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Anti-Bio Gram of the Most Common Dietary Additives (Spices) Against Common Problematic Organisms

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ABSTRACT

Background: In the current scenario, the greater antibiotic toxicity, increasing drug cost, and continually rising multi drug resistance are the main compelling factors for the researchers to focus on natural bioactive sources for safe and natural therapeutic agents as an alternative to allopathic antibiotic. **Objectives:** The main objective of the study is to investigate antimicrobial potential of most commonly used kitchen spices in an attempt to explore the natural bioactive sources for natural therapeutic agents as an alternative to synthetic antibiotics, against which there is a continuous rise in multi-drug resistant pathogens. **Study design:** Experimental/in vitro study conducted (from Sep, 2015 to Feb, 2016) in different departments (of School of Pharmacy, Pathology and Bio-chemistry of UM & DC), The University- of Faisalabad. **Methodology:** Antibiotics assay is performed by using the disc diffusion methods. Clove and Cinnamon extracts are prepared and from these sensitivity discs are prepared. Standard size inoculum is also prepared. Then by using standard disc diffusion method the zones of inhibitions produced by Clove and Cinnamon extracts are measured and recorded, and is compared with positive and negative controls. **Results:** Spice extracts of Clove and Cinnamon tested against test organisms revealed antimicrobial potency with diameter of zone of inhibition (13.33±0.33mm to 20.50±0.72mm) and (12.17±0.31mm to 15.67±0.49mm) respectively while positive control manifested zone of inhibition (19.33±0.21mm to 29.50±0.22mm). Ethanol extracts prepared by soxhlet apparatus showed better results compared with extracts prepared by simple maceration process. Furthermore, overall ethanol extracts showed better results compared with the aqueous extracts. Statistical analysis: Data was analyzed by two-way analysis of variance (SPSS). **Conclusion:** This study reported the presence of natural bioactive compound(s) in Clove and Cinnamon with highly significant broad spectrum antibacterial potential, even against multi drug resistant pathogens.

Keywords: Anti-bio gram, Antimicrobial potential, Clove, Cinnamon, Ciprofloxacin, Multi drug resistance (MDR) pathogens.

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INTRODUCTION

Over the last decade, the substantial rise in emergence of most problematic nosocomial pathogens, multi-drug resistance clones of which have been disseminated worldwide, and becoming the main factor for failure for curing the microbial diseases, and creating the most challenging public health dilemmas for society.[1] Within hospitals about 70% of bacteria that caused ailments are becoming insensitive to at least one of the drugs currently prescribed for treatment.[2] So, the scenario is paving the way for new and reemerging infectious diseases worldwide.[3]

So, to address the ever-increasing health problems, there is an urgent need to put attentions to natural sources (medicine plant, fruits and spices etc.), for cost effective safe and natural therapeutic agents as alternative to antibiotics /modern medicines.[4] Medicinal herbs have long been believed as a source of therapeutic remedy based on sacred and cultural customs. These are considered as natural drugs.[5]

Cinnamon (*Cinnamomum cassia*), a unique multifaceted medicinal plant having unique fragrance for which it is being widely used in drugs, food and cosmetic products. It is valued as one of the world's most important and popular food flavoring spices.[6] It contains a variety of resinous compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and numerous essential oils (cinnamaldehyde, eugenol, cinnamic acid, weitherhin). Its different parts (Inner bark, leaves and oil) are being used for culinary and traditional medicine worldwide.[7] Its various biological activities observed with cinnamon are; neuro protective,[8] antioxidant,[9] coagulant,[3] anti-diabetic,[10] anti-microbial,[11] Cardio protective,[12] anti-inflammatory.[13] It's most important active principle is cinnamaldehyde, which is present in its essential oil, and is responsible for its fragrance and diverse

bioactivity.[14] Further, its cholesterol and lipid lowering activity is also observed.[15] Recently, it's anti plasmid property has also been explored.[16]

