Antibacterial Activity of Locally Available Spices against Common Pathogenic Bacteria

Rabindra Pandit[1]*, Susan Karki[1], Rojina Shrestha[1], Garima Regmi[1], Sabin Karki[1], Kalyan Subedi[1], Rabin Paudyal[2]

[1]Department of Microbiology, Kathmandu College of Science and Technology, Kathmandu, Nepal

[2]Department of Microbiology, Tri-Chandra College, Kathmandu, Nepal

***Corresponding author:** Rabindra Pandit, Department of Microbiology, Kathmandu College of Science and Technology, Kathmandu, Nepal, E-mail: prabindra053@gmail.com

ABSTRACT

Objectives: The objective of this study was to determine the antibacterial activity of spices against common pathogenic bacteria and Minimum Inhibitory Concentration (MIC) of spices extract.

Methods: Dried buds of *Syzygium aromaticum*, bulb of *Allium sativum*, root of *Allium sativum* and rhizome of *Curcuma longa* were collected from different locality in Kathmand, Nepal. They were dried, crushed to powder form and mixed with adequate ethanol and methanol to get the extract respectively. After processing, the extracts were tested against *Bacillus* spp, *Klebsiella pneumoniae, S. aureus* and *Escherichia coli* by disc diffusion on Muellar Hinton Agar plate. Minimum Inhibitory Concentration (MIC) was determined by dilution technique.

Results: All the extract of spices were found effective against *Bacillus* spp, *K. pneumoniae, S. aureus* and *E. coli. However,* the ethanolic extract of clove was found to be highly effective against *E. coli* with Zone of Inhibition (ZOI) of 28 mm. Similarly, the methanolic extract of clove was found to be highly effective against *K. pneumoniae* (ZOI= 22 mm). The MIC against *S. aureus*, *Bacillus* spp, *K. pneumoniae* and *E. coli,* of the ethanolic extract of clove was found to be 1:32, 1:8, 1:4 and 1:16 and methanolic extract was found to be 1:64, 1:16, 1:8 and 1:4, respectively.

Conclusion: Clove was found to be the most effective spice against common pathogenic bacteria. Ehanolic extract was found to be more effective compared to methanolic suggesting ethanol as a suitable solvent. Further study on its antibacterial characteristics may be required to update its medicinal value.

Keywords: Antibacterial activity, spices, ethanolic extract, methanolic extract, MIC, Nepal.

INTRODUCTION

A spice is a dried seed, fruit, root, bark, or vegetables substances primarily used for flavoring, coloring or preserving food. Sometimes a spice is used to hide other flavors. Spices are distinguished from herbs, which are parts of leafy green plants also used for flavoring or as garnish. Many spices have antimicrobial and antioxidant property. This may explain why spices are more commonly used in warmer climates, which have more infectious disease, and why use of spices is especially prominent in meat, which is particularly susceptible to spoiling. There are several reports on development of antibiotic resistance in diverse bacterial pathogens (Gold and Moellering 1996). Spice may have other uses, including medicinal, religious, ritual, cosmetics or perfume production, or vegetables. For example: Turmeric roots are consumed as a vegetables and Garlic as an antibiotic. Due to antimicrobial property of the Spice, Crude extract of Spices are used for the therapeutic treatment. Extraction is the separation of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids, and flavonoids, etc. using

Date of Submission: August 18, 2024
Date of Acceptance: November 29, 2024
Published Online: December 31, 2024
DOI: https://doi.org/10.3126/tujm.v11i1.85512

selective solvents through standard procedures (Handa and Khanuja 2008). The aim of all solvent extraction methods is to separate the soluble plant metabolites, leaving behind the insoluble cellular macromolecules.

Clove, member of family Myrtaceae which is commonly used as spices. The major component of clove is eugenol (76.23%). It also called clove oil which is an aromatic oil extracted from cloves. The study conducted by the Pandey and Singh (2011) has shown Clove to be effective against Staphylococcus aureus, *Pseudomonas aeruginosa* and *E. coli.* According to the Pandey and Singh, Methanolic extract of clove showed good inhibitory activity against *S. aureus* with the maximum Zone of Inhibition (ZOI) of 24mm while minimum was 19 mm against *P. aeruginosa*. Ethanolic extract of clove showed maximum ZOI 20 mm against *P. aeruginosa* while minimum was 18mm against *E. coli*. The antimicrobial activity of clove has been attributed to the presence of Eugenol, the hydrophobicity of the oil enables it to partition the lipids and disrupt the outer membrane of Gram- positive and Gram- negative bacteria (Walsh et al 2003). Eugenol also found to enhance protein leakage of cell membranes in both bacteria (Oyedemi et al 2003).

