ANTIBACTERIAL ACTIVITY OF DIFFERENT SPICES AGAINST *S.AUREUS*, *E.COLI* AND KLEBSELLA

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Abstract

The plant extracts and phytochemicals, with antibacterial properties, have immense importance in therapeutic treatments. A research was conducted to find out the antibacterial activities of plant spices on G+ve and G-ve bacteria like S. aureus, E. coli, and Klebsella.. The plant spices used were: Clove (Syzygium aromaticum), Garlic (Allium sativum),Fennel (Foeniculum vulgare),Poppy seeds (Opium poppy),Coriander (Coriandrum sativum). Disc diffusion assay was employed for antibacterial susceptibility with decoctions prepared with 10g of spices in 100ml and 50ml of distilled water. With 100ml extract, Garlic was most active against S.aureus having inhibition zone of 9mm, Clove was found highly active against E.coli with inhibition zone of 10mm, coriander was most active against Klebsella with zone of inhibition 9mm in decoction of 100ml, and Poppy seeds were found to be most active against S.aureus with zone diameter of 11mm.Using 50ml decoction Poppy seeds have shown high activity against S.aureus with inhibition zone of 11mm.Clove formed the highest zone of inhibition of 11mm against E.coli. A zone of inhibition of 10mm was observed against Klebsella with Poppy seeds and coriander extracts. Hence the study revealed the importance of natural products to control bacteria, which are being a threat to human health.

Keywords: Spice extracts, Antimicrobial activity, E.coli, S.aureus, Klebsella and Zone of Inhibitions.

INTRODUCTION

Spices are used in various forms such as fresh, ripe, dried, whole, broken and powdered. They impart aroma, taste, flavour, color and pungency to food. Spices are frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments. Several investigations have been directed towards their antibacterial properties (Chaudhry and Tariq, 2008). The present study gave an account on the antibacterial activities of aqueous decoctions of some spices; like Clove, garlic, fennel, poppy seeds and coriander.

There are about 70 spices grown in different parts of the world. Many of them possess medicinal properties and have a profound effect on human health. They possess anti–gas activities, anticoagulant activities, antidepressant activities, pain killing activities, antibacterial activities,anti-diabetic activities, anti-Inflammatory activities etc.

The spices used in our study were:

- Clove (*Syzygium aromaticum).*
- Garlic (*Allium sativum).*
- Fennel *(Foeniculum vulgare).*
- Poppy seeds (*Opium poppy*). and
- Coriander (*Coriandrum sativum).*

Clove *Syzygium aromaticum (*L) is a small-to medium-size tropical evergreen tree of the myrtle family native to the Moluccas, eastern Indonesia. Traditional use of clove is for the treatment of worms, viruses, candida, and various bacterial and protozoan infections. The main constituents of essential oil are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde. Several studies have demonstrated potent antifungal, antiviral and antibacterial effects of clove (Saeed and Tariq, 2008).

Garlic, *Allium sativum* L. [Fam. Liliaceae], has being used as a medicine for over 5000 years by many cultures than perhaps any other plant. Traditionally garlic has been a remedy for abnormal growths, bronchitis, pneumonia, digestive problems, tuberculosis, earaches and vascular disorders of many kinds.

It inhibits the growth of both gram-negative and gram-positive bacteria, like molds and yeasts. The ordorless amino acid, alliin, present in the garlic,that acts by totally inhibiting RNA synthesis and partially inhibiting DNA and protein synthesis, suggesting that RNA is the primary target of allicin (Eja *et al*., 2007). Garlic has been reported to inhibit *Aerobacter, Aeromonas, Bacillus, Citrella, Citrobacter, Clostridium, enterobacter, Escherichia, Klebsiella, Lactobacillus, Leuconostoc, Micrococcus, Mycobacterium, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Shigella, Staphylococcus, Streptococcus* and *Vibrio*, (Sivum,2001). In folk medicine, fennel is used to promote menstruation and to enhance lactation. Extracts of fennel have antimicrobial activity against a number of bacteria, fungi and yeasts. It has been found that fennel exhibited a very strong antibacterial activity against bacteria *Escherichia coli, Listeria monocytogenes, Salmonella typhimurium,* and *Staphylococcus aureus* in a vitro study, (Ruberto *et al*., 2000).

