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In Vitro Antibacterial Potentials of Various Extracts of Rosemary Leaf Powder and Black Cumin Seed Powder

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ABSTRACT

The present investigation was undertaken for *in vitro* screening of antibacterial activities of acetone, benzene and ethyl acetate extract of Rosemary leaf powder and Black cumin seed powder. *In vitro* antibacterial efficacy of selected plants was assessed by well diffusion method against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The results showed that ethyl acetate and acetone extract of black cumin seed powder were most effective against the *Staphylococcus aureus.* The ethyl acetate extracts of Rosemary leaf powder was most effective against the *Escherichia coli*, while benzene extract of both herbs remained ineffective against *Escherichia coli*. The ethyl acetate extracts of both herbs were effective against *Pseudomonas aeruginosa*, whereas acetone and benzene extracts of both herbs remained ineffective against *Pseudomonas aeruginosa* respectively. The Present study showed that these plants possess compounds with antibacterial activity.

HIGHLIGHTS

- Rosemary leaf powder extracts showed effective antimicrobial activity against *S. aureus* and *E. coli.*
- Black cumin seed powder extracts showed effective antimicrobial activity *S. aureus.*

Keywords: Rosemary leaf powder, Black cumin seed powder, Antibacteria

Foodborne illness is still a thought for both consumers and the food industry despite the use of various preservation methods. The extreme utilization of antibiotics, improper treatment, long-term consumption and prevention measures are the offenders that accelerate the resistance of different pathogenic microorganisms to commercial antibiotics (Soni and Sosa, 2013). The increasing antibiotic resistance of some pathogens is considered as a global health threat that requires extensive and collaborative researches to find an alternative source of antimicrobial products without any side effects to synthetic chemical treatments (Ugur *et al*., 2016). Plant metabolites have a unique pharmacological value for scientific and clinical research (Shraddha *et al*., 2017; Meena, 2017). Identification of their phytochemical activity against several pathogenic microorganisms results in treatment of various diseases (Gupta *et al*., 2017; Sharma, 2017; Marwaha, 2018). Rosemary leaf have following properties; antioxidative, antimicrobial, antidiabetic (Bakirel *et al*., 2008), antitumour, chemopreventive and anti-inflammatory (Cheung and Tai, 2007) etc. Black cumin seed possess antibacterial activity against Gram positive and negative bacteria (Hanafy and Hatem, 1991), antioxidant (Mariod *et al*., 2009), immune- potentiating (Al-Mufarrej, 2014, Kumar *et al.,* 2017a) activities. The black cumin seed extracts and their oil have a range of other activities, including effects on physio-pathologies, e.g., anti-inflammatory, anti-diarrhoeal properties, and activity in different body systems, e.g., endocrine and immune system (Gilani *et al*., 2004). Keeping in mind the infectious diseases, transmissible diseases, resistant

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pathogenic organism and side effect of antibiotics an effort was needed to determine the antimicrobial activity of phytochemical agents. Therefore, the current study was focused to investigate the effects of acetone, benzene and ethyl acetate extract of rosemary leaf powder and black cumin seed powder against growth of *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Collection of plant material

Rosemary leaf powder and black cumin seed powder were purchased from the shop of herbal medicine. Crude plant extract was prepared by Soxhlet extraction method. Five grams of powdered rosemary leaf powder and black cumin seed powder filled in thimble directly were placed in soxhlet apparatus, and extracted separately using acetone, benzene and ethyl acetate extract for 24 hrs or until the solvent in siphon tube of an extractor become colorless. The extracts were than concentrated in pre-weighted vials on a rotary evaporator below 50°C. Dried extract was weighted and reconstituted with known volume of solvent and were stored in vials at 4°C for further studies.

