Evaluation of Antimicrobial Activity of Kitchen Spices & Condiments Used in West Bengal against Enteropathogens

Madhavi R.[1] T. K. Maity[2]
[1]Professor [2]Student
[1]College of Dairy Technology, SVVU, Tirupati, India [2]West Bengal University of Animal and Fishery Sciences, India

Abstract— In the present study three spices of Cinnamon (Cinnamomum zeylanicum, family Lauraceae), Clove (Eugenia caryophyllus, family Myrtaceae) and Turmeric (Curcuma longa L, family Zingiberaceae), were used at various extracts of concentrations i.e. 20%, 60%, 100% against the three enteropathogens i.e. Escherichia coli-97, Salmonella-98 and Klebsiella pneumonia-34.These were performed for antibacterial activity of the spice extracts at various pH i.e. 5, 6, 7, & 8 at 100% concentration against the enteropathogens , with different temperatures ie. 26°C, 37°C & 100°C by Disc diffusion method. Extracts of Cinnamon powder, three different solvents like hot water, cold water and ethanol and were used in the present study. The spices were washed with distilled water aand pulverized into smooth powder by using a grinder this was added to 20 ml of all the extracts and kept on. The mixture was left to incubate overnight in a water bath (70°C) followed by centrifugation (15minutes, 2000 rpm at 4°C). And filtered through Whatman no.1 filter paper. Among the various combinations used the spices with ethanol extract showed better technological and microbiological quality.

Keywords: Kitchen Spices, Enteropathogens

I. INTRODUCTION

From the last few decades many beneficial effects of the common food spices on the health have been understood. There are also new challenges about food safety due to increasing occurrence of new food-borne disease outbreaks caused by pathogenic micro-organisms. This raises considerable challenges, particularly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit growth of spoilage and pathogenic micro-organisms. Spices can be added to foods in several forms as whole spices, as ground spices, or as isolates from their extracts. Since India is known the world over as "The Home of Spices". In Indian system a large number of medicinal plants have been used for many centuries for treating various diseases, these plants have a wide variety of chemical constituents and some of them have the ability to inhibit the growth of pathogenic micro-organisms. Spices and herbs have an important impact of the human diet. Spices were used to kill such microorganisms or inhibit their growth before they could produce toxins, use of spices might reduce foodborne illnesses and food poisoning.

Gould (1995) proposed the "Natural antibiotic system" that emphasized the use of spices and their derivatives as possible alternatives for mainstream medicine. This system expanded on the synergistic effect of antimicrobial compounds extracted from plants and spices that was expressed in physical testing procedures in the attempt to create inhospitable living conditions for micro-organisms. Spices have been used for not only for flavour& aroma of the foods but also to provide antimicrobial properties (Nanasombat et al., 2002).Food Safety and Standards Act, 2006 allows making nutraceuticals drinks of spices like masala chai, jal jeera, Kalam khatta and kokum sherbet. Usage of spices is prominent in meat, which is particularly susceptible to spoiling by microbes. Moreover plant volatiles have been generally recognized as safe (GRAS) (Newberne et al., 2000). A study was shown that eugenol had stronger bactericidal activity against E. coli and K. pneumonia than some antibiotics (Ouattara et al., 1997). (Bullerman 1974) found out that 1% of cinnamon has significant inhibition on growth of Aspergillers paraciticus spores and aflatoxin production.

As far as the cosmetic field is concerned, (Herman et al., 2013) showed that commercial cinnamon essential oil in a cosmetic emulsion at 2.5% concentration possesses very good antibacterial activity against several contaminants such as P. aeruginosa ATCC 27853, E. coli ATCC 25922, and S. aureus ATCC 29213. Pandey et al. (2014) were screened for acetone and methanol extracts of 5 Indian spices and their antibacterial property; found that the methanol extracts of turmeric, clove, pepper, ajwain and dalchini powder have high antimicrobial activity on all pathogenic organisms. A study from Hili et al. (1997) indicated that Cinnamon oils have potential action against various bacteria (Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli) and yeast (Torulopsisutilis, Schizosaccharomyces pombe, Candida albicans, and Saccharomyces cerevisiae). Doaa et al. (2013) examined the antimicrobial activity of aqueous extracts from cinnamon, cumin and coriander on MRSA and results revealed that the aqueous extract of cinnamon is the most potent activity against Methicillin-Resistance Staphylococcus aureus (MRSA), compared to cumin and coriander.Therfore in the present study we aim to show the antimicrobial activity of the spice extracts against three different enteropathogens.

