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ORIGINAL ARTICLES

Evaluation of antibacterial activity of aqueous extracts of thyme and black pepper against pathogens and probiotics.

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ABSTRACT

Recent studies show great opportunities in food industry for antimicrobial activity of plant extracts on some pathogenic microorganisms. Plant extracts are used in food processing technologies as natural additives substituting synthetic products. The aim of this research subject was to evaluate the antibacterial activity of aqueous extracts of thyme and black pepper against food associated bacteria and some probiotics. Results showed that the concentrations of 3% and 4% aqueous extracts of both tested plants demonstrated antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*. On the other hand, *Salmonella typhi* and *Bacillus cereus* showed a resistance for all tested concentrations of both aqueous extracts of thyme and black pepper. Aqueous extracts of both tested plants did not affect the cultures of tested probiotics in concentrations up to 10%. Based on these findings, aqueous extracts of thyme and black pepper may be used as natural antibacterial preservatives to reclaim the shelf life of food, as well as pharmaceutical and natural plant based products.

Key words: Pathogens–antibacterial activity –thyme –black pepper – probiotics.

Introduction

Recently spices and herbs have been a renewed interest in improving health and fitness through the use of more natural products. Spices and herbs have an important impact of the human diet. They have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, spices and herbs are also known for their preservative and medicinal value (Chaudhry and Tariq, 2006) which forms one of the oldest sciences. Yet, it is only in recent years that modern science has started paying attention to the properties of spices. The extracts from these plants have shown potent antimicrobial effect. Prevention of pathogenic and spoilage microorganisms in food is usually achieved by using chemical preservatives but they are responsible for many carcinogenic and teratogenic attributes, thus the exploration of naturally occurring antimicrobial for food preservations receives increasing attention (Pundir and Jain, 2010). The results of different studies provide evidence that the antimicrobial effect of the medicinal plants are well documented (Holley and Patel, 2005 and Elango *et al*, 2010).

Naturally occurring water-soluble component in most plant materials include various anionic components such as thiocynate, nitrate, chlorides and sulphates, starch and tannins, saponins, terpenoids, polypeptides and lactins (Darout *et al*, 2000). Aqueous extracts of black pepper exhibited activity against *Bacillus megaterium*, *Bacillus sphaericus*, *Bacillus polymyxa* ,*Staphylococcus aureus* and *Escherichia coli* (Ali *et al*, 2007 and Pundir and Jain, 2010). On the other hand, the different concentrations of aqueous extract of leaves of thyme had an inhibitory action *on E.coli, Klebsiella pneumonia, Enterobacter cleacoe, Acinetobacter haemolyticus and S.aureus* but not effect on *Candida albicans*, *Proteus mirabilis* and *Salmonella typhi* (El-Astal, *et al*, 2005).

Probiotics have a known antimicrobial effect. They are very potent against pathogens. There are several proposed mechanisms for antimicrobial action of the probiotic. Bacteriocins, organic acids, hydrogen peroxide ,diacetyl and other inhibitory chemicals released by the probiotic (Abee *et al*, 1995). Probiotics have been an integral part of the human diet for centuries .The major probiotics that are taken in the diets belongs to the genera *Lactobacillus* and *Bifidobacterium* (Shipradeep *et al*, 2012).

The minimal inhibitory concentrations (MICS) of some plant extracts against probiotic microorganisms such as *Bifidobacterium bifidum*, *Bifidobacterium Longum*, *Lactobacillus acidophilus* and *Lactobacillus plantarum* are much higher in magnitude than the pathogen (Hawrelak *et al*, 2009). The above phenomenon makes it possible that probiotics and plant extracts can be combined to form flavored fermented products.

The objective of this study was to evaluate the antimicrobial activity of two plant extracts against food associated bacteria and some probiotics.