Screening of plant extracts for antibacterial activity

Antimicrobial activities of different extracts were studied by the well diffusion method. The pure cultures of bacteria maintained in the nutrient broth medium. The test organisms used are *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa Salmonella typhi.*

Preparation of Inoculums

Stock cultures were maintained at 4°C in nutrient broth. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated without agitation for 24 h at 37°C. Media was prepared by dissolving 0.5% Peptone, 0.3% beef extract/ yeast extract, 1.5% agar, 0.5% NaCl and dissolved in 100 ml distilled water and autoclaved at 121°C for 15 min.

Antibacterial Susceptibility Test

Standard well diffusion method was carried out to screen the antibacterial activity. *In vitro* antibacterial activity was screened by using nutrient agar media. The nutrient agar plates were prepared by pouring 10 ml to 15 ml of molten liquid media into sterile petri plates. The plates were allowed to solidify for a few minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 10 min. Wells were prepared on agar plates and 100 µl extract and solvent in control well was inoculated and the plates were kept for incubation at 37°C for 24 h. At the end of incubation, inhibition zones formed around the wells were measured with transparent ruler in millimeter.

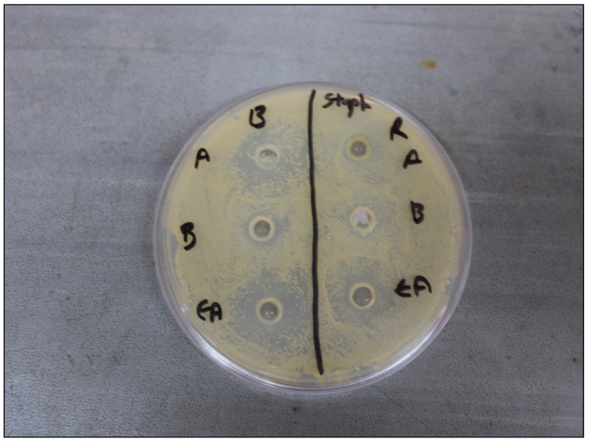
RESULTS AND DISCUSSION

In the present investigation, *in vitro* antibacterial activity of the various crude extracts of Rosemary leaf powder and Black cumin seed powder was qualitatively assessed based on the zone of inhibition. The zones of inhibition in diameter (mm) recorded for acetone, benzene and ethyl acetate extract and have been presented in Table 1 and Fig. 1.

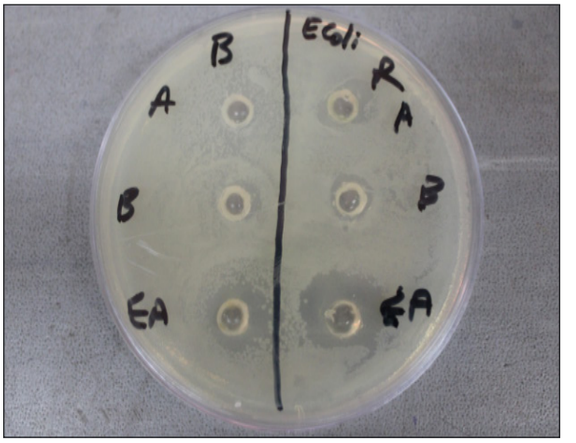
Table 1: Mean inhibitory zone (mm) induced by the various crude extracts of rosemary leaf powder and black cumin seed powder against tested microorganism

Microorganism		*Staphylococcus aureus*	*E. coli*	*Pseudomonas aeruginosa*
Rosemary leaf powder	Acetone	10	5	—
	Benzene	—	—	—
	Ethyl Acetate	13	13	8
Black Cumin seed powder	Acetone	13	5	—
	Benzene	—	—	—
	Ethyl Acetate	14	11	8

Against *Staphylococcus aureus* highest (14 mm) zone of inhibition was observed in ethyl acetate extract and acetone extract (13 mm) of black cumin seed powder followed by ethyl acetate (13 mm) extract and acetone extract (10mm) of Rosemary leaf powder, while no zone of inhibition was observed in benzene extract of Rosemary leaf powder and Black cumin seed powder. The highest (13 mm) zone of inhibition against *Escherichia. coli* was observed in ethyl acetate extract of Rosemary followed by ethyl acetate extract (11 mm) of Black cumin. Against *Escherichia*