Poppy seed oil and basil leaf oil are extremely promising chemopreventive agents. They prevent diarrhea, give Protection from cold and asthma, and helps in relieving abdominal pain. The aqueous decoction of poppy seed exhibited weak antibacterial activity against *Escherichia coli, Citrobacter spp, Alcaligenes spp,* and *Micrococcus roseus,* (Chaudhry and Tariq, 2008).

Coriander is a commonly used spice in domestic remedy. The oil is fungicidal and bactericidal. *Coriandrum sativum* is famous for its essential oils and some important chemical constituents such as coriandol, oxalic acid, ascorbic acid, and carotene. Its important medicinal uses are like anti-diabetic, anti-inflammatory (Hussain *et al*., 2009). The volatile oil found in the leaves have antimicrobial properties, and used for its anti-inflammatory effect. *Coriandrun sativum* was also found to relieves colds, combat diarrhea and intestinal poisoning, (Suliman *et al*., 2008).

MATERIAL AND METHODS

Five medicinal spices; Clove (*Syzygium aromaticum*), garlic (*Allium sativum*), Fennel *(Foeniculum vulgare),* Poppy seeds (*Opium poppy*) and coriander (*Coriandum sativum)* were used in the study. Bacteria used in this study were *E.coli. S.aureus* and *Klebsella*, obtained from Quetta diagnostic lab. Aqueous 100ml extracts of clove, garlic, fennel, poppy seeds and coriander were prepared by boiling 10g of each spice in 100ml sterile distilled water over low flame for 15minutes. The flasks were then plugged and removed from heat and allowed to cool down. After cooling the contents of flasks were filtered to obtain clear decoction. Similarly, water extracts of above mentioned five spices were prepared by boiling 10g of each spice in 50ml sterile distilled water over low flame for 15 minutes. The flasks were then plugged and removed from heat and allowed to cool down. After cooling, the contents of flasks were filtered to obtain required extracts then they were shifted to sterilized bottles which were then stored at 4ºC in refrigerator for further use. Disc diffusion method was employed for antibacterial susceptibility assay. Filter papers were first sterilized (autoclaved) then were cut into small discs of 5mm of diameter. Total of thirty sterilized discs of filter paper were used. They were soaked in 1ml of extracts of 50ml and 100ml of Clove, garlic, fennel, poppy seeds and coriander for 1-2 minutes and then used for screening. Brain Heart infusion agar (BHI) was used as medium that was prepared by dissolving 47g of BHI powder in a liter of distilled water and then autoclaved at 121ºC for 15 min. After autoclaving the media was allowed to cool at room temperature keeping in mind that it is not solidified. It was then poured into sterile petri plates. Then these BHI plates were incubated at 37°C for 24hours. After 24 hrs, a sterile wire loop was used to pick each type of bacteria to inoculate entire surface of BHI plates. Previously soaked discs in aqueous decoctions of 100ml and 50ml of Clove, garlic, Fennel, Poppy seeds and coriander were placed on the surface of inoculated plates with sterile forcep. Two discs per plates were used. All plates were incubated again at 37°C for 24hours and were observed after 24hrs. The diameters of the zones of inhibition appearing around the discs were measured to the nearest millimeter (mm) and recorded.

