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## Formulation and evaluation of Yemeni ginger and thymus as lozenges

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### Abstract

Medicinal plant is a study the effective of plant on some pathogenic microorganisms and, holds a great promise as source of medicines against some diseases to all people in some developing countries, including Africa. Directly, almost 80% of people around the world depends on the traditional medicine for their primarily health care. The result of current study revealed that the highest antimicrobial activity was in ginger against *S. aureus* and *B. subtilis*. This result was disagree with pervious study which reported that the ginger lozenge tablet showed antifungal effective recording inhibition zone diameters of 17, 19, 20, 23, and 25 mm at 5, 10, 20, 30, and 60 min release times, respectively, against the laboratory isolate of *Candida albicans*. The result of this study revealed that (Menthol and thymus) were found on this tablet by using GC.

**Keywords:** Herbal, formulation, evaluation, antibacterial, Yemen, ginger, thymus

### Introduction

Herbal medicines or natural medicines product must be taken as medicines supplements, and might be considered as alternative treating medicine to prevent diseases. Consumers have preferred using the natural therapies. Herbal medicinal plants should be used traditionally as a treatment for different diseases, in humans and animals. Many of essential oils and herbs of plant extracts have studied the effective of antimicrobial and growth of microorganisms [1]. Actually, the people believed that the product of herbal medicinal plants are safe (no side effects) and so much effective more than medicines.

The structure of medicinal plants such as, alkaloids, phenolics, terpenes, saponins, lipids, and carbohydrates are important to prevent various diseases like cancer, tumors high-cholesterol, aging activities [2]. The genes of *Zingiber officinale*, is belong to the *Zingiberaceae* family. The history of medicinal have used 2000 years as one of the most important medicinal plants. This medicinal plants were a wide of antimicrobial effective and a common in different foods and beverages.

Zingiberaceae is a rhizome which is widespread by the most consumed in Southeast Asian countries. This plants were used in cooking long time ago and also as a spice and traditional treatment like vomiting, nausea, pain, arthritis, indigestion, gastro reflux [3].

Moreover, many several uses of ginger tablet was useful in treating, managing, or preventing many diseases like cardiovascular disease, diabetes [4]. Obesity [6], respiratory disease [5], nausea and vomiting induced by chemotherapy [7] and in neurodegenerative diseases; it also in-holds some of the biological properties like antimicrobial [8], anti-inflammation [9], antioxidant [10], anticancer [11], and wound healing properties [12].

In previous researches, there is different active contains of herbal medicinal plants showed different effective on poultry performance.

This present research have been aimed to formulating aqueous extract of ginger to tablet. In this recent research, the tablets of lozenges and ginger was prepared by wet granulation. Ministry of health lab scale tablet compression machine in Yemen did compression of lozenges tablets. This compressed tablets were evaluated for thickness, hardness, friability, antimicrobial activity, assay and identification.

## Materials and Methods

### Selection of Medicinal Plants

#### Ginger

Ginger rhizomes were grown up in Ameen House and it was starting idea for local source in Sana'a.

#### Thymus

All the samples in this study were collected from (Mounts Thamar governor) and transfer to (Egyptian Yemen company) and prepared to start working there.

The thymus amount is very low the hardness of tablets is very low when we increase the hardness, the capping is occur So we increase the amount of thymus to 100 mg we made treatment by vivapure.

#### Preparation of tablet

The fresh sample for *Zingiber officinale* rhizomes were prepared by peeling, washed with purified water, and air-dried to a constant weight and. Then this sample was dissolved in 1 L of ethanol for 48 hours at room temperature with occasional mechanical shaking. Finally, the sample filtrate and concentrated the extract subsequently air-dried.

#### Antimicrobial activity

Almost, some bacteria and one Fungi have been used in this research. In reality, three of gram-positive bacteria that includes of *Staphylococcus aerus*, *Bacillus subtilis*, and *streptococcus*. And two of gram-negative bacteria includes of *Escherichia coli* and *Pseudomonas aeruginosa*. These tested microorganisms obtained with ATCC number and have got from Yemen Egyptian Pharma Company. These microorganisms served for test pathogens of antimicrobial effective test. Using 10% dimethyl sulfoxide (DMSO) the tablets have been used in antimicrobial activity test. The solution of this tablet was prepared just before carrying out the test, which means freshly prepared.