Ginger, a member of family Zingiberaceae which has long been used as naturopathy due to their potential antimicrobial activity against different microbial pathogens. The main bioactive compound in ginger is Gingerol and other phenolic compounds such as eugenol, shogaols, zingerone, gingerdiols (Singh et al 2005). A study conducted by Islam et al (2014) has shown Ginger to be effective against *E. coli, P. aeruginosa, S. aureus, Vibrio cholerae, Klebsiella s*pp. and *Salmonella* spp. The antimicrobial activity of ginger has been attributed to the presence of its constituent monoterpenes and sesquiterpenes, as they are capable of altering the permeability and fluidity of the plasma membrane of microorganisms. The lipophilic character of its hydrocarbon skeleton and the hydrophilic character of some of its functional groups confer this property (Lopez et al 2017).

Garlic, a member of family Lilieaceae has been used for a long time as a spice or traditional medicine. The most significant component of garlic is allicin (diallyl thiosulfinate). Allicin is not present in fresh clove of garlic, but it is released after crushing and chopping with the alliinase enzyme activity. Alliums, component of garlic, include largely cysteine sulfoxides. Conversion of alliinase to allicin by cysteine sulfoxides transforms to thiosulfates, which are volatile and lachrymatory (Block et al 1992). The study conducted by the Strika et al (2017) has shown Garlic to be effective against fungus *Candida albicans, Bacillus subtilis, S. aureus*, Salmonella enteritidis and *E. coli.* This study showed good inhibitory activity against *C. albicans* with the Zone of Inhibition (ZOI) of 62 mm. The antimicrobial activity of Garlic has been attributed to the presence of Allicin, an organosulfur compound, which prevents lipid biosynthesis that damage *C. albicans* cell wall (Ghannoum et al 1988) and cause inhibition of RNA synthesis in bacteria (Feldberg et al 1988).

Turmeric, a member of family Zingiberaceae is spices which is widely used all over world that comes from the root. The active compound in turmeric is curcuminoids. The major curcuminoid is curcumin (diferuloyl methane) which makes up approximately 90% of the curcuminoid content in turmeric followed by demethoxycurcumin and bisdemethoxycurcumin (Ruby et al 1995). *Curcumin* is a pleiotropic molecule that interacts with multiple targets involved in inflammatory reaction such as tumor necrosis factor-alpha (TNFα) and interleukins (ILs) (Aggarwal et al 2013). A study conducted by Pandey et al (2001) has shown turmeric to be effective against a wide range of microorganisms including *E. coli, Bacillus subtilis, and S. aureus.* The antimicrobial property of turmeric has been attributed to the presence of essential oil, an alkaloid, curcumin and other curcuminoids, turmeric oil, turmerol and veleric acid (Cikrikci et al 2008).

Hence, this study was conducted with an objective to evaluate antibacterial activity of spice extracts against common pathogenic bacteria. This study will help to know the antibacterial activity of spices which is used in Nepal. This study will further contribute to expand the existing knowledge on medicinal value of plant spices such that their properties might be further investigated for their use in commercial medical products.

METHODS

Collection of Spices

In this study, four plant sample; Clove, Garlic, Ginger and Turmeric were collected from different places of Kathmandu Valley. The study was conducted in Microbiology Laboratory of Kathmandu College of Science and Technology, Kamalpokhari. This study was done from early of December 2019 to mid 2020.

Preparation of Spices extract

The collected Spices were then dried in the laboratory for 6-7 days avoiding direct sunlight. After complete drying, the Spices were then ground to fine powder. Grinding was done with clean mortar and pestle. After the making of powder, the approximately 10 gm of fine powder was then, mixed with 50 ml of methanol to get methanolic extract and with 50 ml of ethanol to get ethanolic extract. Different powders were mixed with each 50 ml of methanol and ethanol to get methanolic extract and ethanolic extract of every four spices (Sapkota et al 2018).

