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THERAPEUTIC APPRAISAL OF ETHANOLIC AND AQUEOUS EXTRACTS OF CLOVE (*Syzygium aromaticum)* AND GARLIC (*Allium sativum*) AS ANTIMICROBIAL AGENT

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Antibiotic resistance; a threatening scenario to public health, has encouraged the use of herbal medicines in lieu of allopathic drugs. Since ancient times, clove (*Syzygium aromaticum*) and garlic (*Allium sativum*) have been used for culinary and medicinal purpose. The aim of the present study was to evaluate the antibacterial properties of aqueous and ethanolic extracts of *Syzygium aromaticum* (clove) and *Allium sativum* (garlic) against two gram positive (*Methicillin-resistant Staph aureus, Streptococcus spp.)* and three gram negative (*E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*) bacteria. After collection and confirmation, the aqueous and ethanolic extracts of both the herbs were prepared. Agar well diffusion test was employed to determine the antibacterial activity of these herbs. The results of the present study showed that ethanolic extract of clove had greater antimicrobial potential followed by aqueous extract of clove. The ethanolic extracts of garlic delineated moderate activity; while, least antibacterial potential was shown by aqueous extracts of garlic. It was further observed that highest zone of inhibition was produced by ethanolic extract of clove (26±0.5mm) against *K. pneumonia* followed by the zones of inhibition produced by ethanolic extract of clove against MRSA (20±1 mm) at 1.0 µg/mL concentration. The ethanolic and aqueous extract showed 64-128 ug/mL minimum inhibitory concentration (MIC) against all pathogens. The present study concluded that the studied spices had the potential to be used in the production of new antibacterial drugs.
Keywords: *Allium sativum, Syzygium aromaticum*, ethanolic extract, aqueous extract, antimicrobial activity.

INTRODUCTION

Since ancient times, spices like garlic, clove, cinnamon, mint, turmeric etc. have been used as flavor enhancer and preservative in food products. Moreover, it is also important for medicinal purposes in traditional medicinal culture like Ayurveda, Chinese medicine and Western herbalism by human (Kumar *et al.*, 2014). According to an estimate, almost 1500 plants are comprehensively used in local system for medicine (Joshi *et al.*, 2011). Herbal medicine usage is based exclusively on experience gained from trials and errors (Rafieian and Sewell, 2014).

In the history of medicine, antimicrobials are probably one of the most successful forms of chemotherapy. It is not necessary to restate here how many lives they have saved. Antibiotics have great contribution to control the morbidity and mortality rates of infectious diseases (Aminov, 2010; Khan *et al.*, 2019). Globally infectious diseases are leading cause of morbidity and mortality and are responsible for one third causalities as estimated by WHO (Nabavi *et al.*, 2015; Rahman and Mohsin, 2019).

Any therapeutic agent is successful till the development of resistance to it (Ashraf *et al*., 2017; Elsayed *et al*., 2018; Mahmood *et al*., 2018; Mehmood *et al*., 2018; Riaz *et al*., 2019; Khater *et al*., 2020; Salman *et al*., 2020; Zaman *et al*., 2020). Antibiotics are effective against several infectious diseases but due to their excessive use an increasing trend of the antibiotic resistance, use of herbal medicine as an alternative of allopathic drugs for both infectious and non-infectious diseases is suggested (Ibrahim, 2017; Abbas *et al*., 2017a, 2017b, 2018, 2019, 2020; Idris *et al*., 2017; Khater *et al.*, 2018; Fayyaz *et al*., 2019; Lin *et al*., 2020; Zhang *et al*., 2020). Antibiotic resistance has resulted in the development of multi drug resistant bacterial strains, which are associated with the epidemics of human diseases (Rahman *et al*., 2019; Younas *et al*., 2019). Antimicrobial agents obtained from medicinal plants can be used to treat infectious diseases with least side effects, which are often linked to synthetic drugs (Shihabudeen *et al*., 2010; Do *et al*., 2019).

Many commonly used spices have medicinal value. Spices add taste, fragrance and color to our food and also use as preservatives, savory and appetizers by many societies (Gupta, 2010; Mahmood *et al*., 2018). They are of great value among the herbal medicines having greater antimicrobial activity due to presence of different bioactive chemicals like Allicin, flavonoids, terpenoids, tannins, alkaloids etc. (Akhtar *et al.*, 2014). Different parts of plants can be utilized in preparation of drugs like bark, flowers, leaves or seeds etc. (Vaghasiya *et al.*, 2011; Khan *et al*., 2018). Spices like cinnamon, mint, clove, garlic and thyme show antioxidant and antimicrobial activities. Researchers have confirmed the antiviral, anticarcinogenic and antifungal properties of spices (Rojas *et al.*, 2014; Mahmood *et al*., 2018).

