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RESEARCH PAPER

In vitro Evaluation of Antibacterial Activity of Bark and Flower Extracts of *Pimenta officinalis Lindl.*

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ABSTRACT
Methanol, petroleum ether, chloroform, ethyl acetate, acetone extracts of pimenta officinalis stem bark and flower was investigated for in vitro antibacterial activities by agar well diffusion method. Methanol, chloroform and ethyl acetate extracts were found to be more effective than other extracts. The results of the study indicate that the stem bark and flowers have potential antimicrobial activity and is concentration dependent.
***KEY WORDS**: Pimenta officinalis, antibacterial activity, bark, flower extracts, chemical compounds.*

INTRODUCTION

Pimenta officinalis Lindl / *Pimenta dioca* (L) Merr. Family Myrtaceae Common name – Clove pepper, Jamaica pepper, Pimento, Allspice.

The odour of pimento berries is thought to resemble that of a mixture of Cinnamon, cloves and nutmeg. Hence, the name of allspice, by which they are best known in this country. The chief use of pimento is as a spice and condiment; the berries are added to curry powder and also to mulled wine. It is popular as a warming cordial of a sweet odour and grateful aromatic taste. The oil resembles that of cloves, and is occasionally used in medicine and is also employed in perfuming soaps.

Pimenta officinalis is considered an anodyne against rheumatism and neuralgic, powdered fruits are used against flatulence, dyspepsia and diarrhoea. It is also used in the preparation of tonics and purgatives. Pimento berry oil is used as a carminative and stimulant. It shows bactericidal, fungicidal and antioxidant properties. All spice is used as a paste to soothe and relieve toothache and as a mouth wash to freshen the breath. Pimento is an aromatic stimulant and carminative to the gastro-intestinal tract, resembling cloves in its action. It is employed chiefly as an addition to tonics and purgatives and as a flavouring agent.

Food borne pathogens are widely distributed in nature which causes considerable mortality in the population. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi drug resistant pathogens, [1]. Strains of resistant food borne pathogens to a variety of antimicrobials have become a major health concern, [2]. In recent years, food safety concerns have been focused on pathogens. Recently there has been increasing interest in discovering new natural antimicrobials, [3].Plant products with antimicrobial properties notably have obtained emphasis for a possible application in food production in order to prevent bacterial and fungal growth, [4]. Spices and herbs have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives [5, 6 & 7].

Since ancient times, plants have been model source of medicine as they are reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many human disorders. Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new target is a matter of urgency, most of the spices extracted either in water or in organic solvents have bacteriologicaly active compounds, which can be used for food preservation or in the synthesis of potent drugs. In addition to imparting characteristic flavors, certain spices prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bactericidal activity, [8].

The main objective of this study was to determine the effect of phytochemicals of the spice plant *pimenta officinalis* under in-vitro conditions against the test microorganisms. The extracts were used in different concentrations. These results were compared with the potent antibiotic i.e gentamicin that is effective against these pathogens.

MATERIALS AND METHODS

The Plant material

The bark and flowers of *pimenta officinalis* were collected in morning hours from the plants maintained in the Botanical garden of the Department.

Micro organisms and culture

Five food borne and pathogenic bacteria viz., *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Vibrio cholerae* and *Pseudomonas eeruginosa* were purchaged by the IMTECH Chandigarh, India. The strains were cultured at 37^0C on plate count agar medium (PCA). Bacterial strains were maintained in sterile Glycerine stubbs at - 20^0 C.

Preparation of extract

Flowers and bark of *pimenta officinalis* were cleaned and naturally dried. Dried plant samples were further air dried in a ventilated oven at 40^0C for 24h., then ground into a fine powder and passed through a sieve. Powdered sample was extracted using Soxhlet extractor for 24 hours with five different solvents separately the solvents used were methanol, ethyl acetate, chloroform, acetone and petroleum ether. 5gm bark and flower powder was extracted in 200ml of each solvent. Solvent extract was then filtered through whatman filter paper No1. Extracts were kept for evaporation under reduced pressure to yield residue. This residue was collected and different concentrations of extract were prepared using respective solvent. The extracts were stored at 4^0C until use.

