Investigating the Antimicrobial properties of Southeast Asian Herbs and Spices

Group ID: 1 - 12

David Siew 2p2 (3)

Isaac Low 2p2 (9)

Raymus Ong 2P2 (20)

Introduction

Many pathogen-related conditions are increasingly antibiotic-resistant [1] . These include *Mycobacterium tuberculosis* [2] and *MRSA* [3]. New remedies need to be found to counter current and future microbial diseases.

We drew inspiration from the available herbs and spices found in Southeast Asia to investigate potential antimicrobial properties. Some natural remedies include basil, coriander and cloves.

Basil exhibits antimicrobial activity against *Listeria* , *Staphylococcus* and *Salmonella* [4]. In the region, basil oil is commonly used to treat cuts, wounds and infections [5]. Basil is also known to contain many antioxidants [6].

Coriander has many health and medical benefits, helping to lower blood sugar [7] and promote heart [8], brain [9] and promote digestion and gut [10] health. It has antimicrobial properties [11] and contains antioxidants [12]. In folk medicine, it is thought to boost immunity and help fight infections [13].

Cloves has been used as a medicinal spice for generations [14] and is purported to have many medical benefits [15] and health benefits such as maintaining oral health [16], treating diabetes [17], reducing the chance of getting cancer [18] and reducing obesity [19]. It contains eugenol [20] which is used as a local antiseptic and anaesthetic [21].

While these natural remedies are commonly used throughout Southeast Asia, there are contradicting studies in their efficacies as antimicrobial agents.
Hence, we want to study their antimicrobial properties in hopes of finding new cures to illnesses.

Objective

We aim to identify potential new sources of antimicrobial agents from commonly -found herbs and spices found around south-east Asia.

Hypothesis

We hypothesise that basil, cloves and coriander will exhibit antimicrobial properties.

Methodology

Extraction

50% (m/v) extracts of cloves, basil and coriander were prepared by dry blending the spices and herbs, before adding water. These mixtures were centrifuged at 9000rpm at 10°C to obtain a clear supernatant for further testing. The resultant supernatant was microfiltered to remove potential bacterial contamination before being stored at 0°C.

Microorganisms for antimicrobial tests

For the antimicrobial screening, one species of bacterial isolate and one species of fungus isolate were selected. *Escherichia coli* (ATCC 25922) strain was used. *Aspergillus niger* fungus was used as the test organism. The bacterial culture was maintained on nutrient agar while the fungus was maintained on potato dextrose agar, both at 4°C. The fresh cultures were obtained by growing the bacteria at 37˚C overnight, and the fungus at 28°C for 72h.

Zone of InhibitionTest

E. coli was cultured in nutrient broth at 37 ºC overnight and swabbed onto nutrient agar plates. Sterile paper discs were soaked in 5 different extracts and solvents--cloves, basil, coriander, ethanol and sterile water--and placed on different sections of the agar plates. These were incubated at 37°C overnight before zones of inhibition were measured.

Antifungal Tests

Fungus (*A. niger*) was grown on either normal potato dextrose agar (PDA) or PDA spiked with 10% cloves extract This was incubated at room temperature (RT) for 7 days and the extent of fungal growth was measured.

Colony Count Assay

Serial dilution was conducted on the *E. coli* that had been cultured overnight in nutrient broth. The dilutions were plated and incubated overnight. Colony-forming units (CFUs) were counted and bacteria numbers in the original liquid culture were adjusted to 10^6 CFU/ mL. This was mixed with clove extract. The mixtures were sampled at 10-minute intervals, plated and incubated overnight. The number of colonies was then counted and CFU numbers were calculated. Part of the adjusted culture was mixed with sterile water and ethanol as controls and similarly plated. The resultant CFUs were counted and used to calculate bacteria numbers at different time points.