RESULTS

Garlic was found to be most active against S.aureus. (Table1). The zone of inhibition of five spices extracts prepared in 100ml conc against E.coli showed that Clove was found to be most active against E.coli while Poppy seed extracts did not show any activity against E.coli (Table 2). The detail of different spice extract prepared in 100ml of conc are shown in table 3. It was observed that the coriander was highly active against Klebsella with inhibition zone of 9mm.Clove did not show any activity against Klebsella. Spice extracts of 50ml conc exhibit different degree of antibacterial activity than 100ml preparations. Poppy seeds were found to be highly active against S.aureus with inhibition zone of 11mm (Table 4). The table 5 describes the zone of inhibition of five spices extracts prepared in 50ml conc: against E.coli. Clove forms the highest zone of inhibition while the lowest zone of inhibition was observed by garlic but poppy seeds did not show any activity. Extracts, prepared in 50ml concentration, Poppy seeds and coriander showed highest activity against Klebsella with inhibition zone of 10mm while clove did not show any activity against Klebsella. This result is shown in table 6. The extracts of 50ml concentrations of all spices showed slightly higher activity than 100ml concentrations. The highest inhibition zone observed with 100ml concentrations against S.aureus was of 9mm and in 50ml concentrations it was of 11mm, which is greater than 100ml concentrations shown in table 7. After comparison 50ml and 100ml concentrations, clove has the highest antibacterial activity in both concentrations with an inhibition zone of 10mm in 100ml, and 11mm in 50ml. Zone of inhibition of different spice extracts prepared in 100ml and 50ml concentrations formed against E.coli are shown in table 8. Comparison of antibacterial activity of 50ml and 100ml of spice extracts against klebsell is shown in table 9. It was found that extracts prepared in 100ml of concentrations have less antibacterial activity against klebsella while extracts prepared in 50ml of concentrations have the highest antibacterial activity against klebsella

Table 1: Zone of inhibition of 100 ml Spice extracts against *S.aureus*.

S.NO	SPICES (extract)	ZONE OF INHIBITION (mm)
1	Clove	7
2	Garlic	9
3	Fennel	6
4	Poppy seeds	7
5	Coriander	8

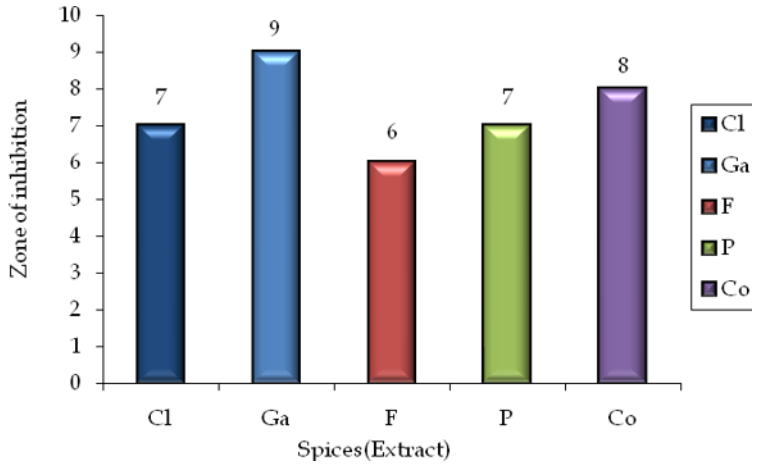

Figure 1: Antibacterial activity of 100ml extract of different spices against *S.aureus*.

S.NO	SPICES(extract)	ZONE OF INHIBITION (mm)
1	Clove	10
2	Garlic	7
3	Fennel	6
4	Poppy seeds	-ve
5	Coriander	9

Table 2: Zone of inhibition of 100 ml spice extracts against *E.coli.*

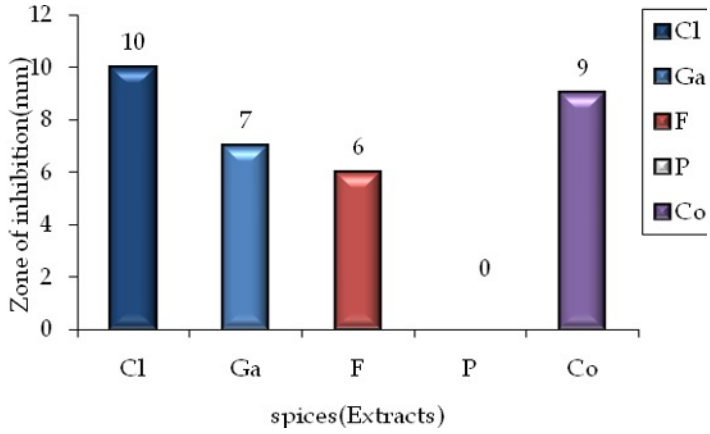

Figure 2: Antibacterial activity of 100ml extract of different spices against *E.coli.*