Determination of antibacterial activity

An agar–well diffusion method was employed for determination of antibacterial activities. The freeze-dried extract samples were sterilized by filtration. Bacteria were suspended in sterile water and diluted. The bacterial suspension (100µl) was spread on to the surface of PCA (Plate count agar) medium. Wells 5.00 mm in diameter were prepared from the agar with a sterile borer and 60µl extract solution was delivered into them. Negative controls were prepared. Gentamicin is used as positive standard to determine the sensitivity of each microbial species tested. The inoculated plates were incubated at 37^0C for 24 hrs. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. All tests were performed in triplicates.

The Minimum Inhibitory Concentration (MIC)

The MIC was defined as the lowest concentration tested samples showing no visible bacterial growth after 24hr incubation period at 37^0C. A loopfull of bacterial cultures from the slant was inoculated into nutrient broth and incubated at 37^0C for 24 hrs. The fresh broth (20ml) was seeded with 0.25ml of 24hr bacterial broth culture. Then 0.2ml of the extract was added to 1.8ml of seeded broth which was the 1st dilution. 1ml of the solution was diluted further with 1ml of the seeded broth to produce 2nd dilution and the procedure was repeated until six dilutions were obtained. A set of tubes containing only seeded broth were kept as control. After incubation for 24 hrs at 37^0C with no visible growth of bacteria was taken to represent MIC of test samples which was expressed in mg/ml. The broth dilution assay was also carried out with in the same way as the extracts and MIC values of Gentamicin were determined (Table 3 & 4).

RESULTS AND DISCUSSION

Bark extracts

The antimicrobial sensitivity of bark and flower extracts was observed using agar well diffusion method by measuring the diameter of zone of growth inhibition. The results were shown in the Table 1 and 2. Gram's Positive and Gram's negative bacteria at a concentration of 0.0625, 0.125, and 0.25 exhibited resistance to all extracts. These pathogens are sensitive at 0.5, 1.0 and 2.0 mg/ml. Methanol and ethyl acetate extracts of *Pimenta officinalis* stem bark has shown high degree of inhibition, DIZ- 12mm (methanol extract) for S. aureus and DIZ-11mm (ethyl acetate extract) for S.typhi. It is found that there was no antibacterial activity exhibited by some concentrations of *Pimenta officinalis* bark crude extract (Table 1) whereas, there were some good antibacterial

activities found from 1.0 to 2.0 mg/ml against *S.aureus, P.eeruginosa, V.cholerae, S.typhi* and *B.subtilis*. The size of the zone increases as the concentration increased indicating concentration dependent effect. The chloroform extract had exhibited no significant antimicrobial activity. Some extracts at lower concentration had no significant antimicrobial activity.

Fig - 1

Fig .1 *Pimenta officinalis* plant (above) and closer view of flowers (below

Flower extracts

The results were shown in the Table.2. Gram's positive and Gram's negative bacteria at concentration of 0.0625, 0.125 exhibited resistance to all extracts of flowers, these pathogens are sensitive at 0.25, 0.5, 1.0 and 2.0 mg/ml. Methanol and chloroform flower extracts of *Pimenta officinalis* has show high degree of inhibition (DIZ- 7mm and 11mm respectively) against the test organisms. Some extracts at lower concentration had no significant antimicrobial activity. The

acetone extract had exhibited no significant antimicrobial activity. It is found that there was no