II. MATERIALS & METHODS

Three spices cinnminon (innamomum zeylanicum), Cloves (Syzygium aromaticum), Turmeric (Curcuma longa L.). Procured from Mohanpur market. And washed with distilled water then dried first in sunlight for two days and finally pulverized into fine powder by a grinder. Three gram negative organisms were used as test organisms in the study.Escherichia coli-97, Salmonella-98, Klebsiella pneumonia-34 procured from Department of Veterinary Microbiology, F/O Veterinary and Animal Science, Belgachia, Kolkata.

Three different solvents like hot water, cold water and ethanol and were used in the present study for Cinnamon. Similarly, distilled water was also used. To

prepare the solvent extracts, four grams each of Cinnamon fine powder was added to 20 ml, ethanol and kept on. The mixture was left to incubate overnight in a water bath (70°C) followed by centrifugation (15minutes, 2000 rpm at 4°C).The supernatant was filtered through Whatman no.1 filter paper and the filtrates were used as extracts. To prepare cool water extract, 4 grams of Cinnamon powder was added to 20 ml of distilled water and kept in deep freeze at 7 °C for 12 h. After 12 hr of, it was filtered through Whatman no.1 filter paper and the filtrate was used as cool water extract. The warm water extract was prepared by adding 4 gm of Cinnamon fine powder to 20 ml of distilled water and heated on water bath until boiling. Then, it was cooled at room temperature and filtered through Whatman no.1 filter paper and the filtrate was used as warm water extract. All the solvent extracts were used to test the antimicrobial activity against selected bacteria. The extracts were stored at 4°C until the use. The filtrate was regarded as 100% concentration spice extract and this was diluted with sterile distilled water by making different concentrations i.e. 60% and 20%. Similar methods used for extractions Clove & Turmeric

Preparation of Sensitivity Disc: Disc was punched using No.1 Whatman filter paper with the diameter of 7mm, and was sterilized by dry heat at 140°C for 1 hour. The Disc were allowed to cool, using screw-capped bottle, different concentrations of the spice extracts i.e.Cinnamon (Cinnamomum zeylanicum), Cloves (Syzygium aromaticum), Turmeric (Curcuma longa L.) were prepared using Distilled Hot water (80-90°C), Distilled Cold water (10°C) and Ethyl alcohol (90%) which arrived at the concentration of 100%, 60% and 20%. 50 pieces of the paper Disc were introduced into 0.5ml of the different concentration of extracts and allowed to stand until the whole concentration was completely absorbed by the filtration Disc, because each Disc is capable of absorbing 0.01ml.

A. Propagation & Maintenance of Test Culture

Pure cultures were maintained for every 3 to4 days on nutrient agar slants and plates on regular basis. The cultures were streaked on sterile nutrient agar plates and kept in incubator for 24 hours at 37°C and stored at 4°C. Inoculum was prepared by growing the pure bacterial culture in nutrient broth over night at 37°C.

B. Preparation of the Culture Broth and Nutrient Agar

Nutrient broth was prepared and was inoculated with the test organism. A loop full of microorganism was taken and inoculated in the Nutrient broth and was incubated at 37°C for 24 hrs to obtain a viscous growth and purity was confirmed by microscopic examination. Nutrient agar was prepared as per manufacturer instructions

C. Preparation of Nutrient Agar (NA)

Nutrient agar (NA) was prepared according to manufactures instructions; 28g NA was dissolved in 1000 ml of distilled water, the suspension was mixed until completely homogenized. The conical flask containing the media were plugged with cotton wool and capped with aluminium foil. Using 16 procedures, the flask was sterilized using lender autoclave at 121°C for 15 minutes, cooled to 30°C and poured into sterile plates.