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Materials and Methods

Source of microorganisms:

Six food-associated bacteria (3 Gram-positive and 3 Gram-negative) strains (*Staphylococcus aureus, Listeria monocytogenes and Bacillus cereus* (Gram-positive); *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginsa* (Gram-negative) and Five probiotic starins (*Lactobacillus casei, L. plantarum, L. acidophilus, Bifidobacterium bifidum* and *Bif. longum*) were provided from Dairy Microbiology Lab., National Research Centre, Dokki, Cairo, Egypt .All probiotic strains had previously been shown to possess properties required of probiotic microorganisms (El-Shafei *et al*, 2010). Additionally, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were supplied from the previous source. All the tested strains were screened against plant extracts.

Plant samples:

The plant samples were collected from local markets at Cairo governorate, Egypt.

Preparation of plant extracts:

The air-dried plant materials were ground into fine powder in grinder. A 100g Sample of each ground plant was soaked in 500 ml distilled water (Vaishnavi *et al*, 2007).

The mixture being stored at 30C and shaken firmly at regular time intervals for 48h, the extract was filtered through a Buchner funnel with whatman filter paper number 1. After filtration, the extracts were evaporated under reducing pressure to dryness at 45^{o} C on a Rota -evaporator (El-Safey and Ali, 2011).

Antibacterial assay:

Disc diffusion method (Vaishnavi *et al*., 2007) was employed for antibacterial susceptibility assay. Discs of filter paper (5mm in diameter) were soaked in 1 ml of aqueous extracts of black pepper and thyme for 1-2 minutes and then used for screening. Four concentrations (1,2,3 and 4% w/v) of each aqueous extract were prepared and tested against all the food-associated bacteria under investigation .Also, the influence of different concentrations of aqueous extracts (1, 2, 4, 6, 8 and 10% w/v) on the growth of probiotic strains were measured using the same diffusion method. Tryptone soya agar (TSA) (Oxoid) was used as antimicrobial susceptibility test medium and Tryptone soya broth (Oxoid) was used for preparation of inoculum. A sterile cotton swab was dipped into the standardized bacterial test suspension and used to inoculate the entire surface of TSA plates. Previously soaked discs in different concentrations of aqueous extracts of black pepper and thyme were placed on the surface of inoculated plates with sterile forceps. All plates were incubated at 37 ^{o}C for 24 hours. The diameters of the zones of inhibition appearing around the discs were measured to the nearest millimeter (mm) and recorded. All assays were performed in duplicate and the results presented are the means of duplicate trails.

Results and Discussion

Antibacterial activity of aqueous extracts of thyme and black pepper on some pathogenic microorganisms:

Figure (1) shows the inhibitory effect of thyme using the disc assay. Different concentrations of tested thyme were used (1, 2, 3 and 4% as aqueous extracts). The results of the 1% and 2% concentrations did not show any inhibitory effect. It can be seen that when its concentration was raised to 3% ,the aqueous extract have a bactericidal effect against *S .aureus, L.monocytogenes, E.coli* and *Ps.aeruginosa* .Moreover ,the growth of *S.typhi* and *B.cereus* was not inhibited at high concentrations (3 and 4% w/v).

The previous results revealed that thyme possess some antimicrobial activity. Though the mode of action of the extract is not known ,its antimicrobial agent include thymol ,terpenes ,eugenal ,flavones ,glycosides of phenolic monoterpeniods and aliphatic alcohols among other elements (Deans and Ritchie,1987 and Nzeako *et al* , 2006). These substances acting alone or in combination may result in a broad spectrum of antimicrobial activity exhibited for bacteria (Nzeako *et al* ,2006) .The results of present study are in harmony with the study carried out by Oral *et al* (2007) in which thyme water extract exhibited broad antibacterial spectrum against the food borne pathogenic bacteria *E.coli* 0157:H7 *,Listeria monocytogenes* and *S. aureus* .Another study ,reported that thyme water extract possessed antimicrobial activity against *S.aureus* (Nzeako *et al* ,2006) This is in contradiction with Tuchila *et al*(2008) .In addition ,Al-Muhna (2010) showed that aqueous extract of thyme has strong antimicrobial activity against *L.monocytogenes* .From the microbial sensitivity side of view ,both of *E.coli* and *S.typhi* proved to be the most resistant among the tested organisms .Similar observations were also shown by Mahmood *et al* (2010) This may due to the presence of outer membrane barriers in gram-negative bacteria.