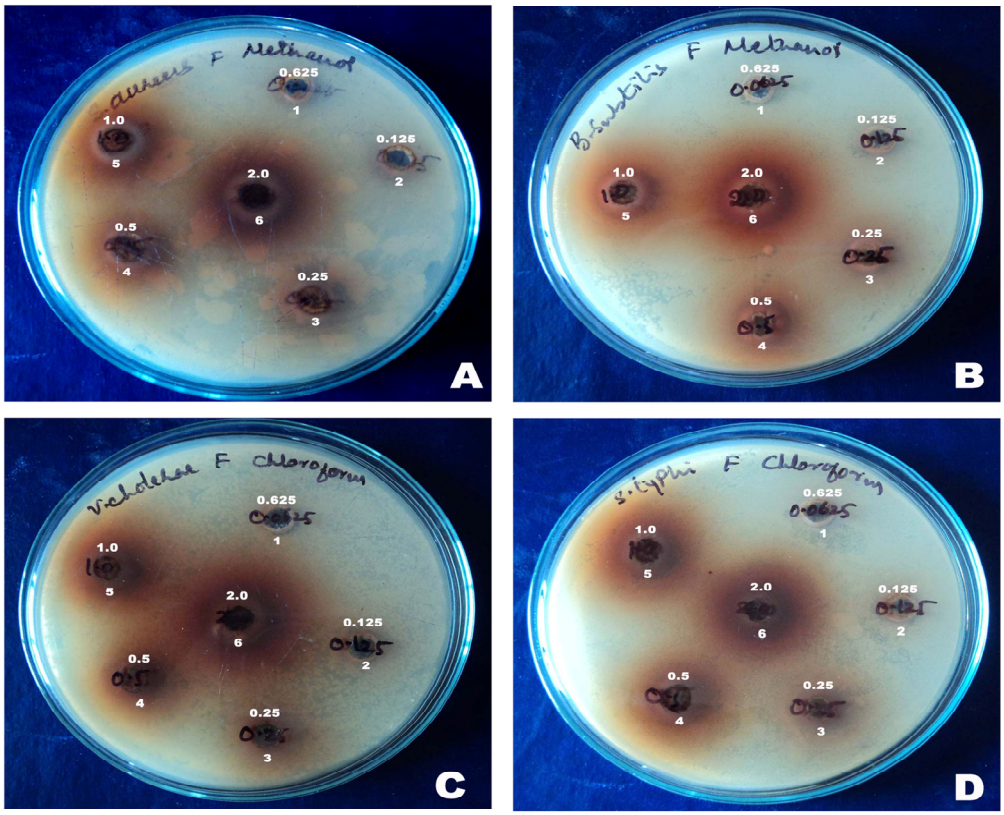

Fig - 2

Fig .2 A. Response of flower extracts (methanol) of *pimenta officinalis* for *S aureus*. 1.0.625 2. 0.125 3. 0.25 4. 0.5 5. 1.0 6. 2.0 mg/ml concentration.
B. Response of flower extracts (methanol) of *pimenta officinalis* for *Bacillus subtilis* 1. 0.625 2. 0.125 3. 0.25 4.0.5 5. 1.0 6.2.0 mg/mlconcentration.
C. Response of flower extracts (chloroform) of *pimenta officinalis* for *V.cholerae*. 1. 0.625 2. 0.125 3. 0.25 4. 0.5 5. 1.0 6. 2.0 mg/ml concentration.
D. Response of flower extracts (chloroform) of *pimenta officinalis* for *S. typhi*. 1. 0.625 2. 0.125 3. 0.25 4. 0.5 5. 1.0 6. 2.0 mg/ml concentration.

antibacterial activity exhibited by some concentrations of *Pimenta officinalis* flower crude extract (Table 2) whereas, there were some good antibacterial activities found from 0.25-2.0 mg/ml against *V.cholerae, S.typhi* and *B.subtilis* (Table 2). However these antibacterial activities against these bacteria were shown to be equal or less activity when compared to the control Gentamicin. This differential action of antibacterial property of crude extract of *P.officinalis* may be depending upon the active compounds on the specific bacteria. These observations suggest that the bioactive compounds responsible for the activity are polar and can be extracted through the organic solvent medium.

The effectiveness of the plant extracts was not due to one constituent, but to the combined action of other chemical compounds involved in it [9]. Bioactive compounds like alkaloids, flavonoids, triterpinoids, thymol and other phenolic compounds are classified as antimicrobial compounds [10]. The present study shows the effect of the extracts on pathogenic bacterial agents which really shows the presence of biological principles.