D. Antibacterial Sensitivity Testing using Disc Diffusion Method

Filter paper Disc of 7mm diameter using Whatman no. 1 filter paper was prepared and sterilized. The test microorganisms were transferred from nutrient broth to sterile Muller Hinton/Nutrient agar plates with the help of sterile cotton swabs. Using an ethanol dipped and flamed forceps the Discs were aseptically placed over the Muller Hinton/ Nutrient agar plates seeded with the test microorganisms .10-µL of the various spice extracts i.e. pure extract, ethanol extract and aqueous extract were aseptically transferred to each Disc at all dilutions that were made in triplicate. Plates were incubated in an upright position at 37°C for 24hours. 10 µL of 95% ethanol was added in sterile filter paper Disc as negative control. Triplicate sample of each dilution was tested. After 24hrs the diameter of zone of inhibition were measured in mm and results were recorded. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active (Junior and Zanil, 2000)

E. Effect of Temperature and pH on Antibacterial Activity of Spices

The effect of temperature pH on its antibacterial activity is determined by taking aqueous and solvent extracts of cinnamon, clove and Turmeric in sterile test tubes and kept at room temperature (25°C, 37°C) and other boiled at higher temperature (100°C) and pH by adjusting the pH (5. 6, 7, and 8) at room temperature with 1N NaOH and allowed it to stand for 2 hours. Then the antibacterial activity was tested by Disc diffusion method. (Anees Ahmed et al., 2015).

F. Statistical Analysis

All result's collected during the present investigation were analysed using one way Analysis of Variance (ANOVA) with the general linear model and univariate data from the Statistical Analysis System software package version SPSS 10.0 with the significance level set at $P \leq 0.05$ and $P \leq 0.01$

III. RESULTS & DISCUSSIONS

A. Antimicrobial Activity of Cinnamon at Different Concentrations against Various Enteropathogens by Disc Assay Method

From Fig 1 It is observed that the ethanol extract of cinnamon bark, the zone of inhibition was very much active for E.coli-97, partially active for salmonella-98 and inactive for Klebsiella-34 at different concentrations. The inhibition zones ranged from 9-17mm for ethanol extract of Cinnamon. In the cool water extract lowest zone of inhibition were registered for cold water extract against all the enteropathogens (7-10mm). Hot water extract showed the average zone of inhibition for all enteropathogens (8-12mm). From the figure (4.1a, 4.1b and 4.1c), It was observed that ethanol extract showed the highest zone of inhibition followed by hot water extract. The cold water

extract exhibited the lowest zone of inhibition. The results of the present findings demonstrate that Ethanol extract have shown better results as compared to the aqueous extracts (hot and cold) at different concentrations. Similar findings was reported by Abdelfadel et al. (2015) who observed that aqueous extracts of cinnamon showed inhibition ranging 9-12mm. Ismail et al. (2012) found that, clove, thyme and cinnamon extracts showed broad spectrum antimicrobial activities.

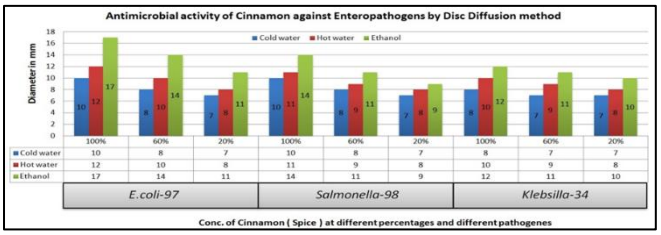

Fig. 1: Antimicrobial Activity of Cinnamon at Different Concentrations against various Enteropathogens by Disc Assay Method

*Including the diameter of filter paper Disc 7mm. Results are the average of three replicates. Cold water extraction: 10(°C), Hot water extraction 80-90 (°C).

B. Antimicrobial Activity of clove at Different Concentrations against Various Enteropathogens by Disc Assay Method

The results of the antagonistic capacity of the clove extracted with cold water, hot water and ethanol at various concentration viz. 100%, 60% and 20% against enteropathogenic strains of Escherichia coli- 97, Salmonella-98, and Klebsiella-34 are presented in Fig 2. Statistical analysis revealed that highly significant results (P≤ 0.01) were among the three spice extracts i.e. Hot water, cold water and Ethanol extracts as well as among the different concentrations of spice extracts of clove.Studies showed that ethanol extracts of Clove revealed highest antimicrobial activity against different pathogens i.e. E.coli-97, Salmonella-98 and Klebsiella-34 (9-18mm) followed by hot water extract (8-15 mm) and cold water extract (7 -13 mm). Also, results showed that, hot extract of clove showed a highest antagonistic activity against E.coli with inhibition zone 10-15mm and moderately active against Salmonella-98 and Klebsiella-34 with inhibition zone 9-14mm and 8-12mm respectively at different concentrations. From the figure it was observed that the ethanol extract of clove exhibited highest antimicrobial activity followed by hot water extract and cold water extract. Similar findings was also recorded by Saeed and Tariq (2008) who found that clove extracts showed antimicrobial activity against Escherichia coli and Pseudomonas aeruginosa.