Results and Discussion

Compounds in Cloves inhibit bacterial growth of Gram-negative bacteria

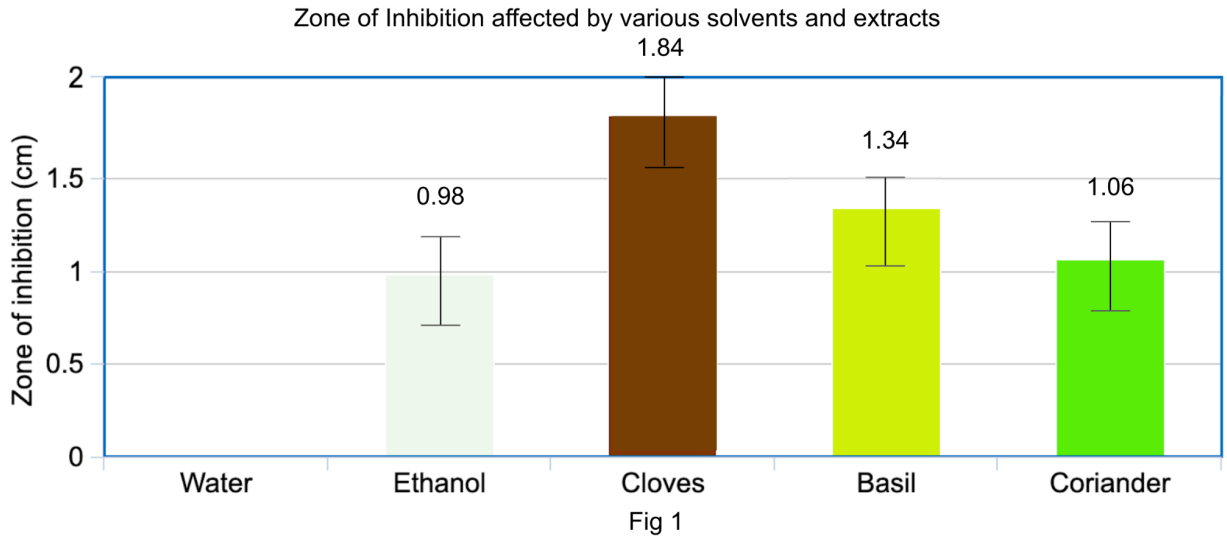
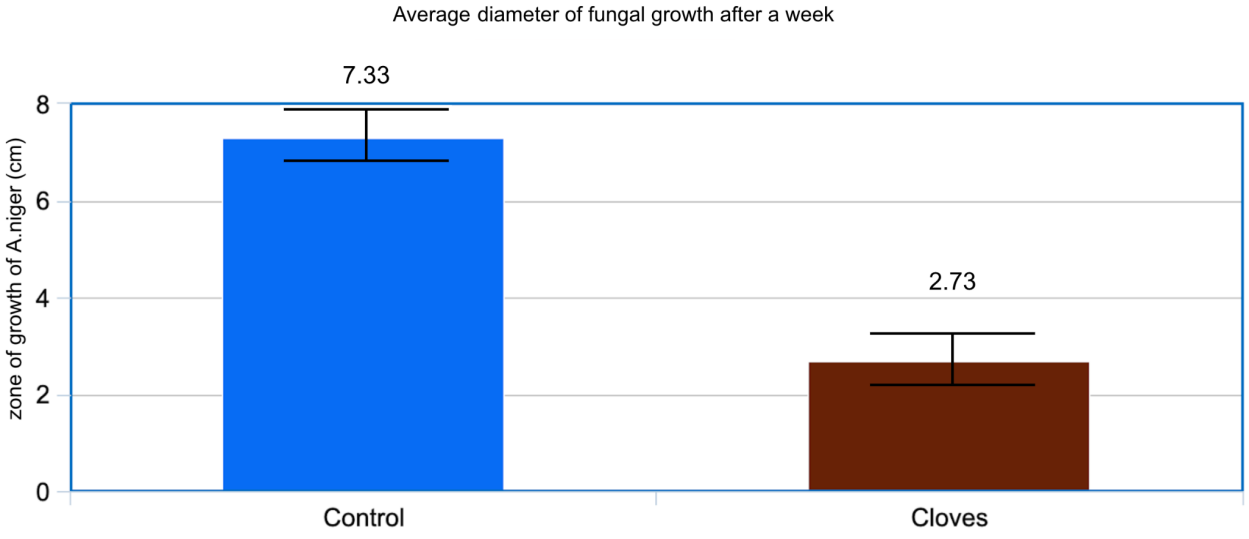