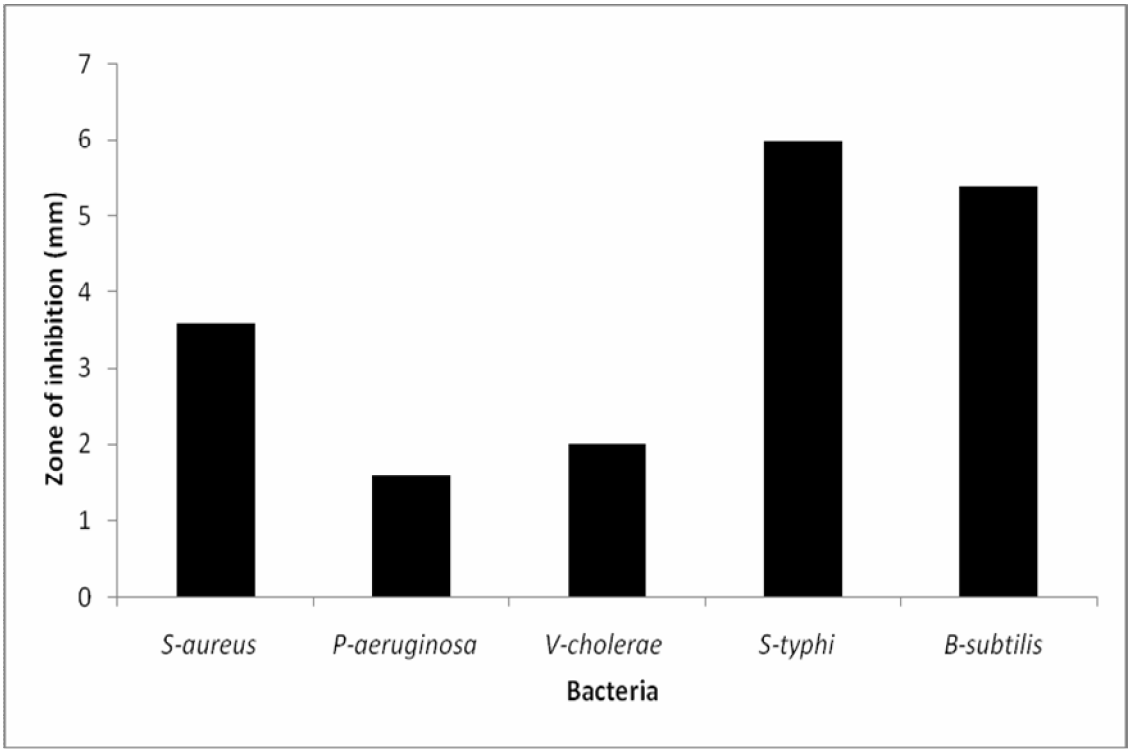

Fig -3 *Pimenta officinalis* bark extract on Different Bacterial Strains.

