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Evalution of Antibacterial Activity of *Piper nigrum* Extract against *Streptococcus mutans* and *Escherichia coli*

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Abstract

Medicinal plants are very popular in different traditional systems of medicines due to their diverse pharmacological potentials and lesser side effects in biological systems. Black pepper **(*Piper nigrum* L.)** is a natural plant medicinal agent used to treat many diseases . The spicy tang of pepper is due to the presence of piper amides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit. Among them piperine and its isomers are the major factors responsible for the pungency and irritant action of black pepper. In the present study Black pepper was evaluated for its antimicrobial activity against ***Streptococcus mutans*** isolated from patients with tooth decay and inflammation of the teeth and ***Escherichia coli*** isolated from patients with diarrhea, By use agar well diffusion method and microtiter plate method. The results of the study showed that the maximum inhibitory region was against Streptococcus mutans (29mm) and the minimum was against Escherichia coli (8mm). As conclusion significant activity of Black piper that it be used as a natural antimicrobial agent.

Keywords: *Piper nigrum*; *Streptococcus mutans* , *Escherichia coli*, Antimicrobial activity.

INTRODUTION

Piper nigrum, commonly known as ``Black-pepper``, has gained a global consideration because of its volume in the spice industry. (1) . It The aromatic or pungent vegetable substances used in flavouring foods and food preservations, medicinal preparations, cosmetics , bakery goods and various other products(2). where Pepper's pungency was found in 1821 . Historically, it has been thought to cure many illnesses such as cancer, malaria and cholera (3). and also nausea, fever, migraine headaches, poor digestion, strep throat and even coma (4).

Piperine is an alkaloid found naturally in plants belonging to the pyridine group of family **Piperaceae**, such as ***P. nigrum***.(5) and contains some of the antimicrobial components such as Terpinene, α-pinene, β-pinene, Linaleol and Terpineol (6), (7), Piperine, pipene, piperamide and piperamine it have possess diverse pharmacological activities (8),(9). Piperine has been found to enhance the therapeutic efficacy of many drugs, vaccines and nutrients by increasing oral bioavailability by inhibiting various metabolising enzymes(10),(11),(12). And there is preclinical evidence that it may have modest immune system Yenhancing properties.(13),(14).

Pepper based irritants and searing chemicals are utilized in a variety of liniments and mouth gargles. And commercial use as chemical carminatives (15).

Studied the antimicrobial activity of black pepper fruit extract against some microorganisms by (16) and found that phenolic compounds present in the black pepper fruit extract. They found that black pepper fruit extract control the microorganisms. (17)

In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Extracts of plants contain variety of phenolic compounds and essential oils which may inhibit the growth of some microorganisms. In the last few years, antimicrobial properties of plants essential oils (EOs) have been investigated through several observations and clinical studies which purpose them as potential tools to overcome the microbial drug resistance problem (18). such as used alcoholic extract and essential oil of Black pepper and effective against growth/activity of ***E. coli*** and ***S. aureus*** where piperine and oleic acid were major components in phytochemical analysis. (19).

Escherichia coli is a member of the normal flora of the human and animal gastrointestinal tract, and its several pathogenic types can cause different diseases. *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC) strains have emerged in recent years as important human pathogens associated with a spectrum of diseases ranging from diarrhea to hemorrhagic colitis and hemolytic-uremic syndrome (HUS). The production of Shiga toxins by *E .coli* O157:H7 has a major role in pathogenesis, particularly in the pathogenesis of HUS.(20),(21). On the other hand ***S.mutans*** is considered one of the primary causative agents of dental caries and can also be a source of infective endocarditis. The main virulence factors associated with cariogenicity include adhesion, acidogenicity, and acid tolerance. Each of these properties works coordinately to alter dental plaque ecology. The ecological changes are characterized by increased proportions of ***S.mutans*** and other species that are similarly acidogenic and aciduric. The selection for a cariogenic flora increases the magnitude of the drop in pH following the fermentation of available carbohydrate and increases the probability of enamel demineralization.(22). The present study was aimed to study the Antibacterial Activity of the *P.nigrum* extract against some bacteria such as *S. mutans* and *E. coli*.

METHODS :-

Bacterial isolates

Three bacterial isolates were selected for each bacterial species isolated from patients with tooth decay, tooth disease and diarrhea to test the Antibacterial activity of the *P. nigrum* extract.