Fig 1

Our results show that cloves affected the widest zone of inhibition (1.84 ±0.18 cm), indicating that it contained the most potent antimicrobial active compounds. In contrast, basil and coriander did not affect the zone of inhibition as much, but still more so than ethanol. A previous study [22] showed that the antibacterial activity of clove oil against E. coli showed a zone of inhibition 1.9cm. On the contrary, another study [23] showed that clove oil did not show any activity against *E. coli*, having a negative effect. Our results definitively demonstrates that clove has antibacterial properties.

Compounds in Clove extract inhibit fungal growth



We decided to focus on clove, as it was the most promising of the three candidate herbs. Clove exhibited strong antifungal properties. Our results showed that clove concentration of 10% caused 72% inhibition of *A. niger* growth after 7 days. This is in line with previous work [24] which demonstrated that clove concentration of 10% effected 31% inhibition after 5 days, while 20% concentration effected 100% inhibition over 3 days.

Compounds in Cloves are bactericidal against Gram-negative bacteria

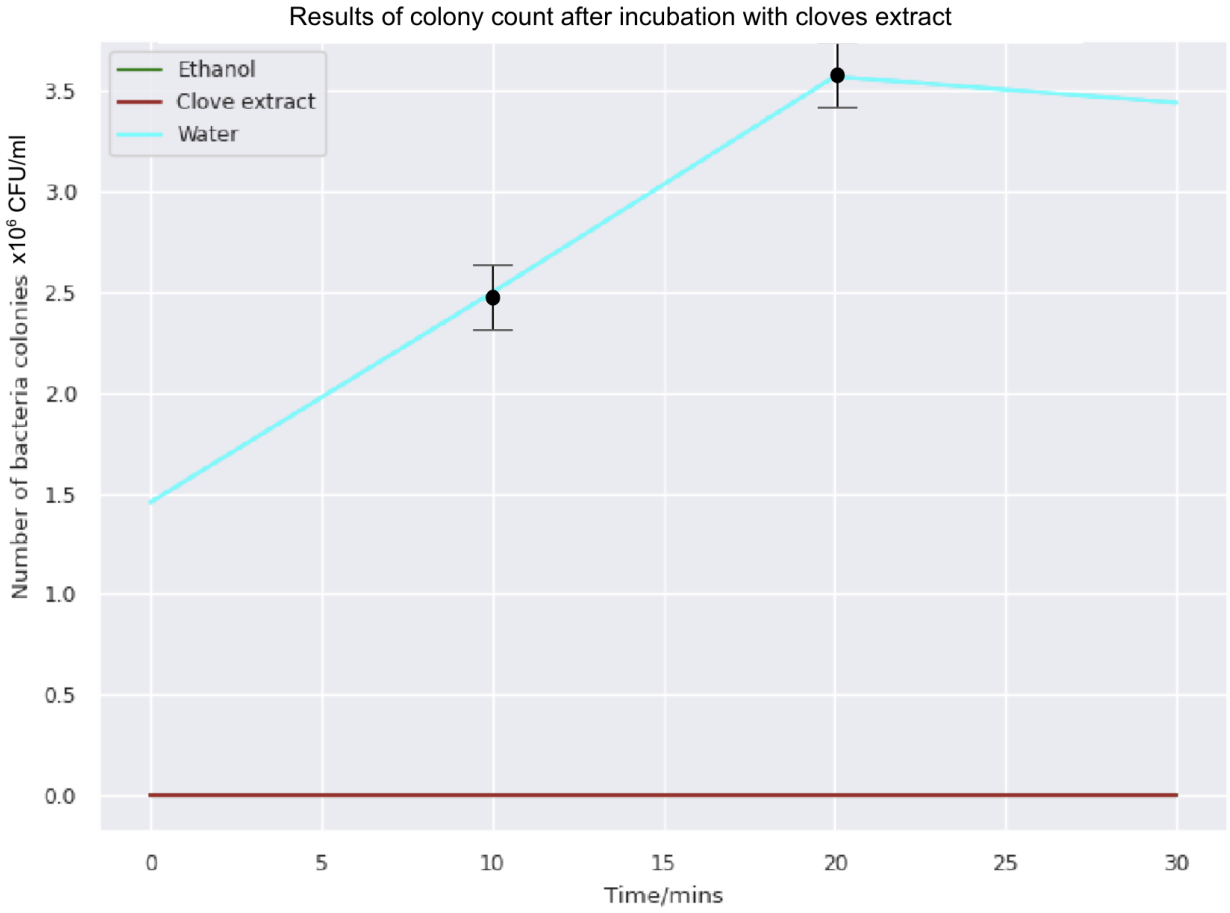


Fig 2

While the well diffusion test showed us whether the clove extract has antibacterial properties, it did not tell us whether the cloves extract was bactericidal or bacteriostatic. We therefore conducted a colony count assay to quantify the bacterial counts of *E. coli* over time, following exposure to active compounds if clove. Compounds of clove effectively eliminated all live bacteria in 10 minutes, preventing any CFUs from manifesting subsequently (Fig 2), proving that it was bactericidal against the Gram-negative bacteria. Such potent bactericidal activity was equal to ethanol, a well-known antimicrobial. Because cloves is regularly consumed by humans,

we know that it is non-toxic and is possibly safer for use compared to ethanol. Several types of commonly-used antibiotics are bacteriostatic, such as Glycylcyclines, Tetracyclines and Lincosamides. The fact that the aqueous extract of clove contains extracts which are bactericidal shows its potential to be developed as an antibacterial drug. Critically, clove extract kills bacteria 10 minutes after application, preventing future pathogenesis by fast mutating of bacteria.

