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Research Article

In Vitro and *in Vivo* Evaluation of Antimicrobial Potential of Herbal Throat Lozenges

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Abstract

The objective of the present research work was to evaluate invitro and invivo antimicrobial activity of herbal throat lozenges (Curcumin lozenges and Ginger lozenges) formulated in the laboratory. Antimicrobial activities were evaluated using the agar dick method against *Staphylococcus aureus, Corynebacterium diptheriae, Streptococcus pyogenes, Klebsiella pnemoniae, Pseudomonas aeruginosa* and *Candida spp.* Strepsil lozenges were used as standard. Inhibition of growth of micro-organisms were noted and the minimum inhibitory concentration were found out for each lozenges. From all the above results it can be concluded that both the lozenges under study i.e., Ginger lozenge and Curcumin logenze, exhibits antimicrobial properties against the selected set of microorganisms.

1. INTRODUCTION

Lozenges are flavored medicated dosage forms intended to be sucked and held in the mouth/pharynx. These preparations are used for local effect as well as systemic effect[1].They are intended to be allowed to dissolve on the back surface of the tongue to provide drug delivery locally to the mouth, tongue , throat, etc, to minimise systemic and maximise local drug activity[2]. Advantages of the lozenges as dosage forms include bypass of first pass metabolism, increase in bioavailability, reducing gastric irritation, and improves onset of action. The new design to this area always benefits for the patient, physician and drug industry[3].

Curcumin (diferuloylmethane) is a polyphenol derived from *Curcuma longa* plant, commonly known as turmeric. Curcumin has been used extensively in ayurvedic medicine for centuries, as it is nontoxic and has a variety of therapeutic properties including antioxidant, analgesic, anti-inflammatory, antiseptic activity, and anticarcinogenic activity[4,5]. Ginger (*Zingiber officinale* Roscoe) has been used widely as a food spice and an herbal medicine. In particular, its gingerol-related components have been reported to possess antimicrobial and antifungal properties, as well as several pharmaceutical properties[6]. The objective of the present research work was to evaluate invitro and invivo antimicrobial activity of herbal throat lozenges (Curcumin lozenges and Ginger lozenges) formulated in the laboratory.

2. MATERIALS AND METHODS

2.1 Materials

Sterile Mueller Hinton agar was procured from Hi Media, Mumbai. Curcumin and dry Ginger powder were purchased from the local supplier.Lozenges of Curcumin and Ginger (Content: 300mg each) were developed in the laboratory using inert excipients which were tested initially for the microbial load. Strepsil lozenges were purchased from local medical shop.

2.2 Test micro-organisms used : Following clinical isolates were obtained from Hospitals and were used in the present study.
Gram positive bacteria: *Staphylococcus aureus, Corynebacterium diptheriae and Streptococcus pyogenes*
Gram negative bacteria : *Klebsiella pnemoniae and Pseudomonas aeruginosa*
Fungus : *Candida spp.*

2.3 Evaluation of *invitro* antimicrobial activity

The Agar Ditch method was used to carry out the primary screening of the test compounds (Curcumin. This method allows a single compound to be evaluated against a number of organisms simultaneously. A rectangular ditch of 8 cm x 1.5 cm was made in the agar medium. The compound to be tested was mixed with sterile agar and introduced in the ditch in required concentration. Test organisms were streaked across the ditch and on incubation their inhibition was observed.

Procedure to test the activity

1) Sterile Mueller Hinton agar plates were prepared and a ditch of 8cm x 1.5 cm was cut from the centre of the plate aseptically.
2) The weighed amount of the drug (Concentration of drug used: 6% of drug in Molten MH agar) was dissolved in 5 ml of sterile molten Mueller Hinton agar butts, aseptically. It was mixed thoroughly and aseptically poured into the ditch of the plates such that the surfaces of the agar in the medium and that in the ditch were even.
3) The plates were allowed to solidify completely and dried to remove any moisture present.
4) The test organisms were streaked perpendicular to the ditch and parallel to each other.
5) The plates were incubated at 37˚C for 24 hrs.
6) Next day, the growth of the culture along the streak line on the ditch and near the ditch was observed.