Table 3: Zone of inhibition of 100 ml spice extracts against *Klebsella.*

S.NO	SPICES	ZONE OF INHIBITION (mm)
1	Clove	-ve
2	Garlic	7
3	Fennel	7
4	Poppy seeds	8
5	Coriander	9

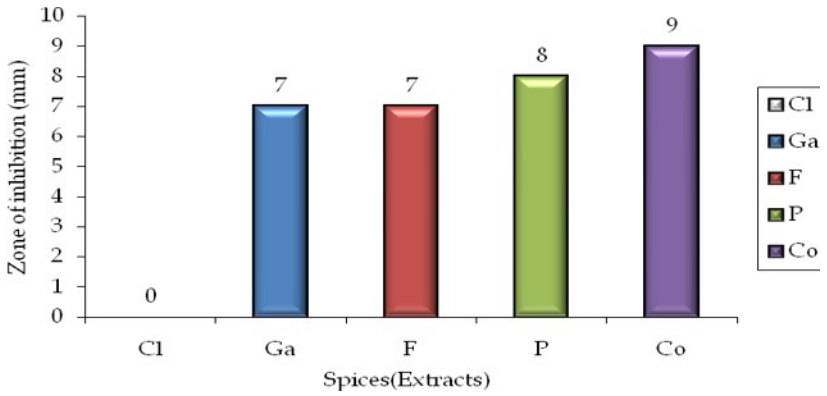

Figure 3: Antibacterial activity of 100ml extract of different spices against Klebsella

Table 4: Zone of inhibition of 50 ml Spice extracts against *S.aureus.*

S.NO	SPICES	ZONE OF INHIBITION (mm)
1	Clove	9
2	Garlic	9.5
3	Fennel	7
4	Poppy seeds	11
5	Coriander	8

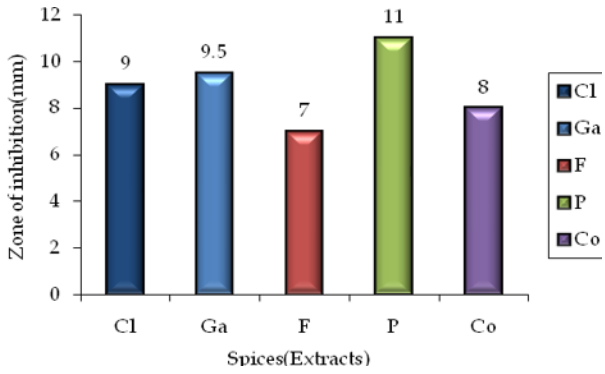

Figure 4: Antibacterial activity of 50ml extract of different spices against *S.aureu*

Table 5: Zone of inhibition of 50 ml Spice extracts against *E.coli.*

S.NO	SPICES(Extracts)	ZONE OF INHIBITION (mm)
1	Clove	11
2	Garlic	8
3	Fennel	9
4	Poppy seeds	-ve
5	Coriander	10

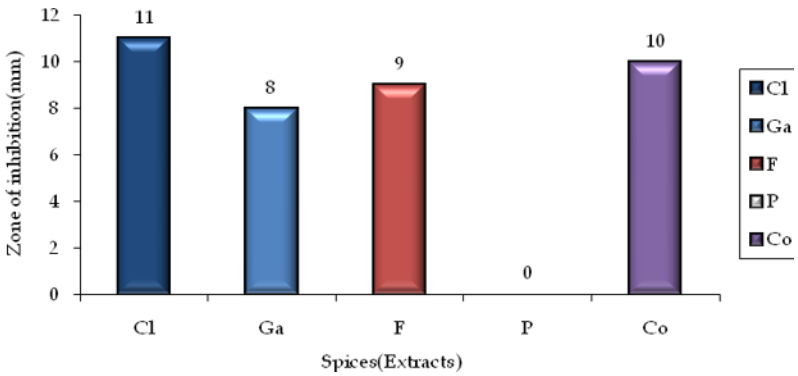

Figure 5: Antibacterial activity of 50ml extract of different spices against *E.coli.*