The solutions in 8 different test tubes then kept at room temperature for 3 successful days. During day time the tubes were shaken hourly. The extract was filtered through Whatman filter paper (Figure 1). The filtrate was then kept at incubator at 50°C for about 36 hours to remove methanol and ethanol by vaporizing them out. This way the desired plant extracts were prepared.

Preparation of test organism

Different test organisms *Bacillus spp, K. pneumoniae, S. aureus* and *E. coli* against whom the antibacterial activity of proposed four spices were to be seen, were prepared on laboratory. Adequate colonies of organism were taken from their culture and were sub cultured to get fresh colonies. Fresh colonies of test organism were emulsified in normal saline. The turbidity was matched with 0.5 MacFarland standard which is equivalent to 108 cfu/ml. McFarland standard was prepared as described by Cockerill and Franklin (2012).

Preparation of antibacterial disc

Initially, the Whatmann no 1 filter paper were punched to the size of 6 mm with punching machine. Then the discs were autoclaved for making them organisms free, sterilized. Then, the sterilized discs were dipped in plant extract to make them antimicrobial disc (Kirby et al 1957).

Inoculation and incubation

For screening of antibacterial activity of plants' extract, Mueller Hinton Agar (MHA) was used. The media was prepared according to the manufacturer's instruction (Hi-Media). The preparation of MHA plate is presented in Appendix C. The prepared MHA plates were taken. The cotton swab was taken and then it was dipped to the test organisms' inoculation. The swab was pressed against wall of tube to leave the excess amount in the tube. A uniform lawn of the test organism was prepared to determine the antibacterial activity of plant extracts against them. With the new swab, the excess flow of inoculation at corner of plates were removed.

Antibacterial discs were then inoculated into MHA plate with sterile forceps, maintaining sterile condition in the lab. Antibacterial discs of methanol extracts were inoculated onto a single MHA plate with one of the test organism and disc of ethanol extracts were inoculated onto another single MHA plate with one of the test organism. In this way, discs were inoculated onto various plates with test organisms. To identify the antibacterial disc in the plate, appropriate labeling was provided in the plate below the site of disc placement.

Then, after inoculation of test organisms and antibacterial disc in the MHA plates, they were incubated at 37°C for 24 hours.

Observation and Analysis

The incubated plates were observed for ZOI around the disc. The ZOI of each methanolic extract and ethanolic extract of spices were measured and recorded. The activity of each extracts against common pathogenic bacteria were then compared with each other.

Determination of MIC

For the calculation of MIC, the extract with highest antibacterial activity was diluted up to 7 level dilution; 1:2, 1:4, 1:8, 1:16, 1:32, 1 :64 and 1:128. Two MHA plates were taken in which the well were bored with borer of size 6 mm. The extracts of various dilutions were poured into the well on MHA plate with labeling on backside of plate. Then the plates were incubated at 37°c for 24 hours. After observing the ZOI by different dilutions, MIC was detected (Wayne 2015).

RESULTS

Antibacterial activity of ethanolic extract of clove, garlic, ginger, and turmeric against common pathogenic bacteria

The maximum activity of ethanolic extract of clove was seen against *Escherichia coli* (28mm) followed by *Klebsiella pneumoniae* (19mm), *Bacillus* spp. (14mm). *S. aureus* was least susceptible 8mm ZOI. The highest activity of ethanolic extract of Garlic was seen against *Escherichia coli* (27mm) followed by *Klebsiella pneumoniae* (19mm). *S. aureus* and *Bacillus* spp were least susceptible with 12mm ZOI. Maximum activity of ethanolic extract of Ginger was seen against *Escherichia coli* (22mm) followed by *Bacillus* spp (14mm), and *Klebsiella pneumonia* (13mm). *S. aureus*

(9mm) was the least susceptible with 9mm ZOI. The highest activity of ethanolic extract of Turmeric was seen against *Bacillus* spp (17mm) followed by *S. aureus (*15mm), *K. pneumonia* (11mm). *E. coli* was least susceptible (10mm) (Figure 1, 2, 3)

MIC of ethanolic extract of clove against common pathogenic bacteria

MIC of the ethanolic extract of clove against *S. aureus*, *Bacillus* spp*, K. pneumoniae* and *E. coli* was found to be 1:32, 1:8, 1:4 and 1:16 respectively (Table 1).