Clove (*Syjygium aromaticum*), is commonly used as a spice in Subcontinent, Near and Middle East, Africa and so many other countries of the world. It is one of the most valuable spices that has been used since ancient times as food preservative and also for many medicinal purposes.[17] Currently pharmacological effects are being actively investigated. In vitro studies demonstrate that it has antiviral,[18] anti-nociceptive,[19] antifungal,[20] antioxidant,[21] antimicrobial,[22] anticancer,[23] Insecticidal,[24] pest repellent[25] and anti plasmid activity.[16]

Test Organisms: Most common problematic pathogens, one from gram positive and other from gram negative bacteria, are selected as test organisms.

Salmonella typhi: Gram negative bacterium, many strains of which are rapidly adopting multi drug resistance capabilities,[26] is responsible for typhoid global burden/typhoid fever; which is still on rise, especially in high risk areas of the world.[27,28]

Staphylococcus aurous: This very notorious gram-positive bacterium is major pathogen for human that is rapidly acquiring multi drug resistance and can cause suppuration in any organ/system.[29]

Objectives:

To investigate the anti-microbial potential of Clove and Cinnamon that may lend more weight to general acceptability of plant products (crude extracts) for therapeutic use in general and may provide preliminary information(s) for further development of more potent broad-spectrum antibiotics effective against multi resistant pathogens, particularly against *Salmonella* (responsible for deadly typhoid fever), which is sensitive only to very few standard antibiotics presently.

METHODOLOGY

Period: September 2015 to February 2016.

Antibiotic assay is performed by using the disc diffusion methods.

Extract Preparation: The spices were grinded to make fine powder. Fine powder of spices, Clove and Cinnamon, were taken. 30gram of each spice was mixed with 100ml of ethanol and 100ml of water separately. Extracts were prepared with the help of Soxhlet apparatus as well as simple maceration process. Then 10µl of each extract was used for anti-bio gram.

Inoculum Preparation: The test organism of each strain was sub cultured on nutrient agar medium (by incubating at 37 degree overnight), and from this fresh culture, standard size (10^8 to10^9 CFU per ml) inoculum is prepared in used.

Preparation of Disc: What Mann No. 1.6mm filtered paper antibiotic (extract) discs were prepared and sterilized by autoclaving.

Inoculum and Testing: Antimicrobial activity of the extracts was tested using the disc diffusion method 10µl of each extracts was impregnated into empty sterilized antibiotic disc. Each Muller Hinton agar plate was inoculated with the standard inoculum suspension by soaking a swab and rotating it over the agar plate. The paper antibiotic discs were placed over the inoculated agar. After 24 hours of incubation at 37˚c, zones of an inhibition were measured and recorded in millimeter.

Reference Drugs:

Ciprofloxacin 5µg of composed disc was used as positive control.

Ethanol and distilled water were used as negative control.

Antimicrobial Bioassay: Antimicrobial activities of different extracts were determined by Agar Diffusion method.[30] For this, nutrient agar was used as culture media, Cotton swabs were dipped in the standard size inoculum and were swabbed on the solidified media surface. Discs were placed aseptically over the standard size inoculum on the nutrient agar plates along with positive and negative controls and incubated at 37^0c for 24 hours. All treated petri plates were immediately placed in incubator at 37^0c. Sterile, blank paper discs impregnated with only sterile water and ethanol were used as negative control each time. Standard Ciprofloxacin was used as positive control for comparison of antibacterial activity. After 24 hours incubation, all the plates having discs for alcoholic extracts and water extracts were observed for their zone of inhibition. The zone of inhibition around the discs were measured (in mm) by venire caliper. The data of zone of inhibition of spice extracts, having two types of solvent extracts (water and ethanol) and a control against two bacterial pathogens was recorded with six times repeats to confirm the reproducible results of plant extracts. Since all the observations in negative control were zero, therefore data in negative control was not used for statistical analysis.

