Antibacterial Activity of *Cuminum Cyminum* L. Oil on Six Types of Bacteria

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Abstract: The *Cuminum Cyminum* (cumin) oil was extracted from cumin seeds by distillation process. The extracted cumin oil was used to assess its effectiveness as antibacterial that through testing on six types of bacteria; two of them were bacteria gram-negative (E. coli and S. typhi) and the remainders were bacteria gram-positive (*Proteus Vulgaris*, *Klebsiella Pneumonae*, *Enterococcus Feacalis* and *Staphylococcus Aureus*). Four concentrations (12.5%, 25%, 50% and 100%) of cumin oil were used for screening fulfillment by using the cup-plate agar diffusion method and gentamicin (10µg) as the positive control. According to different concentration the inhibition area, minimum inhibition zones diameters (MIZD) in mm and the relative percentage inhibition of the test with respect to positive control were calculated. The results showed that all tested concentrations of cumin oil showed antibacterial activity against gram positive and gram negative bacteria.

Keywords: Cumin, Gram-negative, Gram-positive and Gentamicin

1. Introduction

*Cuminum Cyminum* L. is an herbaceous and medicinal crop and one of the oldest and popular seed spice worldwide after black pepper [1]. The term ‘spice’ originated from the Latin word ‘species’, meaning of specific kind. A closely related term, ‘herb’, is used to distinguish plant parts finding the same uses but derived from leafy or soft flowering parts. The two terms may be used for the same plants in which the fresh leaves are used as herbs, while other dried parts are used as spices [2]. Cumin is popularity spread from Latin America to Africa and all over Asia [3]. Although cumin was originally cultivated in Iran, where it is one of the most important export crops, and Mediterranean region but today it is also grow in Uzbekistan, Tajikistan, Turkey, Morocco, Egypt, India, Syria, Mexico, Bulgaria, Cyprus and Chile, where India is the largest producer and consumer of cumin seed in the world [4].

Cumin is a small flowering herbaceous plant belonging to the Apiaceae, in the genus. The cumin plant grows to 30–50 cm tall and is harvested by hand. It is an annual plant, with a slender, branched stem 20–30 cm tall. It has blue-green linear leaves and finely separated. The white or pink flowers are borne in small compound umbels [6]. Nutrient contents of cumin (in 2 g of seeds) are included: water 0.16 g; some calories: protein 0.36 g, carbohydrates 0.88 g, dietary fiber 0.22 g, total fat 0.44 g, saturated fat 0.04 g, monounsaturated fat 0.28 g, polyunsaturated fat 0.06 g, Ash (g) 0.16, some vitamins such as vitamin A 25.40 IU, thiamin (B₁) 0.02 mg, niacin (B₃) 0.10 mg, niacin equiv 0.10, vitamin C 0.16 mg, vitamin E 0.02 mg, folate 0.20μg and vitamin K 0.11μg; in addition it contained some minerals: eg; calcium 18.62 mg, copper 0.02 mg, iron 1.32 mg, magnesium 7.32 mg, manganese 0.06 mg, phosphorus 9.98 mg, potassium 35.76 mg, selenium 0.10μg, sodium 3.36 mg and zinc 0.10 mg [7].

Phytochemicals analysis showed that *Cuminum Cyminum* contained: alkaloid, anthraquinone, coumarin, flavonoid, glycoside, protein, resin, saponin, tannin and steroid [8]. Cumin also contains very good amounts of B-complex vitamins such as thiamin, vitamin B₆, niacin, riboflavin, and other vital anti-oxidant vitamins like vitamin E, vitamin A and vitamin C. The seeds are rich source of many flavonoid phenolic anti-oxidants such as carotenes, zeaxanthin and lutein [9]. It also contains safrole, a mutagen, which is degraded by cooking [10]. Organic acids (aspartic, citric, malic, tartaric, propionic, ascorbic, oxalic, maleic and fumaric acids) were isolated from seeds of cumin [11].

