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ORIGINAL ARTICLE

Antibacterial Activity of *Cuminum cyminum* and *Piper nigrum* against antibiotic resistant *Klebsiella pneumonia*

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ABSTRACT

Herbal medicines are the major remedy in traditional medical systems from thousands ofyears and made a great contribution in maintaining human health and in preventing many infectious diseases. The present study was carried out to determine the potential antibacterial effect of ethanol extracts and of Cuminum cyminum and Piper nigrum against Klebsiella pneumoniae which is antibiotic resistant. All 20 isolates of Klebsiella Pneumonia isolated from urine culture of hospitalized patients suffered from urinary tract infections were evaluated. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plants extract against bacteria were determined using micro dilution broth method. The result show that the least MIC value of alcoholic extract of C.cyminum was 1.25 mg/ml and the highest MBC value of alcoholic extract of C.cyminum were 10 mg/ml. The least MIC and MBC value of alcoholic extract of P. nigrum were 0.62 mg/ml.

Keyword: Antibacterial activity, Cuminum cyminum, Piper nigrum, Klebsiella pneumonia

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INTRODUCTION

Medicinal plants is very important one in human health, it will act as an antibactericide activity against the bacterial pathogens, this is followed from ancient times (1).Plant extracts have numerous health-related effects such as antibacterial, antimutagenic anticarcinogenic, antithrombotic and vasodilator activities (2.) Among the plants investigated to date, one showing enormous potential is the pepper family otherwise known as Piperaceae (3). The fruit contain 1% volatile oil, resin, a waxy alkaloid. It is used for several medicinal properties. It has much pharmacological action such as antifungal, anti-inflammatory, antioxidant and anti- cancer effect (4) and it is known to have insecticidal activity against mosquitoes and flies. *Piper nigrum* fruits are also used to produce white pepper and green pepper and are valued due the presence of piperine including its different isomers (5). Black pepper can be used for different purposes such as human dietaries as medicine, as preservatives, as biocontrol agent (6 & 7). *P. nigrum* fruits are anthelmintic, antiasthmatic and used to treat pain, piles,insomnia, and epilepsy (8*). Cuminum cyminum a* member of the family Apiaceae is an herbaceous annual plant with short leaves of about 5-10 cm. The fruit contains a single seed which is yellow brown in colour. Seeds are used in mixed soups, pickles, cheese, meat dishes and candies (9). The essential oil yield of cumin is 2.5 to 4% of the weight of the fruits and the main constituent is cuminol. The present study was carried out to determine the potential antibacterial effect of ethanol extracts and of *Cuminum cyminum* and *Piper nigrum* against *Klebsiella pneumoniae* which is antibiotic resistant.

MATERIAL AND METHOD

***Plant materials**:*

Theseed of *Cuminum cyminum* and fruit of *Piper nigrum* were collection in the region of Iran and dried at room temperature .Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of extracts:

Plants were properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethanol95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4^0C in air tight screw-cap tube.

Isolation of bacteria:

All 20 strains of K. *pneumoniae*isolated from urine culture of hospitalized patients suffered from urinary tract infections were evaluated. Isolated bacteria were identified by Gram's stain.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts:

The broth microdilution method was used to determine MIC and MBC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/ml to 10.00 mg/ml. To each well, 10 µl of indicator solution (prepared by dissolving a 10-mg extract in 2 ml of DMSO) and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^4 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The colour change was then assessed visually. The lowest concentration at which the colour change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

RESULTS AND DISCUSSION

In the study result show that different inhibitory effects of alcoholic extract and essential oil from *c.cyminum against* most *K. pneumoniae* isolates were demonstrated in table 2, 3.The results in tables 1 and 2 showed that ethanol extract of *C. cyminum and P.nigrum* had inhibitory effect against most isolated plates. The least MIC value of alcoholic extract of *C.cyminum was* 1.25 mg/ml and the highest MBC value of alcoholic extract of *C.cyminum were* 10 mg/ml. The least MIC and MBC value of alcoholic extract of P. nigrum were 0.62 mg/ml. The study of Soniya, the result show that plant extracts showed strong antibacterial action against pathogens among the plant extracts the methanol extracts of d *Cuminum cyminum* against E. coli while aqueous extracts of *Piper nigrum* gave maximum inhibition against Proteus sp(10). The study of Stefanini, the essential oil of *Cuminum cyminum* was active against the gram - organisms *E. coli*, *P. aeruginosa* and *Salmonella* sp., with inhibitory zones of 18 mm, 10 mm and 23 mm, respectively (11). The study of Keskin, the methanol extracts showed no inhibition against R. rubra like ethyl acetate extracts. But the acetone extracts did not inhibit Faecalis and Rubra(12). It can be suggested that the inhibitory effect of cumin might be due to carvone and carvacrol contaiened in its volatile oil as reported by Ouattara et al. (13). The inhibitory effect of cumin extract on E. coli 0:157 demonstrated *in vitro* (14). According to Shetty *et al.* (15), fungi and yeast were more sensitive to cumin essential oil as compared to bacteria.The study of Erturk, the result show that, P. aeruginosa was the most sensitive bacterial strain to P. nigrum and E. arborea extracts among both Gram-positive and Gram-negative bacteria tested with MIC of 5 mg/mL (16). Aqueous decoction of black pepper has maximum effect against Staphylococcus aureus (17). The study of Karsha, the results indicate excellent inhibition on the growth of Gram positive bacterial like Staphylococcus aureus, followed by Bacillus cereus and Streptococcus faecalis. Among the Gram negative bacteria Pseudomonas aeruginosa was more susceptible followed by Salmonella typhi and *Eschericha coli* (Karsha et al., 2010).

Table1: Minimum inhibitory concentration C.cyminum extract of against Klebsiella pneumonia

10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.62mg/ml	0.3mg/ml	
0	40%	50%	10%	0	0	MIC
25%	60%	15%	0%	0	0	MBC

Table2: Minimum inhibitory concentration P.nigrum extract of against Klebsiella pneumoniae

10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.62mg/ml	0.3mg/ml	
0	20%	35%	25%	20%	0	MIC
20%	30%	20%	20%	10%	0	MBC

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