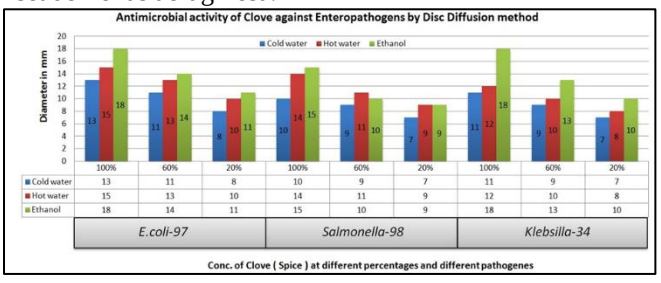

Fig. 2: Antimicrobial Activity of Clove at Different Concentrations against Various Enteropathogens by Disc Assay Method

*Including the diameter of filter paper Disc 7mm. Results are the average of three replicates. Cold water extraction: 10(°C), Hot water extraction 80-90 (°C).

C. Antimicrobial Activity of Turmeric at different Concentrations against various Enteropathogens by Disc Assay Method

From the figure 3 it is observed that all the extracts of turmeric at various solvent used in study have an antagonistic activity against all the Enteropathogens. Highest activity was observed by Ethanol extracts forming a maximum zone of 9 -15 mm against E.coli-97, 10-16 mm against Salmonella-98 and 10-17 mm against Klebsiella-34, while aqueous extracts (Hot and cold) of turmeric showed lowest antimicrobial activity (7-13mm) of all enteropathogens. Ethanol extracts showed highest antagonistic activity followed by hot water and cold water. The findings of this investigation are in agreement with the work of Sana and Ifra (2012). In that study the ethanol extract of turmeric showed better results against B. subtilis DSM3256 and E. coli ATCC25922 as compared to the aqueous ones.

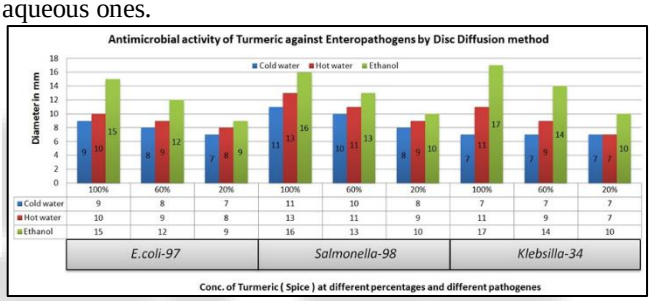

Fig. 3: Antimicrobial Activity of Turmeric at different Concentrations against various Enteropathogens by Disc Assay Method

*Including the diameter of filter paper Disc 7mm. Results are the average of three replicates. Cold water extraction: 10(°C), Hot water extraction 80-90 (°C).

D. Antibacterial Activity Aqueous Extract of three Spices at different pH against Enteropathogens by Disc Diffusion Method

From Fig 4 it is observed that a significant decrease in antibacterial activity of cinnamon as the pH increases. Highest antagonistic activity was observed against all pathogens at pH 5 (20-22mm). All the organisms are highly resistant at pH 8 (8-12mm) against the Cinnamon extract. Statistical analysis revealed that highly significant (P≤ 0.01) differences were observed among the pH and significant (P ≤ O.O5) among pathogens. There is a significant decrease in antibacterial activity of clove as the pH increases (Fig 5). The antibacterial activity was greater in the acidic pH 5.The findings of investigation are similar with Tynecka et al.(1999) and Srinivasan Durairaj (2009) in which the antimicrobial activity of Allium ursinum juice and garlic extract was decrease with increase in pH. Statistical analysis revealed that highly significant variation (P≤ 0.01) were observed among different pH and significant (P ≤ O.O5) among pathogens. Statistical analysis revealed that all the

results are significant (P ≤ O.O5) among the pathogenic strains and highly significant among the pH (P≤ 0.01).There is a decrease in antibacterial activity of Turmeric as the pH increases, which indicates high antimicrobial activity against all tested pathogens (Fig 6). Highest antibacterial activity was observed at pH 5 against all the enteropathogens (20-22mm) whereas lowest antibacterial activity was noticed in alkaline condition of turmeric extract (8-9mm).