Antibacterial activity of methanolic extract of clove, garlic, ginger, and turmeric against common pathogenic bacteria

The maximum activity of methanolic extract of clove was seen against *K. pneumoniae* (22mm) followed by *S. aureus* (16mm), *E. coli* (9mm). *Bacillus* spp was the least susceptible with 8mm ZOI. Highest activity of methanolic extract of garlic was seen against *S. aureus* (14mm). *K. pneumoniae* was the least susceptible to methanolic extract of garlic (5mm) whereas no ZOI towards *Bacillus* spp and *E. coli.* The maximum activity of methanolic extract of ginger was seen against *Bacillus* spp (8mm). *S. aureus* and *E. coli* was least susceptible to methanolic extract of ginger i.e (7mm) whereas no zone of inhibition towards *K. pneumoniae*. The highest activity of methanolic extract of turmeric was seen against *S. aureus* (16mm) followed by *E. coli* (15mm). *K. pneumoniae* (10mm). *Bacillus* spp was the least susceptible with 9mm ZOI.

MIC of methanolic extract of clove against common pathogenic bacteria

MIC of the ethanolic extract of clove against *S. aureus*, *Bacillus* spp*, K. pneumoniae* and *E. coli* was found to be 1:64, 1:16, 1:8 and 1:4, respectively (Table 2).

Figure 1: Filtration of methanolic/ ethanolic extracts

Figure 2: ZOI shown by ethanolic extract of selected spices against *K. pneumoniae* (1: Garlic, 2: Clove, 3: Ginger and 4: Turmeric)

Figure 3: ZOI shown by ethanolic extract of selected spices against *Bacillus* spp (1: Garlic, 2: Clove, 3: Ginger and 4: Turmeric)

Figure 4: ZOI shown by ethanolic extract of selected spices against *S. aureus* (1: Garlic, 2: Clove, 3: Ginger and 4: Turmeric)

Table 1: MIC of ethanolic extract of clove against common pathogenic bacteria

Extract concentration	Zone of inhibition (mm)			
	S. aureus	*Bacillus* spp	*K. pneumoniae*	*E. coli*
1:128	-	-	-	-
1:64	-	-	-	-
1:32	7	-	-	-
1:16	9	-	8	-
1:8	10	9	10	-
1:4	12	11	11	12
1:2	16	18	13	14

Table 2: Antimicrobial activity of different extraction of cinnamon against test bacterial strains

Extract concentration	Zone of inhibition (mm)			
	S. aureus	*Bacillus* spp	*K. pneumoniae*	*E. coli*
1:128	-	-	-	-
1:64	3	-	-	-
1:32	5	-	-	-
1:16	8	2	-	-
1:8	10	7	8	-
1:4	15	10	9	11
1:2	19	15	11	15

DISCUSSION

In this study, the ethanolic extract of Garlic (*Allium sativum*) was found to be highly effective against *E. coli* (27mm) whereas least activity was seen in the case of *S. aureus* (12mm) and *Bacillus* spp (12mm). In case of methanolic extract of Garlic was found to be highly effective against *S. aureus* (14mm) whereas least activity was seen against *K. pneumoniae* (5mm). Garlic contains allicin, a thiosulfinate with two allyl groups as carbon chains (diallyl thiosulfinate). Therapeutic effect of garlic is possible because of its oil and water soluble organosulfur compounds. Allicin combats fungal infections and parasites, lowers blood cholesterol, treats atherosclerosis and promotes circulatory function (Block 2010). However, in the study conducted by Gongacul (2010) showed weaker antimicrobial activity against Gram negative bacteria *E. coli* and *S. enteritidis* than the tested Gram positive bacteria (*S. aureus*, MRSA and *B. subtilis*).