Allium sativum (garlic) belongs to family *Liliaceae,* native to Central Asia has been use over centuries for culinary and medicinal purposes (Farías-Campomanes *et al.*, 2014). Allium family especially garlic has broad antimicrobial activity because of its thiosulphate compounds, among them Allicin is of main value found only in fresh crushed garlic (Ibrahim, 2017). It has various biological role being as antimicrobial, antifungal, antithrombic, anticancerous (Jasamai *et al.*, 2016), antihyperlipidemic, antihypertensive and antiviral (Farías-Campomanes *et al.*, 2014).

Garlic consists of sulfur compounds, but the most attractive one is allicin. It is produced from allinin by an enzyme mediated reaction. Antibacterial activities of garlic have been proved against both Gram-negative and Gram-positive bacteria such as *E. coli*, *Salmonella spp., Klebsiella spp, Staph aureus* (Ibrahim, 2017), *H. pylori*, *Shigella senteriae, P. aeruginosa*, *Streptococcus spp. and Proteus mirabilis* (Khashan, 2014) and also against *Candida albicans* (Moore and Atkins, 2018).

Syzygium aromaticum(clove) is the dried aromatic flower buds of an evergreen tree belonging to family *Myrtaceae* used as spice, carminative, to reduce gastric irritation, and in herbal medicines (Singh *et al.*, 2015). Clove buds and its oil has to be known as antibacterial, antioxidant, antiparasitic, antimutagenic and antithrombic (Kumar *et al.*, 2014) antifungal effect (Giordani *et al.*, 2004). Active ingredient of clove include Eugenol, acetyl eugenol, chavicol, acetyl salicylate, humulenes (Joshi *et al.*, 2011) tannin, and vitamin B (Gupta, 2010). According to a rough estimate 89% of clove oil is eugenol. Many other volatile compounds like limonene, farnesol, benzaldehyde, 2-heptanone ethyl hexanoate and β-pinene are also present in lower concentration (Rojas *et al.*, 2014). Antibacterial activity of clove has been tested against *E. coli*, *Yersinia enterolitica Salmonella typhimureum*, *Listeria monocytogenes*, *Bacillus cereus* (Dussault *et al.*, 2014) and *Acinetobacter baumannii* and *Enterococcus faecalis* (Siddiqua *et al.*, 2015).

Because of the increasing resistance of pharmaceutical products research of plants is needed to be done as an alternatives of synthetic drugs and these two spices clove and garlic shows good potential to be used as antibacterial agents, therefore this study was designed to determine the antibacterial activity of clove and garlic on different bacterial species and to compare the antibacterial activity of ethanolic and aqueous extract of clove and garlic against prevalent bacteria.

So, in the present study two commonly used spices *i.e.*, clove (*Syzygium aromaticum*) and garlic (*Allium sativum*) were taken into consideration to explore their antimicrobial potential in the form of ethanolic and aqueous extracts.

MATERIALS AND METHODS

***Collection of herbs*:** The fresh form of clove (*Syzygium aromaticum*) and garlic (*Allium sativum*) herbs used in this study were collected from local market and identified and confirmed from a botanist. The fresh herbs were washed twice with distilled water, made into pieces, air dried and made into powdered form using pestle and mortar.

***Aqueous extract*:** Total five grams (5gm) of the powdered herbs and 20mL of distilled water were added into conical flasks. The flasks were covered with a wooden cork and contents of the flasks were mixed thoroughly. The flasks were placed at shaker adjusted at 100 rpm overnight. The mixtures were then filtered through a muslin cloth and centrifuged at 2000rpm for 5 min and supernatant was transferred into sterile falcon tube after filtration and stored at 4°C in refrigerator.

***Ethanolic extract*:** For preparation of ethanolic extract, 5gm of powdered herbs were mixed in 20mL of 95% ethanol in a flask and sealed with a cork. Similar procedure was adopted for mixing, shaking for 24 hours and filtering by muslin cloth. Centrifugation was performed at 2000rpm for 5 min and supernatant was decanted. The pellet was discarded and supernatant was filtered followed by concentration using rotary evaporator. The extract was then stored in sterile falcon tube at 4°C in refrigerator till further use.