Statistical Analysis: Data was analyzed by two-way analysis of variance. The data collected for each experiment are subject to statistical analysis. Data was analyzed using (SPSS 20.0)

RESULTS

The study has been analyzed to investigate the antibacterial activity of Clove and Cinnamon against two bacteria by using two different methods (Soxhlet

and Maceration). Antimicrobial activity of each plant was determined by agar diffusion method. After 24 hours of incubation at 37º C the data has been recorded in the zone of inhibition (mm) of Clove and Cinnamon extracts under two bacterial pathogens using six repeats. The present study assessed/investigated the anti-biogram of Clove and Cinnamon. Extracts were prepared by two methods; soxhlet and maceration, using two different solvents (ethanol and distilled water).

Regarding the;

Ciprofloxacin (reference drug) showed zone of inhibition ranging from 19.33±0.21mm (*S.aureous*) to 29.50±0.22mm *(S.typhi).*

Plant extracts: All extracts manifested varying but significant degree of activity against two tested bacteria.

Solvents: Among the solvents ethanol showed better extraction power than distilled water.

Methods: Soxhlet method proved more effective.

Bacteria: Both bacteria tested proved susceptible to the plant extracts evaluated. Among these tested organisms, *S.typhi* showed highest zone of inhibition.

Table 1: Botanical information of the spices used

Botanical Name of Plant	*Cinnamomum verum*	*Syzygium aromaticum*
Common Name (English)	Cinnamon	Clove
Common Name (Urdu)	Dar Chini.	Laung
Family	Laureaceae	Myrtaceae
Part Used.	Barks	Buds

Table 2: Comparison of Antimicrobial activity (in millimeter) of alcoholic crude extract (by soxhlet extraction) of Clove and Cinnamon spices with ciprofloxacin against test organisms

Plant	**Bacterial Pathogens**		**Mean**
	S. typhi	***S. aureus***	
Clove	20.50±0.72b	18.67±0.42b	19.58±0.48B
Cinamon	15.67±0.49c	14.83±0.40c	15.25±0.33C
Standard drug	29.50±0.22a	19.33±0.21b	24.42±1.54A
Mean	21.89±1.42A	17.61±0.52B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 3: Comparison of Antimicrobial activity (in millimeter) of alcoholic crude extract (by maceration extraction) of Clove and Cinnamon spices with ciprofloxacin against test organisms

Plant	**Bacterial Pathogens**		**Mean**
	S. typhi	***S. aureus***	
Clove	19.50±1.15	18.50±0.56	19.00±0.63B
Cinamon	14.00±0.45	13.83±0.70	13.92±0.40C
St. drug	24.33±0.21	21.50±0.22	22.92±0.45A
Mean	19.28±1.10A	17.94±0.82B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Table 4: Comparison of Antimicrobial activity (in millimeter) of water crude extract (by maceration) of Clove and Cinnamon spices with ciprofloxacin against test organisms

Plant	**Bacterial Pathogens**		**Mean**
	S. typhi	***S. aureus***	
Clove	16.50±0.76c	13.33±0.33d	14.92±0.62B
Cinamon	12.67±0.49d	12.17±0.31d	12.42±0.29C
St. drug	21.50±0.22a	19.33±0.21b	20.42±0.36A
Mean	16.89±0.92A	14.94±0.78B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5: Analysis of variance

Source	**DF**	**SS**	**MS**	**F**	**P**
Extract (E)	2	279.02	139.51	88.21**	0.000
Plant (P)	2	1373.02	686.51	434.09**	0.000
Bacteria (B)	1	171.26	171.26	108.29**	0.000
E x P	4	22.37	5.59	3.54*	0.010
E x B	2	43.46	21.73	13.74**	0.000
P x B	2	97.02	48.51	30.67**	0.000
E x P x B	4	82.59	20.65	13.06**	0.000
Error	90	142.33	1.58		
Total	107	2211.07			