Conclusion

From our experiments, cloves do contain compounds with potent antibacterial and antifungal properties, thus validating our hypothesis stated above. Future work would focus on the elucidation and characterisation of the activities compounds in cloves responsible for such antimicrobial effects. Doing so will pave the way for systematic drug discovery towards a potential new antimicrobial drug.

References

[1] Ellyatt, H. (2016, May 19). 'Superbugs' could cost $100 trillion - and millions of lives - by 2050: REPORT. Retrieved March 08, 2021, from https://www.cnbc.com/2016/05/19/superbugs-could-cost-100-trillion--and-millions-of-lives--by-2050-report.html

Spellberg, B., & Gilbert, D. N. (2014). The future of antibiotics and resistance: A tribute to a career of leadership by John Bartlett. *Clinical Infectious Diseases*, *59*(suppl 2), S71–S75. https://doi.org/10.1093/cid/ciu392

[2] Gygli, S. M., Borrell, S., Trauner, A., & Gagneux, S. (2017). Antimicrobial resistance in mycobacterium tuberculosis: Mechanistic and evolutionary perspectives. *FEMS Microbiology Reviews*, *41*(3), 354–373. https://doi.org/10.1093/femsre/fux011

Gygli, S. M., Borrell, S., Trauner, A., & Gagneux, S. (2017). Antimicrobial resistance in mycobacterium tuberculosis: Mechanistic and evolutionary perspectives. *FEMS Microbiology Reviews*, *41*(3), 354–373. https://doi.org/10.1093/femsre/fux011

[3] Turner, N.A., Sharma-Kuinkel, B.K., Maskarinec, S.A. *et al.* Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol* 17, 203–218 (2019). https://doi.org/10.1038/s41579-018-0147-4

[4] RATTANACHAIKUNSOPON, P., & PHUMKHACHORN, P. (2010). Antimicrobial activity of basil (ocimum basilicum) oil againstsalmonellaenteritidisin vitroand in food. *Bioscience, Biotechnology, and Biochemistry*, *74*(6), 1200–1204. https://doi.org/10.1271/bbb.90939

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agricultural and Food Chemistry*, *51*(11), 3197–3207. https://doi.org/10.1021/jf021038t