Procedure for determination of minimum inhibitory concentration (MIC)

A rectangular ditch of 8 cm x 1.5 cm was made in the agar medium. The compounds to be tested were mixed with sterile agar in various concentrations and introduced in the ditch in required concentration. Test organisms were streaked across the ditch and on incubation their inhibition was observed. The ditch with various concentration of drug ranging from lower to higher can be used to determine the minimum inhibitory concentration of drug to inhibit specific organism

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2.3 *In vivo* antimicrobial evaluation of herbal throat lozenges on Human volunteers

1. Eight human volunteers were consented and used for studies.
2. Salivary sample from each volunteer were collected initially to obtain initial count.
3. The collected samples were spread on sterile plate count agar plate and incubated at at 37˚C for 24 hrs.
4. In the sets the volunteers were given drug sample to be kept in mouth, while one set was maintained as control.
5. Salivary samples were collected after 5 and 10 minutes respectively.
6. The collected samples were spread plate on a sterile plate count agar plate and incubated at at 37˚C for 24 hrs.
7. After incubation, colonies were counted and reported accordingly.

3. RESULTS AND DISCUSSION

3.1 *In vitro* antimicrobial activity

a) Ginger Lozenges

The Ginger Lozenge as well as its active ingredient showed good antibacterial activity against Gram positive bacteria Staphylococcus aureus, Corynebacterium diptheriae, Streptococcus pyogenes, and Gram Negative bacteria Klebsiella pneumoniae. But shown no effect against Pseudomonas aeruginosa (Gram negative bacteria). No effect was observed against Candida spp. (fungus).The results of Invitro antimicrobial activity of ginger powder and ginger lozenges are summarized in table-1.

Table 1: *In vitro* antimicrobial activity of ginger powder and ginger lozenges

Sample	*Staphylococcus aureus*	*Corynebacterium diptheriae*	*Streptococcus pyogenes*	*Klebsiella pnemoniae*	*Pseudomonas aeruginosa*	*Candida spp.*
Ginger Lozenges	**-**	**-**	**-**	**-**	**+**	**+**
Dry Ginger powder	**-**	**-**	**-**	**-**	**+**	**+**

Note: + → Growth, – → Inhibition

The MIC of the Ginger drug was found to be 1% wherein it inhibits all the test pathogens. The results of MIC of ginger powder and ginger lozenges are summarized in table 2.

Table 2: MIC of ginger powder and ginger lozenges

Concentration of drug in %	***Staphylococcus aureus***	***Corynebacterium diptheriae***	***Streptococcus pyogenes***	***Klebsiella pneumoniae***
0.3	+	+	-	-
0.6	+	+	-	-
0.9	-	+	-	-
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
6	-	-	-	-

Note: + → Growth, – → Inhibition

b) Curcumin Lozenges

The Curcumin Lozenge as well as its active ingredient showed good antibacterial activity against Gram positive bacteria Staphylococcus aureus, Corynebacterium diptheriae, Streptococcus pyogenes, and Gram Negative bacteria Klebsiella pneumoniae. But shown no effect against Pseudomonas aeruginosa (Gram negative bacteria). No effect was observed against Candida spp. (fungus).

The results of invitro antimicrobial activity of curcumin powder and curcumin lozenges are summarized in table-1.

Table 3: Invitro antimicrobial activity of curcumin powder and curcumin lozenges

Sample	Staphylococcus aureus	Corynebacterium diptheriae	Streptococcus pyogenes	Klebsiella pnemoniae	Pseudomonas aeruginosa	Candida spp.
Curcumin Lozenge	**-**	**-**	**-**	**-**	**+**	**+**
curcumin powder	**-**	**-**	**-**	**-**	**+**	**+**

Note: + → Growth, – → Inhibition

The MIC of the Curcumin drug was found to be 3% wherein it inhibits all the test pathogens. The results of MIC of curcumin powder and curcumin lozenges are summarized in table-4.

