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FORMULATION AND EVALUATION OF EFFECT OF YEMENI *ALLIUM SATIVUM* (GARLIC) EXTRACT IN TREATMENT OF ORAL CANDIDIASIS

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ABSTRACT

Background: *Allium Sativa* is species of bulbous flowering plant that is very common in many countries, especially in Yemen. It has many uses, principally as food flavouring and traditional medicines. **Objectives:** The study aimed to test the effect of garlic on the treatment of oral ulcers and what dosage form will be suitable for using in mouth. **Methods:** The present study was carried out by extraction of active constituents of garlic bulbs and test their activity against fungi (Candida Albican) that regards one of the essential causes in mouth ulceration and healing of oral ulcers. Also formulating the garlic extract in suitable dosage form and performing some tests for stability of dosage form. **Results:** In the proposed studies, two garlic extracts (ethanol and distilled water) were highly effective against Candida Albican, the inhibition zone of two extract were 45 mm and 29 mm respectively, these results were more effective than standard antifungal nystatin with inhibition zone 23 mm, due to the high efficacy of ethanol extract, it was selected to prepare suitable oral dosage form to treat mouth ulcer and candidiasis. **Conclusions:** Based on the results obtained from this study, the two Yemeni garlic extracts were highly effective against Candida Albican, this efficacy was more than standard antifungal nystatin. The ethanol extract was more effective than water extract, so, this extract was selected to prepare suitable dosage form to treat mouth ulcer and mouth candidiasis.

KEYWORDS: Allium Sativa, Candidiasis, Formulation, Garlic Extracts.

INTRODUCTION

Medicinal plant is defined as any plant which in one or more of its parts contains substance that can be used for therapeutic purpose or as precursors for the synthesis of useful drugs.[1]

Garlic (Allium sativum) is one of the oldest cultivated plants. It has been used as a spice, food, and folklore medicine for over 4000 years.[2]

Garlic (Allium sativum L.) is a common spice with many health benefits, mainly due to its diverse bioactive compounds, such as organic sulfides, saponins, phenolic compounds, and polysaccharides.[3-5] Garlic is commonly consumed and has a long history of being utilized as a traditional medicine in China. [6] The height of this plant reaches 4 feet but varies in different species. The product of garlic is a tuber or bulb that is composed of several small tubers called cloves. Each garlic tuber includes about 12 cloves as showed in figure 1. This plant is rich in nutrients as 100 grams of garlic contain 61% water, 30% hydrocarbonate, 2% protein, 1% fat, and some amounts of sugar and vitamins A and C.[7,8]

Fig. 1: Picture of *Allium Sativum* bulbs.

Garlic is one of the plants recommended in the Quran as a nutrient (Surah of Al-Baqarah, verse 61). Prophet Mohammad (PBUH) said that garlic is a cure for seventy diseases. His statements on the medicinal properties of some plants, such as garlic, have been confirmed by medical research. The accuracy of these statements was proven after fourteen centuries.[9-13]

Through the tries to confirm the benefit of allium sativum as antifungals, several previous studies establish that the allium sativum have antifungal effects. [14-21], and The formulation of medicinal plant in suitable dosage form as oral gel was established in other previous studies.[20, 22-24]

This study was aimed to extraction of Allum Sativum and evaluate the effect of this extract in treatment of oral candidiasis in comparing to standard drugs and formulate suitable dosage form.

MATERIALS AND METHODS

Materials

Allium sativum bulbs were collected from Sana'a region. Ethanol 96%, **(**Pure Chem *Pvt. Ltd.* India***)***, Dextro-sabourude agar (Himedia-Lab. USA) Carbomer, Glycerine, Phosphoric acid, Sodium hydroxide and Peppermint oil were purchased from the market, Antifungi standard Nystatin (MCE®- MedChem) was purchased from the drug marked.

