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Preparation of a Medicinal Formula as Emulsion Spray from Oil Extracts of Thyme, Spearmint and Clove to Treat Oral Ulcers in Animals and FMD in Cattle

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Abstract

Oral ulcers are common occurrences in animals for many reasons, including accidental injuries that occur during the consumption of feed or food, and may be bacterial and fungal or viral causes such as foot-and-mouth disease, which is usually accompanied by ulcers in the mouth, udder and foot (FMD), which leads to the animal's abstinence from food and then death so to find ways that solve this problem a pharmaceutical formula had been prepared from extract oils mint, thyme and cloves called it (mint vet). The oils were extracted by water evaporation then examined by GC-MS and attended many formulations until reaching the until reaching the perfect consistency and stability where the pharmaceutical form was white emulsion kept inside a spray bottle given in the form of a mouthwash. The biological test stability was conducted on the product for six months at two temperatures one of them cold degree 4℃ and the other is room temperature 25℃ to determine the expiration date and the best storage conditions, it has been compare with stander Gentamicin and Nystatin the inhibition zone of formula was higher than stander inhibition zone, high temperature was avoided because the product contains essential oil, the expiry date was calculated two years and store at less 15℃. The formula has been tasted in veterinary hospitals in Al-Rashedia and Baghdad, the report had proved the efficiency of the mouth wash and there are significant responses, when it's used to treat Foot and Mouth Disease FMD without any side effect.

1. Introduction

A mouth ulcer is the erosion of part of the delicate tissue that lines the inside of the mouth (mucous membrane), the most common cause is injury (such as accidentally biting the inside of cheek), and other causes include bacterial, fungal and viral infections. The mucous membranes of the lips, oral cavity, gingiva, tongue and pharynx, are repeatedly subject to varying degrees of trauma. This is due to their location at the entrance to the gastrointestinal tract and their role in mastication and transport of foodstuffs and other objects of varying consistencies to the lower parts of the alimentary tract, in herbivores, the coarse nature of pasturage and roughage and foreign objects that may be inadvertently ingested with them can cause laceration and abrasions of the mucosa. The possibility of traumatic injuries to the mouth in young dogs is more likely due to retaining in baby teeth.

Dental diseases can be observed in adult dogs and can be developed in early age through certain breeds such as poodles and yorkshire terriers. The most serious complications such as tooth loss, trauma, damage teeth due to hard chewing, infections all these causes severe pain so the animals stop eating [1]. Bovine are infected by one of the most important disease, Foot-and-mouth disease (FMD), is a contagious viral infection which is s a highly infectious viral disease of cattle, pigs, sheep, goats caused by FMD virus which has 7 major serotypes: A, O, C, Asia1 and SAT caused by an Aphthovirus of the family Picornaviridae. The disease is characterized by fever, salivation and vesicles in the mouth, muzzle, dental pad, tongue, teats and feet. The rupture of the vesicles results in marked painful swelling of the coronary band, depression, salivation, lameness, severe mastitis and abortions [2]. Nowadays the control and treatment of FMDV have become a worldwide economic problem and a challenge for the society Essential oil is consisting of several chemical compounds offering different biological properties and activities. It could reduce foodborne pathogens and diminished the use of synthetic and semisynthetic antimicrobial compounds. Essential oil extracted from medicinal plants that are obtained by natural concentrated by steam distillation or through solvent extraction [3]. The main components of Spearmint essential oil are 55.0 % Carvone, while Thyme oil contains 40% Thymol and Carvacrol, and Clove oil consist of 75.0 - 88.0% of Eugenol [4]. Spearmint is a common aromatic and medicinal herb that is used in traditional medicines in the world for its antimicrobial and antioxidant properties [5]. Spearmint is a medicinal plant that has received greater attention in both the food and pharmaceutical industries due to its health benefits to human society. The addition of 25µg/mL spearmint essential oil enhanced endogenous antioxidant defense, the protective effect is comparable to that of supplementation of 10 µg/mL of ascorbic acid [6].The results of one of the research carried out on meat broilers necrotizing enter colitis type showed the treatment with spearmint oil at a concentration of 0.5 ml /L - 0.25 ml /L of drinking water is a clear reduction in the number of mortality rate and in the lesions of bacteria colonies isolated from the intestinal tissue in addition to an increase in the growth performance [7]. Thyme oil contains thymol, a phenolic compound that efficiently kills a variety of bacteria and it has strong bactericidal effects on the A.Pleuropneumoniae that thymol could rapidly disrupt the cell walls and cell membranes of A.Pleuropneumoniae causing leakage of cell contents and cell death.In the same way the treatment of infected mice with pleural lung disease germs by giving them thymol (20 mg / kg) has protected them from the lethal dose of these microbes [8]. Furthermore, thyme oil has antibacterial activity with inhibition zones ranging from 16 to 22 mm [9]. Also it has been tested against clinical bacterial strains of Staphylococcus aureus and Enterococcus aureus that isolates from patients with infections.