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 6: Overall Comparison of Antimicrobial activity (in mm) of Ethanolic extract (by soxhlet), Ethanolic extract (by maceration) and water extract (by maceration) against test organisms

Extract	**Bacterial Pathogens**		**Mean**
	S. typhi	***S. aureus***	
Ethanolic Soxhlet	21.89±1.42a	17.61±0.52c	19.75±0.83A
Ethanolic Maceration	19.28±1.10b	17.94±0.82c	18.61±0.68B
Water Maceration	16.89±0.92c	14.94±0.78d	15.92±0.62C

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 7: Overall Comparison of Antimicrobial activity (in millimeter) of Ethanolic extract (by soxhlet), Ethanolic extract (by maceration) and water extract (by maceration) of Clove and Cinnamon spices with ciprofloxacin (standard drug) against test organisms

Plant	Extract	Bacterial Pathogens		Mean
		S. typhi	*S. aureus*	
Clove	Ethanolic (Soxhlet)	20.50±0.72cd	18.67±0.42de	19.58±0.48B
	Ethanolic (Maceration)	19.50±1.15cd	18.50±0.56de	19.00±0.63B
	Water (Maceration)	16.50±0.76ef	13.33±0.33ghi	14.92±0.62C
Cinamon	Ethanolic (Soxhlet)	15.67±0.49fg	14.83±0.40f-i	15.25±0.33C
	Ethanolic (Maceration)	14.00±0.45f-i	13.83±0.70ghi	13.92±0.40CD
	Water (Maceration)	12.67±0.49hi	12.17±0.31i	12.42±0.29D
Standard drug	Ethanolic (Soxhlet)	29.50±0.22a	19.33±0.21cd	24.42±1.54A
	Ethanolic (Maceration)	24.33±0.21b	21.50±0.22c	22.92±0.45A
	Water (Maceration)	21.50±0.22c	19.33±0.21cd	20.42±0.36B

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

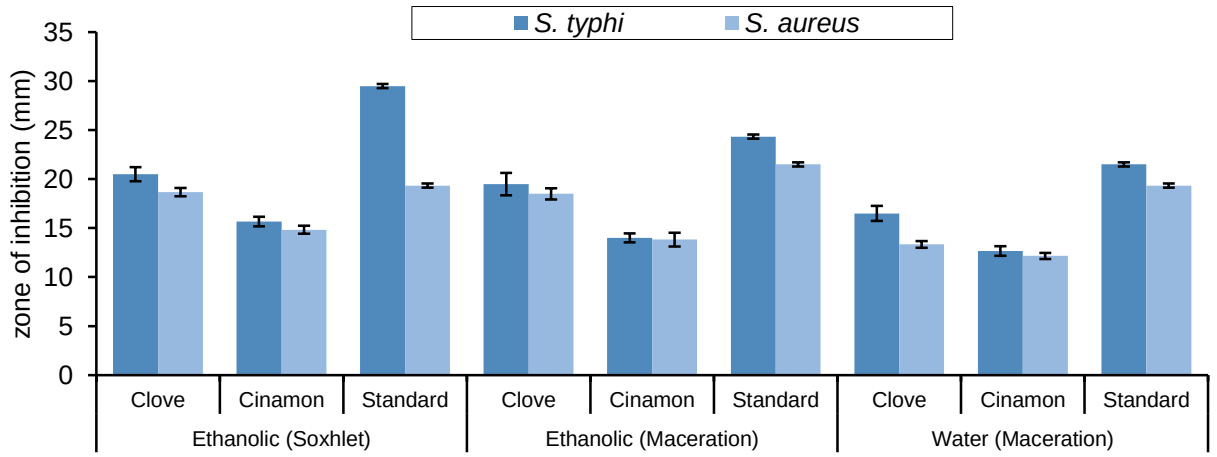


Figure 1: Overall Comparison of Antimicrobial activity (in millimeter) of Ethanolic extract (by soxhlet), Ethanolic extract (by maceration) & water extract (by maceration) of clove and cinnamon spices with ciprofloxacin (standard drug) against test organisms.