***Sterility test of extracts*:** Sterility of all the extracts was examined on nutrient agar. 1ml of each extract were inoculated in nutrient agar plates and incubated for 24 hours at 37°C. Bacterial growth was observed to check the contamination. No growth in the plates indicated that the extracts were sterile.

***Antimicrobial susceptibility testing*:** All the bacterial isolates including two gram positive (*Methicillin-resistant Staph aureus, Streptococcus spp.)* and three gram negative (*E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae)* used in this study were characterized following Singh *et al*., (2015). The agar well diffusion method was performed for the antimicrobial susceptibility testing according to clinical and laboratory standard institute (CLSI) guidelines. Bacterial suspensions were prepared with comparison of 0.5 McFarland standards and inoculated on Mueller-Hinton agar (Oxoid) plates. Steel borer (12mm) was used to form wells on agar plate. The wells were filled with 0.1mL, 0.5mL and 1.0mL of each extract. The central well was kept as negative control and for the positive control Ciprofloxacin (10 µg) was used. Triplicate sample of each dilution was tested. ZOI (Zone of inhibition) were measured after 24 hours incubation at 37ºC (Mukhtar and Ghori, 2012). Antibacterial activity of extracts depends on the diameter of ZOI, larger ZOI denotes the high activity of extracts and smaller or no ZOI indicate the no activity of extracts.

***Minimum inhibitory concentration (MIC) of clove and garlic extract*:** For the determination of minimum inhibitory concentration (MIC) of clove and garlic previously reported broth dilution method by (Eloff, 1998) was modified. In micro titration plate 50 uL nutrient broth was added upto 12 well, followed by 50 uL extract added from 1st well to 10th well by making 2-fold serial dilution. Then 20uL bacterial suspension (0.5 McFarland) were added up to 12th well, 11th (nutrient broth+ extract) and 12th (broth+ extract) wells were positive and negative control respectively and incubated at 37ºC for 24 hours. Results were determined by observing the growth on nutrient agar plates.

RESULTS

Ethanolic extract of garlic showed no antibacterial activity against any of the bacterial isolates with the exception of *K. pneumoniae* towards which it showed mild antibacterial activity (7.30±0.5mm, 8.70±0.5mm and 9.00±1mm) at 01 mL, 0.5 mL and 1.0mL of 50 µg/mL, respectively as showed in Fig. 1. The results indicated that aqueous extract of garlic showed no antibacterial activity at any concentration against all the bacterial isolates. The zone of inhibition produced by aqueous and ethanolic extracts of garlic are mentioned in Table 1.

In the present study it was found that ethanolic extracts of clove exhibit a good antimicrobial activity than the aqueous extracts. Both extracts of clove showed antibacterial activity against all bacterial species with highest activity against *K. pneumonia* (12±0.5mm, 18±1 mm and 26±0.5mm) and MRSA (12±0.5mm and 20±1 mm). The aqueous extract had better antibacterial activity against *K. pneumoniae* (10±0.5mm and 16±0.5 mm) and no antibacterial effect against MRSA and *P. aeruginosa* as (Fig. 2 and Fig. 3). The zone of inhibition produced by aqueous and ethanolic extracts of clove are mentioned in Table 2.

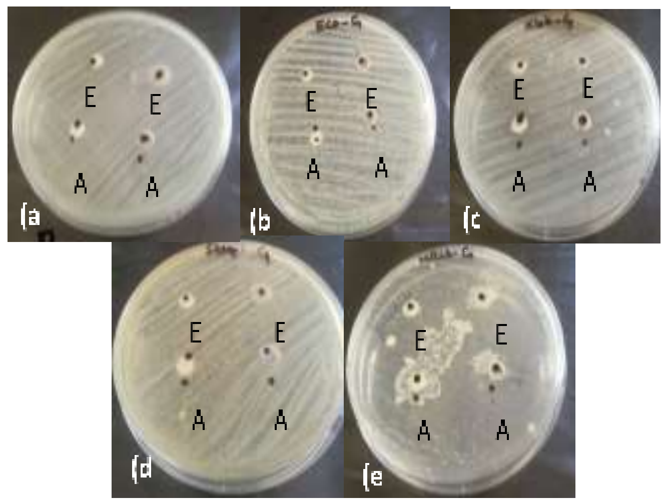

Figure 1. Antibacterial activity of ethanolic (denoted by E) and aqueous (denoted by A) extract of garlic on (a) *Pseudomonas aeruginosa* (b) *E. coli* (c)

