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Antibacterial activity of *Cuminum cyminum* Linn. On gram positive bacteria

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Abstract

Cuminum cyminum Linn is an herbaceous and medicinal crop and one of the oldest and popular seed spice worldwide after black pepper. This study was conducted to determine the anti-bacterial effect of *Cuminum cyminum in vitro* test and aims to Identify what organisms are highly susceptible to cumin, and to determine the concentration of cumin against Gram-positive bacteria that possess anti-bacterial property (25%, 50%, 75%, 100% were used as treatments), and compare to antibiotic effect of Amoxicillin and Streptomycin (Served as Control) for Gram positive bacteria as control. The *Corynebacterium diphtheriae* bacteria in Gram-positive bacteria was observed as the high susceptible in antibacterial activity of cumin. 100% concentration of cumin treatment have a significant effect on Gram-positive bacteria compared to 75%, 50% and 25% concentration but, no significant difference on the antibacterial effect of Amoxicillin (Control) to invade bacteria.

Keywords: *Cuminum cyminum* Linn, amoxicillin, streptomycin and *Corynebacterium diphtheriae*

Introduction

Cumin (*Cuminum cyminum* Linn.), an important commercial seed spice from the Umbellifereae family, is valued for its flavor as well as for curative and therapeutic effects. The plant is indigenous to the Mediterranean area, where it is widely cultivated. This annual herb, sometimes called zeera or jeera in Hindi, has been used as a spice and medicine since the dawn of time. Second only to black pepper in importance, it is regarded as one of the most significant spices (Divakara and Anandara, 2013) [1]. Cumin is mostly produced and consumed in India. Cumin seeds and their many value-added derivatives, including oleoresins and oil, are also exported from India. Anise (*Pimpinella anisum*) is commonly known as sweet cumin, which should not be mistaken with this spice. The plant known as black cumin, or *nigella sativa*, is not related to cumin.

Cuminum cyminum included alkaloids, anthraquinones, coumarins, flavonoids, glycosides, proteins, resins, saponins, tannins, and steroids, according to a phytochemical examination (Chand *et al.*, 1999) [2]. Experimental evidence supports cumin's widespread medical applications. Cumin seeds are used for treating moderate digestive problems, diarrhea, dyspepsia, flatulence, colic, abdominal distension, edema, bronchopulmonary disorders, puerperal disorders, analgesic, and as a cough treatment in Ayurveda. They are also regarded to be carminative, eupeptic, antispasmodic, and astringent. The effects of cumin include improving vision, stamina, and lactation. It has been said that cumin seeds have been used in traditional medicine to cure jaundice, indigestion, toothaches, and epilepsy. It exhibits a range of pharmacological properties, including anti-diabetic, immunologic, anti-tumor, and antibacterial activities (Belal *et al.*, 2017; Mohammad Reza *et al.*, 2015) [3, 4].

Cumin essential oil functions as an effective analgesic, anti-inflammatory, antibacterial (Jufri, and Natalia, 2014) [5], haemolytic, anti-enzymatic, sedative, stimulant, diuretic (Lahlou *et al.*, 2007) [6], and stomachic whether used externally or internally. Cumin is regarded as a stimulant, carminative, and astringent in Iranian traditional medicine, and its therapeutic benefits on gastrointestinal, gynaecological, and respiratory illnesses have also been noted. It is also used to cure toothaches, diarrhoea, and epilepsy.

Cumin is used as a purgative, bitter tonic, and antiseptic in Italy whereas cumin is regarded as an abortive, galactagogic, antiseptic, and antihypertensive herb (Tahraoui *et al.*, 2007) [7] in traditional Tunisian medicine.

Therefore, it is clear that cumin as feed additives have many beneficial effects due to its aforesaid properties. Research has showed that the use of chemical additives especially antibiotics in animal nutrition may result in the accumulation of chemical residues in animal products. Therefore, the use of organic feed additives in animal nutrition has gained more attention during recent years due to concerns about food safety and human health issue. Cumin is extremely good for digestion and related problems. The very smell (Aroma) of it, which comes from an aromatic organic compound called Cumin aldehyde, the main component of its essential oil, activates our salivary glands in our mouth (The mouth-watering flavour), facilitating the primary digestion of the food.