DISCUSSION

The important findings of present study are:

- All spice extracts revealed significant antimicrobial activity against both pathogens.
- Magnitude of antimicrobial activity of different extracts varied significantly according to the solvent used, as well as method of extraction applied.
- Highly significant findings were, that spice extracts exhibited almost comparable antimicrobial activity with the Ciprofloxacin (positive control).

Syzygium aromaticum (clove): Clove extract exhibited great antimicrobial activity almost comparable to reference drug ciprofloxacin (currently used medicine). The results of this study are in harmony to those reported by earlier researchers.[31,32,33] The results are quite encouraging as this spice exhibited marked antimicrobial activity against both pathogens, thus opening up the possibility for the search of new antimicrobials as alternative to the antibiotics from this spice extracts as a potential source of bioactive materials for analyzing and, subsequently synthesizing new and natural antibiotics. This study may serve a preliminary bio data for further analytical research

work, hence may contribute in synthesizing '*novel molecules*' with more desirable bioactivities.

Cinnamomum verum (Cinnamon): Cinnamon, a favorite spice around the world, significantly inhibited both G negative and G positive test bacteria, showing great antimicrobial potential. The finding of the study are in agreement with previous studies,[34,35] which showed remarkable inhibition against variety of tested bacterial and fungal strains. The result of the present work is also consistent with the work done,[36] who showed different effective concentration of extracts against three food spoilage and water born bacteria including staphylococcus aureus. Recently dried plant extract of cinnamon has proved to possess marked anti plasmid activity [16,] and thus, it could definitely be helpful to reduce the spread of antibiotic resistance plasmid in eco system. In a recent study [16], both of the above used spices (cinnamon, clove) proved to have remarkable R. plasmid curing activity. Present study findings are significant not only with reference to fast increasing multiple drug resistance (MDR) pathogens, but also for the S.typhi (responsible for dreadful typhoid fever), for which a limited number of effective drugs are available at present. Existing global scenario is now inclining toward the nontoxic medicinal plant products, for synthesis of the natural drugs that could normalize and reinstate the physiological system(s) of the body, that at times become malfunctioning. So, discovery of new drugs with more novel mechanisms of action are urgently required for dealing with the alarmingly increasing new reemerging infectious and non-infectious diseases.[3] In the present scenario, where fast emerging multi drug resistance (MDR) pathogens, are posing very serious global and future challenge to the chemotherapy or drug discovery program, the findings of significant broad spectrum antimicrobial activity in plants (under investigation) having R. plasmid curing property, might prove these dietary additives, '*the bullets of medicine',* against dreadful multi drug resistance microbes. Then the spices would be the future '*home remedy'* and '*elixir of life'*. Soxhlet method proved to be better because different conditioning factors (temperature, pressure and pH etc.) can properly/effectively be controlled by this apparatus.

Solvents: In present study, the ethanol proved to be better extractor, for this selected plant, as were the cases of previous studies in number of other different plants.[37,38,39] But in other studies, Methanol proved to be a better extractor for *psidium guajava, nigella sativa, citrus sinensis, valerian jutamansi* and *cucurbita papa,*[40] Chloroform for *Foenicolum valgare Mill,*[41] distilled water for *Lawsonia innernis Linn,*[42] and petroleum ether for *Trigonella foenum-graecum*[43] proved more effective than other solvents. Differential extraction ability/power of solvents may be because of different solubility of different active principles of different plants in different solvents. So, it appears to be related with differential solubility of active principles of plants for different solvents. It is thus concluded that in preparation of extracts, the solvents used are very important factor. So, different solvents should be probed to find out the best solvent for particular plant.

