Original Article

Effect Of Aromatic Oil Of Three Plants Of *Labiatae* Gene On Microorganism By Using In Preservation Of Minced Meat

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Abstract

The effect of oil extracts of three plants of *Labiatae* gene, Mentha *longifolia, M. spicata and Ocimum basilicum,* by the agar well diffusion method, were studied, the total count of the bacteria responsible for spoilage meat was kept at 4-6˚C for different periods (1, 72,192) hours. The results showed that the oils extract were inhibited all the treatment that were studied and more affected inhibition of the growth of *Staphylococcus aureus* than the growth of other microbes. The total count of the bacteria responsible for meat spoilage decrease in all treatments extracted oils for all different periods. Statistic analysis results showed high significant variations at the level P≤ 0.01 of all the treatment and control for all reservation periods.

INTRODUCTION

Natural medicinal materials have been considered as a divine blessing and have been used throughout history. Man has recognized that some wild plants have curing potential for diseases (1).
Medicinal plants contain many active compounds such as the alkaloids, tannins, resins, glucosides, saponins, coumarines and volatile oils (2).

The volatile oils of such plants contain compounds that can be described to have antioxidants and microorganisms inhibiting functions. Many of the plants volatile oils have been used to prolong the shelf life of foods or as flavouring components in many food products. Garlic, for example, has been used to supress the *Bacillus cereus* (3). furthermore, tilia, sage and black tea have been used as microbial suppressants in various foods and studied the effect of cinnamon oil on suppressing pathogenic microbes that also damage minced meat and they reported that the oils improved the shelf life of the meat(4).

In another similarly study (2) has reported that the aqueous and oil extracts of *Rosmarinus officinalis* can suppress a number of bacteria and yeasts in foods. Furthermore, (5) the suppressive function of the alfalfa extract studied on meat contamination microbes.

A number of studies have focused on improving the mint plant to increase its oil yield for medicinal uses as well as in the food industry (6). The mint oil contains antioxidants that are effective in treating many diseases (7). In another research (8) reported that the oil extracted of mint leaves suppresses the *Salmonella* and the *Staphylococcus* bacteria. The oil yields and action against the microbes are season related where the summer harvest is superior to the winter harvest (9).

Another study (10) found that horse mint oil has moderate anti oxidation function. On the other hand, (11) has identified 21 active compounds in the horse mint oil that can suppress the *E. coli* bacteria.

In another study found that sun drying or hot air drying produce 48% a menthone compounds whilst drying in an electric oven produces 41% Limonene compounds (12).
It has been reported that the extract of basil leaves has an antioxidant function as well as anti-internal-parasites function(13,14), and (15) has defined the minimum inhibition concentration (MIC) for some microbes that ruin food products; whilst (16) showed that the alcohol extracted of horse mint oil is superior to the aqueous extraction in suppressing microorganisms. It has also been reported (13, 14) that the basil aqueous extract has antioxidant potential.

The *Mentha pipertia* plant extracts suppress *E.coli* bacteria better than the *M. longifolia* and *M. aquatica;* it has also been found that the oil suppresses the hydroxide ion (17).

The present work aimed at studying the effect(s) of the extracts of three aroma plants of the *Labiatae* family in suppressing the growth of microbes that, infect, the minced meat, and the possibility of using these extracts as substitutes for the chemical preservatives that are commonly used to preserve foods.

MATERIALS AND METHODS

1. Extraction of plant oils

The leaves of the three plants were dried in a thermal oven and ground before extracting the volatile oils through the aqueous distillation method described previously (18) using Clevenger machine. Each sample (50g) was extracted for 2.5 hr.

The upper oil layer was removed into special containers with tight fit lids and kept until use.

2. Physical properties of the oils:

- The specific gravity of the volatile oils was determined by weighing 1 ml of the oil on a sensitive balance at 25°C in triplicates.
- The refractive index value of the volatile oils was determined by the Refract meter at 25°C
- A polarimeter was used to determine the light rotation index at 25°C according to the method described previously (19).

3. The active chemical components in the plants leaves were determined according to by a qualitative chemical method described elsewhere (20).

4. The efficacy of the volatile oil extracts in suppressing the microbial activities were evaluated by the Ager Well Diffusion Method that included observing the effect of 0.1 ml of oil on 4 isolates of bacteria that can contaminate food products (*Pseudomonas spp*., *Bacillus subtilus, Staphylococcus aureus* and *E. coli*) and 2 types of yeast (*Torulopsis* and *Saccharomyces*).

Plant count agar laced with 0.2 ml of the microbe suspension (1×10^{5} cells/ml) was placed in plastic dish. Equally spaced wells were made on the agar, and 0.1 ml of the oil extract was placed in each well except one that was considered as a control. Triplicates were prepared for each microbe species, and the dishes were incubated at 37°C for 48 hr. The suppression potential of each oil was determined by measuring the radius of the suppression zone in each plate and calculate the average of the triplicate sample values.

