



ANTIMICROBIAL ACTIVITY OF HERBAL FORMULATION

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ABSTRACT: With the increasing incidence of diseases and resistance to diseases, the alternative, safe, effective, and economical products are the need of time. The formulation prepared is an elixir with herbal composition of *Cuminum cyminum* and *Allium sativum* having antimicrobial activity. In the present study, Elixirs showed antimicrobial activity against various strains of micro-organisms used. Minimum Inhibitory Concentration of *Cuminum cyminum* and *Allium sativum* ranged from 0.1mm to 0.7mm. The present study discusses the estimation of the antimicrobial activity of the elixir.

KEYWORDS: Antimicrobial activity, Elixir, *Cuminum cyminum* and *Allium sativum*

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INTRODUCTION

Natural products of animals, plants and microbial sources have been used by man for thousands of years either in the pure forms or crude extracts to treat many diseases. Garlic (*Allium sativum* L.) is one of those plants used for centuries to fight infectious diseases. Garlic is used as source of medicine in many ways in human beings in their day to day life¹. As a result, researchers from various disciplines are now directing their efforts towards discovering the medicinal values of garlic on human health. The main interest of researchers in the medicinal values of garlic is its broad-spectrum therapeutic effect with minimal toxicity^{2,3}. Garlic extract has antimicrobial activity against many genera of bacteria, fungi and viruses. Garlic contains a higher concentration of sulfur compounds which are responsible for its medicinal effects. Its antibacterial activity is mainly due to the presence of allicin produced by the enzymatic activity of allinase on alliin^{4,5}.

The aqueous extract of Cumin (*Cuminum cyminum*) is reported to inhibit the growth of many pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species, *Bacillus cereus* and *Aspergillus niger* due to presence of polyphenols. Composition, concentration of the constituents and extraction procedure are some factors which affect the efficiency of the extract¹. In the present study, antimicrobial activity of the aqueous ethanolic extract of Garlic and Cumin against various

species of microbes were examined for zone of inhibition.^{6,7,8}

EXPERIMENTAL PROCEDURES:

The herbs used for study were obtained from Jadhavji Lallubhai and Co, 245, Kalbadevi road, Mumbai-2.

1. EXTRACTION:

Herbal extracts of *Allium sativum* and *Cuminum cyminum* were extracted by maceration.

Procedure:

Maceration: 50g of herbs were weighed, crushed and powdered with the help of mortar pestle and then grinded to fine powder. 150ml of ethanol was added to each and kept at room temperature for 48 hours. Extracts were then filtered. 100ml of ethanolic extract was obtained for each.

2. FORMULATION OF ELIXIR:

Herbal formulation prepared was a medicated elixir.

METHOD TO PREPARE ELIXIR:

- Ethanolic extract of 17ml was taken and dissolved separately in the vehicle.
- To this solution, sucrose- a sweetening agent was dissolved in it, 0.8 gm of citric acid was added which acts as a buffering agent, then volume was made by 12% of ethanol.
- Filter the preparation.

FORMULA: (25ml) Ten different batches were made of the *Allium sativum*, *Cumin cyminum* and *Alium*

sativum -*Cumin cyminum* Elixir, final batch of 25ml was selected.

ELIXIR FORMULA

Table 1: Elixir formula

ETHANOLIC EXTRACT	17ml
SWEETENING AGENT	5ml
CITIRC ACID	0.8gm
ALCOHOL	q.s...25ml

3. ANTIMICROBIAL ACTIVITY OF ELIXIRS:

Following Strains were used for the study:

Bacillus subtilis-(Strain no NCIM 2063, ATCC No-6633)

Pseudomonas aeruginosa--(Strain no NCIM 5031, ATCC No-25619)

Staphylococcus aureus--(Strain no NCIM 2079, ATCC No-6538)

Escherichia coli --(Strain no NCIM 2256, ATCC No-9002)

Nutrient Agar was used as medium for antimicrobial study.

Antimicrobial activity of herbal extract was determined by the following methods^{9,10,11}:

1. Agar well diffusion method:

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly, to the procedure used in disk-diffusion method, the agar plate surface

is inoculated by spreading the microbial inoculum such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile corkborer, and a volume 50 µL of the extract solution was introduced into the well. Then, agar plates were incubated at 37° for 24 hours¹². The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microorganisms.

2. Disc agar diffusion method:

The disk-diffusion agar method determines the effectiveness of antimicrobial agents on a specific microorganism. An agar plate is first spread with each of the microbial suspension of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, then Whatman filter paper discs (about 6 mm in diameter), containing the

extracts, are placed on the agar surface. The Petri dishes are incubated at 37° for 24 hours. Antimicrobial agent diffuses into the agar and inhibits the growth of microorganisms and the average diameter of zone of inhibition is measured.

RESULTS AND DISCUSSION:

1. EXTRACTION-BY MACERATION:

100ml of Ethanolic extracts were obtained

by maceration for Cumin cyminum, Allium sativum and combined form of extracts.

2. FORMULATION OF ELIXIR:

An elixir was prepared for both the extract. Cumin cyminum elixir showed significant antimicrobial activity than Allium sativum elixir. A combined extract elixir showed weak effect. Hence no synergistic effect was observed for combined elixir.

3. ANTIMICROBIAL EVALUATION OF ELIXIR:

Table 2: Antimicrobial activity of herbal formulation

Antimicrobial activity of Elixirs			
HERBAL FORMULATION	MICRO ORGANISM	ZONE OF INHIBITION (Disc diffusion)	ZONE OF INHIBITION (Well diffusion)
<i>Cumin cyminum elixir</i>	<i>E.coli</i>	0.4 mm	0.3mm
	<i>Bacillus subtilis Pseudomonas</i>	0.5 mm	0.4mm
	<i>aeruginosa Staphylococcus</i>	0.7 mm	0.5mm
	<i>aureus</i>	0.3 mm	0.3mm
<i>Allium sativum elixir</i>	<i>E.coli</i>	0.3 mm	0.2mm
	<i>Bacillus subtilis Pseudomonas</i>	0.2 mm	0.1mm
	<i>aeruginosa Staphylococcus</i>	0.2mm	0.1mm
	<i>aureus</i>	0.1mm	0.2mm
<i>Combined elixir</i>	<i>E.coli</i>	0.1 mm	0.2mm
	<i>Bacillus subtilis Pseudomonas</i>	0.1 mm	0.1mm
	<i>aeruginosa Staphylococcus</i>	0.1 mm	0.1mm
	<i>aureus</i>	0.2 mm	0.2mm



Fig. 1: Antimicrobial evaluation of Elixir

The Elixirs showed antimicrobial activity against microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*. Cumin cuminum Elixir showed maximum zone of inhibition as compared to *Allium sativum* and combined elixir of *Allium sativum* and Cumin cuminum. The results are the values in triplicates.

Disc diffusion method shows maximum zone of inhibition as compared to Well diffusion in all the three elixir formulations.

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