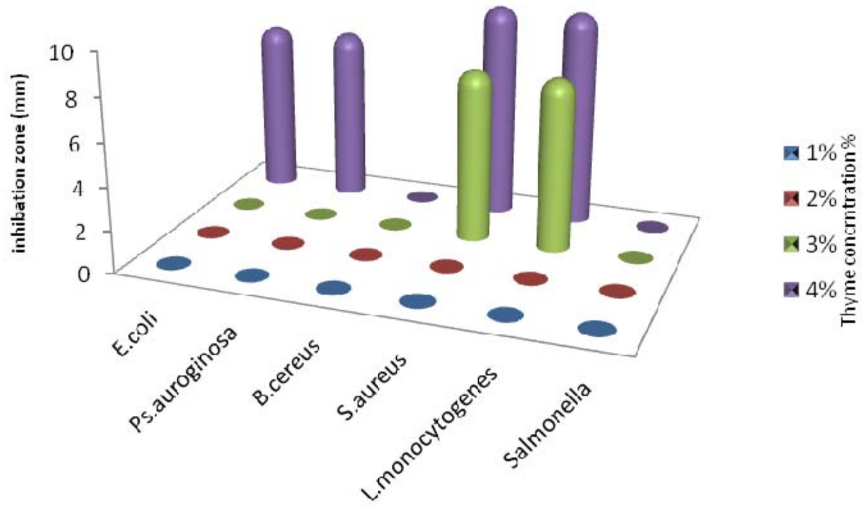

Fig. 1: Inhibitory effect of Thyme on some pathogenic microorganisms.

In the present study, as shown in Fig. (2), 4% concentration of the black pepper aqueous extract exhibited antibacterial activity against *S.aureus* ,*E.coli*,*Ps aeruginosa* and *L.monocytogenes.* In contrary, it was found to be ineffective against *B.subtilis* and *S.typhi* and there was no zone of inhibition.

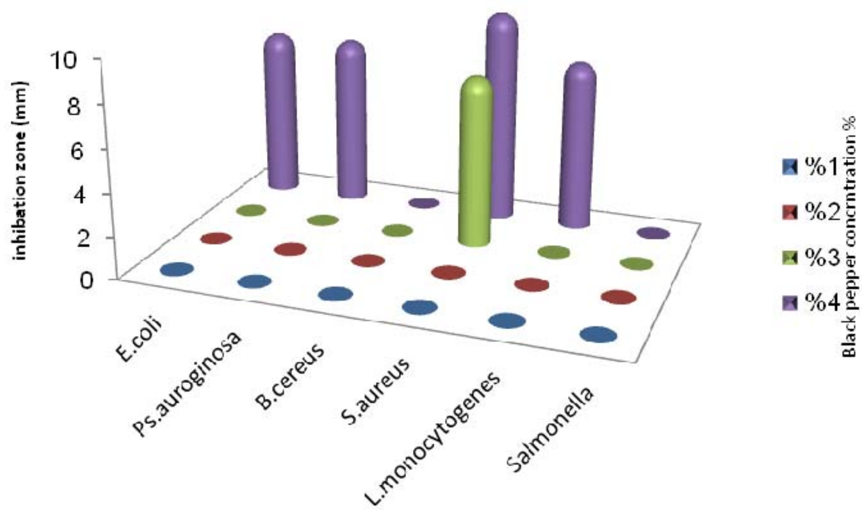

Fig. 2: Inhibitory effect of Black pepper on some pathogenic microorganisms.