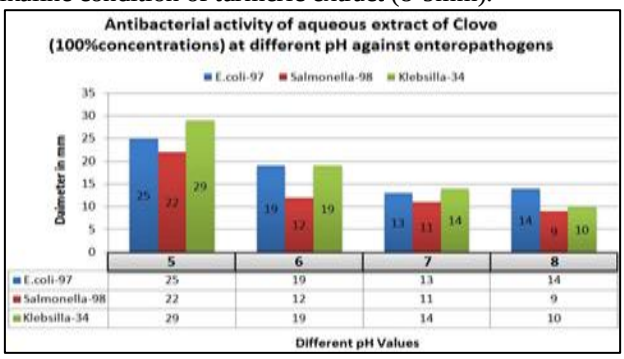

Fig. 4: Antimicrobial activity of clove at different ph against various Enteropathogens by Disc assay method

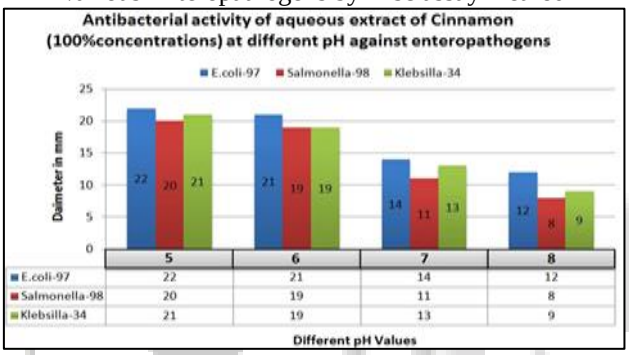

Fig. 5: Antimicrobial activity of cinnamon at different pH against various Enteropathogens by Disc assay method

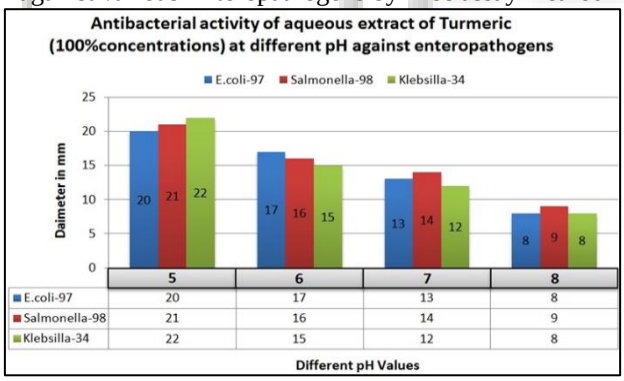

Fig. 6: Antimicrobial activity of Turmeric at different pH against various Enteropathogens by Disc assay method

E. Antibacterial Activity Aqueous Extract of Three Spices at Different Temperatures against Enteropathogens by Disc Diffusion Method

The results of the antibacterial activity of cinnamon are highest in 37°C compared to the 25°C and 55°C. From the figure 7 it was also observed that cinnamon exhibited highest antimicrobial activity against enteropathogens. Results revealed that 37°C is the best temperature for the antagonistic activity of the spice Cinnamon. The temperature 55°C exhibited the lowest antibacterial activity. Statistical analysis revealed that highly significant difference (P≤0.01) were observed among different temperature. Highest antibacterial activity was observed at 37°C against all the pathogens. Lowest antagonistic activity was recorded at 25°C.

From the figure 8 it was also observed that clove exhibited highest antimicrobial activity against enteropathogens. Results revealed that 37°C is the best temperature for the antagonistic activity of the spice clove. The temperature 25°C exhibited the lowest antibacterial activity. Statistical analysis revealed that highly significant difference (P≤0.01) were observed among different temperature. Highest antibacterial activity was observed at 37°C against all the pathogens. Lowest antagonistic activity was recorded at 25°C.