Instrumentations

Rotary evaporator (BUCHI Labortechnik AG-Switzerland), Mixture (JJ-1mixer, China), Electric balance (Radwag, Poland), pH Measure (China), Water bath (HH-4, China), Centrifuge (China), Hot Oven (Labline Stock Centre) India**,** Morter and Pestle, Sheaker (Himedia-India), Blood Glucose Measuring Apparatus (ACCU-CHEK- Switzerland), UV spectrophotometer (Mettle Toledo spectrometers- Switzerland), Digital Viscometer (China), Incubator (China) and Filter paper (AU 480, Beckman Coulter, American).

Methods

Preparation of extracts

The extraction of Allium Sativum was carried out by two solvents (ethanol 96% and distilled water). The sample was extracted by using maceration method.

Procedure: Weight 2 quantities of 20 g of garlic bulbs, put each 20 g of garlic in the mortar and start crushing them by pestle till the juice of garlic gets out. Then pour each one into separated conical flask. Add 200 ml of distilled water into the first conical flask and add the same quantity of ethanol into second conical flask. Put the two conical flasks on the shaker for 3 hours. After that filter the two mixtures by filter papers into beaker. Finally, the extract was obtained.

Microbial Study

Collection of fungi samples

Samples of fungi were collected in Al-Awlagi laboratory in Sana'a city. Fungi sample was Candida Albican.

Test of two extracts on Candida Albican

The two extracts were experimented as antifungal on *Candida Albican* in compared to standard antifungal drug Nystatin.

Culture preparation

The culture medium of sabouraud dextrose agar (SDA) was prepared then sterilized by heating in autoclave at 118 ^{0}C for I hour, cultures from growing colony of *Candida Albican* were transferred and incubated at 36 ^{0}C for 24 hours.

Preparation of suitable dosage form

Formulation of ethanol extract of Allium Sativum (garlic) as oral gel

Five concentrations (7.7%, 8.33%, 10%, 15.4%, 20%) of extract in gel dosage form were prepared, formulated and tested their activity against fungi.

Preparation of gel

Using the following as gel base:

Table 1: Materials used in preparation of gel base.

Material	Amount
Carbomer	0.45 g
Glycerin	5 ml
Water	to 100 ml
NaOH 20%	4 drops

Study of Extreme isothermal stability of gel dosage form

The Study of stability was carried out through the following tests:

1- Accelerated stability testing studies designed to increase the rate of chemical degradation and physical change of a drug by using exaggerated storage conditions as part of the formal stability testing program.

Accelerated stability study was carried out through test the gel efficacy after put the gel in oven for 1 hours at temperature 70 and 90 ^{0}C.

2-Density

3-pH test by using pH mater

4-Viscosity by using viscometer

5-Grittness: this test was carried out by experiment the gel on 5 volunteers and take their feeling toward grittiness of gel.

RESULTS AND DISCUSSION

From the present study, the yield percents of ethanol extract and distilled water extract of *Allium Sativum* as showed in Table 2.

Table 2: Results of yield of fresh ethanol and distilled water extracts for *Allium Sativum(garlic)*.

No.	Plant name	Site of collection	Weight of fresh plant	Solvent used	Weight of extract yield	Yield percentage
Sample 1	*Allium Sativum*	Sana'a	20 g	Ethanol 96%	1.01 g	5.05%
Sample 2	*Allium Sativum*	Sana'a	20 g	Distilled Water	0.8 g	4%

The ethanol and distilled water extracts of *Allium Sativum* bulbs were tested against Candida Albican in comparison with standard antifungal nystatin and the results obtained were positively effective against fungi in comparing to standard antifungal nystatin where the ethanol extract has more antifungal activity than both distilled water extract and nystatin while distilled water extract has also more activity than nystatin.

From the present study, the ethanol extract of Allium Sativum was appeared with antifungal activity against Candida Albican more than water extract. This was comparable to reported studies that confirm the present study[25-28] and other studies that different and opposite the present study.[29, 30] The antifungal activity of ethanol and water extracts (in vitro) were more than standard anti-fungal nystatin and the results (inhibition zone) were 45 mm, 29 mm and 23 mm respectively as showed in Table 3 and Fig. 2,3.

Table 3: Result of antifungal activity of *Allium Sativum* extract.