Table 4: MIC of curcumin powder and curcumin lozenges

Concentration of drug in %	***Staphylococcus aureus***	***Corynebacterium diptheriae***	***Streptococcus pyogenes***	***Klebsiella pneumoniae***
0.3	+	+	+	+
0.6	+	+	+	+
0.9	+	+	+	+
1	+	+	+	-
2	-	+	+	-
3	-	-	-	-
6	-	-	-	-

Note: + → Growth, – → Inhibition

The MIC of the strepsils (standard) drug is found to be 1% wherein it inhibits all the test pathogens. The results of MIC of Strepsil lozenges are summarized in table-5.

Table 5: MIC of Strepsil lozenges

Concentration of drug in %	*Staphylococcus aureus*	*Corynebacterium diptheriae*	*Streptococcus pyogenes*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida spp.*
0.3	-	+	-	-	+	+
0.6	-	-	-	-	+	+
0.9	-	-	-	-	+	+
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
6	-	-	-	-	-	-

The comparative MIC results of all the lozenges are depicted in table-6.

Table 6: Comparative MIC of all the lozenges used in the study

MIC of Ginger Lozenges (Test sample)	MIC of Curcumin Lozenges (Test sample)	MIC of Strepsil Lozenges (Standard Sample)
1%	3%	1%

3.2 *In vivo* antimicrobial evaluation of Herbal throat lozenges on Human volunteers

The results of Invivo antimicrobial evaluation of Herbal throat lozenges on Human volunteers are summarized in table -7.

Table 7: Invivo antimicrobial evaluation of Herbal throat lozenges on Human volunteers

Sample	Average CFU/ml Initial	Average CFU/ml after 5 minutes	Percentage change after 5 minutes compared to Initial count	Average CFU/ml after 10 minutes	Percentage change after 10 minutes compared to Initial count
Ginger Lozenge	1390	1830	31.65 % ↑	840	**39.56 %** ↓
Curcumin Lozenge		1850	33.09 %↑	980	**29.50 %** ↓
Strepsils		230	**83.45%** ↓	150	**89.20 %** ↓
Control		2990	115.1 % ↑	2120	**52.50 %** ↑

Note:-↓ - Decrease in count ↑- Increase in count, CFU/ML : Colony Forming Units per ml