Plants: Generally a plant is consider to be active/effective against pathogenic microbes when the zone of inhibition is greater than 6mm [44]. Results showed/indicated that both spice extracts tested, significantly inhibited the growth of two tested pathogen bacteria at varying degrees. The maximum zone of inhibition 20.50±0.72mm was found with ethanol extract of spice (against *S.typhi*) and the minimum zone of inhibition (12.17±0.31mm) was found with aqueous extract of spice (against *S.aureous*). Results obtained from in vitro antimicrobial activity showed that both plant extracts exhibited a substantial/significant inhibitory effects against two tested bacteria. Ethanol extracts of plant extract from soxhlet method proved superior in suppressing the bacterial growth. This was followed by water extracts. Ethanol extracts exhibited promising antibacterial activity against two tested bacteria (both gram negative and positive), may be due to presence of phenols and flavonoids (active principles/ingredients) which are better extracted with ethanol. The lesser activity seen with other solvent (water) may be due to low solubility of active constituents/principles in this solvent. Varying degrees of antimicrobial activities may be due to different;

i. Active principle(s) within the different plant (spice) extracts.
ii. Solubility of active principles in different solvents.
iii. Conditioning factors (temperature, pressure and pH etc.) during processing.

Academic Value of study: This study provides;

iv. Scientific prove/base to traditional healers claim.
v. Bases for a full-scale investigation of the therapeutic potential, application and commercialization of various pharmacognostic, phytochemical and pharmacological profiles of cinnamon and clove.

CONCLUSION

Crude extracts of plants under investigation, significantly inhibited common medically important isolates (both Gram positive & Gram negative),

proving that these plants have potential as an alternate source of broad spectrum antimicrobial agents affective against multi drug resistant (MDR) pathogens including life-threatening *S.typhi.*

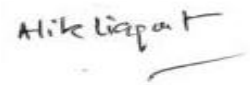
Suggestion: There is need to isolate the bioactive components (bio-principles) that are responsible for the ethno-pharmacological properties of this medicinal plant. This is might be accomplished after proper purification, quality chemotherapeutic index and pharmaceutical analysis of this plant extract.