Table 6: Zone of inhibition of 50 ml Spice extracts against *Klebsella.*

S.NO	SPICES(Extracts)	ZONE OF INHIBITION (mm)
1	Clove	-ve
2	Garlic	8
3	Fennel	9
4	Poppy seeds	10
5	Coriander	10

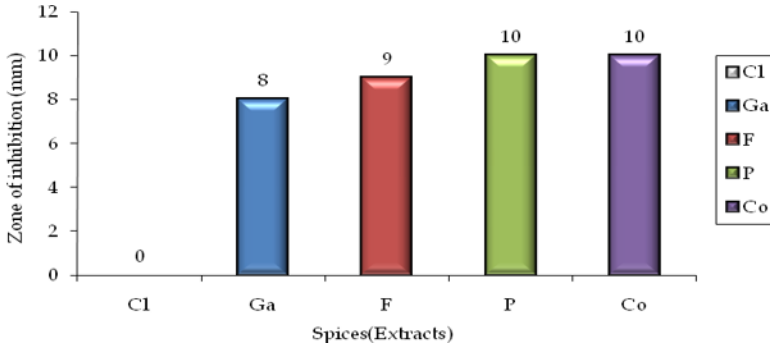

Figure 6: Antibacterial activity of 50ml extract of different spices against *Klebsella.*

Table 7: Comparison of 50 ml and 100ml extracts of different spices against *S.aureus.*

S.NO	SPICES(Extracts)	ZONE OF INHIBITION(mm) 100ml	50ml
1	Clove	7	9
2	Garlic	9	9.5
3	Fennel	6	7
4	Poppy seeds	7	11
5	Coriander	8	9

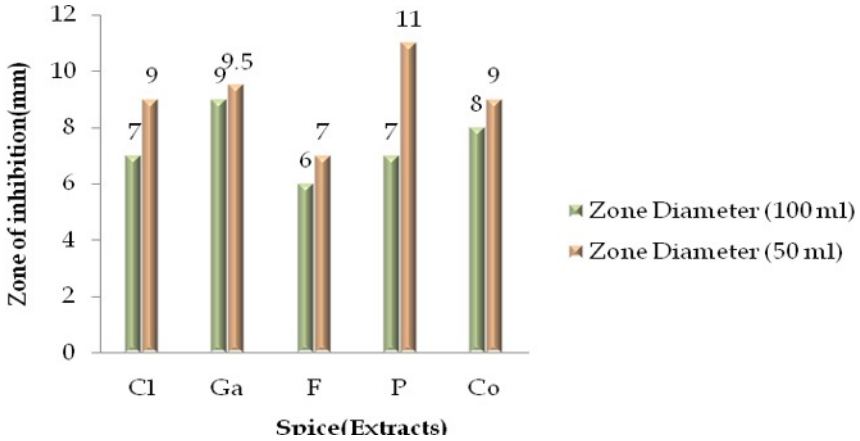

Figure 7: Comparison of antibacterial activity of 50ml and 100ml extracts of different spices against *S.aureus*.