Staphylococcus aureus in acetone (A), benzene (B) and ethyl acetate (EA) extracts of black cumin and rosemary



Escherichia coli in acetone (A), benzene (B) and ethyl acetate (EA) extracts of black cumin and rosemary

Pseudomonas aeruginosa in acetone (A), benzene (B) and ethyl acetate (EA) extracts of black cumin and rosemary

Fig. 1: A figure showing antibacterial activity of Rosemary leaf powder and Black cumin seed powder

coli lowest zone of inhibition (5 mm) was recorded in acetone extract of both Rosemary and Black cumin seed powder, while no zone of inhibition was observed in benzene extract of both Rosemary leaf powder and Black cumin seed powder. Against *Pseudomonas aeruginosa,* the highest zone (8 mm) of inhibition was observed in ethyl acetate extract of both Rosemary and Black cumin seed powder, while no zone of inhibition was observed in benzene and acetone extract of both Rosemary leaf powder and Black cumin seed powder.

The result of antimicrobial study showed that ethyl acetate and acetone extract of Black cumin and Rosemary were most effective against the *Staphylococcus aureus,* while the ethyl acetate extract of the same was also effective against the *Escherichia. coli* and *Pseudomonas aeruginosa*. Benzene extract of both the herbs remained ineffective against *Escherichia coli* and *Pseudomonas aeruginosa.* Acetone extract of both the herbs remained ineffective against *Pseudomonas aeruginosa,* respectively. Several workers had reported the anti-microbial activity of various extracts of Rosemary and Black cumin against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas Aeruginosa* such as (AbdEl-Hamied *et al.* (2012), Abdel-Raouf *et al.* (2014), Kumar *et al.* (2019), and Gazwi *et al.* (2020) which support the present findings.

Ahead, the results are in line with the results of AbdEl-Hamied *et al.* (2012) who reported that the essential oil of cumin had the best inhibitory effect against Gram-negative bacteria (*Escherichia coli O157H7, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi and Serratia marcescens*) and Gram-positive bacteria (*Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus and Streptomyces sp.*). Meanwhile, the essential oil of Rosemary exhibited good antibacterial effect against all of these bacteria except *Listeria monocytogenes and Pseudomonas aeruginosa* Gazwi *et al*. (2020) evaluated antibacterial activity of rosemary against *Bacillus cereus* and *Vibrio fluvialis*, *Escherichia coli*, *Enterococcus faecalis, Staphylococcus aureus, and Candida albicans, whereas* no inhibition zone was reported against *Pseudomonas aeruginosa*. Erdogrul *et al.* (2009) find out antibacterial activity of ethyl acetate, methanol and hexane extract of black cumin seed against *Staphylococcus aureus*, Similarly, ethyl acetate and hexane extract also have antibacterial activity against *Pseudomonas aeruginosa.* Abdel-Raouf *et al.* (2014) showed that the ethanolic extract of black cumin was moderately effective against *Salmonella typhi* and *Staphylococcus aureus*, whereas, no inhibition zone was observed against *Pseudomonas aeruginosa.* Kumar *et al.* (2019) was found that benzene and ethyl acetate extract of black cumin seed powder were most effective against the *Salmonella typhi*, while the ethyl acetate extracts of black cumin seed powder was most effective against the *Staphylococcus aureus*, whereas benzene extract of Black cumin seed powder and the all three extracts of the herbs remained ineffective *against Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively.

Phytochemicals in herbs alter fatty acid composition, which may influence surviving ability of microbes. The components permeate the cell membranes and mitochondria of the microorganism and inhibit the membrane bound electron flow hampering energy metabolism. The antibacterial activity against microorganisms obtained in present investigation might be due to any one or more phytochemicals present in rosemary leaf powder and black cumin seed powder.

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