Table 1. Antimicrobial activity of ethanolic and aqueous extracts of clove (*Syzygium aromaticum)* and garlic (*Allium sativum*)

Organism	Concentration of Aqueous extract of garlic and zone of Inhibition 50µg/mL			Organism	Concentration of ethanolic extract of garlic and zone of Inhibition 50µg/mL			Positive control (Ciprofloxacin)
	0.1Ml	0.5 mL	1.0 mL		0.1mL	0.5 mL	1.0 mL	5 µg
MRSA	Resistant	Resistant	Resistant	MRSA	Resistant	Resistant	Resistant	23 mm
P. aeruginosa	Resistant	Resistant	Resistant	*P. aeruginosa*	Resistant	Resistant	Resistant	20 mm
K. pneumoniae	Resistant	Resistant	Resistant	*K. pneumoniae*	7.30±0.5mm	8.70±0.5mm	9.00±1mm	20 mm
S. pyogenes	Resistant	Resistant	Resistant	*S. pyogenes*	Resistant	Resistant	Resistant	20 mm
E. coli	Resistant	Resistant	Resistant	*E. coli*	Resistant	Resistant	Resistant	20 mm

Table 2. Zones of inhibition produced by aqueous and ethanolic extracts of clove

Organism	Concentration of Aqueous extract of clove and zone of Inhibition (50µg/mL)			Organism	Concentration of ethanolic extract of clove and zone of Inhibition (50µg/mL)			Positive control (Ciprofloxacin)
	0.1mL	0.5 mL	1.0 mL		0.1mL	0.5 mL	1.0 mL	5 µg
MRSA	Resistant	Resistant	Resistant	MRSA	Resistant	12±0.5mm	20±1 mm	23 mm
P. aeruginosa	Resistant	Resistant	Resistant	*P. aeruginosa*	Resistant	Resistant	10±1 mm	20 mm
K. pneumoniae	Resistant	10±0.5mm	16±0.5 mm	*K. pneumoniae*	12±0.5mm	18±1 mm	26±0.5mm	20 mm
S. pyogenes	Resistant	Resistant	11±0.5 mm	*S. pyogenes*	Resistant	8±1 mm	14±1mm	20 mm
E. coli	Resistant	Resistant	8±1 mm	*E. coli*	Resistant	Resistant	10±1mm	20 mm

Table 3. Minimum inhibitory concentration of Garlic and Clove extract

Bacterial strain	Garlic		Clove	
	Ethanolic (µg/mL)	Aqueous (µg/mL)	Ethanolic (µg/mL)	Aqueous (µg/mL)
E. coli	64	128	64	64
P. aeruginosa	128	--	64	128
K. pneumoniae	64	128	64	128
MRSA	128	--	128	--

Klebsiella pneumonia **(d)** ***Streptococcus pyogenes*** **(e) MRSA.**

Minimum inhibitory concentration (MIC) was determined by broth dilution method, ethanolic and aqueous extract of garlic and clove showed 64-128 µg/ml range, detail description (Table 3).

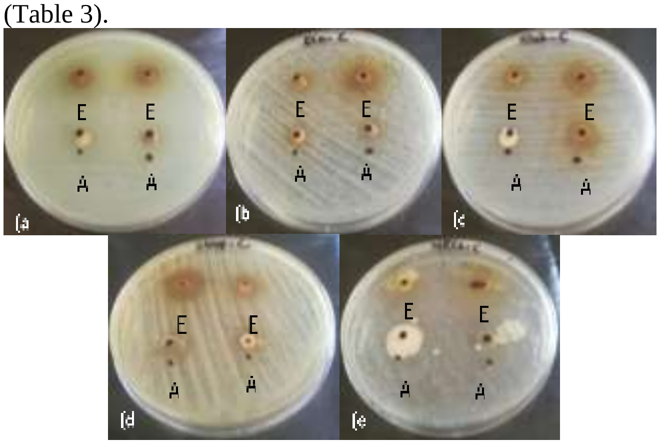

Figure 2. Antibacterial activity of ethanolic (denoted by E) and aqueous (denoted by A) extract of clove on (a) ***Pseudomonas aeruginosa*** **(b)** ***E. coli*** **(c)** ***Klebsiella pneumonia*** **(d)** ***Streptococcus pyogenes*** **(e) MRSA.**