From the figure 9 it was observed that antagonistic activity of turmeric was highest against Salmonella-98 irrespective of any temperature .Highly significant results was found among the enteropathogenic strains as well as among the various temperature ((P≤ 0.01)

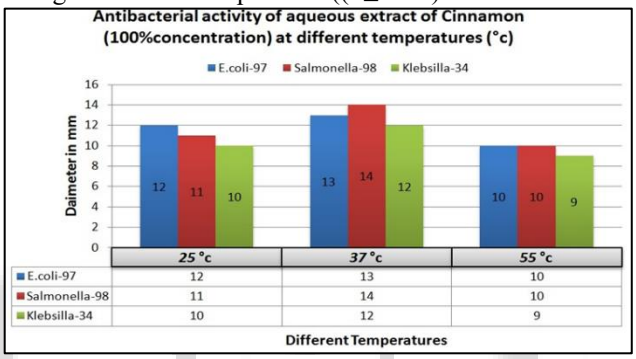

Fig. 7: Antimicrobial activity of cinnamon at different temperatures against various Enteropathogens by Disc assay method

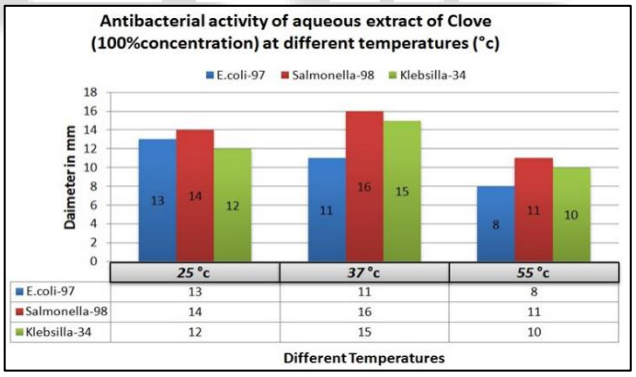

Fig. 8: Antimicrobial activity of clove at different temperatures against various Enteropathogens by Disc assay method

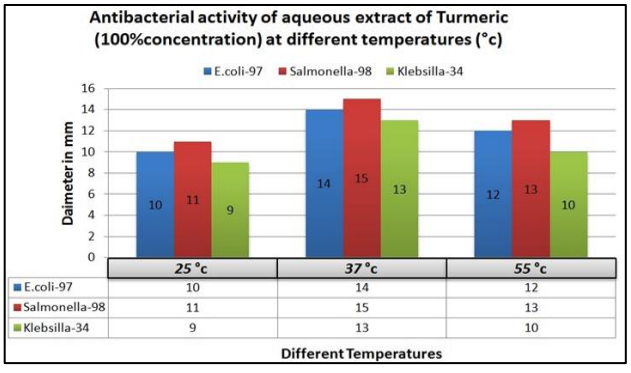

Fig. 9: Antimicrobial activity of turmeric at different temperatures against various Enteropathogens by Disc assay method

IV. CONCLUSION

The study provides a new perspective on the specific use of spices against enteropathogens. Spices in different milk and milk products at different concentrations coupled with fortification strategy may be used to overcome the antimicrobial activity against different pathogens in humans.

V. CONFLICT OF INTEREST

The authors declare no competing financial interests.

ACKNOWLEDGEMENTS

The authors acknowledge the contribution of Director ICAR for the financial support and providing a necessary facility for carrying out this study, This research did not reveal any specific grant from the funding agenciesnin the public,commercial or not – for-profit sectors