PREPARATION OF THE MEAT SAMPLES

Minced meat (360 g) of local steer meat purchased from local butcher shops that were selected randomly. The sample was divided into 36 subsamples (10 g each); three treatments for oil extract and one sub sample was used as a control with three replicate of each treatment. Total bacteria count was done after 1, 72 and 192 hours of incubation at 4-6°C by the dish count method and using 1×10^{5} dilution.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was performed by using spss 12 computer programme.

RESULTS AND DISCUSSION

The extraction results showed that mint produced more oil than the other two plants (Table 1). The yield was 1.6%, 0.6% and 0.57% for the mint, basil and horse mint respectively. The specific gravity of the oils was 0.903, 0.924 and 0.921 for the mint, basil and horse mint oil respectively. The results are in agreement with previously reported values that showed that the specific gravity of oils from almost all aroma plants range between 0.824 and 1.172 (7, 14).

The results of the chemical characterization showed that the leaves of the three plants contained glycosides, alkaloids, tannins, resins, saponines, flavones and phenols (Table 2). The results are in agreement with those reported in the literature (21, 22, 23).

The extracts of the three plants clearly suppressed the growth of some bacteria and yeasts that contaminate foods especially on *Staphylococcus aureus* where the suppression diameter was 19, 18 and 16 mm for the oil of basil, horse mint and mint respectively. Furthermore, the effect of the 3 oils varied with the type of microbes that were used in this study. For example, the suppression diameter of the basil oil on *E. coli* was 7 mm, whilst that of the horse mint was 14mm on *Pseudomonas spp*. Previous studies (15, 17, 21) have reported similar results, and showed that these plants contain antibacterial, especially the *Staphylococcus aureus*, compounds such as the Carvone (C10H14O) in mint oil, the

Piperitenone oxide (C10H14O2) in horse mint oil, and Linalool (C10H18O) and Methyl eugenol (C10H12O2) in basil oil.

There was a clear reduction in the average total count of bacteria after one hour of treatment with the three plants oils compared with the control. The difference was especially obvious between the control (6.33×10^5) and that of the horse mint oil (4.33×10^4). This difference gap increased to become 6.33×10^8 for the control vs. 7.66×10^5 for the horse mint oil after 72 hr; and 3.33×10^{10} for control treatment and 2.66×10^7 for horse mint oil respectively after 192 hours (Table 4).

The analysis of data showed that all the observed differences between the oil treated samples and the controls were statistically significant ($p \leq 0.01$). This was true for all the incubation periods. We have observed that the horse mint oil was superior in its bacterial suppression effect when compared to the basil and mint oils. This observation is in agreement with previous findings (9, 17).

Table 1: The physical properties of oils extracted from the leaves of the three plants

Oil type	Specific gravity	Light rotation	Refractive Index	Oil yield%
Basil oil	0.924	-6.3	1.55	0.6
Mint oil	0.903	-26	1.46	1.6
Horse mint oil	0.925	-7.4	1.50	0.56

Table 2: Qualitative chemical characteristics for the active compounds in the volatile oils in the aromatic plants

Active compounds	Chemical tests	Basil oil	Mint oil	Horse mint oil
Glycosides	Fehling reagent (solution A+ solution) Benedict reagent (CuSo4 + Na2Co3 + Na citrate)	++	++	++
♀Alkaloids	Mayer s reagent (HgCl2 + KI) Wagner reagent (Iodine + KI) Dragendorff reagent (BsNo3 + KI)in AcOH Picric acid	++	++	++
Coumarins	Sodium hydroxide, filtration + U.V.	++	++	++
Phenols	1 %Ferric chloride	++	++	++
]Flavones	Ethanol + Potassium hydroxide	++	++	++
Tannins	1%Lead acetate 1%Ferric chloride	++	++	++
Resins	Ethanol + Hydrochloric acid	++	++	++
Saponins	HgCl2	++	++	++

Table 3: Average suppression of growth diameter (mm) of some bacterial and yeast species by using 10 µl/ml concentrations of essential oils of the three plants

Microorganism	Basil oil	Mint oil	Horse mint oil
Staphylococcus aureus	19	16	18
Escherichia coli	7	8	12
Pseudomonas spp.	11	12	14
Bacillus subtilus	8	8	10
Saccharomyces cerevisiae	12	10	8
Torulopsis spherical	12	8	8

Table 4: The effect of the essential oils of three aromatic plants of the Labiatae genus on the total bacterial count during different incubation periods.

Principle oils	1 hour	72 hours	192 hours
Control	6.33×10^5	6.33×10^8	3.33×10^{10}
50µl/gm basil oil	4.66×10^4	8.33×10^5	3.0×10^7
50µl/gm mint oil	4.33×10^4	8.66×10^5	4.0×10^7
50µl/gm horse mint oil	4.33×10^4	7.66×10^5	2.66×10^7
LSD	4.5×10^5	4.48×10^8	6.3×10^8

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