The ethanolic extract of Ginger (Zingiber officinale) was most active against *E. coli* (22mm) as lowest antibacterial activity was seen against *S. aureus* (9mm). In case of methanolic extract of Ginger was found to be highly effective against *Bacillus* spp (8mm) whereas least activity was seen against *E. coli* (7mm) and *S. aureus* (7mm). Ginger contains 3% of an essential oil that causes the fragrance of the spice (Hara et al 1998). The main constituents of ginger are sesquiterpenoids with zingiberene as the main component. Other components include Bsesquiphellandrene, bisabolene and farnesene, which are sesquiterpenoids and trace monoterpenoid fraction (B-sequiphellandrene, cineol and citral) (Hara et al 1998). Ginger has a sialagogue action, which stimulate the production of saliva and can be used to distinguise the taste of medicines (Hara et al 1998). However, in the study conducted by Onyeaba et al. (2004) found the synergistic effect of ethanol extract of ginger and garlic against *Bacillus* spp and *S. aureus*. They also found the antimicrobial activity of the ethanol extract ginger, lime and garlic against broad range of bacteria including *Bacillus spp, S. aureus, E. coli* and *Salmonella* spp. Okiki et al (2015) carried out a study by soybean oil extract of ginger showed highest zone of inhibition against *Salmonella* spp and lowest zone of inhibition against *E. coli*.

In this study, the ethanolic extract of Turmeric (Curcuma longa) was found to be highly effective against *Bacillus* spp (17mm) whereas least activity was seen in the case of *E. coli* (10mm). In case of methanolic extract of Turmeric has maximum activity against Staphylococcus aureus (16mm) whereas minimum activity was seen in the case of *Bacillus* spp (9mm). Turmeric contains curcuminoids, which include mainly curcumin which are believed to be the most important fraction responsible for the biological activities of *Curcuma longa*. The antimicrobial property of Turmeric has been attributed to the presence of essential oil, an alkaloid, curcumin and other curcuminoids, turmeric oil turmerol and veleric acid (Cikrici et al 2008). Similarly, In the study conducted by Maharjan et al (2019), among *S. aureus, E. coli, S. enterica* Typhi, *P. aeruginosa* used in this study, the maximum antibacterial activity was seen against *E. coli* (15mm) and lowest antibacterial activity against *S. aureus* (14mm) at 100mg/ml concentration whereas, no activity was seen against other organisms. According to Chandrana et al (2005) who studied antimicrobial activity of Turmeric reported that it was effective against *E. coli, B. subtilis* and *S. aureus* and suggested that the activity is due to the presence of Curminoi, a phenolic compound.

The MIC of clove (*Syzygium aromaticum*) extract was tested against selected pathogens. *S. aureus* was found to be sensitive in highest dilution (1:32) in ethanolic extract of clove. Similarly, in case of methanolic extract of clove, *S. aureus* was found to be sensitive in highest dilution (1:64). The study only focuses on pathogenic organism by using four bacterial isolates and limited number of spices from the beginning to the end of research, tests were performed using crude extract without conforming its purity. The extracts of these plants should be further analyzed to isolate the specific antibacterial properties in them. Clinical trials should be carried out to explore the potential of these plant extracts in the treatment of the infectious disease.

Conclusion

Based on the study, clove has been found to be the most effective spice against common bacterial pathogens. In comparison between ethanolic and methanolic extract, ethanolic extract was found to be more effective. Thus, ethanol was found to be suitable solvent than methanol. Further study on its antibacterial characteristics may be required to update its medicinal value.

ACKNOWLEDGEMENTS

The authors are very thankful to Kathmandu College of Science and Technology, Kamalpokhari, Kathmandu for providing necessary laboratory arrangement for the successful completion of the study. We are also thankful to the supervisors for the proper guidance, support, and encouragement throughout the project.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Aggarwal BB, Gupta SC, Sung B (2013). Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. Br J Pharmacol. 169(8): 1672–1692. 10.1111/bph.12131

Block E (2010). Garlic and other Allium: The Lore and the Science. The Royal Society of Chemistry Publishing, 22-32.

Block E, Naganathan S, Putman D and Zhao SH (1992). Allium chemistry: HPLC analysis of thiosulfonates from onion, garlic, wild garlic, leek, scallion, shallot, elephant garlic, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. Journal of Agriculture and Food Chemistry 40(12): 2418–2430.

Chandarana H, Baluja S and Chanda S (2005). Comparison of antibacterial activities of selected species of Zingerberaceacae family and some synthetic compounds. Turk J Bio 29: 83-97.

Cikricki S, Mozioglu E and Yylmaz H (2008). Biological activity of curcuminoids isolated from Curcuma long. Rec. Nat. Prod.2; 19-24.

Cockerill and Franklin R (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI, 12.

Feldberg RS, Chang SC and Kotik AN (1988). In vitro mechanism of inhibition of bacterial cell growth by allicin. Antimicrobial Agents and Chemotherapy 32(12): 1763–1768.

