ml (**Table 5**).

Table 5. MIC µg \ml of plants extracts (treatments) against tested bacteria

Tests	*Pseudomonas* spp.	*Streptococcus* spp	*Bacillus subtilis*
Ex1	400	400	400
Ex2	400	400	200
Ex3	100	100	200
Con.	200	200	200

The (MIC) findings for treatments and the control treatment against the investigated microorganisms were 400 µg \ml at Ex1. However, when compared to the control and other treatments, Ex3 was clearly the best treatment for all tested bacteria, including Pseudomonas spp., Streptococcus spp., and Bacillus subtilis, at 100, 100, and 200 µg \ml, as shown in **Table 5.**
Some studies were supportive of these fallouts and argued that the antibacterial activity of the phenolic compounds could be affected by the extraction technique and the solvent's configuration [24, 25]. However, the disc's charge also affected the antibacterial activity, and the drying and grinding conditions of the plant, along with the oxidation and breakdown of thermolabile molecules, influenced the effectiveness of the active components [26-28]. Glycosides, tannins, flavonoids, and phenolic compounds must be extracted in a way and with a solvent that preserves their biological characteristics in order to maximize the value of these active substances [29, 30]. According to this study, the best methods for extracting phenols from plants were aqueous and organic, and the extract's antimicrobial activity demonstrated a moderate inhibitory effect against some bacteria that cause food spoilage. This paper also highlighted the abundance of phenolic compounds in both plants, underscoring the significance of their uses in traditional medicine as antimicrobial agents for food safety.

4. Conclusion

The FTIR test showed that the Origanum majorana and Matricaria chamomilla extracts have bioactive chemical groups and secondary metabolites that give them their healing properties. In some ways, it could be a viable strategy for sustainability to use the naturally occurring alternative antimicrobial agents from these plants' bioactive components as a source of antibiotic characteristics, which pharmaceutical firms can then use for drug formulation.

Acknowledgment

Many thanks to Market Research and Consumer Protection Center, University of Baghdad for facilitating the work of the practice parts in this article.

Conflict of Interest

There is no conflict of interest

Funding

There is no funding for the article

Ethical Clearance

This research was subject to ethical considerations, and the research was approved by the Scientific Committee at the Market Research and Consumer Protection Center /University of Baghdad.

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