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Antibacterial Activity of Piperine and Black Pepper Oil

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Black pepper (*Piper nigrum* L.) is known as king of spices and it's sharp taste is due to the presence of piperine which is the main bioactive alkaloid in the fruit. In the present study both of piperine and black pepper oil in different concentrations evaluated for their antimicrobial activity against *Staphylococcus aureus* (G+ coccoid shaped bacteria), *Bacillus subtilis* (G+ long spore forming bacteria), *Salmonella sp* and *E.coli* (G- short rod bacteria). The inhibition activity was measured by using agar well diffusion method. Piperine and black pepper oil showed antibacterial activity with all tested Gram positive bacteria with zones ranged from 8.23-18.1mm and 3.14-10.43,respectively. The results showed that piperine is an excellent antibacterial agent with all tested bacteria.

Keywords : Antimicrobial, spices, herbs, black pepper seeds, black pepper oil and piperine.

Food products should be protected against microorganisms during storage to prevent contamination. Synthetic preservatives have serious health problems with repeated use. So,consumers nowadays concern their efforts to use new natural food additives. Spices and herbs have been used for countries by many cultures enhance the flavor and aroma of foods. In addition, they have used for preserving foods and as natural antimicrobials[11]. Pepper is most commonly used in the prescription of ayurvedic and other traditional medicinal systems[6]. The oleoresin of black pepper ranged between 10 and 15 percent and it contains volatile oil non volatile oils,resins,fixed oil, colors,sugar etc. The volatile oil lies between between 15 to 35 ml/100g. Pepper ethanol extracts and volatile oils can preserve food from spoilage[12,14]. Piperine found naturally in plants belonging to the Piperaceae family,such as *Piper nigrum* L, commonly known as black pepper . It is the major alkaloid compound accumulate in the skin and seeds of the fruit. It is a solid substance essentially insoluble in water[1,7] Figure 1:

Alkaloids such as piperine have been investigated for their biological and antibacterial activities[9,16]. The essential oil of black pepper oil was evaluated for potential inhibition of spoilage moulds in sunflower oil which coated cheeses[17].

MATERIALS AND METHODS

Materials

Plant Material

Black pepper (*Piper nigrum* L.) seeds were collected from Mansoura markets, Dakahlia, Egypt.

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Microbial l Cultures

Four pathogenic bacterial strains were used. These strains were four bacterial strains namely *Staphylococcus aureus* (G+ coccoid shaped bacteria)*, Bacillus subtilis* (G+ long spore forming bacteria), *Salmonella sp* and *E.coli* (G- short rod bacteria).

Methods

Isolation of piperine from black pepper

10 gm black pepper were ground well and extracted with 150 ml 95% ethanol in a soxhelt extractor for 2 hours , the solution is filtered and concentrated in vacuum on a water bath at 60Úc . 10 ml of 10% alcoholic KOH are added to the filtrated residue. The alcoholic solution is left overnight , where upon yellow needles of piperine are deposited,the yield is a bout 0.3 gm[5].

Extraction of oil

The seeds were sieved , dried at 120°C for 2hours and then the oil was extracted by mechanical pressing.

Assessment of antimicrobial activities

The antibacterial activity of both piperine and pepper oil was evaluated by using agar well diffusion method. Bacterial cultures are mixed in nutrient agar medium and poured in petriplates. Wells of 5mm size were made with sterile borer into agar plates containing the bacterial inoculum. 2mg of crude piperine was completely dissolved in 2ml of Di Methyl Sulfoxide(DMSO). Antibacterial activity was measured by adding 0.05 ml from both of piperine and pepper oil described by[13] with different concentrations as 0.2 ,0.3 and 0.5% into the wells and left them for one hour to allow diffusion, then incubated at appropriate temperature and period of time. At the end of incubation period, the inhibition zones of microbial growth were measured and recorded[2]**.**

RESULTS AND DISCUSSION

The effect of piperine and black pepper oil as antibacterial agents were listed in the Tables

Table 1. Inhibition effect of black pepper oil extract on bacteria *in vitro*

Concentrations	Procaryotic cells			
	G+		G-	
	Staphylococcus aureus	*Bacillus subtilis*	*Salmonella sp*	*E.coli*
0.2%	8.34	8.23	3.4	5.0
0.4%	12.3	9.0	5.23	6.42
0.5%	18.1	14.15	9.11	8.4

•Values expressed the diameter of inhibition zone in Cm
• G+:Gram positive cells
• G-: Gram negative cells

Table 2. Inhibition effect of piperine extract on bacteria *in vitro*

Concentrations	Procaryotic cells			
	G+		G-	
	Staphylococcus aureus	*Bacillus subtilis*	*Salmonella sp*	*E.coli*
0.2%	3.14	5.42	-	-
0.4%	5.22	6.13	-	-
0.5%	10.43	8.34	-	
-				

