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Original Article

Chemical Composition, Essential Oil Characterization and Antibacterial activity of Cumin (*Cuminum cyminum*)

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ABSTRACT

Family Apiaceous, an important source of bioactive compounds, used for the treatment of many diseases for ages. **Objective:** To explore active components of *Cuminum cyminum* essential oil. Endeavor of the current study was to investigate the phytochemical contents and antibacterial assessment of essential oil against gram positive and negative bacteria. **Methods**: Essential oil was extracted from seeds by hydro distillation, dried and stored at -4°C. Physical and chemical characterization was done. Antibacterial activity was also determined. **Results**: GC-MS analysis revealed major components culminal 35%, γ-terpinene 32%,γ-terpinene-al 7%, γ-terpinene 4.45%, daucene 4.3%, and trans-caryophyllene 5.342%, some trace components like myrcene 0.12%, 1-8 cineole, and γ-terpinene-7-al were also present. **Conclusion:** Chemical components (culminal, turpentine, daucene, caryophyllene) present in cumin essential oil are responsible for its biological activities.

INTRODUCTION

Belonging to the family *Apiaceous*, this plant is commonly termed as zeera, Camino or cumin, in varying culture and ethnicity. Its origin is Iran, Egypt, and the Mediterranean areas. Cumin is greenish-brown in its color, has some primary and less prominent secondary ridges with a hairy body, and bears a short stalk . Cumin is native to Mediterranean regions but has also been found in Asia since the 2nd century. The evidence of its presence is found in Rome and some parts of Africa. The annual production of cumin varies from 0.5 to 2.0 hundred thousand tones. Asia, Chile, Tajikistan, Uzbekistan, Burma, India, Central Asia, and Asian countries are the largest producers of cumin nowadays. Loamy, Fertile, and organic soil is needed, along with a proper irrigation system for its growth. Phytochemical analysis of the *Cuminum cyminum* confirmed anthraquinone, alkaloid, coumarin, glycoside, flavonoid, protein, saponin, steroid, tannin and resins. Organic acids such as citric, aspartic, tartaric, malic, ascorbic, propionic, oxalic, fumaric, and maleic acids were also isolated from the cumin seeds. Furthermore, there are 10% fixed oil and 2.5-4.5% EOs also present in cumin fruit. The chemical components of *Cuminum cyminum* essential oil were found to be different, depending upon the area from where the samples were collected. Major chemical constituents *Cuminum cyminum* are γ-terpinene, cuminaldehyde, β-pinene, limonene, and o-cymene . While the major chemical constituents of Egyptian cumin essential oil were tetradecane (12.25%), aldehyde (35.25%), β-ocimene (9.72%), γ-terpinene (12%), α-terpinyl acetate (5.32%), p-mentha-2-en-ol (9%), α-terpinolene (3%), myrcene (0.2%), limonene (0.5%), α-pinene (0.19%) and β-pinene (0.9%) . It has been reported by some researchers that the oil extracted from the cumin seeds and its alcoholic extract can inhibit the Klebsiella pneumonic growth and its clinical isolates by an improvement of capsule expression, decreasing the urease activity and altering cell morphology. The compound which is responsible for this activity is the Cuminaldehyde. Eugenol, α- pinenes, β-pinenes, limonene, and some other minor chemical constituents were found in the essential oil

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of cumin, and these compounds are suggested as primary active antimicrobial agents . However, phytoestrogens present in cumin oil has been reported for anti-osteoporotic effects . Oil extracted from the cumin is also observed to have an anti-epileptic effect, which involved in decreasing the frequency of the impulsive movement in experimental animals that are induced by the pentylenetetrazol (PTZ) . It was also found to act as a substantial analgesic by the formalin test in experimental rats. As it is involved in the suppression of morphine tolerance. Furthermore, it also reverses morphine dependence. With the use of Cumin EOS, the catalytic activity of Ca^{2+}-ATPase was found to be lowered or altered in the uterine tissue of ovariectomized rats that indicates the absence of any oxytocic/ ecobolic effect and this is an important property for the good uterine stimulating agent. Furthermore, studies have shown the estrogenicity of Cumin seed extracts. Further experimentation showed that a higher dose (250 mg) is more effective in eliciting this response in the uterine tissues . Further studies revealed that it also can improve the serum insulin as well as glycogen (liver and skeletal muscle content) in streptozotocin and alloxan-induced diabetic rats Cumin oil has strong antitussive and antioxidant potential. Cumin oil is needed for inhibition of lipid hydroxyl, scavenging superoxide radicals and inhibition of lipid peroxide in low quantity as compared to that of ascorbic acid. The EOs of cumin have high antioxidant activity primarily due to monoterpene alcohol, which is the main chemical constituent of cumin oil . Cumin, at an optimized dose (0.25g/kg) for forty-five days in rats, exhibited protection against the alcohol-induced iatrogenic hepatotoxicity. Cumin seeds are a rich source of dietary fibers. It has been assessed as a replacement supply of dietary fiber for its chemical characteristics, application potential and quality.