Table 8: Comparison of 50 ml and 100ml extracts of different spices against *E.coli.*

S.NO	SPICES(Extracts)	ZONE OF INHIBITION(mm) 100ml	50ml
1	Clove	10	11
2	Garlic	7	8
3	Fennel	6	9
4	Poppy seeds	-ve	-ve
5	Coriander	9	10

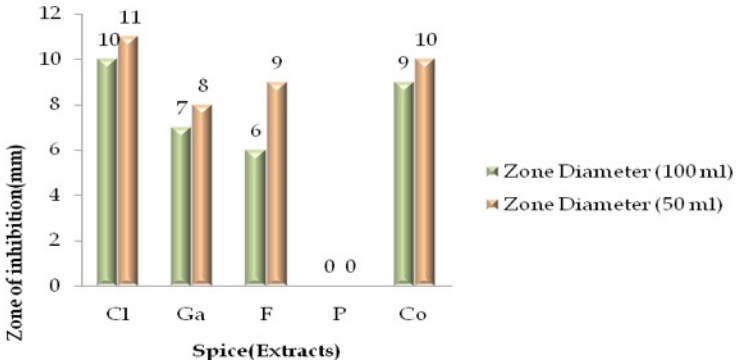

Figure 8: Comparison of antibacterial activity of 50ml and 100ml extracts of different spices against *E.coli*.

Table 9: Comparison of 50 ml and 100ml extracts of different spices against *Klebsella.*

S.NO	SPICES(Extracts)	ZONE OF INHIBITION	
		100ml	50ml
1	Clove	-ve	-ve
2	Garlic	7	8
3	Fennel	7	9
4	Poppy seeds	8	10
5	Coriander	9	10

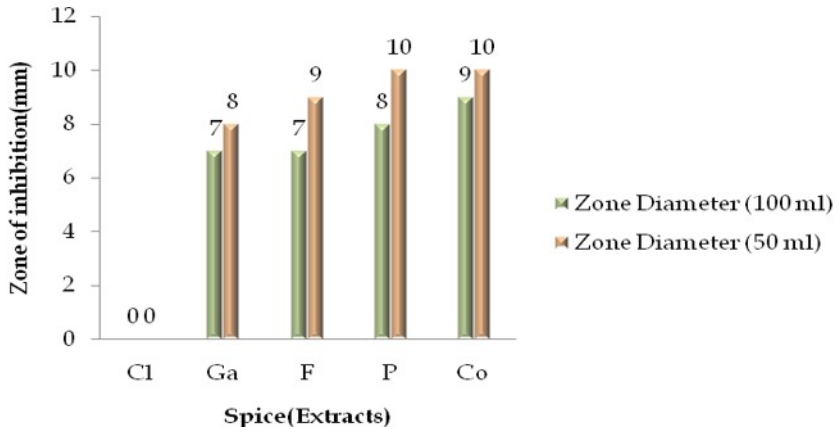

Figure 9: Comparison of antibacterial activity of 50ml and 100ml extracts of different spices against *Klebsella.*

DISCUSSION

Our study indicated variation in antibacterial activities of aqueous decoctions against selected spices. In this study the aqueous decoction of clove of 100ml and 50ml exhibited maximum antibacterial activity against *E.Coli.* The results of the present study are in harmony to those reported by Saeed and Tariq (2008) that clove was found effective against non-toxigenic strains of *E. coli* O157:H7. Clove was also active against S.aureus but did not show any activity against *Klebsella*. *K. ozaenae, K. pneumoniae, S. marcescens, S. typhi, S. dysentriae* and *V. cholerae* were found resistant to aqueous decoction of clove. In another study clove was found active against foodborne Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*E. coli*). Clove extract showed inhibitory effect against *S. aureus* (Saeed and Tariq, 2008).

In present study the aqueous decoction of garlic of 100ml and 50ml exhibited maximum activity against S.aureus. Garlic extract was also active against *Klebsella* and *E.coli*. Garlic exhibited broad spectrum activity against gram-positive and gram negative bacteria (Eja et al.,2007).
In this study it was also found that coriander was most active against *Klebsella.* Coriander also showed activity against *E.coli* and *S.aureus*. The results of the present study are in harmony to those reported by Al-Jedah et al.(2000) that coriander has strong antibacterial activity against *S. aureus, S. typhi* and *E. coli.*

While this result is contrary to the work of Bakhiet et al.(2006) that *Coriandrum sativum* seed extracts showed no inhibition zone to the microorganisms tested which is in fair correlation with the study carried out by Chaudhry and Tariq (2006) who found that decoction of *C. sativum* did not show antiBacteria potential against Gram positive and Gra negative bacteria.