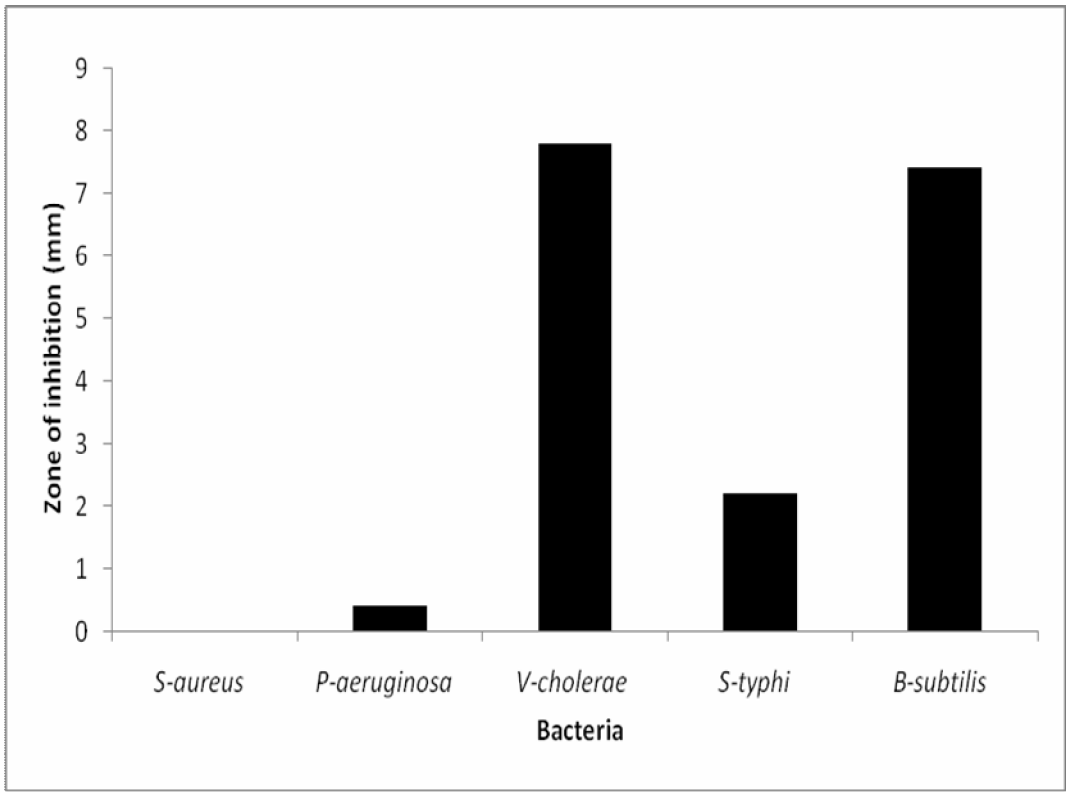

Fig -4 *Pimenta officinalis* flower extract on Different Bactrial Strains

Table 1. Antimicrobial activity of *pimenta officinalis* bark extracts.

Test organism	Extraction medium	Zone of inhibition (mm) in various concentrations						
		0.0625mg/ml	0.125mg/ml	0.25 mg/ml	0.5 mg/ml	1.0 mg/ml	2.0 mg/ml	MIC mg/ml
S-aureus +ve	Mehanol	-	-	-	-	3	12	1
	Petroleum ether	-	-	-	-	-	-	>2
	Chloroform	-	-	-	-	-	-	>2
	Ethyl-acetate	-	-	-	-	-	3	2
	Acetone	-	-	-	-	-	-	>2
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	13	18	21	25	27	34	<25
P-aeruginosa -ve	Mehanol	-	-	-	-	-	1	2
	Petroleum ether	-	-	-	-	-	2	2
	Chloroform	-	-	-	-	-	1	2
	Ethyl-acetate	-	-	-	-	-	2	2
	Acetone	-	-	-	-	-	2	2
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	-	-	1	3	8	14	100
V- cholerae -ve	Mehanol	-	-	-	-	-	-	>2
	Petroleum ether	-	-	-	-	-	3	2
	Chloroform	-	-	-	-	-	-	>2
	Ethyl-acetate	-	-	-	-	2	5	1
	Acetone	-	-	-	-	-	-	>2
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	13	15	18	21	23	27	<25
S-typhi -ve	Mehanol	-	-	-	-	3	5	1
	Petroleum ether	-	-	-	-	1	1	1
	Chloroform	-	-	-	-	-	1	2
	Ethyl-acetate	-	-	-	2	5	11	0.5
	Acetone	-	-	-	-	-	1	2
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	2	13	16	21	25	27	25
B- subtilis +ve	Mehanol	-	-	-	-	3	6	1
	Petroleum ether	-	-	-	-	-	-	>2
	Chloroform	-	-	-	-	-	-	>2
	Ethyl-acetate	-	-	-	2	4	7	0.5
	Acetone	-	-	-	-	1	4	1
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	8	10	15	19	22	25	<25

* Values are the average of triplicate, includes the well diameter (5mm), '-' no zone of inhibition