Materials and Methods

Procurement of freshly ground Cumin powder

Fresh seeds were collected and appropriate amounts of seeds of cumin were grounded by using mixer grinder. The cumin powder was placed in the clean sterile bottle.

Preparation of the different concentration of cumin powder: Cumin powder was weighed out on a balance to create 2.5 grams of powder, which was then combined with 7.5 milliliters of distilled water in a beaker to create a 25% concentration.

Cumin powder was weighed out in a weighing scale to produce 5 grams of powder, which was then combined with 5 milliliters of distilled water in a beaker to get a 50% concentration. Cumin powder was weighed out in a weighing scale to produce 7.5 grams of cumin powder, which was then combined with 2.5 milliliters of distilled water in a beaker to create a 75% concentration. Cumin powder was measured in a weighing balance to produce 10 grams of powder with a 100% concentration. As 10grams of powder is used as 100% concentration then the other concentrations are calculated by

$$\text{Formula: } \frac{\% \text{ of concentration} \times 10}{100}$$

Diffusion Method

Sterile cotton-tipped swabs were dipped into a pure culture of bacterial suspension and were inoculated into Brain Heart Infusion broth. The inoculate of the Mueller-Hinton Agar Plate. The pure culture of bacteria coming from Brain Heart Infusion broth was refused using a cotton swab and then inoculate on the Mueller-Hinton Agar, thus was done by streaking back and forth across the medium rotating the plate 60 degrees and then streak again to give a uniform inoculum to the entire surface. Lastly, the control disc was soaked into the Amoxicillin and Streptomycin antibiotics. The plates were incubated at 35 degrees Celsius for 18 hours. The distinct zones of growth inhibition were noted around the disc that contains different kinds of concentration of the cumin powder. The control disc that contains the antibiotic is also measured for zones of inhibition. Zones of inhibition around the disc were measured with a millimetre ruler. The measurement of the zone of inhibition involves taking a reading across the centre of the zone to include the diameter of the disc.

Parameter range (Range of susceptibility)

The distinct zone of growth inhibition around the disc that contain different kinds of concentration of cumin powder were noted after 18 hours of incubation including the control disc, expressed in millimetres and categorized as follows: If the growth inhibition is below 0-14mm, it is resistant; 15mm, it is less susceptible; 16-20mm, it is moderately susceptible and if 21mm above- it is high susceptible.

Table 1: Disc diffusion setup

Replicates	Concentration of cumin powder
1	25% (25% powder, 75% distilled water)
2	50% (50% powder, 50% distilled water)
3	75% (75% powder, 25% distilled water)
4	100% (100% powder)
5	Control (Amoxicillin-Streptomycin)

Measurement of zone of inhibition

The zones of growth inhibition at the different concentration of cumin seed extract had been noted after 18hours of incubation including the control disc. Distinct zones of growth of inhibition was expressed in millimetre. Zone of inhibition around the disc were measured with a calliper across the centre of the zone to include the diameter of the disc.

Treatment of data

To determine any significant difference among the different concentration and when compared with concentrations of known antibiotics in terms of the antibiotic effect or level of susceptibility, Least Significant Test (LST), was conducted for gram positive bacteria. It was used for comparing two or more independent samples of equal or different sample sizes.

Results and Discussion

Gram-positive bacteria

Table 2 presents the susceptibility of selected Gram-positive bacterial specimen after inoculation with various concentration of cumin, which are: *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Lactobacillus spp*, *Bacillus subtilis*. These were inoculated and tested in the laboratory with different rates of cumin range from 25%, 50%, 75% and 100% were compared with that of the susceptibility of the same test organisms to Amoxicillin as positive control.

Table 2: Qualitative Assessment Results of bacterial inoculation of positive control and four concentrations of cumin on Gram-positive bacteria

Gram-positive bacteria spp	25%	50%	75%	100%	Amoxicillin (control)
<i>Streptococcus pneumoniae</i>	04	08	10	10	12
<i>Staphylococcus epidermidis</i>	05	07	08	10	14
<i>Corynebacterium diphtheria</i>	08	15	18	20	23
<i>Lactobacillus spp</i>	02	06	10	15	17
<i>Bacillus subtilis</i>	02	08	12	15	18

Table 2 shows the results of the inoculation of sample Gram-positive bacteria with cumin, which were the level of susceptibility of the selected bacteria to different concentrations of cumin. Cumin was effective at 100% concentration.