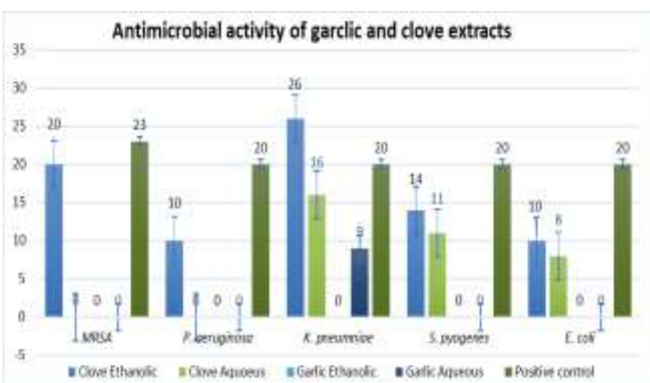

Figure 3. Comparison of antibacterial activity of ethanolic and aqueous extracts of clove and garlic at 1.0mL concentration.

DISCUSSION

The aim of this study was to check the antibacterial activity of clove and garlic extracts and to compare the activity of ethanolic and aqueous extracts of both spices. Natural antibacterial agents are being more economic with least toxicity and side effects have gained the attention of modern world. Researchers have confirmed the antioxidant, antiviral, anticarcinogenic, antibacterial and antifungal properties of spices (Rojas *et al.*, 2014). In the present study, highest antibacterial activity was shown by ethanolic extract of clove against *Klebsiella pneumoniae*. Diameter of zone of inhibition was recorded as 26±0.5mm around *K. pneumonia*, similarly highest zone of inhibition produced by aqueous extract of clove was also against *K. pneumoniae* with a diameter of 16±0.15mm at 1.0mL concentration. Poorest performance in terms of growth inhibition was shown by aqueous extracts of garlic. All tested bacteria were found resistant to it. Ethanolic extracts of garlic showed antibacterial activity against *K. pneumoniae*. All other bacteria were found resistant to it too. According to the results of study, ethanolic extract of clove was found most effective among all bacterial isolates of the study.

In an earlier study by Kumar *et al*. (2014) it has reported that clove buds and its oil have antibacterial, antioxidant, antiparasitic, antimutagenic and antithrombic and Giordani *et al*. (2004) reported their antifungal effects. Eugenol, acetyl eugenol, chavicol, acetyl salicylate, humulenes are the active ingredients in clove (Joshi *et al.*, 2011), and the clove contains tannin, and vitamin B as well (Gupta, 2010). Clove has also been reported to contain many volatile compounds like limonene, farnesol, benzaldehyde, 2-heptanone ethyl hexanoate and β-pinene are also present in lower concentration (Rojas *et al.*, 2014). Antibacterial activity of clove has been tested against *E. coli*, *Yersinia enterolitica Salmonella typhimureum*, *Listeria monocytogenes*, *Bacillus cereus* (Dussault *et al.*, 2014) and *Acinetobacter baumannii* and *Enterococcus faecalis* (Siddiqua *et al.*, 2015).

The antibacterial activity of garlic is due to its active ingredient named as Allicin (Karuppiah and Rajaram, 2012). Antimicrobial activity of garlic extract is highly associated with allicin content. If it is removed from the extract, no antimicrobial activity is shown by the extract. In a study the effectiveness of synthetic allicin and fresh garlic extract was compared. Fresh extract presented antimicrobial activity twice then the synthetic allicin against *Staphylococcus aureus*. This study concluded that there is a synergistic relation between allicin and other phytochemicals of garlic extract. So the source of allicin should be given prime importance in every study (Harris *et al.*, 2001). Allicin inhibits bacterial growth by partially inhibiting the synthesis of DNA. RNA is the primary target of Allicin, its synthesis is totally inhibited thus no protein production (Karuppiah and Rajaram, 2012).

The antibacterial activity of clove is due to its component named as Eugenol (4-allyl-2-methoxyphenol). It is commonly used in perfumery, dentistry and food industry. Eugenol inhibits cox-2 expression, prostaglandins synthesis and nuclear factor-κB (NF-κB) activation induced by tumor necrosis factor-α (TNF-α). This is the basis of all type of antimicrobial activity of clove (Chaieb *et al.*, 2009). Eugenol inhibits bacterial growth by disruption of cell membrane. This results in increased nonspecific permeability of antibiotics. Many other effects are also exerted by clove extract at sub-lethal level. As the nonspecific permeability increases on treatment with clove extract, so clove have the ability to sensitize the bacterial cells towards various groups of antibiotics. This indicates that clove is very nonspecific and general in its nature. Pretreatment of cells with clove extract, makes them more vulnerable to many nonspecific antibiotics like penicillin, vancomycin and oxacillin (Mohamed, 2010).