REFERENCES

1. Hornsey M, Phee L, Longshaw C, Wareham DW. In vivo efficacy of telavancin/colistin combination therapy in a Galleria mellonella model of Acinetobacter baumannii infection. Int J Antimicrob Agents. 2013;41(3):285-7.
2. Purkayastha S, Narain R, Dahiya P, Evaluation of antimicrobial and phytochemical screening of Fennel, Juniper and Kalonji essential oils against multi drug resistant clinical isolates. Asian Pac J Trop Biomedicine. 2012;2(3): S1625-S1629.
3. Hussein N, Zahra Z, Abolfazl M, Mahdi S, Ali K. "Effect of Cinnamon zeylanicum essence and distillate on the clotting time," J Medicinal Plants Res. 2013;7:1339–43.
4. Dua A, Garg G, Mahajan R. Polyphenols, flavonoids and antimicrobial properties of methanolic extract of fennel (Foeniculum vulgare Miller). European J Exp Biol. 2013;3(4):203-8.
5. Gaur S. Medicinal and therapeutical potential of Nigella sativa. Journal of Biomedical and Pharmaceutical Research. 2015;4(1):1.
6. Huang TC, Fu HY, Ho CT, Tan D, Huang YT, Pan MH. "Induction of apoptosis by cinnamaldehyde from indigenous cinnamon Cinnamomum osmophloeum Kaneh through reactive oxygen species production, glutathione depletion, and caspase activation in human leukemia K562 cells," Food Chemistry, 2007;103(2):434–43.
7. Bairwa R, Sodha RS, Rajawat BS. Trachyspermum ammi Pharmacogn, 2012;6(11):56–60.
8. N. Cho., K. Y. Lee., and J. Huh, et al., "Cognitive-enhancing effects of Rhus verniciflua bark extract and its active flavonoids with neuroprotective and anti-inflammatory activities," Food and Chemical Toxicology. 2013;58:355–61.
9. Georgiev L, Chochkova M, Totseva I. et al. "Anti-tyrosinase, antioxidant and antimicrobial activities of hydroxycinnamoylamides," Medicinal Chemistry Research, 2013;22:4173–82.
10. Lee SC, Xu WX, Lin LY, Yang JJ, Liu CT. Chemical composition and hypoglycemic and pancreas-protective effect of leaf essential oil from indigenous cinnamon (Cinnamomum osmophloeum Kanehira). J Agric Food Chem. 2013;61(20):4905-13.
11. Parthasarathy H, Thombare S. Evaluation of antimicrobial activity of Azadirachta indica, Syzygium aromaticum and Cinnamomum zeyalnicumagainst oral microflora. Asian J Exp Sci 2013;27(2):13-6.
12. Song F, H Li, J Sun, Wang S. Protective effects of cinnamic acid and cinnamic aldehyde on isoproterenol-induced acute myocardial ischemia in rats. J Ethnopharmacol. 2013;150(1):125-30.
13. Stoner G, Wang L.S. Natural products as anti-inflammatory agents. Obesity, Inflammation and Cancer. 2013:341–61.
14. Yeh HF1, Luo CY, Lin CY, Cheng SS, Hsu YR, Chang ST. Methods for thermal stability enhancement of leaf essential oils and their main Constituents from Indigenous Cinnamon (Cinnamomum osmophloeum). J Agric Food Chem. 2013;61(26):6293-8.
15. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evidence-Based Complementary and Alternative Medicine. 2014:10;2014.
16. Mujumdar SS. et al. Herbal Extraction and Plasmid Curing (Curing of Multiple Drug Resistance in Bacteria Using Herbal Extracts). J Agric Food Chem 2016;5:456-9.
17. Cortés-Rojas DF, de Souza CR, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pac J Trop Biomed. 2014;4(2): 90–6.
18. Kurokawa M, Hozumi T, Basnet P, Nakano M, Kadota S, Namba T, Kawana T, Shiraki K. Purification and characterization of eugeniin as an anti-herpesvirus compound from Geum japonicum and Syzygium aromaticum. J Pharmacol Exp Ther. 1998;284(2):728–35.
19. Daniel AN, Sartoretto SM, Schimidt G, Caparroz-Assef SM, Bersani-Amado CA, Cuman RK. Anti-inflamatory and antinociceptive activities of eugenol essential oil in experimental animal models. Rev Bras Farmacogn. 2009;19(1A&1B):212–7.
20. Rana IS, Rana AS, Rajak RC. Evaluation of antifungal activity in essential oil of the Syzygium aromaticum (L.) by extraction, purification and analysis of its main component eugenol. Braz J Microbiol. 2011;42(4):1269–77.
21. Gülçin I, Elmastaş M, Aboul-Enein HY. Antioxidant activity of clove oil-A powerful antioxidant source. Arab J Chem. 2012;5(4):489–99.
22. Hill LE, Gomes C, Taylor TM. Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT-Food Sci Technol. 2013;51(1):86–93.
23. Nam H, Kim MM. Eugenol with antioxidant activity inhibits MMP-9 related to metastasis in human fibrosarcoma cells. Food Chem Toxicol. 2013; 55:106–12.
24. Barbosa JD, Silva VB, Alves PB, Gumina G, Santos RL, Sousa DP, et al. Structure-activity relationships of eugenol derivatives against Aedes aegypti (Diptera: Culicidae) larvae. Pest Manag Sci. 2012;68 (11):1478–83.
25. Zhang QH, Schneidmiller RG, Hoover DR. Essential oils and their compositions as spatial repellents for pestiferous social wasps. Pest Manag Sci. 2013;69(4):542–52.