Type of product	Inhibition zone against Candida Albican
Ethanol extract	45 mm
Distilled water extract	29 mm
Standard nystatin	23 mm

Fig. 2: Inhibition zone of ethanol extract of Allium Sativum in comparison to standard nystatin against *Candida Albican.*

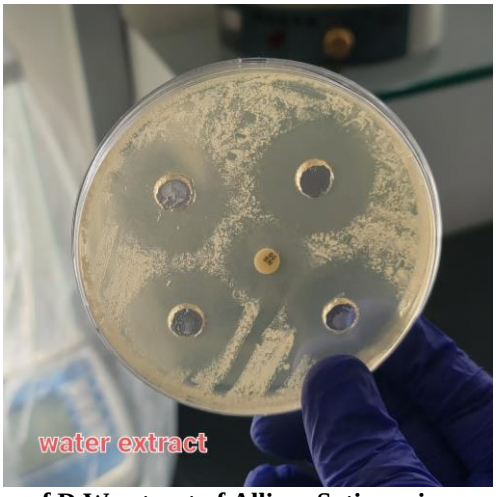

Fig. 3: Inhibition zone of D.W extract of Allium Sativum in comparison to standard.

So, the ethanol extract was selected to complete the research. From the present study, the determination of the best concentration in comparing to nystatin was carried out by the preparation of four different concentrations, these preparations were carried out through dilution of crude ethanol extract to 50%, 33%, 10% and 5% and using the ethanol as a solvent**.** The prepared different concentrations of ethanol extract were tested against Candida albican and comparing the activity of these concentrations to standard antifungal nystatin, the best concentration of ethanol extract was 10% concentration with activity near to the activity of nystatin as showed in Table 4.

Table 4: Results of efficacy of different concentrations of ethanol extract in comparing to nystatin through their efficacy against *Candida Albican*.

Type of product	Concentration	Efficacy against *Candida Albican* (inhibition zone)
Ethanol extract	Crude	45 mm
	50%	40 mm
	33.3%	37 mm
	10%	26 mm
	5%	18 mm
Nystatin		**23 mm**

In the proposed study, suitable dosage form was prepared that can be used to treat mouth ulcer and mouth candidiasis, five gel formulas from ethanol extract were prepared and tested as antifungal. From these five formulas, the formula 3 was the best one according to their antifungal activity against Candida and their devoid from water which lessen probability of microbial growing as showed in Table 5. The selected formula 3 was tested to stability as showed in Table 6.

Table 5: Results of efficacy of different formulations of ethanol extract as antifungal.

Formula No.	Concentration %	Amount of gel	Ethanol extract	Water	Activity (zone inhibition)
Formula 1	7.7%	10 ml	1 ml	2 ml	20 mm
Formula 2	8.33%	10 ml	1 ml	1 ml	33 mm
Formula 3	10%	10 ml	1 ml	No	30 mm
Formula 4	15.4%	10 ml	2 ml	1 ml	29 mm
Formula 5	20%	10 ml	2 ml	No	34 mm

Table 6: Results of Extreme isothermal stability tests for formula 3.

Parameter	Extreme isothermal stability		Density (kg/m^3)	pH	Viscosity (Pas*s)	Grittiness (5 volunteers)	
Gel	70 ^{0}C	90 ^{0}C	0.9895	5.3-5.5	1.353	Garlic gel	Sucrate gel
Efficacy as antifungal	(IZ) 25 mm	(IZ) 26 mm				5	7.5

IZ: Inhibition zone.

CONCLUSIONS

Based on the results obtained from the present study, the two Yemeni garlic extracts are highly effective against *Candida Albican*, this efficacy is more effective than standard antifungal nystatin. The ethanol extract is more effective than water extract, so, this extract is select to prepare suitable dosage form to treat mouth ulcer and mouth candidiasis. The formulation of 10% ethanolic garlic extract as carbomer gel prepared in this study is a promising formulation to produce oral gel pharmaceutical product in large scale.

AUTHORS' CONTRIBUTIONS

The reporting author considered the idea, developed the theory, and performed the calculations for the presented work. All authors participated in conducting the experiments, discussing the results, and contributing to the final manuscript.

DECLARATIONS

Conflicts of Interest

The authors declare that there are no conflicts of interest of publishing this article.

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