[5] Orafidiya, L. O., Agbani, E. O., Abereoje, O. A., Awe, T., Abudu, A., & Fakoya, F. A. (2003). An investigation into the wound-healing properties of essential oil OF Ocimum gratissimum linn. *Journal of Wound Care*, *12*(9), 331–334. https://doi.org/10.12968/jowc.2003.12.9.26537

[6] Li, H., Ge, Y., Luo, Z., Zhou, Y., Zhang, X., Zhang, J., & Fu, Q. (2017). Evaluation of the chemical composition, antioxidant and anti-inflammatory activities of distillate and residue fractions of sweet basil essential oil. *Journal of Food Science and Technology*, *54*(7), 1882–1890. https://doi.org/10.1007/s13197-017-2620-x

Jayasinghe, C., Gotoh, N., Aoki, T., & Wada, S. (2003). Phenolics composition and antioxidant activity of sweet basil (ocimum basilicuml.). *Journal of Agricultural and Food Chemistry*, *51*(15), 4442–4449. https://doi.org/10.1021/jf034269o

[7] Chithra, V., & Leelamma, S. (1999). Coriandrum sativum — mechanism of hypoglycemic action. *Food Chemistry*, *67*(3), 229–231. https://doi.org/10.1016/s0308-8146(99)00113-2

Aissaoui, A., Zizi, S., Israili, Z. H., & Lyoussi, B. (2011). Hypoglycemic and hypolipidemic effects of CORIANDRUM SATIVUM L. in Meriones shawi rats. *Journal of Ethnopharmacology*, *137*(1), 652–661. https://doi.org/10.1016/j.jep.2011.06.019

Eidi, M., Eidi, A., Saeidi, A., Molanaei, S., Sadeghipour, A., Bahar, M., & Bahar, K. (2009). Effect of coriander seed (coriandrum sativuml.) ethanol extract on insulin release from pancreatic beta cells in streptozotocin-induced diabetic rats. *Phytotherapy Research*, *23*(3), 404–406. https://doi.org/10.1002/ptr.2642

[8] Jabeen, Q., Bashir, S., Lyoussi, B., & Gilani, A. H. (2009). Coriander fruit exhibits gut modulatory, blood pressure lowering and diuretic activities. *Journal of Ethnopharmacology*, *122*(1), 123–130. https://doi.org/10.1016/j.jep.2008.12.016

R. Vasanthi, H., & P. Parameswari, R. (2010). Indian spices for healthy heart - an overview. *Current Cardiology Reviews*, *6*(4), 274–279. https://doi.org/10.2174/157340310793566172

[9] Mani, V., Parle, M., Ramasamy, K., & Abdul Majeed, A. B. (2010). Reversal of memory deficits BY CORIANDRUM sativum leaves in mice. *Journal of the Science of Food and Agriculture*, *91*(1), 186–192. https://doi.org/10.1002/jsfa.4171

[10] Vejdani, R., Shalmani, H. R., Mir-Fattahi, M., Sajed-Nia, F., Abdollahi, M., Zali, M. R., Alizadeh, A. H., Bahari, A., & Amin, G. (2006). The efficacy of an herbal MEDICINE, Carmint, on the relief of abdominal pain and bloating in patients with irritable bowel Syndrome: A pilot study. *Digestive Diseases and Sciences*, *51*(8), 1501–1507.https://doi.org/10.1007/s10620-006-9079-3

[11] Silva, F., Ferreira, S., Queiroz, J. A., & Domingues, F. C. (2011). Coriander (Coriandrum sativum l.) essential oil: Its antibacterial activity and mode of ACTION evaluated by flow cytometry. *Journal of Medical Microbiology*, *60*(10), 1479–1486. https://doi.org/10.1099/jmm.0.034157-0

Sambasivaraju, D., & ZA, F. (2016). Evaluation of antibacterial activity of CORIANDRUM Sativum (l.) against gram positive and gram negative bacteria. *International Journal of Basic and Clinical Pharmacology*, 2653–2656. https://doi.org/10.18203/2319-2003.ijbcp20164141