METHODS

Cumin seeds, collected from local market of Faisalabad, were dried and stored for essential oil extraction. Small batches of plant materials (1.5 Kg) were introduced in the Lab-scale hydro-distillation apparatus while in the pilot plant, a batch of 3 Kg plant samples was used. The experimental parameters, like temperature and time, were optimized for hydro and steam distillation during the present study . Extracted essential oil was dried and stored in dark colored glass bottles for further experimentation. Percentage yield, color, odor, density, optical activity, and viscosity were determined for evaluation of the quality of the essential oils .The phytochemical analysis was carried out to determine the alkaloids, flavonoids, and phenols .Chemical composition of essential oils as well as the major, minor and trace components were determined by GC-MS coupled with FID. GC only separates the components in this method, while MS analyses the components in complete .

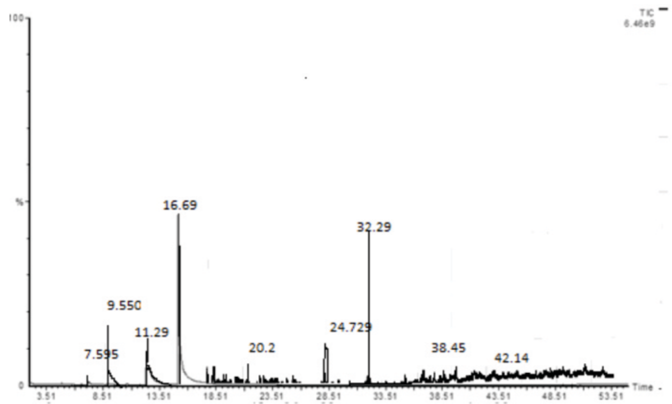

Figure 1: Gc chromatograph of cumin essential oil

Antibacterial activity, A) Disc Diffusion method: The goal of the study was to check the antibacterial action of cumin essential oil, the inhibition zone was determined using the method of disk diffusion. Nutrient agar was prepared autoclaved then poured into sterilized petri dishes. Bacterial strains *(S. aureus, B. subtilis, E.coli, P. aeruginosa)* were added to the agar in a homogeneous fashion, and in solidified media. Small, 6 mm in diameter filter paper disks, soaked 10µL of individual samples of essential oils. Standard antibiotics (Amoxicillin and fluconazole) used for effective regulation. Then the prepared petri-dishes were incubated at room temperature for 24 hours. After incubation, the Inhibition Zone was established on the media, measured in millimeters with digital Vernier caliper . MIC value was also determined using dilution method.. Bacterial Strains utilized for antimicrobial action, *Staphylococcus aureus (S. aureus)* ATCC 2592, *Pseudomonas aeruginosa* (P. aeruginosa) NCTC 1, *Bacillus* subtilis (B. subtilis) NCTC 10400, *Escherichia coli (E. coli)* ATCC 11078.`

RESULTS

Cumin essential oil is nearly colorless with a pleasant smell. Its yield is 2-3%, its insoluble in water but soluble in organic solvents like CCl_4 and alcohol etc.

Species	Color in daylight	Odor	Yield %	Specific gravity Kg/m3	Solubility In water	Solubility in alcohol
Cuminum cyminum cumin	Nearly Colorless	Pleasant and spicy	2.01±0.86	0.78±0.02	Insoluble	soluble

Table 1: Physical characterization of cumin essential oil

*Values represented as the mean± standard deviation. Cumin essential oil has medium thick consistent having viscosity 38.72, optical rotation is 35.5 at 25oC and refractive index value is 1.34 . Different tests were

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performed for checking the alkaloids, phenol, proteins, flavonoids and carbohydrates. Results showed the presence of all the phytochemicals[26].

Scientific Name	Oil Consistency	Viscosity kg/m/s	The optical rotation at 25°C	Refractive index value at 30°C
Cuminum cyminum	Thick	38.72	+35.5◦± 0.03	1.341±0.004

Table 2: Physicochemical characteristics of cumin seed essential oil

No	Phytochemical	quality	Test	Colour
1	Alkaloid	+	Dragendorff's	Orange
2	Phenol	+	FeCl3	Green
3	Protein	+	Biuret test	Purple color
4	flavonoids	+	Harborne	Yellowish
5	carbohydrates	+	Benedict test	Deep red

Table 3: Phytochemical contents of essential oil: Antibacterial action of Cumin essential oils against selected panel of microorganism

Essential oil and standard drug were tested against *Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Pseudomonas aeruginosa (P.aeruginosa) and Escherichia coli (E. coli).* Cumin essential oil showed significant antibacterial activity against both gram-positive and gram-negative bacterial strains. Standard drug data showed that it was effective against *S .aureus* and least against *E.coli* which is a resistant gram negative strain.