of the oral cavity, respiratory tract, genitourinary system, and from the hospital enviroment, indeed the results of the experiments were that thyme oil possessed extremely strong activity against all clinical strains [10]. It has been observed that the addition of thyme oil at a concentration of 600 mg per kg in feed for quails raised in high density fields improves feed consumption, increases the food conversion factor and reduces the adverse effects of stress associated with intensive breeding [11]. Essential oils mixed with water are called hydrosol-. That mix up more type of the herb, have inhibitor effects against pathogenic bacteria, such as mixing oils of thyme, mint, sage (at ratio 1: 1) had partially higher antibacterial activity against Bacillus subtilis in same time this combination also shows significant activity against Salmonella enteritidis contrast with single plant hydrosols, therefore, these hydrosols are considered natural food or animal feed [12]. Clove oil has antibacterial activity with inhibition zones ranging from 16 to 20 mm [9]. Along with has effective against fungal pathogens of opportunistic infections such as Candida albicans and Cryptococcus neoformans and Aspergillus fumigatus, etc. the oil was found to be extremely successful in the treatment of experimental murine vaginitis in model animals, on evaluating various formulations, topical administration of the clove oil was found to be most effective against treatment of vaginal candidiasis [13]. One of the studies confirmed the effectiveness of clove oil as a natural antimicrobial and suggests the capability of consumed it as a promising alternative product for control of infectious diseases caused by Streptococcus suis in animal and human patients. [14]. Moreover, one study showed that injecting clove oil into a calf horn bud would suppress horn growth [15]. The addition off essential oil at different stages during the viral infection cycle and at the maximum non-cytotoxic concentration, decreased plaque formation by more than 90% when thyme oil was incubated against herpes simplex virus. The results showed that the essential oil affected the herpes simplex virus before adsorption mainly through interaction with the viral envelope [16]. The aim of the research is to develop a mouthwash for the treatment of oral ulcers and ulcers of FMDV, which has great therapeutic and economic importance, and the formula was given the name Mint Vet.

2. Experimental Procedure

1. The perfect way that used to extract the essential oils from Spearmint, Thyme and Clove oil are steam distillation using Clevenger -type apparatus. The oils were kept at 4℃ and protect from light.
2. Weight 150g from dried herb then add 1.5 L of distilled water, after three hours of steam distillation the yield was approximately 3ml of thyme oil and 4ml of clove oil but in case of Spearmint oil the amount was too little so the oil was brought from India. Then evaluation extracted oils by gas chromatography.
3. Determine the required Hydrophilic–Lipophilic Balance (HLP) values for spearmint, thyme and clove oil.
4. 1-Prepare 100 ml of the formulation constituents to spray mint mouth wash and weigh accurately all ingredient presented in Table (1).

Table (1). List of ingredients

Item No.	Constituent
1	Spearmint oil
2	Thyme oil
3	Clove oil
4	D.W.

5. In suitable pyrex beaker transfer emulsifying agents and distilled water.
6. In another pyrex beaker transfer all essential oils.
7. Mixed and homogenize (4) + (5) for 30 min.
8. Send a sample to the biological control section.
9. Fill in 50 ml of suitable spray container with nitrogen gas and kept in refrigerator.

Results: White emulsion packaged in plastic spray bottle 50 ml. pH: 8.3.
Working conditions: All equipment are sterilized and temperature of emulsion should be in two phase.

3. Results and Discussion

In this study formula of emulsion was prepared according to specification of united state pharmacopeia 2005 [4]. A white homogenous emulsion of acceptable consistency was produced and physically formula showed excellent stability at temperature 4&25℃. The (pH) measurements were done by using a digital type of pH meter by dipping the electrode glass into the emulsion. The pH is 8.3, at other hand pH of saliva is range in ruminant species between 8.3 to 8.5 [17], while in canine is 8.5 [18], so the formula will not hurt the mouth cavity. The biological test was conducted on the product for six months to determine the stability of formula and the best storage conditions. This test was applied on S. epidermis, S. mutans and C. albicans which are brought from Biotechnology Department at two degrees' temperature 4C˚&25C◦.Biological test was carried out for six months to determine its stability and the best storage conditions. At tow temperature 4 - 25˚C, at a temperature of 4C the diameter of the inhibition zone ranged from 15 to 25 mm for epidermis S. comparison with stander, gentamicin, is 17 mm, while in S. mutans, the inhibition zone ranges from 16 to 32. 32 mm compared to the same Stander (Table 2), Figures (1 & 2).

Table (2). Diameter of Inhibition zone at 4℃.