Preparation of extracts (Extraction of piperine from Black pepper)

Plant materials (black pepper seeds)

Plant material ,The seeds of *P. nigrum* were collected from local market. Then they were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The seeds were subsequently dried in a hot air oven at 40℃ for 48h, Then The dried seeds were grinded in a mill which is an electrically operated to produce fine particle size (powdered) which used for extraction. Extraction and isolation were performed according to the method described by (23), (17)

Preparation of crude extract 50gm of dry powdered fruits of *P.nigrum* were extracted successively with ethanol (95%) (each 400ml.) in a Soxhlet apparatus for 3 hours . Then collected solutions were filtered through Whatman No-1 filer paper, and concentrate under vacuum on water bath at 60ºC, Then (10ml of 10%) alcoholic potassium hydroxide KOH are added to filtrate residue with constant stirring and filter. Allow the alcoholic solution to stand overnight whereupon needles of piperine separate out. Piperine was found to be soluble in various nonpolar solvents but insoluble in polar solvent like water. The proposed method for extraction and characterization lead to rapid (15) ,The extracts were evaporated to dryness under reduced pressure at 900C by Rotary vacuum evaporator to obtain the respective extracts and stored in a freeze condition at −180C until used. (5). Yielding (1.5gm) of piperine alkaloid.

Thin Layer Chromatography and HPLC a particularly valuable qualitative methodes for determination of small amount of impurities and frequently used for evaluating medicinal plant materials. (15)

Agar Well Diffusion Method:

The diffusion method was used to detect the inhibitory activity of the piper extract against *S.mutans* and *E. coli*.

1- The bacterial strains were diluted with the Normal saline to make solution equal to McFarland standard tube (24)

2- 100µL of bacterial suspention was spread on surface of **Blood Agar** medium in 100µL/per dish using sterile cotton swabs and leave 15 min. in room temperature .

3- A cork borer was used to make holes (6 mm) on the surface of the agar. Each hole was filled with 50µL of each concentration (5, 2.5,1.25)% . Two walls used as a control , one of them fill with 95% methanol (negative control) and other fill with antibiotic solution as (positive control).

4- The dishes were incubated at 37Cº for 24 hours.

5- Inhibition zones were measured around the holes and compared with the control coefficient containing 95% methanol alcohol (25) ,(26).

Microtiter plate method(96 well)

1-The bacterial suspension was compared with the McFarland standard tube and each constration repated (replicated) three time (24).

2-The concentrations (5, 2.5, 1.25) were prepered from the stock solution of (pepper extract) and each was sterilized by millipore filter 0.22 µm.

3- (180µL) of Mullur-Hinton broth was in each hole and then (10µL/) of extract and 10 microliters of bacterial suspension were added to the previous well. The plate was incubated for 24 hours at 37 C^0 and attended with a repeat rate of each concentration with antibiotic ampicillin 32% use as a positive control treatment and 95% methanol alcohol as a negative control treatment (27).

Statistical Analysis

The data obtained were analyzed by using SAS software version 9.1, 2003,USA

RESULTS

Well diffusion method

In the present study the antibacterial effect of piperine is showed in table**(1)** Piperine showed antibacterial activity against test Gram positive bacteria ***S.mutans*** with zone of inhibition ranged from (11mm-29mm) According to the concentration of the extract , And showed table**(2)** antibacterial activity against Gram negative bacteria ***E.coli*** with zone of inhibition ranged from (8mm-14mm) According to the concentration of the extract. for all the bacterial cultures. It indicates that the inhibition zone of increases as the concentration of piperine increased. And Fig1: showed comparison between inhibitory areas of *P. nigrum* extract against *E. coli* and *S. mutans* .

Microtiter plate 96well method

The concentration(5,2.5,1.25)% was used in the microtiter platel. The test showed that the minimum inhibitory concentration was at concentration (2.5)% for *S.mutans* while was (5)% concentration for *E.coli* **(Fig 2).** And table**(3)** showed The minimum inhibitory concentration in the Microtiter plate method.

Table (1) Diameters of the inhibitory zones of the piper extract against *Streptococcus mutans*

Con	Diameter of inhibition zones ***S. mutans***
5	29
2.5	24
1.25	11
Control+	5
L.S.D(0.05)	6.9818

Table (2) Diameters of the inhibitory zones of the piper extract against *Escherichia coli* in well diffusion method.