Fennel was also active against *S.aureus, E.coli and Klebsella,* but was less active than other spices. Fennel showed an inhibitory effect only against *S. aureus*, which is an important pathogen in food poisoning. The effect of fennel was the lowest amongst other spices (Agoglu *et al.,* 2007).

In this study aqueous extract of 50ml and 100ml of poppy seeds did not show any activity against *E.coli.* The aqueous decoction of poppy seed exhibited weak antibacterial activity against *Escherichia coli* (Chaudhry and Tariq,2008*)* .While Poppy seeds(50ml extract) showed the highest antibacterial activity against *S.aureus.* Poppy seeds were also found to be active against *Klebsella.*

CONCLUSION

The aqueous decoctions showed variation in antibacterial activities against bacteria (*S.aureus, E.coli* and *klesella)*. Garlic, coriander and fennel were active against all bacteria but poppy did not show any activity against *E.coli,* clove was not active against *klebsella*. A maximum activity against *E.coli* was observed with clove while garlic was most active against *S.aureus.* Extract of 50ml poppy seeds showed highest activity against *S.aureus.* The present study has revealed the importance of natural products to control bacteria which are being a threat to human health.

REFERENCES

- **Agoglu,S.,Dostbil,N., and Alemdar, S**.(2007). Antimicrobial activity of some spices used in the meat industry. *Bull Vet Inst Pulaway*. **51**, 53-57.
- **Al-Jedah, J.H., Ali, M.Z., and Robinson, R.K**. (2000). The inhibitory action of spices against pathogens that might be capable of growth in a fish sauce (Mehiawah) from the Middle East. *Int.J.Food Micro.* **57**, 129-133.
- **Bakhiet,A.O., Mohammad,S.D., Samia., El Badwi,M.A.,Warda.,Gadir,S.A., Alkhatim, A.G.H and Adam,S.E.I.** (2006). Antimicrobial activity of *Petroselinum sativum*. *Res.J.Micro*. **1(4)**, 346-35
- **Chaudhry,N.M.A., and Tariq, P**. (2006). Bactericidal activity of black peeper, bay leaf, aniseed and coriander against oral isolates. *Pak. J. Pharm. Sci*., 19, 214-218.
- **Chaudhry,N.M.A., and Tariq,P**. (2008). In vitro antibacterial activity of kalonji, cumin and poppy seeds. *Pak.J.Bot*. **40(1)**, 461-467.
- **Eja,M.E., Asikong, B.E., Abriba, C., Arikpo, G.E.,Anwan,E.E.,and Enyi-Idoh,K.H**. (2007).A comparative assessment of the antimicrobial effects of garlic (*Allium sativum*) and antibiotics on diarrheagenic organisms.*SAJTMPH*. **38(2)**, 343-347.
- **Hussain,J., Khan, A.L.,Rehman, N.,Zainullah.,Khan, F.,Hussain, S.T., and Shinwari,Z.K.** (2009). Proximate and nutrient investigation of selected medicinal plants species of Pakistan. *Pak.J.Nutri*. **8(5)**, 620-624.
- **Ruberto,G., Baratta,M.T., Deans,S.g., Dorman,H.J.** (2000). Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum marithmum* essential oils. *Planta Med*. **66(8),** 687-693
- **Saeed,S., and Tariq,P**. (2008). In vitro antibacterial activity of clove against gram negative bacteria. *Pak. J.Bot*. **40(5)**, 2157-2160.
- **Sivum,G.P.** (2001) Protection against *Helicobacter pylori* and other bacterial infections by garlic. *J of Nutri*. **131**, 1106-1108.
- **Suliman,S.H., Elmahdi,B., and Abuelgasim,A.I**. (2008).The effects of feeding *coriandrum sativum* fruits powder on the plasma lipids profile in cholesterol fed rats. *Res.J.Ani and Vet Sci*. **3,** 24-28.