REFERENCES

[1] Abdelfadel M.M., Khalaf H. H., Sharoba A.M. and Assous M. T. M. (2015).Effect of extraction methods on antioxidant and antimicrobial activities of some spices and herbs extracts.International Journal of Advanced Research, 3(12):165 – 179.
[2] Abdul Rahman, M.S., Thangaraj, S., Mohamed Salique, S., Feroz khan, k. and EsathNatheer, S. (2010). Antimicrobial and Biochemical Analysis of Some Spices Extract against Food Spoilage Pathogens. Internet Journal of Food Safety, 12:71-75.
[3] Abhishek Sharma., Sankhla, B.,Parkar, M.S. and Mathur, S. (2014). Antimicrobial Activity of Clove and Ginger Powder Extracts on Streptococcus mutans.Scholars Academic Journal of Biosciences, 2(12):953-956. species.Biochemical and Biophysical Research Communications,13:183-188. al of food science,49:737-809.
[4] Shete, H.G., and Chitanand, M.P. (2014). Antimicrobial activity of some commonly used Indian Spices. International journal of current microbiological and applied sciences. 3(8):765-770.
[5] Shobana.S. and Akhilender Naidu, K.(2000). Antioxidant activity of selected Indian spices Prostaglandins Leukotrienes andEssential Fatty Acids, 62(2):107–110.
[6] Shukla, Y., Arora, A. and Taneja, P.(2002).Antimutagenic potential of curcumin on chromosomal aberrations in Wistar rats.Mutation Research,515:197–202.
[7] Singh, A.K., Dhamanigi, S.S, and Asad, M. (2009). Anti-stress activity of hydro-alcoholic extract of Eugenia caryophyllus buds (clove). Indian Journal of. Pharmacology,41:28-31
[8] Singh, G., Maurya, S., Marimutha, P., Murali, H. S. and Bawa, A. S. (2007). Antioxidant and antibacterial investigation on essential oils and acetone extracts of some spices. Natural Product Radiance, 6(2):114-121.
[9] Singh, S. and Aggarwal, B. B. (1995). Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane). Journal of Biological Chemistry, 270:24995–25000.
[10] Singh, S. and Aggarwal, B. B. (1995). Activation of transcription factor NF- kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. Journal of Biological Chemistry, 270:24995-5000.
[11] Song,E., Li., H., Sun,J. and Wang, S.(2013). Protective effects of cinnamic acid and cinnamic aldehyde on Isoproterenol-induced acute myocardial ischemia in rats.Journal of Ethnopharmacology, 150(1):125–130.
[12] Souza, E. L., Stamford, T. L. M., Lima, E. O., Trajano V. N. and Filho, J. B. (2005). Antimicrobial effectiveness of spices: an approach for use in food conservation systems.Brazilian Archives of Biology and Technology, 48:549-558.
[13] SrinivasanDurairaj., SangeethaSrinivasan, P. AndLakshmanaperumalsamy.(2009). In vitro Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature Electronic. Journal of Biology, 5(1):5-10.
[14] Srivastava, R., Dikshit, M., Srimal, R.C. and Dhawan, B.N. (1985).Antithrombotic effect of curcumin.Thrombosis Research, 40:413–417.
[15] Stoner, G. and Wang, L. S. (2013). "Natural products as anti-inflammatory agents," in Obesity, Inflammation and Cancer:341–361,
[16] Suganthi, R., Rajamani, S., Ravichandran, M. K. and Anuradha, C. V. (2007). Effect of food seasoning spices mixture on biomarkers of oxidative stress in tissues of fructose-fed Insulin-resistant rats, Journal of Medicinal Food, 10 (1):149–153.
[17] Suhaj, M. (2006). Spice antioxidants isolation and their antiradical activity: a review, Journal of Food Composition and Analysis, 19(6-7):531–537.
[18] Sunilson, J. A. J., Suraj, R., Rejitha, G., Anandarajagopal, K., Kumari, A. V. A. G. and Promwichit, P. (2009). In vitro antimicrobial evaluation of Zingiberofficinale, Curcuma longa and Alpinia galangal extracts as natural food preservatives. American Journal of Food Technology, 4(5):192-200.
[19] Surh, Y. J., Chun, K. S., Cha, H. H., Han, S. S., Keum, Y. S., Park, K. K. and Lee, S. S. (2001). Molecular mechanism underlying chemopreventive activities of anti-inflammatory phytochemicals: down regulation of COX-2 and iNOS through suppression of NF-kB activation. Mutational Research, 243–268.
[20] Syed Abdul Rahman, M., Thangaraj, S., Mohamed Salique, S., Feroz khan, k. and EsathNatheer, S. (2010). Antimicrobial and Biochemical Analysis of Some Spices Extract against Food Spoilage Pathogens. Internet Journal of Food Safety, 12:71-75.
[21] Tabak, M., Armon, R., Potasman, I. and Neeman, I. (1996). In vitro inhibition of Helicobacter pylori by extracts of thyme. Journal of Applied Bacteriology, 80:667–72.
[22] Taher, M. M., Lammering, G., Hershey, C. and Valerie, K. (2003).Curcumin inhibits ultraviolet light induced human immunodeficiency virus gene expression.

Journal ofMolecular Cellular and Biochemistry, 254:289–297.
[23] Tajkarimi, M. M., Ibrahim, S. A. and Cliver, D. O. (2010).Antimicrobial herb and spice compounds in food.Food Control, 21:1199–1218.
[24] Tajuddin, Ahmed, S., Latif, A. and Qasmi, I. A.(2004). Effect of 50% ethanolic extract of Syzygiumaromaticum (L.) Merr. & Perry.Clove on sexual behaviour of normal male rats.BMC Complementary and Alternative Medicine, 4:17.