Con%	Diameter of inhibition zones (***E. coli***)
5	14
2.5	9
1.25	8
Control+	4
L.S.D(0.05)	3.3943

Table(3)The minimum inhibitory concentration in the Microtiter plate method

Con%	*S. mutans*	*E. coli*
5	_	_
2.5	_	+
1.25	+	+
Control+	+	+

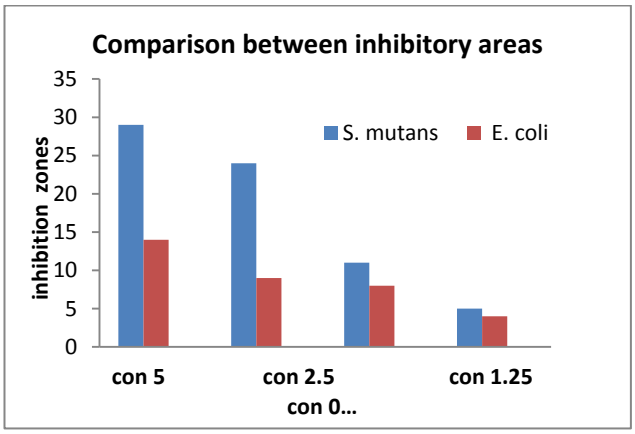
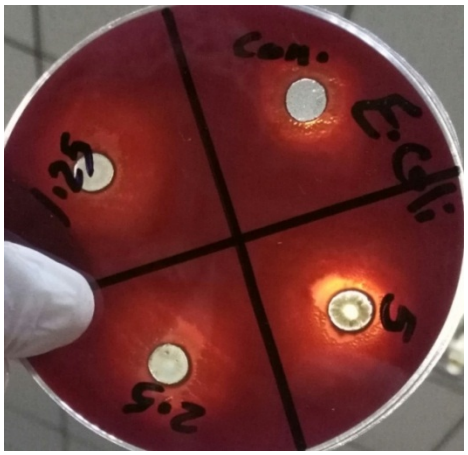


Fig1: Comparison between inhibitory areas of *Piper nigrum* extract against *E. coli* and *S. mutans* .

A



B

Fig2:Diameters of the inhibitory zones to A-Gram positive bacteria B- Gram negative bacteria

DISCUSSION

It is known that the quality of the solvent affects on the degree of inhibitory effectiveness of the extract. Ethanol is an organic solvent, so it is efficient in dissolving the organic compounds present in the medicinal plant (28), and liberation of the antimicrobial components. (15).

The effect of the extract may be due to the structure of the gram-positive bacteria wall, as it lacks a layer of external membranes that makes the permeability of the material into the cell larger, and through from the results the plant is effective against gram positive bacteria. This is consistent with the researchers by inhibiting the formation of the cell wall of the microorganism, inhibition of the synthesis of some of the basic proteins in it and the formation of complexes with the cellular wall impede the regularity of permeability and inhibition of some enzymes with the important metabolic role in the growth and reproduction and the disruption of cellular membranes and change function. (30), (31).

The results showed that Gram positive bacteria were more sensitive to pepper extract in compared with gram negative bacteria and this is consistent with (32) ,(33). the variation in the inhibition among the gram positive and gram negative bacteria is due to the cell wall and cell membrane compositions. According to (32). The effect of anti-bacterial peppers may be due to analysis of the plasma membrane and cellular wall (33), and this is consistent with (30),(31),(29). However, there are reports of occurrence of antibacterial activity against *S. aureus, E. coli, B. megaterium, B. sphaericus, B. polymyxa* .

Antimicrobial activity of piperine was increases when the concentration increases against both for bacteria and fungi. It is also supports the earlier investigations. (34),(35),(29). This is agree with (36) Which he mentioned that Secondary metabolites from *P. nigrum* play defensive role against infections by microbes, insects and animals Efforts have been made in screening these chemicals against different pathogenic species of microorganisms (37),(1) and agree with study that was reported Black pepper in to slow the growth of a variety of types of bacteria isolated from human volunteers. (29).

Also (38) reported that piperine present in black pepper fruit has antimicrobial activity. Also with (39) which he mentioned that black pepper (aqueous decoction) showed strongest antibacterial activity and at the concentration of 10µL/disc in research against different bacterial isolates from oral cavity of two hundred individual volunteers.

The proposed method of extraction and characterization leads to a fast, accurate and sensitive method requiring less solvent. To use is an active composite Against bacterial microbes.

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