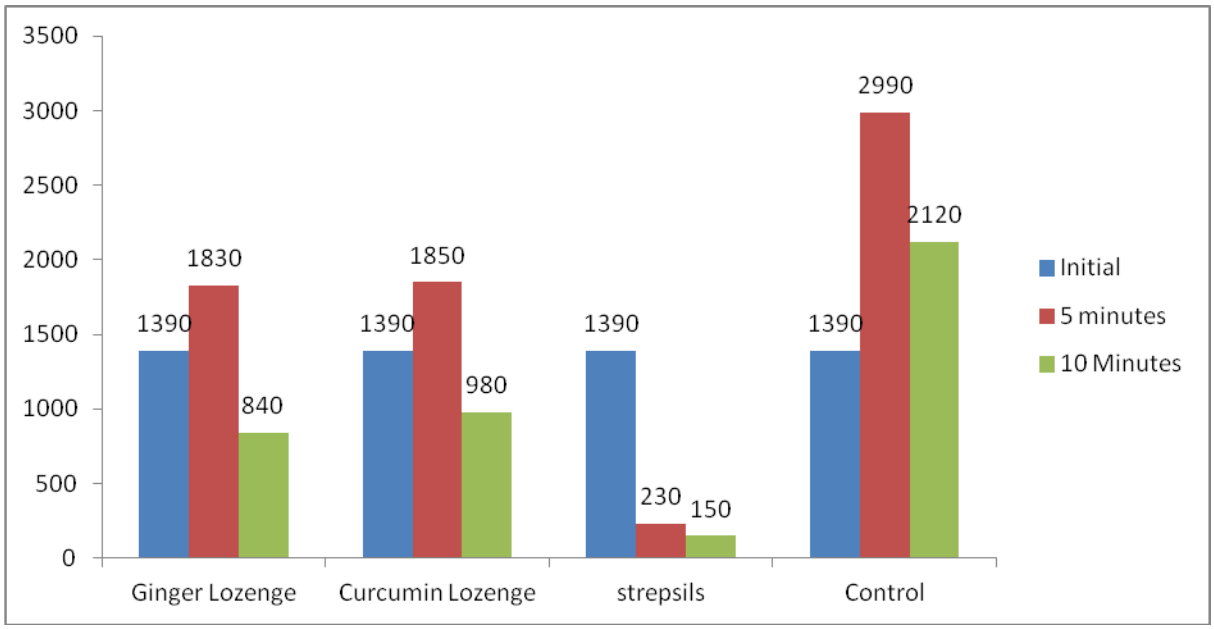

Fig. 1: Graphical representation of CFU/ml

As An average initial microbial count of 1390 CFU/ml was recorded from all the 8 volunteers. Each set of two volunteers was administered with Ginger Lozenge, Curcumin lozenge and Strepsils, while the last set of volunteers acted as control set. On administration of the respective drugs, after 5 mins, the oral microbial flora changed with different degrees.In case of Ginger lozenge, there was a 31.65% increase in the microbial count taking it to 1830 CFU/ml. Similarly in case of Curcumin lozenge, there was an increase of 33.09% taking the count to 1850 CFU/ml.

Strepsils showed a significant decrease of 83.45% within 5 mins, taking the count to 230 CFU/ml.And, as the control set had no inhibitory drug, the microbial count increased considerably by 115.1 %, wherein the count is 2990 CFU/ml.

After 10 mins, the microbial flora was again determined. The results were much positive after 10 mins of administration of the drugs.

In case of Ginger lozenge, there was a 39.56 % decrease in the microbial count with values of 840 CFU/ml. In comparison, Curcumin lozenge showed only 29.50 % decrease in the microbial count with values of 980 CFU/ml. Strepsil continued its strong

activity and extended its inhibition from 83.45 % to 89.20 % lowering the count to 150 CFU/ml.

The growth rate of microbial flora in the control sets was reduced to 52.50% (2120 CFU/ml) from 115.1 % (2990 CFU/ml). The reason for this could be the repetitive washing of the mouth.

From the above results, it is clear that the test sample i.e., Ginger lozenge and Curcumin lozenge, both doesn't show any significant activity in the initial 5 mins of administration. The reason could be the solubility of the products.

However, by the end of 10 mins, both the lozenges showed decent inhibitory activity. Ginger lozenge showed comparatively better activity than Curcumin lozenge.Strepsils, acting as standard drug, showed significant activity at both 5 and 10 mins intervals.

From the above results obtained, the Ginger Lozenge and Curcumin lozenge showed decrease in count of oral flora by 40% and 29.5 % respectively after 10 minutes. Thus it can be concluded that Lozenge shows inhibitory action against the microorganisms *In Vivo*.

4. CONCLUSION

From all the above results it can be concluded that both the lozenges under study i.e., Ginger lozenge and Curcumin logenze, both exhibit antimicrobial properties against the selected set of microorganisms. Ginger lozenge showed MIC value of 1%. On *in vivo* testing, ginger lozenge showed 39.56% inhibition of microbial flora after 10 mins of consumption. Curcumin lozenge showed MIC value of 3%. *In vivo* testing of curcumin lozenge showed 29.50% inhibition of microbial flora after 10 mins of consumption. Strepsils, used as standard drug for the studies, showed MIC value of 1%. On *in vivo* testing the, strepsils showed 83.45 % inhibition of microbial flora within 5 mins of consumption. The activity of strepsils is much superior, the reason for it being the two active ingredients of strepsils i.e., Amylmetacresol and 2, 4-dichloro benzyl alcohol (both synthetic antiseptic drugs). Most synthetic drugs are designed from the reductionist point of view where-by one chemical is to influence one receptor in the human body. However, with the ever increasing resistance amongst pathogens, there are chances that within the next few years microorganisms might develop resistance against one or both of these active ingredients and might reduce the effectively of the product. Herbal medicines are a composition of a multitude of different constituents, all arranged in harmonious ways, all contributing to a different duty and, most importantly, doing this all at the same time.

Both the lozenges under study are herbal in nature and are composed of multiple, comparatively sober and diverse active ingredients. Although the activity is less as compared to standard drugs, the effects could be long lasting. Because of multiple active ingredients, the chances of development of resistance against these herbal lozenges are very less.

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