Suspensions of bacteria contain 10<sup>7</sup> CFU/ml of bacteria and should be widespread on petri dishes with a sterile swab with bacterial suspension. Five wells in each plate by using a standard corn borer (7 mm) were cut out. In plate's wells, approximately 70 µl of each tablet solution was added and duplicate each concentration. As a negative control, DMSO was used. In addition, control Positive on wells of antibiotic were placed in the plate using antibacterial and antifungal. The incubated of plates were for 24 hr at 37°C. After incubation period time, the effective of tablets solution evaluated by measuring the inhibition zone. Two antibiotics standard was performed, antibacterial such as Gentamycin. The antifungal (Nystatin) (10 mcg) were used to be as reference to determine the sensitivity for the effective of bacteria and fungi.

#### Test microorganisms

In this present study two methods of microbial activity at two stage were used Antimicrobial activity at zero time for bulk product (ginger): This stage was used for antimicrobial activity of ginger, which used five tested microorganisms such as (*Bacillus*, *Saureus*, *E. coli*, *streptococcus* and *P. aeruginosa*).

#### Second stage (ginger and thymus)

##### Zero time of finish product

Ginger and thyme were test at zero time for the above two substance used five tested microorganisms like (*Bacillus*, *S.*

*aureus*, *E. coli*, *streptococcus* and *P. aeruginosa*). This stage used antimicrobial activity test to show the sensitivity of product on the all tested microorganisms.

#### After 6 months stage of finish product

After six months of stability study chamber, ginger and thyme were tested again used the same tested microorganisms which are (*Bacillus*, *Saureus*, *E. coli*, *streptococcus* and *P. aeruginosa*). The antimicrobial activity test was used to show the sensitivity of these microorganisms. All microorganisms showed in table 1.

**Table 1:** Showed the name of bacteria and ATCC number

No	Name of bacteria	ATCC number
1	<i>Staphylococcus aureus</i>	ATCC 6538
2	<i>Streptococcus</i> sp	ATCC 12228
3	<i>Bacillus subtilis</i>	ATCC 6633
4	<i>Escherichia coli</i> ,	ATCC 8379
5	<i>Pseudomonas aeruginosa</i>	ATCC 9027
6	<i>Candida albicans</i>	ATCC 10231

#### Assay by Gas Chromatography (GC)

In this research assay of menthol was done by GC at limit N.M.T 110%. This method was used for menthol test in the product. Taken amount of pure Menthol and keep it as standard Inject 9.8 mg of pure Menthol in GC instrument.



**Fig 1:** Gas chromatography of volatile materials.

#### Identification of thymus by thin layer chromatography

Mill one tablet by mortar and put the milled tablet in flask 50 ml Then add methylene chloride to dissolved weight 100 mg of thymus (Raw material) & put it in flask 50 ml. Add methylene chloride until 50 ml and put the flasks in ultrasonic water path half hour clean & dry bakers after that add 2 mg of sod. Sulphate anhydrous and add the previous materials to the bakers. Finally filtrate via filter paper 0.45 micron taken 5 micron from each sample then inject to TLC plate prepare container of TLC add 150 ml Di chloro methan leave it one hour and half to reach to saturation state put the plate in UV to see the result.

#### Results

This chapter describe the results of formulation, evaluation of Yemeni ginger and Thymus as lozenges. Also, describe many tests had made for ginger. All these tests will be described in the next chapter.

**Antimicrobial activity:** For antimicrobial activity the tablet of ginger and thymus have been tested against many

microorganisms like (*Bacillus*, *S. aureus*, *E. coli*, *streptococcus* and *P. aeruginosa*).

May be after 24 hr. incubation period, the effective of bacteria and fungi of the tablet solution of sample and the dilutions were placed on agar medium to evaluate the presence or absence of inhibition zone. By measuring the inhibition zone diameter around the wells. Commonly, the effective of bacteria in ginger tablet display to be more inhibitory against all tested microorganisms.

The present study made the antimicrobial effective and these results showed a more sensitivity with ginger at zero time of bulk product against *Bacillus* sp and *S. aureus*. These results

express that the effect of this extract depend on the cell wall of these bacteria which break down of bacteria easily and allow the extract to penetrate and inhibit growth of bacteria, but showed no activity against other tested microorganisms. The test of antimicrobial activity of ginger and thyme was repeat again of finish product at zero time and 6 months. This recent study revealed a high antimicrobial activity against all tested microorganisms at zero time and after 6 months of finish product. This activity for all bacteria means that the two substances (ginger and thyme) made complex and high activity against all tested microorganisms. All these results showed in the table 2 and figure 2.