Values expressed the diameter of inhibition zone in Cm
Data given are mean of triplicates
G+:Gram positive cells
G-: Gram negative cells

(1 and 2) and Figures (2 and 3). It could be noticed that antibacterial activity of piperine and black pepper oil increase as the concentrations increases. It is supporting the view of[3,4]. On the other hand, the maximum zones of inhibition were recorded with both of piperine and black pepper oil at the concentration of 0.5% against all Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis)* with zones of 18,1,14,15;10.43 and 8.34mm, respectively. Black pepper oil with all concentrations gave no zones of inhibition against all the Gram negative species and that was inagreement with[10] who confirmed that spices like turmeric and ginger extracts have potent antibacterial activity against Gram positive more than Gram negative bacteria through individually and by mixtures. The variation in the inhibition among the Gram positive and Gram negative bacteria is due to the cell wall and cell membrane

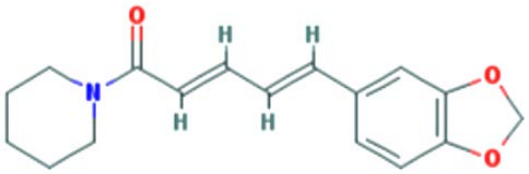

Fig. 1. Piperine molecular Formula: $C_{17}H_{19}NO_3$

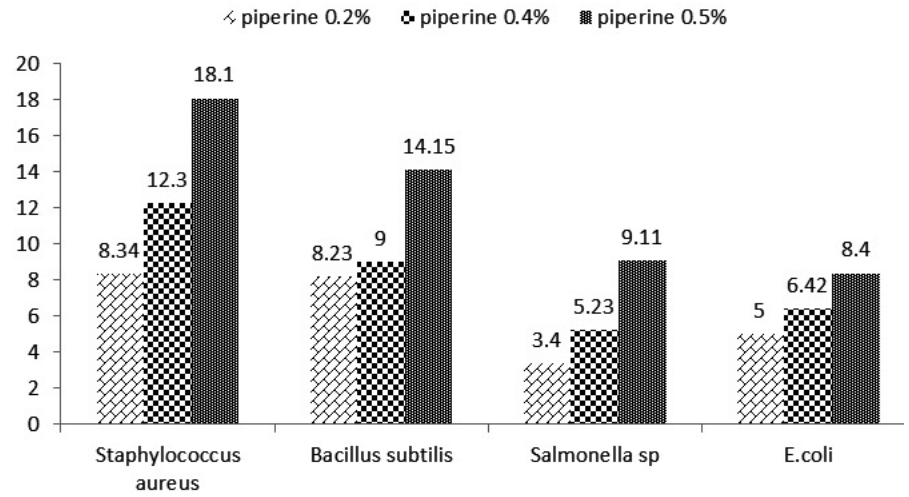

Fig. 2. Inhibition effect of piperine extract on bacteria *in vitro*

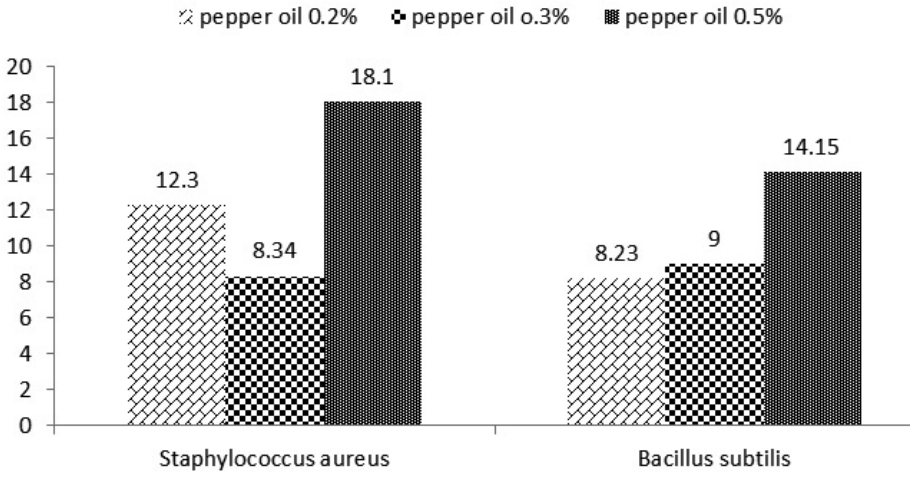

Fig. 3. Inhibition effect of black pepper oil extract on bacteria *in vitro*

compositions[15]. The sensitivity of *E.Coli* in compare other Gram negative bacteria to piperine when it used with lowest concentrations resulting from the highly permeable membrane. On the other hand, piperine alteration in the permeability of the cell wall which contain high level of lipid material[8].

CONCLUSIONS

Piperine and black pepper oil have potent activity as antibacterial agents especially piperine with both Gram positive and Negative bacteria strains.

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