Analysis of variance result

The analysis shows that there is a highly significant difference

among treatments but, treatment with Amoxicillin (control), and 100% cumin powder have no significant difference but, a high significant difference compares to three remaining treatments the 25%, 50% to 75% cumin concentration. The data observed that only the 100% concentration of cumin can be comparable to Amoxicillin (control), in the absence of antibiotic Amoxicillin 100% treatment with cumin can be used against Gram-positive bacteria.

Table 3: Analysis of variance result

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
TREATMENT	4	470.2400	117.5600	8.53	0.0003**
Error	20	275.6000	13.7800		
Total	24	745.8400			
Highly significant CV (%) (33.50) POS Mean (11.08)					

ANOVA table shows that the level of probability of the test was 0.0003 which is lower than 0.05 level of significant. Highly significant difference among treatments were observed.

Table 3 shows the susceptibility to cumin of gram-positive bacteria which are: *Streptococcus pneumonia*, *Staphylococcus epidermidis*, *Corynebacterium diphtheria*, *Lactobacillus spp*,

Bacillus subtilis.

Based on the statistical analysis of mean presented in the table 4 treated gram positive bacteria with amoxicillin (control) shows with moderately resistant of 16.80 mm, followed by 100% cumin (100% powder) with 14.00 mm resistant, followed by 75% cumin (75% powder, 25% distilled water) with 11.60 mm resistant, followed by 50% cumin (50% powder, 50% distilled water) with 8.80 mm resistant, and 25% cumin (25% powder, 75% distilled water) were as recorded with highly resistant measures of 4.20 mm. The results of the present investigation agree with those of Sheikh *et al.*, 2010 [8], who reported that the cumin oil showed an antibacterial activity against Gram-positive.

Table 4: Gram-positive bacteria

Treatment	means	N group
Amoxicillin (Control)	16.80	5 a
25% (25% powder, 75% distilled water)	4.20	5 d
50% (50% powder, 50% distilled water)	8.80	5 cd
75% (75% powder, 25% distilled water)	11.60	5 bc
100% (100% powder)	14.00	5 ab

Means with the same letter are not significantly different.

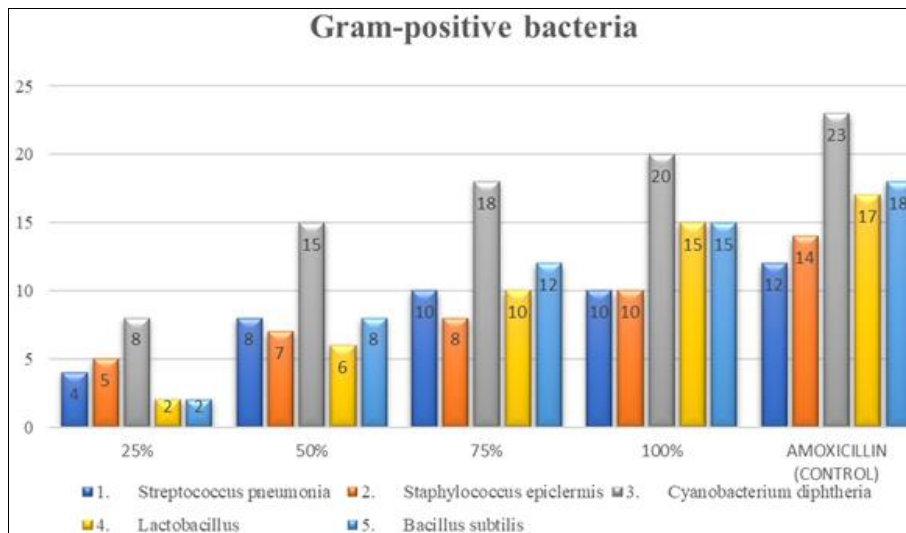
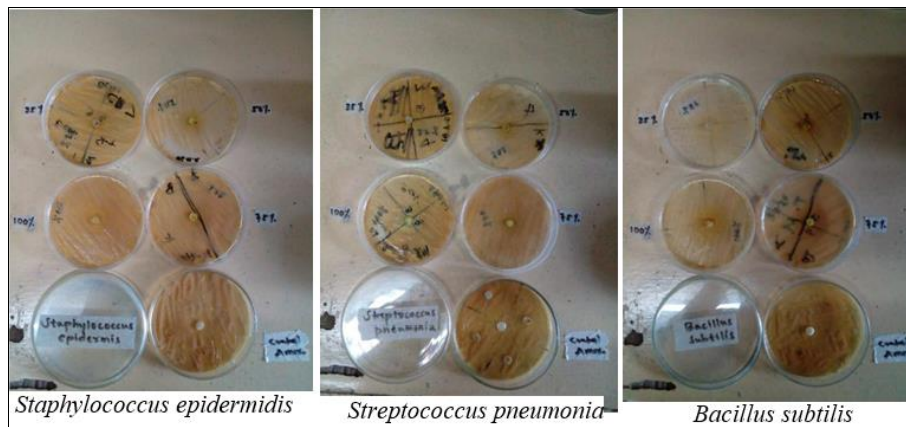