Findings of the present study are similar to those reported by Pundir and Jain (2010). They observed that aqueous extracts of black pepper did not exhibit antibacterial activity against *B.subtilis*, while showed a good antibacterial activity against *S.aureus.* In another study the aqueous decoction of black pepper exhibited maximum effect against *S.aureus* and found to be most active antibacterial agent against *Enterobacter aerogenes, E.coli, Ps. aeruginosa* but did not find any bactericidal effect against *S.typhi* (chaudhry and Tariq, 2006). This substantiate the findings of Al-jedah *et al* (2000), who had been reported that black pepper and other spices able to exert static effect on all assayed bacteria except on *S.aureus*. According to Pundir and Jain (2010), the antimicrobial activity of black pepper is due to the presence of essential oil (3%), whose aroma is dominated by menoterpenes hydrocarbons: sabinen, B-pinene and limonene. Furthermore, terpinene α-pinene, myrcene and monoterpene derivatives like borneol, carvone, carvacrol, 1,8-cineol and linalool are also present. The mechanism of action of terpene is no fully understood but is speculated to involve membrane disruption by lipophilic compounds (Ahmed *et al*, 1993).

Antibacterial activity of aqueous extracts of thyme and black pepper on some probiotics:

As seen in Table (1) aqueous extracts of both thyme and black pepper at concentrations up to 10% have no inhibitory effect against probiotic bacteria. Our results in the line of Shipradeep *et al* (2012) they recorded that essential oils of medicinal plants have very high MIC (minimal inhibitory concentration) against beneficial probiotic bacteria, while it is effective is much lesser concentration against the pathogen. In this concern ,Kivanc *et al* (1991) mentioned that starter cultures (*Lactobacillus plantarum* and *leuconostoc mesentroides*) are relatively resistant to toxic effect of spices and derivatives and that some spices have exerted stimulatory effect on these microorganisms resulting in enhanced acid production.

Table 1: Antibacterial activity of Thyme and Black pepper on some probiotic bacteria.

Strains	Black pepper %						Thyme %					
St.thermophilus	1	2	4	6	8	10	1	2	4	6	8	10
L.acidophilus	-	-	-	-	-	-	-	-	-	-	-	-
L.casei	-	-	-	-	-	-	-	-	-	-	-	-
Bif. bifidium	-	-	-	-	-	-	-	-	-	-	-	-
L.bulgaricus	-	-	-	-	-	-	-	-	-	-	-	-
Bif..longum	-	-	-	-	-	-	-	-	-	-	-	-

Lactic acid bacteria has a number of properties, which render them highly suitable as vehicles for the delivery to the mucosa of compounds that are of pharmaceutical interest. Large populations of LAB inhibit the proximal regions of the digestive tracts of pigs ,fowl ,and rodents. Some gastrointestinal strains of LAB have the ability to adhere to and colonize the surface of stratified squamous epithelium in the oesophagus ,crop ,or stomach. Other *Lactobacillus* strains appear to be inhabitants of the gastrointestinal lumen-LAB, whether shed from epithelial surfaces or multiplying in ingested food, permeate all regions of the digestive tract in these human beings and animals. Many strains of the genus *Lactobacillus* are capable of colonizing specific regions of the body, e.g. the oral cavity and the gastrointestinal and uro-genital tract, where they play an important role in the competitive exclusion of pathogens. Moreover, LAB have been used for many centuries in food fermentation processes and are considered as GRAS organisms that can safely be used for medical and veterinary applications (Arici *et al* 2004 and Sacgdic *et al*, 2005). Therefore, the inhibition of LAB by spice extracts is undesired.

The present results show that the variations in inhibitory potency of the tested aqueous extracts seems to be affected by type and composition of the plant used in the extract ,amount used and type of microorganism. In addition, the antimicrobial property of spices might differ depending on the forms of spices added, such as fresh, dried, or extracted forms (El-kholie *et al*, 2012).

Conclusion:

The results of the present study demonstrate that both the extracts can be used as a potential source of natural antibacterial compound which are becoming a threat to human health and if applied to food products. This scientific information can serve as an important platform for the development of inexpensive, safe and effective natural medicines and nutraceutical foods.

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