**Table 2:** Showed the results of ginger against tested microorganisms

Bacteria	Concentration			Standard gentamycin	placebo
	150 mg	100 mg	50 mg		
<i>S. areas</i>	15 cm	14.5 cm	14 cm	17 cm	NG *
<i>Bacillus subtilis</i>	17 cm	16 cm	14 cm	18 cm	NG *
<i>Pseudomonas aeruginosa</i>	8 cm	7	5	10 cm	NG *
<i>E- Coli</i>	6 cm	5.5	4	11 cm	NG *
<i>Staphylococcus epidermidis</i>	9 cm	7	6.5	12 cm	NG *
<i>Candida albicans</i>	6 cm	5	4.5	13 *	NG *

**Note:** standard of *Candida albicans* was Nystatin NG: Negative



**Fig 2:** Showed antimicrobial activity of ginger and thymus.

**Menthol**

**Assay by gas chromatography GC (3900 FID)**

The result of recent study showed the value reality which is 107% of 110% so that the limit of this not more than 110% this result was calculate by

$$\text{Assay of menthol} = \frac{\text{High sample}}{\text{High standard}} \times 100$$

**Thyme**

**Identification GC (3900 FID)**

Rotation time of standard was the same rotation time of sample so that inject thyme purity was the same rotation of sample. So, thyme was inside the sample.

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**Discussion**

Knowledgeably, the antimicrobial characteristics of herbal recourse was up because of their possibility uses in several chronic disease and infectious. This recent study was highlighted in particular on the focused on Yemeni plants. This present study was used to testing this tablet solution of plant against tested microorganisms and evaluated the effective of tablet solution for effective of microorganisms using agar well diffusion assay.

The current research displayed that the highest antimicrobial effective was with ginger against *S. aureus* and *B. subtilis*. This research was disagree with pervious study who reported that the ginger lozenges demonstrated pronounced antifungal activity recording inhibition zone diameters of 17, 19, 20, 23, and 25 mm at 5, 10, 20, 30, and 60 min release times, respectively, against *Candida albicans* [13]. There was no previous studies were agree with this study, so this result is a great results have done on this tablet solution. Large tablets of lozenges are must be dissolved slowly in the saliva, thus releasing the active ingredient over a relatively prolonged period thereby producing a local effect on the mouth or the throat.

Therefore the expected of lozenges formulation containing garlic-ginger extract to qualify as a suitable base for the ginger tablet, it should be allowed and promote the release of the antimicrobial extract from the dosage form [13].

Test of antimicrobial activity for this extract was made again for finish product at zero time and after 6 months. So, this result showed the highest activity against all tested microorganisms which are (*Bacillus*, *Saureus*, *E. coli*, *streptococcus* and *P. aeruginosa*).

This recent study has done about of ginger and thymus by using Gas chromatography and TLC. Worked on this equipment to evaluate menthol and thyme to determine volatile substances. The result of this study revealed that (menthol and thymus) were found on this tablet by using GC. But this study evaluate the tablet to found this materials by using TLC. So, TLC result using thin layer showed there



was ginger and thyme in the tablet. This study was agree with other studies conducted by <sup>[14]</sup>. The study also mad pre-formulation of this extract it made as tablet.

### Conclusion

The results of current study showed the highest antimicrobial activity with ginger against *S. aureus* and *B. subtilis*. This research was disagree with pervious study who reported that the ginger lozenges demonstrated effective of antifungal activity recording inhibition zone diameters of 17, 19, 20, 23, and 25 mm at 5, 10, 20, 30, and 60 min release times, respectively, against *Candida albicans* <sup>[13]</sup>. There was no previous studies were agree with this study, so this result is a great results have done on this tablet solution. Therefore the expected of lozenges formulation containing garlic-ginger extract to qualify as a suitable base for the ginger tablet, it should be allowed and promote the release of the antimicrobial extract from the dosage form.

### Recommendation

1. We recommend the formula #3 must manufacturing (250 R \ 10 tab.)
2. We recommend to registration this product in ministry of health
3. There is no Yemeni pharmacopeia why?

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