Fig 1: Gram positive bacteria susceptibility (mm)

Figure 1: Shows the Gram-positive bacteria susceptibility, the *Corynebacterium diphtheria* bacteria were observed as the high susceptible followed by *Bacillus subtilis* and *Lactobacillus spp*, and the least susceptible are *Streptococcus pneumoniae*, and *Staphylococcus epidermidis*.

Base on the level of concentration of treatments as compared to Amoxicillin (Control), the 100% cumin concentration was observed with almost the same effect of susceptibility measures on different gram-positive bacteria subjected in this study



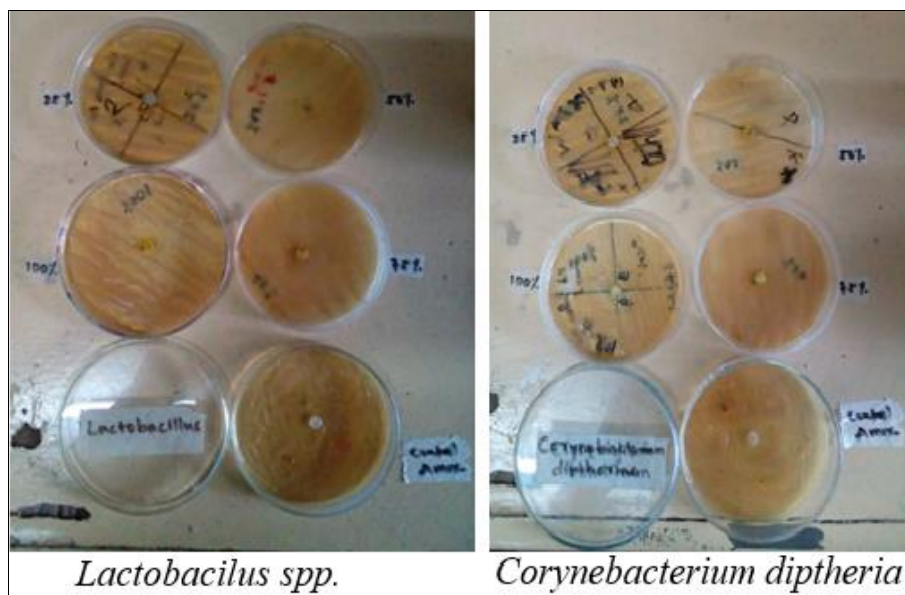


Fig 2: Gram-positive bacteria

Conclusion

Based on the findings of the study the researcher observed that cumin has the significant effect on the Gram-positive bacteria, however this can only be observed at high concentrations. Moreover, the researcher concluded that cumin became less effective to invade Gram-positive bacteria in 75% concentration and beyond 50%, 25% concentrations but, 75% and 100% became more effective and have comparable effect on commercial antibiotics the streptomycin and amoxicillin for Gram-positive bacteria.

Extracts of Cumin (*Cuminum cyminum* L.). Agriculture Conspectus Scientific. 2010;75:39-44.

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