Conclusion: This study was conducted to compare the activity of ethanolic and aqueous extracts of clove and garlic and it is concluded that ethanolic extracts are more effective as compared to aqueous extracts. Reason may be that ethanol is organic in nature thus it has strong interaction with bacterial cell on the basis of rule "like dissolves like". So the organic nature of ethanol makes it more effective as a solvent.

REFERENCES

Abbas, A., R.Z. Abbas, S. Masood, Z. Iqbal, M.K. Khan, M.K. Saleemi, M.A. Raza, M.S Mahmood, J.A. Khan and Z.D. Din. 2018. Acaricidal and insecticidal effects of essential oils against ectoparasites of veterinary importance. Boletín Latinoamericano Y Del Caribe De Plantas Medicinales Y. Aromáticas. 17:441-452.

Abbas, A., Z. Iqbal, R.Z. Abbas, M.K. Khan, J.A. Khan, Z.D. Sindhu, M.S. Mahmood and M.K. Saleemi. 2017. In vivo anticoccidial effects of *Beta vulgaris* (sugarbeet) in broiler chickens. Microb. Path. 111:139-144.

Abbas, A., Z. Iqbal, R.Z. Abbas, M.K. Khan and J.A. Khan.2017. Immunomodulatory activity of *Pinus radiata* extract against coccidiosis in broiler chicken. Pak. Vet. J. 37:145-149.

Abbas, A., R.Z. Abbas, M.K. Khan, M.A. Raza, M.S. Mahmood, M.K. Saleemi, T. Hussain, J.A. Khan and Z.D. Sindhu. 2019. Anticoccidial effects of *Trachysper mumammi* (Ajwain) in broiler chickens. Pak. Vet. J. 39:301-304.

Abbas, R.Z., M.A. Zaman, Z.D. Sindhu, M. Sharif, A. Rafique, Z. Saeed, T.U. Rehman, F. Siddique, T. Zaheer, M.K. Khan, M.S. Akram, A.J. Chattha, U. Fatima, T. Munir and M. Ahmad. 2020. Anthelmintic effects and toxicity analysis of herbal dewormer against the infection of *Haemonchus contortus*and *Fasciola hepatica* in goat. Pak. Vet. J. 40:455-460.

Akhtar, M.S., B. Degaga and T. Azam. 2014. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review. Issues Biol. Sci. Pharm. Res. 2:1-7.

Aminov, R.I. 2010. A brief history of the antibiotic era: Lessons learned and challenges for the future. Front. Microbiol. 1:1–7.

Ashraf, M.U., M.S. Mahmood, A. Rafique, R.Z. Abbas, Z. Iqbal, M. Younus, S.A. Sadiq, M. Usman, M.O. Asghar and M.U. Ishaq. 2017. Factors responsible for the continuous persistence and evolution of low pathogenic avian influenza virus (H9N5). World's Poult. Sci. J. 73:791-802.

Chaieb, H., Z.T. Hafedh and K. Mahmoud Rouabhia. 2009. the chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (*Syzigium aromaticum* L. Myrtaceae). Phytother. 1213:1205-1213.

Do K.H., J.W. Byun and W.K. Lee. 2019. Serogroups, virulence genes and antimicrobial resistance of $F4^+$ and $F18^+$ *Escherichia coli* isolated from weaned piglets. Pak. Vet. J. 39: 266-270.

Dussault, D., K.D. Vu and M. Lacroix. 2014. *In-vitro* evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. Meat Sci. 96:514-520.

Elsayed, M.S.A.E., A. Awad, R. Tarabees and A. Marzouk, 2018. Virulence repertoire and antimicrobial resistance profile of Shiga toxin-producing *E. coli* isolated from Sheep and Goat farms from Al-buhayra Egypt.Pak. Vet. J. 38:429-433.

Eloff, J.N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64: 711-713.

Farías-Campomanes, A.M., C.N. Horita, M.A.R. Pollonio and M.A.A. Meireles. 2014. Allicin-rich extract obtained from Garlic by pressurized liquid extraction: quantitative determination of allicin in Garlic samples. Food Public Heal. 4:272–278.