Table 2. Antimicrobial activity of *pimenta officinalis* flower extracts

Test organism	Extraction medium	Zone of inhibition (mm) in various concentrations						
		0.0625mg/ml	0.125mg/ml	0.25 mg/ml	0.5 mg/ml	1.0 mg/ml	2.0 mg/ml	MIC mg/ml
S-aureus +ve	Methanol	-	-	-	-	-	-	>2
	Petroleum ether	-	-	-	-	-	-	>2
	Chloroform	-	-	-	-	-	-	>2
	Ethyl-acetate	-	-	-	-	-	-	>2
	Acetone	-	-	-	-	-	-	>2
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	13	18	21	25	27	34	<25
P-eeruginosa -ve	Methanol	-	-	-	-	-	2	>2
	Petroleum ether	-	-	-	-	-	-	>2
	Chloroform	-	-	-	-	-	-	>2
	Ethyl-acetate	-	-	-	-	-	-	>2
	Acetone	-	-	-	-	-	-	>2
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	-	-	1	3	8	14	100
V- cholerae -ve	Methanol	-	-	-	1	3	5	0.5
	Petroleum ether	-	-	-	-	-	1	2
	Chloroform	-	-	1	3	7	11	0. 25
	Ethyl-acetate	-	-	-	-	1	3	1
	Acetone	-	-	-	-	1	3	1
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	13	15	18	21	23	27	<25
S-typhi -ve	Methanol	-	-	-	-	2	4	1
	Petroleum ether	-	-	-	-	-	1	2
	Chloroform	-	-	-	-	-	1	2
	Ethyl-acetate	-	-	-	-	1	2	1
	Acetone	-	-	-	-	-	-	>2
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	2	13	16	21	25	27	25
B- subtilis +ve	Methanol	-	-	1	2	5	7	0.25
	Petroleum ether	-	-	-	-	-	1	2
	Chloroform	-	1	4	5	7	1	0.125
	Ethyl-acetate	-	-	-	-	1	2	1
	Acetone	-	-	-	-	-	-	>2
		25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
	Gentamicin	8	1	15	19	22	25	<25

* Values are the average of triplicate, includes the well diameter (5mm), '-' no zone of inhibition.

Table 3: Minimum inhibitory concentration of *P. officinalis* bark extracts on bacteria

Organism	*P.officinalis* bark extract	Gentamicin
1. S.aureus	1 mg/ml	25µg/ml
2. P.eeruginosa	2 mg/ml	100µg/ml
3. V.cholerae	1 mg/ml	25µg/ml
4. S.typhi	0.5 mg/ml	25 µg/ml
5. B.subtilis	0.5 mg/ml	25 µg/ml

Table 4: Minimum inhibitory concentration of *P.officinalis* flower extracts on bacteria.

Organism	P.officinalis flower extract	Gentamicin
1. S.aureus	2mg/ml	25µg/ml
2. P.eeruginosa	2mg/ml	100µg/ml
3. V.cholerae	0.25mg/ml	< 25µg/ml
4. S.typhi	1mg/ml	25µg/ml
5. B.subtilis	0.125mg/ml	<25µg/ml

REFERENCES

[1] Bandow, J.E., Brotz, H., Leichert, LIO, Labischinskitt and Hecker. (2003). Proteomic approach to understanding antibiotic action antimicrob. Agents. *Chemotherp*. 47: 948-955.

[2] Kessling, C.R., Cutting J.H., Luttis, M.K., Kissling V.N. Data, A.R. and Sofos, J.N. (2002). Antimicrobial resistance of food related *Salmonella* isolates. *Journal of Food protection*. 65:603-608.

[3] Sagdic, O., Karahan, A.G. and Ozean, M. (2003). Effect of some spices extracts on bacterial inhibition. Food Scien. and Technol. 9: 353-359.

[4] Lanciotti, R., Bianotti, A., Patrignani, N., Bellet, N., Buexoni, M.E., and Gaurdini, F. (2004). Use of natural aroma compounds to improve shelf- life of minimally processed fruits. *Trends in Food Scien. and Technol*. 15: 205-208.

[5] Beuchat, L.R. (1994). Antimicrobial properties of spices and their essential oils. In Dillon, Y.M., Board, R.G. (Eds.), Natural Antimicrobial Systems and Food Preservation. CAB International, Oxon, PP. 167-179.

[6] Nakatani, N. (1994). Antioxidative and antimicrobial constituents of herbs and spices. In: charablambous, G. (Ed.), Spices, Herbs and Edible Fungi. Elsevier Science, New York, PP. 251-271.

[7] Cutler, H.G. (1995). Natural product flavor compounds as potential antimicrobials, insecticides and medicinal. *Agro- Food –Industry Hi-Tech*. 6, 19-23.

[8] Beuchat, L.R., Golden, D.A. (1989). Antimicrobials occurring naturally in foods. *Food technology* 43, 134-142.

[9] Bai, D. (1990). Traditional Chinese Material: A respect and prospect. Planta Media 50: 502.

[10] Rojas, A., Hernandez, L., Pereda Mirands, R. and Meta, R. (1992). Screening of antimicrobial activity of crude drug extracts and pure products form Mexican medicinal plants. *J. Ethanopharmacol*. 35: 275-283.