26. Goldrick Barbara. Emerging Infections: Foodborne Diseases". AJN 2003;103(3):105–6.
27. Murray PR, Rosenthal KS, Pfaller MA. Medical Microbiology (6th Ed.). Philadelphia, PA: Mosby Elsevier. 2009. p. 307.
28. World Health Organization. Traditional medicine Media center. 2008. apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf
29. Umamaheswari B, & Rajalakshmi S. invitro Antibacterial Activity of four Indian spices against some pathogenic organisms. Int J Bio Trends Technol. 2015;9:630-9.
30. Rad JS, Alfatemi SMH, Miri A, Rad MS. A study of Antibacterial potentiality of some plants extracts against multi-drug resistant human pathogens. Ann Biol Res. 2013;4:35-41.
31. Khafagi I, Dewedar A, Farouk S. In vitro cytotoxicity and antimicrobial activities of some common essentials oils. Egyptain J Biol. 2000;2:20-7.
32. Shoaib A, Saeed G, Ahmad S. Antimicrobial activity and chemical analysis of some edible oils (clove, kalonji and Taramira) Afr J Biotechnol. 2014;13(46):4347-54.
33. Zeeshan Alam, Khan M, Kumar Y, Agarwal S, Srivastava A, Kumar S, Agarwal G. Antibacterial activity of Clove (Syzygium aromaticum) and Garlic (Allium sativum) on different pathogenic bacteria Int. J. Pure App. Biosci. 2014;2(3):305-311.
34. Ates DA, Erdogrul OT. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol. 2003, 27:157-62.
35. McCann J, Herbal Medicine Handbook. 2003. 2nd Edition. Philadelphia: Lippincott. pp 1133-95
36. Ramalingam C, Rakshit M. Health Benefits of Spices with Special Reference to Antimicrobial Activity and Bio Active Components. Journal of Experimental Sciences. 2010;1(7):203-15.
37. Jude BS, Ibrahim TA, Ola O. Comparing The Antibacteria Activity Of Ethanolic Extract Of Vernonia amygdalina and Occimum gratissimum with some Antibiotics of choice. IJTM 2009;6(2):1-4.
38. Bakht J, Muhammad T, Ali H, Islam A, Shafi M. Effect of different solvent extracted sample of Allium sativum (Linn) on bacteria and fungi. Afr J Biotechnol. 2011;10(31):5910-15.
39. Thirupathy S, Malayaman V, Sisubalan N, Basha MG. Antibacterial Activity of Tephrosia Hookeriana Wight and ARN Leaf Explants. Int J App Biol Pharma Technol. 2013;5:85-9.
40. Ejaz R, Ashfaq UA, Idrees S. Antimicrobial potential of Pakistani medicinal plants against multi-drug resistance Staphylococcus aureus. J Coastal Life Med. 2014;2(9):714-20.
41. Faaz RA, Jabar HS. Evaluation of anti-bacterial activity of foenicolumvulgare (l.) Mill extracts on some species of pathogenic bacteria. Bas J Vet Res. 2006;5:780-92.
42. Saadabi MA. Evaluation of Lawsonia inermisLinn. (Sudanese henna) leaf extracts as an anti-microbial agent. Res J Biolo Sci. 2007;2(4):419-23.
43. Mawahib et al, Antimicrobial activities and phytochemical screening of callus and seeds extracts of fenugreek (Trigonella foenum graecum). Int J Current Microbiol Applied Sci. 2015;4(2):147-57.
44. Muhammad HS, Muhammad S. The use of Lawsonia inermis Linn. (henna) in the management of burn wound infections. Afr J Biotec. 2005;4:934-7.

AUTHORSHIP AND CONTRIBUTION DECLARATION

AUTHORS	Contribution to The Paper	Signatures
Atika Liaqat Lecturer of Pharmacy The University of Faisalabad, Faisalabad	Article writing, Data collection	
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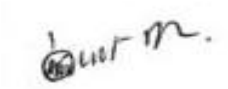
Prof. Dr. Liaqat Ali Professor of Anatomy University Medical and Dental College Faisalabad	Online search, Data analysis	
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