[12] Zhang, C.-R., Dissanayake, A. A., Kevseroğlu, K., & Nair, M. G. (2015). Evaluation of coriander spice as a functional food by using in vitro bioassays. *Food Chemistry*, *167*, 24–29. https://doi.org/10.1016/j.foodchem.2014.06.120

[13] Önder, A. (2018). Coriander and ITS Phytoconstituents for the beneficial effects. *Potential of Essential Oils (Coriander)*. https://doi.org/10.5772/intechopen.78656

[14] Banham, E. K. (2018, December 13). *Earliest discovery OF Clove and Pepper from ANCIENT south Asia*. Earliest discovery of clove and pepper from ancient south Asia. https://www.ucl.ac.uk/news/2018/dec/earliest-discovery-clove-and-pepper-ancient-south-asia.

Cortés-Rojas, D. F., de Souza, C. R., & Oliveira, W. P. (2014). Clove (Cloves): A precious spice. *Asian Pacific Journal of Tropical Biomedicine*, *4*(2), 90–96. https://doi.org/10.1016/s2221-1691(14)60215-x

[15] Liu, H., Schmitz, J. C., Wei, J., Cao, S., Beumer, J. H., Strychor, S., Cheng, L., Liu, M., Wang, C., Wu, N., Zhao, X., Zhang, Y., Liao, J., Chu, E., & Lin, X. (2014, August 14). *Clove extract inhibits tumor growth and promotes cell cycle arrest and apoptosis*. Oncology research. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4132639/.

Kovács, J., Felső, P., Makszin, L., Pápai, Z., Horváth, G., Ábrahám, H., Schneider,. (2016, October 15). Antimicrobial and virulence-modulating effects of clove essential oil on the foodborne pathogen campylobacter jejuni. Retrieved from https://aem.asm.org/content/82/20/6158

Liu, Q., Meng, X., Li, Y., Zhao, C.-N., Tang, G.-Y., & Li, H.-B. (2017). Antibacterial and antifungal activities of spices. *International Journal of Molecular Sciences*, *18*(6), 1283. https://doi.org/10.3390/ijms18061283

[16] Kothiwale, S. V., Patwardhan, V., Gandhi, M., Sohoni, R., & Kumar, A. (2014). A comparative study of antiplaque and antigingivitis effects of herbal mouthrinse containing tea tree oil, clove, and basil with commercially available essential oil mouthrinse. *Journal of Indian Society of Periodontology*, *18*(3), 316. https://doi.org/10.4103/0972-124x.134568

[17] Ghaffar, S., Afridi, S. K., Aftab, M. F., Murtaza, M., Hafizur, R. M., Sara, S., Begum, S., & Waraich, R. S. (2017). Clove and its active compound attenuate free fatty acid-mediated insulin resistance in skeletal muscle cells and in mice. *Journal of Medicinal Food*, *20*(4), 335–344. https://doi.org/10.1089/jmf.2016.3835

Adefegha, S. A., Oboh, G., Adefegha, O. M., Boligon, A. A., & Athayde, M. L. (2014). Antihyperglycemic, hypolipidemic, hepatoprotective and antioxidative effects of dietary clove (szyzgium aromaticum) bud powder in a high-fat diet/streptozotocin-induced diabetes rat model. *Journal of the Science of Food and Agriculture*, *94*(13), 2726–2737. https://doi.org/10.1002/jsfa.6617

[18] Kumar, P. S., Febriyanti, R. M., Sofyan, F. F., Luftimas, D. E., & Abdulah, R. (2014). Anticancer potential of Cloves L. in MCF-7 human breast cancer cell lines. *Pharmacognosy Research*, *6*(4), 350. https://doi.org/10.4103/0974-8490.138291

Liu, H., Schmitz, J. C., Wei, J., Cao, S., Beumer, J. H., Strychor, S., Cheng, L., Liu, M., Wang, C., Wu, N., Zhao, X., Zhang, Y., Liao, J., Chu, E., & Lin, X. (2014). Clove extract Inhibits tumor growth and promotes cell cycle arrest and apoptosis. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics*, *21*(5), 247–259. https://doi.org/10.3727/096504014x13946388748910