Ghannoum MA (1988). Studies on the anticandidal mode of action of Allium sativum (garlic). Journal of General Microbiology 134(11): 2917–405.

Gold SG and Moellering RC (1996.) Antimicrobial drug resistance. England Journal of Medicines 335: 1445-1453.

Goncagul G (2010). Antimicrobial effect of garlic (Allium sativum). Recent Pat Antiinfect Drug Discov. 5 (1), 3-91.

Handa SS and Khanuja SPS (2008). Extraction Technologies for Medicinal and Aromatic Plants. Trieste, Italy: Earth, Environmental and Marine Sciences and Technologies, 22-28.

Hara OM, Keifer D, Farrel K and Kemper K (1998). A review of 12 commonly used medicinal herbs. Archives. Fam. Med. (7): 523-536.

Islam K, Rowsni AA, Khan MM and Kabir MS (2014). Antimicrobial activity of ginger (*Zingiber officinale*) extracts against food-borne pathogenic bacteria. International Journal of Science, Environment and Technology 3(3): 867-871.

Kirby WM, Yoshihara GM, Sundsted KS and Warren JH (1957). Clinical usefulness of a single disc method for antibiotic sensitivity testing. Antibiot Annu. 892–897.

Lopez EIC, Balcazar MFH, Mendoza JMR, Ortiz ADR, Melo MTO and Parrales RS (2017). Antimicrobial activity of essential oil of Zingiber officinale roscoe (Zingiberaceae). American Journal of Plant Sciences 8(07):1511.

Maharjan D, Singh A, Lekhak B,Basnyal S and Gautam LS (2011). Study on Antibacterial Activity of Common Spices. Nepal Journal of Science and Technology 12: 312-317.

Maharjan R, Thapa S and Acharya A (2019). Evaluation of antimicrobial activity and synergistic effect of spices against few selected pathogens, TUJM 6(1): 10-18.

Okiki PA, Oyetunji O and Oso B (2015). Antibacterial activity of Ginger (Zingiberofficinale) against isolated bacteria from the respiratory tract infections. Journal of Biology Agriculture and Healthcare 5(19): 131-138.

Onyeagba RA, Ugbogu OC, Okeke CU and Iroakasi O (2004). Studies on theantimicrobial effects of garlic (Allium sativum Linn), ginger (Zingiber officinale Roscoe) and lime (Citrus aurantifolia Linn). African Journal of Biotechnology 3(10):552-554.

Oyedemi SO, Okoh AI, Mabinya LV, Pirochenva G and Afolayan AJ (2009). Biotechnol. The proposed mechanism of bactericidal action of eugenol, -terpineol and γ-terpinene against Listeria monocytogenes, Streptococcus pyogenes, Proteus vulgaris and Escherichia coli 8(7): 1280 – 1286.

Pandey A and Singh P (2011). Antibacterial activity of Syzygium aromaticum (clove) with metal ion effect against food borne pathogens. Asian Journal of Plant Science and Research 1(2): 69-80.

Pandey J (2001). Antimicrobial activity of Curcuma longa against different species. International Journal of science Environment and Technology 3(3): 867-871.

Ruby J, Kuttan G, Babu KD , Rajashekhharan KN and Kuttan R(1995). Antitumor and oxidant activity of natural curcumnoids. Cancer Lett. 94:79-83.

Sapkota P, Bhattarai S, Bajracharya AM, Lakhe PB and Shrestha N (2018). Antimicrobial Screening of Some Medicinal Plants Against selected Bacterial Species, 20-21.

Singh G, Maurya S, Catalan C and De Lampasona MP (2005). Studies on essential oils, part 42: Chemical, antifungal, antioxidant and sprout suppressant studies on ginger essential oil and its oleoresin. Flavour and Fragrance Journal 20(1):1-6.

Strika I, Basic A and Halilovic N (2017). Antimicrobial effects of garlic (Allium sativum L. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina 47: 17-20.

Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL and Bartolo RG (2003). Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. Appl. Microbiol.94(2): 240 –7.

Wayne PA (2015). Performance standards for antimicrobial susceptibility testing: 25th informational supplement (M100-S23), Clinical and Laboratory Standard Institute (CLSI) 40(1): 42-104.