Cumin had some reputation as a drug but its chief medicinal use now days in veterinary medicine. This spice is
also used as a homeopathic treatment for a variety of conditions. Due to its numerous medicinal properties, cumin is used as an ingredient in many home remedies and ayurvedic preparations [12]. In traditional herbal medicine cumin was used to treat hoarseness, jaundice, dyspepsia and mixed with other ingredients to treat diarrhea and colic. The absolute is superior to the oil in flavoring is cuminaldehyde, the chief constituent of cumin oil. This will not only add taste in food, but will also act as one of the herbal remedies against many health disorders [13].

An anti microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoa [14]. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth of another microorganism [15]. Of course, today common usage the term antibiotic is used refer to almost any drug that attempts to ride your body of bacterial infection. Antimicrobial include not just antibiotics, but synthetically formed compound as well [16].

Despite the development of antibiotics, bacterial and fungal infections are still a major issue in medicine, and the presence of numerous drug resistant strains poses a new challenge. Herbal drugs have been extensively used in this field for many centuries [17]. Essential oils of plant are known for their antimicrobial capability and have the potential to control plant diseases caused by bacteria and, in particular, eradicate bacteria from seeds [18]. Recently, there has been a growing interest in natural products due to their availability, fewer side effects or toxicity as well as better biodegrade ability as compared to the available antibiotics and preservatives [19]. The exploration of naturally-occuring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance toward conventional preservatives [20].

Cumin contains fatty oil and has an antimicrobial effect. A powder suspension of the cumin has diverse inhibitory effects [21]. Numbers of investigations have shown the antimicrobial activity of cumin, the antibacterial action was assessed against a range of useful and pathogenic gram-positive and gram-negative bacteria strain. Cumin seed oil extract inhibited the growth of Klebsiella pneumoniae and its clinical isolates and caused improvement in cell morphology, capsule expression and decreased urease activity [22]. This property was attributed to cuminaldehyde, carvone, limonene and linalool, whereas limnonene, eugenol, -pinene and some other minor constituents have been suggested to contribute to the antimicrobial activity of cumin oil [23].

2. Sample and Specimen Preparation

Cumin seeds were purchased from Shendi local market obtained in the crop season 2015 – 2016. Six types of bacteria (four gram-positive and two gram-negative) were prepared in the medical laboratories college, Shendi University.

3. Material and Methods

Water-steam distillation was applied to obtain the cuminoil of cumin. Extracted oil was subjected to determine its chemical components by using gas chromatography mass spectrometer (GC-MS).

The extracted oil of Cumin Cuminum was used to assess its effectiveness as antibacterial that through testing on six types of bacteria; two of them were bacteria gram-negative (Escherichia Coli– Salmonella Typhi) and the remainders were bacteria gram-positive (Proteus Vulgaris, Klebsiella Pneumonae, Enterococcus Feacalis and Staphylococcus Aureus). The screening was carried out using the cup-plate agar diffusion method at four different concentrations (12.5%, 25%, 50% and 100%). The oil was dissolved in dimethyl sulfoxide (DMSO). The commercial antibiotic, gentamicin (10µg) was used as the positive control. The effects of the extracts were considered after 24 hours by measuring the inhibition zone diameter of each treatment. Three replicates were carried out for each extract/ fractions and control against the test organisms. The relative percentage inhibition of the test with respect to positive control was calculated by using the following formula:

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\text{Relative percentage inhibition of the test extract} = \frac{(X-Y) \times 100}{(Z-Y)}
\]

Where:

- X: Total area of inhibition of the test extract.
- Y: Total area of inhibition of the solvent.
- Z: Total area of inhibition of the standard drug.

4. Results

The GC- MS analysis of cumin oil showed that eleven hydrocarbon monoterpenes which were α-Thujene α-pinene, β-pinene, β-myrcene, α-phellandrene, p-cymene and γ-terpinene and four oxygenated monoterpenes which were cumin aldehyde, Carboxaldehyde, 2-Caren-10-al and cumin alcohol.