Fayaz, M.R., R.Z. Abbas, A. Abbas, M.K. Khan, M.A. Raza, M. Israr, J.A. Khan, M.S. Mahmood, M.K. Saleemi, T. Rehman, M.A. Zaman and Z.D. Sindhu. 2019. Potential of botanical driven essential oils against *Haemochus contortus* in small ruminants. Bol Latinoam Caribe Plant Med Aromat. 18:533-543.

Giordani, R., P. Regli, J. Kaloustian, C. Mikaïl and L. Abou. 2004. Antifungal effect of various essential oils against

Candida albicans. Potentiation of antifungal action of amphotericin B by essential oil from *thymus vulgaris*. Phytotherapy Res. 12:990–995.

Gupta, M. 2010. Pharmacological properties and traditional therapeutic uses of important indian spices: A review. Int. J. Food Prop. 13:1092–1116.

Harris, J.C., S.L. Cottrell, S. Plummer and D. Lloyd. 2001. Antimicrobial properties of *Allium sativum* (garlic). Appl. Microbiol. Biotechnol. 57:282-286.

Ibrahim, E.A. 2017. *In-vitro* Antimicrobial activity of *Allium sativum* (Garlic) against wound infection pathogens. Afr. J. Med. Sci. 2:1-8.

Idris, M., R.Z. Abbas, S. Masood,T. Rehman, U. Farooq, W. Babar, R. Hussain, A. Raza and U. Riaz. 2017. The potential of antioxidant rich essential oils against avian coccidiosis. World's Poult. Sci. J. 73: 89-104

Jasamai, M., C.S. Hui, N. Azmi and E. Kumolosasi. 2016. Effect of *Allium sativum* (garlic) methanol extract on viability and apoptosis of human leukemic cell lines. Trop. J. Pharm. Res. 15:1479.

Joshi, B., G. Prasad Sah, B. Bahadur Basnet, M. Raj Bhatt, D. Sharma, K. Subedi, J. Pandey and R. Malla. 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). J. Microbiol. Antimicrob. 3:1-7.

Karuppiah, P. and S. Rajaram. 2012. Antibacterial effect of *Allium sativum* cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian Pac. J. Trop. Biomed. 2:597-601.

Khan, A.U., M.Y. Tipu, M. Shafee, N.U. Khan, M.M.T. Kiani, M. Rafeeq and S.I.A. Shah. 2018. *In-ovo* antiviral effect of *Nigella sativa* extract against Newcastle Disease Virus in experimentally infected chicken embryonated eggs. Pak. Vet. J. 38:434-437.

Khan A., A.Z. Durrani, A. Yousaf, J.A. Khan, M. Chaudhry, Z. Fatima and A. Khan. 2019. Epidemiology and antimicrobial susceptibility of Methicillin-resistant *Staphylococcus aureus* in cattle of Pothohar region, Pakistan. Pak. Vet. J.39:438-442.

Khashan, A.A. 2014. Antibacterial activity of garlic extract (*Allium sativum*) against *Staphylococcus aureusin-vitro*. Glob. J. Bio-Sciencie Biotechnol. 3:346-348.

Khater, H.F., A.M. Ali, G.A. Abouelella, M.A. Marawan, M. Govindarajan, K. Murugan, R.Z. Abbas, N.P Vaz and G. Benelli. 2018. Toxicity and growth inhibition potential of vetiver, cinnamon, and lavender essential oil and their blends against larvae of the sheep blowfly, *Lucillia sericata*. Int. J. Dermatol. 57: 449-457.

Khater, H.F., H. Ziam, A. Abbas, R.Z. Abbas, M.A. Raza, K. Hussain, E.Z. Younis, I.T. Radwan and A. Selim. 2020. Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. Agrobiological Records 1:11-25

Kopaei, R.M. and R.D.E. Sewell. 2014. The history and ups and downs of herbal medicines usage. J. HerbMed. Pharmacol. 3:1-3.

Kumar, Y., S. Agarwal, A. Srivastava, S. Kumar, G. Agarwal, M. Zeeshan and A. Khan. 2014. Antibacterial activity of Clove (*Syzygium aromaticum*) and Garlic (*Allium sativum*) on different pathogenic bacteria. Int. J. Pure Appl. Biosci. 2:305-311.