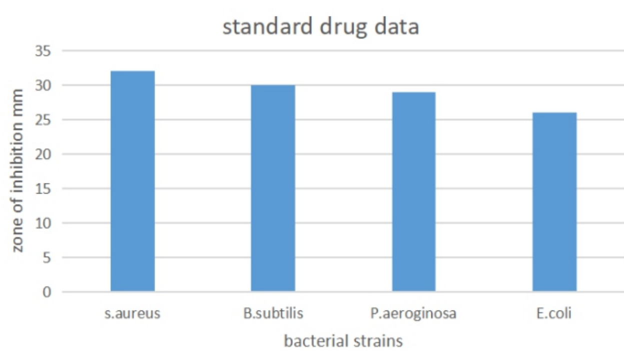

Figure 1: Standard drug against selected bacteria

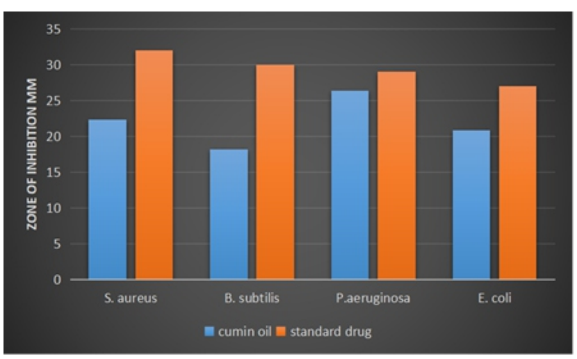

Figure 2: Comparison of antibacterial activity of standard drug and cumin E oil

DISCUSSION

Cumin oil showed the highest antibacterial activity against P. aeruginosa 22.3 ± 1.2mm value and lowest against Bacillus subtilis. Fractions of cumin were also effective against staphylococcus aureus, and their zone of inhibition values was lower to the values obtained by a standard drug used. The members of *Apiaceae* plants show intense antimicrobial effect against all the strains of bacteria (gram-positive and gram-negative). Their defense action is due to the property of essential oil being hydrophobic and disrupting the cell membrane. It is generally observed from results that gram-positive are more susceptible to the attack of essential oil, and gram-negative are more resistant. But *Apiaceae* plant members have proven that they are broad-spectrum and can inhibit the growth of bacteria at even lower concentrations. The resistance of gram-ve bacteria can be due to the complexation of the bacteria's outer wall with an outer membrane, thereby preventing the passage of the bacterial wall by lipophilic essential oil. In a study on cumin essential oil, it was revealed that *Cuminum cyminum* possess significant antimicrobial action against pathogenic microbes with the same concentration of extracts (0.5 mg / mL) . Another research, the essential oil showed marked inhibition of the Gram-positive Staphylococcus aureus bacteria, Bacillus and Gram-negative *Escherichia coli* bacteria, and the findings were compared with standard values. The antibacterial effect of the cumin extract was tested against some pathogens. *E. coli, S. aureus,* and *S. fecalis* is prone to different concentration of the oil. Which were immune to Pseudomonas aeruginosa and Klebsiella pneumoniae. In another research work average antibacterial/ antifungal activity was shown by essential oil, comparable to standard drug . *C. cyminum* established effectiveness against definite bacterial and fungal strains, respectively. Gram-positive *B. subtilis* with the largest zone of inhibition of 27.3 mm followed by a minimum MIC value of 1.10 mg / mL. The action was greatly improved than the antibiotic action of Rifampicin, that show the 10.8 mm and 1.72 mg / mL inhibition region. In a different study, in vitro examination of antibacterial activities of different essential oils were carried out against particular microorganisms, and it was found that *C. cyminum* essential oil was the most successful inhibitor after oregano, showing inhibition zones ranging from 31,23 mm in *Lactobacillus sakei* to 38,17 mm . Cumin oil demonstrated greater potential as an effective antibacterial agent against Vibrio spp. The oil retarded the growth of these strains and the diameter of inhibition zone was from 12-24 mm and MBC & MIC values (0.078–0.31 mg / ml) to (0.34–1.31 mg / ml), respectively.

CONCLUSION

Cumin seed essential oil has dietary fibers, alkaloids,

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steroids, carbohydrates and many chemical components present.

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