Type of bacteria	Inhibition zone diameter (mm)			
	Control Gentamicin	Zero time	After two months	After 6 months
S. epidermedis	17	25	17	15
S. mutans	17	32	17	16

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Figure (1). Inhibition zone of *S. epidermedis* at 4℃.

Figure (2). Inhibition zone of *S. mutan* at 4℃ symbolize by litter B while C is control symbolize by letter B while C is control.

At 25 degrees Celsius the inhibition zone is from 15 - 27 mm epidermis S. while in S. mutans the diameter of the inhibition zone is 17-19 mm (Table 3, Figure 3, 4) compare with the inhibition zone of stander (gentamicin) is 17 mm.

Table (3). Diameter of Inhibition zone at 25°C.

Type of bacteria	**Inhibition zone diameter (mm)**		
	Gentamicin	**After two months**	**After 6 months**
S. epidermidis	17	27	15
S. mutans	17	19	17

Figure (3). Diameter of Inhibition zone at 25℃for *S. epidermedis.*

Figure (4). Diameter of Inhibition zone at 25℃ *S. mutans* Symbolize by no.1.

In C. *albicans* at 4℃ the diameter of inhibition zone was 16 to 22 mm there nearly to the control Nystatin which are 16mm, while at 25C˚the diameter inhibition zone was 15-22mm at the same limited control (Table 4, Figure 5, 6). In other hand, the physical properties including color, consistency and pH was stable at these two temperature 4℃ and 25℃.

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Table (4). Inhibition zone diameter (mm) for C. *albicans*.

Inhibition zone diameter (mm) for *C. albicans*				
Temperature degree(C°)	**Control (Nystatin)**	**Zero time**	**After two months**	**After 6 months**
4	16	22	16	16
25	16	22	19	15

Figure (5). Inhibition zone for *C. albicans* at 4℃.

Figure (6). Inhibition zone for *C. albicans* at 25℃ by litter B, while C is control.

Through these three Tables the diameter of inhibition zone at the limit range of the stander (Gentamicin and Nystatin) [19, 20].

GC-MS analysis

When passing the extracted clove oil in GC-MS the results showed that it contained an amount of eugenol which is the main active ingredient attributed to its effectiveness against bacteria, viruses and fungi, where at the time of 19.02 the area of the substance was 79.74% of the total of the rest substances (Figure 7). While the thyme oil passed thymol appeared at the time of the appearance of the curve 13.862 and the percentage of the area of thymol was 80.44%(Figure 8), whereas in spearmint oil the percentage of carvone is 57. 67% and the time of appearance of the curves in 83.13, 13.48, 13.52 and also appearance the limonene in7.43 with percentage 21.33 % (Figure 9) all these ingredients within normal limits [21]. The result of the stability study showed that the mouthwash emulsion has good stability at two temperatures (4, 25) degrees Celsius, however, to maintain the vitality of the oils, the formula should be stored at a temperature below 15 degrees Celsius. Essential oils of thyme, mint and clove have shown good antibacterial and antifungal efficacy with inhibition ranges at 15-25.

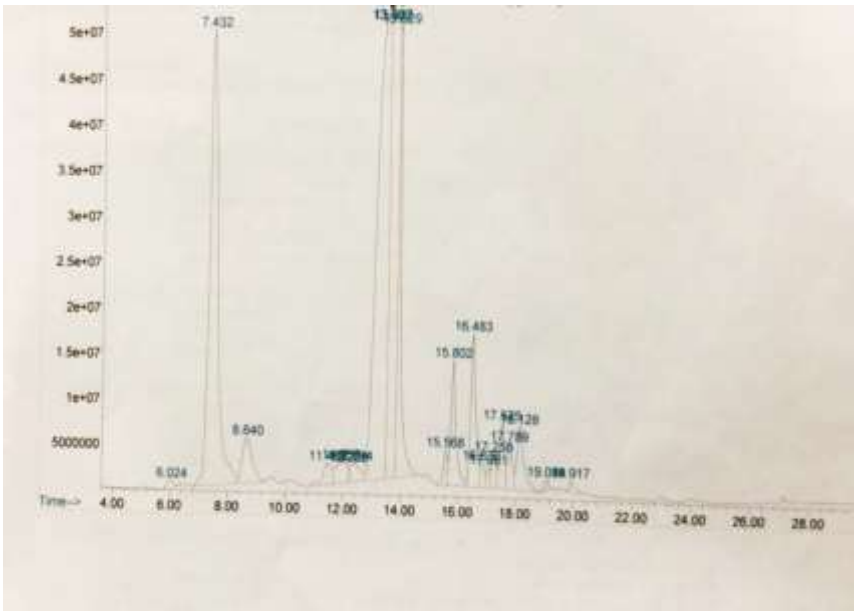

Figure (7). GC-MS spearmint oil.