The inhibition area and minimum inhibition zones (MIZD) and percentage were calculated according to different concentration (12.5%, 25%, 50% and 100%) and represented in tables 1 & 2 and figure 1 below.

| Test Bacteria          | Type of Bacteria | MIZD in mm |
|------------------------|------------------|------------|
|                         |                  | 12.5% 25% 50% 100% |
| Enterococcus fecalis   | Gram-negative    |            |
| Klebsiella pneumoniae  | Gram positive    | 8  18  25  100 |
| Proteus vulgaris       | Gram positive    | 25  20  15  13 |
| Proteus vulgaris       | Gram positive    | 20  15  13  16 |
| Staphylococcus aureus  | Gram positive    | 10  12  13  16 |
| Escherichia coli       | Gram-positive    | 23  30  20  30 |

Table 1. Inhibition area of antibacterial activity of different concentration of cumin oil on the some types of bacteria.
5. Discussion

The results showed that all tested concentrations of cumin oil showed antibacterial activity against gram positive and gram negative bacteria, only Enterococcus fecalis was resistance at the concentrations 12.5% and 50%. In addition the concentration 100% inflicted the highest antibacterial activities except in the cases of Klebsiella pneumoniae and Proteus vulgaris. The most susceptible bacteria strains was Salmonella typhi with highest inhibition zone values (30mm) at concentration 25% and 100%. On the other hand, the tested concentrations 12.5% and 25% of cumin oil showed high inhibition zone against Klebsiella pneumoniae and Proteus vulgaris (25mm) compared with tested concentrations 50% and 100%.

Generally all of the cumin oil concentrations were found to be active against the gram positive bacteria. Enterococcus fecalis revealed the weakest results at the higher concentration (10mm), followed by Staphylococcus aureus which caused inhibition zone, ranged from lower to moderate (8-15mm), while Klebsiella pneumoniae and Proteus vulgaris revealed moderate to high inhibition zone (18 - 25 mm and 13 - 25) respectively. In contrast the results obtained by gram negative bacteria clearly showed that Salmonella typhi has the highest inhibition zone (20 - 30 mm) compared with Escherichia coli which was caused inhibition zone, ranged from 10 - 16 mm. These results show that cumin oil has activity against Gram positive and Gram negative bacteria. The results of the present investigation agree with those of Sheikh [24], who reported that the cumin oil showed an antibacterial activity against Gram-positive and Gram-negative bacteria.

These results were comparable with standard antibiotic gentamicin which was showed inhibition zone ranged from 30-40mm. The study revealed the highest bacteria sensitive compared with standard antibiotic (Gentamicin) was Salmonella typhi (64.38%) followed by Klebsiella pneumonia (62.65%) while Enterococcus fecalis (16.67%) revealed high resistance compared with antibiotic. According to Lorenzetti, [25] myrcene has an analgesic effect and is likely to be responsible for the medicinal properties of lemon grass tea. It has anti-inflammatory properties through prostaglandin E2. P-cymene may have a potential anti-inflammatory action [26]. Both compounds (myrcene and p-cymene) were found in the cumin oil. The essential oils of herbs and spices are widely known for their strong antioxidant, antimicrobial and antifungal activities [27].

6. Conclusion and Recommendation

Antibacterial activity of extracted cumin oil was studied by selecting six types of bacteria (two gram negative and four gram positive) and found that the oil has an evident effect on
bacteria. Higher effect of cumin oil was appeared on Salmonella typhi and less impact was on Enterococcus Fecalis. The overall evaluation of this study concludes that the cumin have a good antibacterial activity potential. As cumin extract contains antiseptic, analgesic anti-inflammatory and anti-bactericidal constituents so that can be used in the preparation of medicinal drugs, principally, the study was showed it has high impact on the bacteria that cause typhoid disease.

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