[19] Ding, Y., Gu, Z., Wang, Y., Wang, S., Chen, H., Zhang, H., Chen, W., & Chen, Y. Q. (2017). Clove extract functions as a natural fatty acid synthesis inhibitor and prevents obesity in a mouse model. *Food & Function*, *8*(8), 2847–2856. https://doi.org/10.1039/c7fo00096k

[20] Cortés-Rojas, D. F., de Souza, C. R., & Oliveira, W. P. (2014). Clove (Cloves): A precious spice. *Asian Pacific Journal of Tropical Biomedicine*, *4*(2), 90–96. https://doi.org/10.1016/s2221-1691(14)60215-x

[21] Park, C.-K., Kim, K., Jung, S. J., Kim, M. J., Ahn, D. K., Hong, S.-D., Kim, J. S., & Oh, S. B. (2009). Molecular mechanism for local anesthetic action of eugenol in the rat trigeminal system. *Pain*, *144*(1), 84–94. https://doi.org/10.1016/j.pain.2009.03.016

Chung G., Oh S.B. (2013) Eugenol as Local Anesthetic. In: Ramawat K., Mérillon JM. (eds) Natural Products. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-22144-6_171

[22] In vitro Evaluation of Antibacterial Activity of Clove Bud Oil on Bacterial Pathogens, Nor Syakirah Binti Shahroom*, Vishnu Priya V, Geetha RV, Int. J. Pharm. Sci. Rev. Res., 36(2), January – February 2016; Article No. 11, Pages: 59-60

[23] Packyanathan, J. S., & Prakasam, G. (2017). Journal of Pharmaceutical Sciences and Research. *Antibacterial Effect of Clove Oil against Clinical Strains of Escherichia Coli.*, *9*(7), 1203–1204.

[24] Avasthi, Shubhi & Gautam, Ajay & Bhadauria, Rekha. (2010). Antifungal activity of plant products against Aspergillus niger: A potential application in the control of a spoilage fungus. Biol. Forum Int. J.. 2.

Appendix:

Table of values:

Zone of inhibition test (Fig. 1)

	Ethanol/cm	Water/cm	Cloves/cm	Basil/cm	Coriander/cm
Plate 1	1 ± 0.1	0	1.7 ± 0.2	1.4 ± 0.1	1.1 ± 0.1
Plate 2	1 ± 0.1	0	1.8 ± 0.2	1.2 ± 0.1	1 ± 0.1
Plate 3	0.9 ± 0.09	0	1.8 ± 0.2	1.5 ± 0.2	1.1 ± 0.1
Plate 4	1 ± 0.1	0	2 ± 0.2	1.3 ± 0.1	1 ± 0.1
Plate 5	1 ± 0.1	0	1.9± 0.2	1.3 ± 0.1	1.1 ± 0.1
Average	0.98 ± 0.1	0	1.84 ± 0.2	1.34 ± 0.1	1.06 ± 0.1

Antifungal test (Fig. 2)

	Potential growth of fungus (*A. Niger)* on Control Plates/cm	Potential growth of fungus (*A. Niger)* Plates with Cloves extract/cm
Plate 1	8.5 ± 0.9	4.2 ± 0.4
Plate 2	5.5 ± 0.6	1.5 ± 0.2
Plate 3	8 ± 0.8	2.5 ± 0.3
Average	7.33 ± 0.7	2.73 ± 0.3

Colony Count Test (Fig. 3)

	Cloves (Colonies)				Average	**Ethanol** (Colonies)		Average	**Water** (Colonies)		Average
0 min	0	0	0	0	0	0	0	0	1456 ± 150	Nil	1456 ± 150
10min	0	0	0	0	0	1	0	0.5	2736 ± 270	2260 ± 230	2498 ± 250
20min	0	0	0	0	0	0	0	0	4304 ± 430	2836 ± 280	3570 ± 360
30min	0	0	0	0	0	0	0	0	3440 ± 340	Nil	3440 ± 340