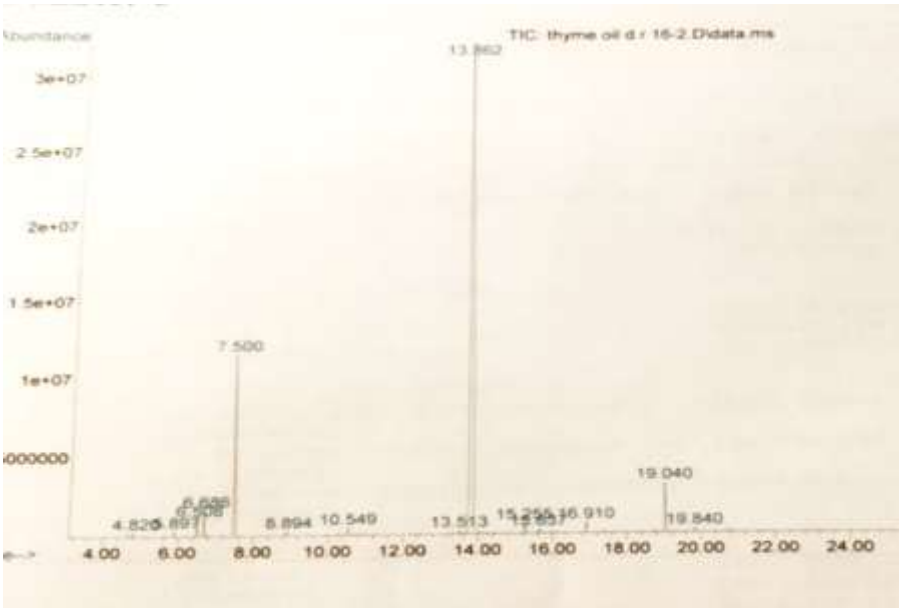

Figure (8). GC-MS Thyme oil.

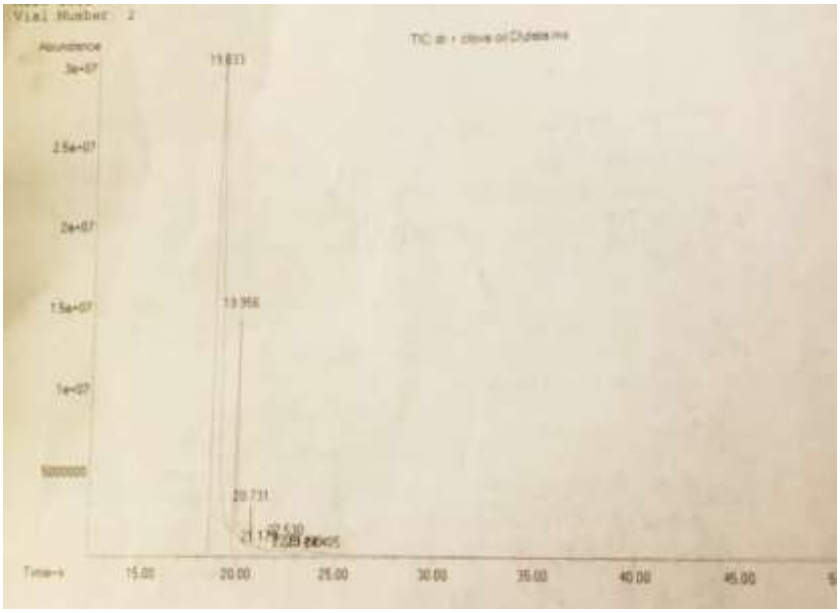

Figure (9). GC-MS of Clove oil.

mm, 16-20 mm and 15-16 mm respectively. These findings are consistent with those of the researchers [22]. Several studies have shown that the activity of oils can be due to the properties of essential oils and their components due to their water permeability, which enables them to split lipids in the bacterial cell membrane, disturbing cell structures and making them more permeable., The extensive leakage of bacterial cells or the exit of critical molecules and ions will lead to death cells [23]. According to the biological study the expiration date was estimated at least one year from the date of manufacture. The formula was tested (Figure10) in veterinary hospitals in Baghdad and Rashidiya on sheep and cows suffering from signs of foot-and-mouth disease and they have sores in the mouth, nipple and foot, when using the treatment, a clear response is observed within two days and the animal's appetite returned to normal (Figure11).

Figure (10). Udder treatment with Mint Vet spray.

Figure (11). Two pictures demonstrate a cow had ulcer in nozzle the lesion disappears after several days from used mint spray by veterinarian.

4. Conclusions

These results indicate that the essential oils from aromatic plants of mint, thyme and cloves have the potential to be used as effective antiviral agents and eco-friendly medicine, which have great therapeutic and economic importance.

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