Lin, X., M. Mohsin, R.Z. Abbas, L. Li, H. Chen, C. Huang, Y. Li, M.U. Goraya, Z. Huang and G. Yin. 2020. Evaluation of immunogenicity and protective efficacy of *Eimeria maxima* immune mapped protein 1 with EDA adjuvant in chicken. Pak. Vet. J. 40:209-213

Mahmood M.S., H.W. Amir, R.Z. Abbas, B. Aslam and A. Rafique. 2018. Evaluation of antiviral activity of *Azadirachta indica* (Neem) bark extract against Newcastle disease virus. Pak. Vet. J. 38:25-28.

Mehmood, K., R.M. Bilal and H. Zhang. 2020. Study on the genotypic and phenotypic resistance of tetracycline antibiotic in *Escherichia coli* strains isolated from free ranging chickens of Anhui Province, China. Agrobiological Records. 2:63-68.

Mohamed, E.F. 2010. Antiviral Properties of garlic cloves juice compared with onion bulbs juice against Potato virus Y (PVY). J. Am. Sci. 6:302-310.

Moore, G.S. and R.D. Atkins. 2018. The fungicidal and fungistatic effects of an aqueous garlic extract on medically important. Mycologia. 69:341-348.

Mukhtar, S. and I. Ghori. 2012. antibacterial activity of aqueous and ethanbolic extract of garlic , cinnamon and tumeric against *Escherichia coli* ATCC 25922 and *Bacillus subtilis*. DSM, 3256:131-136.

Nabavi, S.M., A. Marchese, M. Izadi, V. Curti, M. Daglia and S.F. Nabavi. 2015. Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. Food Chem. 173:339-347.

Rahman S.U. and M. Mohsin. 2019. The under reported issue of antibiotic-resistance in food-producing animals in Pakistan. Pak. Vet. J. 39:323-328.

Rahman S.U., S. Ahmad and I. Khan, 2019. Incidence of ESBL-producing-*Escherichia coli* in poultry farm environment and retail poultry meat. Pak. Vet. J. 39:116-120.

Riaz A., S. Noureen, M.F. Qamar, I. Liaqat, M. Arshad and N. Arshad. 2019. Characterization of bacteriocin like inhibitory substances from *Enterococcus ratti* MF183967. Pak. Vet. J. 39: 1-6.

Rojas, C.D.F., C.R.F. de Souza and W.P. Oliveira. 2014. Clove (*Syzygium aromaticum*): A precious spice. Asian Pac. J. Trop. Biomed. 4:90-96.

Salman, M., R.Z. Abbas, M. Israr,A. Abbas, K. Mehmood, M.K. Khan, Z.D. Sindhu, R. Hussain, M.K. Saleemi and S. Shah. 2020. Repellent and acaricidal activity of essential oils and their components against *Rhipicephalus* ticks in cattle. Vet. Parasitol. 283:109178.

Shihabudeen, S.M.H., H.D. Priscilla and K. Thirumurugan. 2010. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Int. J. Pharma. Sci. Res. 1:430-434.

Siddiqua, S., B.A. Anusha, L.S. Ashwini and P.S. Negi. 2015. Antibacterial activity of cinnamaldehyde and clove oil: effect on selected foodborne pathogens in model food systems and watermelon juice. J. Food Sci. Technol. 52:5834-5841.

Singh, R., R. Lawrence, K. Lawrence, B. Agarwal, R.K. Gupta and S. Dar. 2015. antioxidant and antibacterial activity of *Syzigium aromaticum*, *Zingiber officinale* and *Cinnamomum zeylanicum* essential oils. Chem. Sci. Trans. 4:239-245.

Vaghasiya, Y., R. Dave and S. Chanda. 2011. phytochemical analysis of some medicinal plants from western region of India. Res. J. Med. Plant. 5:567-576.

Younas, M., S.U. Rahman, S. Shams, M.M. Salman and I. Khan, 2019. Multidrug resistant Carbapenemase-producing *Escherichia coli* from chicken meat reveals diversity and Co-existence of Carbapenemase encoding Genes. Pak. Vet. J. 39:241-245.

Zaman, M.A., R.Z. Abbas, W. Qamar, M.F.Qamar, U. Mehreen, Z. Shahid and M. Kamran. 2020. Role of secondary metabolites of medicinal plants against *Ascaridia galli*. Worlds Poul. Sci. J. 76:639-655.

Zhang, K., X. Li, C. Na, A. Abbas, R.Z. Abbas and M.A. Zaman. 2020. Anticoccidial effects of *Camellia sinensis* (green tea) extract and its effect on Blood and Serum chemistry of broiler chickens. Pak. Vet. J. 40:77-80.

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