MS.ID-IJPBS-9528
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2024) 14 (2): 01-07
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Comparative Study of Chemical Constituents of *Syzygium aromaticum* Ball and Calyx Through GC-MS

Richa Thawani[1], Sapna Sharma[1], and Ekta Menghani[2*]
[1]Department of Chemistry, JECRC University, Jaipur, Rajasthan, India.
[2*]Department of Biotechnology, JECRC University, Jaipur, Rajasthan, India

Received: 10 Jan 2024 / Accepted: 8 March 2024/ Published online: 01 April 2024
*Corresponding Author Email: ekta.menghani@jecrcu.edu.in

Abstract

Background: *Syzygium aromaticum* commonly known as Clove may be looked upon as a champion of all the spices. It has been used for centuries as one of the most important spices. Clove belonging to family Myrtaceae originates from Indonesia. It possesses many medicinal properties along with many other beneficial uses. Clove calyx and clove ball are the main parts to be considered for study in this article. Clove is rich in many phytochemicals like hydrocarbon, monoterpenes, sesquiterpenes, and phenolic compounds. Eugenyl acetate, eugenol, and β-caryophyllene are the most significant phytochemicals in clove oil. **Aim:** This study aimed at identifying chemical compounds in the clove calyx and clove ball and indicated their biological activities in the treatment of many maladies**.** In the present work, efforts were made to juxtapose and scientifically validate the results of the concentration of various chemical components in clove ball and calyx to check the presence of main component. **Setting:** Sample material clove was collected from local market in Malviya Nagar, Jaipur. The sample was authenticated and was provided with identification number and submitted the sample to Ethno-medicinal Herbarium, Centre with Potentials for Excellence in Biotechnology funded by DST, to JECRC, Jaipur, India. **Methods**: Analysis of biochemical components in clove ball and calyx was conducted by using GC-MS (Gas chromatography – Mass spectrometer) technique. Compounds were identified based on NIST database (National Institute Standard and Technology). **Results**: Major classes of compounds found in clove ball are Methyl salicylate, Caryophyllene, Ascorbic acid derivative, Anethole. While in clove calyx Eugenol, chemical was found. Eugenol is the main and essential component in clove. **Conclusion**: Eugenol is present in clove calyx and absent in clove ball. So, calyx is the part which should be used and not the ball part. Eugenol, being the most essential constituent in the clove, is used as an antiseptic, analgesic, perfumery and as a flavoring agent.

Keywords
Clove (*Syzygium aromaticum*), GC-MS analysis, Eugenol.

*****

INTRODUCTION:

India has richness of species diversity, genetic diversity, and habitat diversity (Joseph and Raj, 2011; Mehmood *et al* 1999). Nature has provided a very rich botanical wealth and many diverse types of plants growing wild in different parts of our country. Extensive use of costly synthetic drugs can be controlled by making use of drugs extracted from plants (Joseph and Raj,2011). Plants have provided numerous varieties of drugs which are effective on various maladies and the natural products derived from plants, are extensively utilized in

pharmaceutical biology. Most of the drugs which are utilized resemble either structurally to the naturally occurring molecules or have structures that are fully or in part derived from natural patterns (Yates,2002). Plants rich in bioactive phytomedicine compounds such as alkaloids, flavonoids, tannins, and polyphenols have been used to cure illnesses because of their various pharmacological properties. India is always known to be rich depository of medicinal plants and various forms of herbal medicine practices are considered as "living tradition" (Prasath Kumar *et al*, 2021). The traditional herbal practitioners are even today known to follow the herbal medicine system in rural areas, using approximately 2500 plants for treating basic illness which has been considered as one of the best methods in Indian medical practices (Panmei *et al*, 2019). Complex phytochemical mixtures of aromatic compounds with many applications in food, pesticides, pharmaceutical and fragrance industries are known as Essential oils (Eo) (Hossain *et al*,2014; Isman,2000; Calo *et al*,2015; Shaaban *et al*,2012; Thao *et al* 2013). FID (Flame Ionization Detector and GC-MS are the two chromatographical analytical techniques to spot and quantify unknown parts in Eos. Out of these two, GC-MS is the most used technique to spot and quantify unknown parts in Eos (Waseem and Low, 2015).

GC-MS is costly and time consuming despite the extension of devices (Kah & Low, 2015). During the study of pure essential oils, only a dilution is required using a suitable organic solvent. When essential oils are incorporated into a product, they undergo an extraction step before analysis. It is a time-consuming process. The application of organic solvents needs to be limited in relation to their toxicity for humans and the environment. However, GC-MS is a very sensitive method, which is powerful in the analysis of compounds present in traces with high accuracy and to early evidence the formation of new components responsible to off-flavor. Another means to quantify and measure the release of EOs encapsulated in complex matrices is the use of spectrophotometric techniques (Natrajan *et al*,2015). However, most of the aroma compounds present in EOs lack chromophores. Therefore, the worldwide quantification of the EO could not be specifically studied by spectrophotometric techniques (Kfoury *et al*, 2016).

Clove (Syzygium aromaticum) a widely known spice for its distinctive aroma, flavor and potential medical advantages and ancient uses belongs to the dicot family (Amelia *et al*,2017). Clove may be looked upon as a champion of all the spices. Clove is native of Indonesia but nowadays is cultured in several parts of the world including Brazil in the state of Bahia (Diego *et al*,2014).

Clove trees are all the time cultivated in coastal areas at maximum altitudes of 200 m higher than the sea level. The proffering of flower buds, that is commercialized part of this tree, starts after four years of plantation. Flower buds are assembled in the maturation phase simply before flowering. The assembled flower buds might be done manually or chemically mediated employing a natural growth regulator that liberates ethylene in the vegetal tissue, manufacturing advanced maturation. There are 3 types of cloves oil; bud oil, leaf oil and stem oil. Bud oil is pulled from the flower-buds of genus Eugenia caryophyllus. It consists primarily of 60-90% eugenol, caryophyllene and alternative inconsequential constituents. Leaf oil is extracted from the leaves of genus Eugenia caryophyllus. It primarily has 82-88% eugenol, very little quantity of eugenyl acetate, and other minor constituents (Kamatou *et al*, 2012).

Description of the morphology:

The morphology of the clove defines its distinct components i.e., a protracted scroll that further terminates to the four spreading sepals and 4 sealed petals that turn to be a tiny low central ball. Clove oil seems to be a colorless or yellowness color essential oil that is obtained through stem, bud, and plant leaves. Clove oil is extracted from the clove by different methods like steam distillation, hydro distillation, and solvent extraction technique (Guan *et al*,2007). Clove oil is a combination of various constituents; the active ingredients are acetyl eugenol, caryophyllene and eugenol (Rodríguez Luis *et al*.,2014).

Except these major constituents there are some minor constituents found in volatile oil like methyl salicylate oil, chavicol, α- copaene, α-amorphene, tannins, and other substances mainly methyl furfural and dimethyl furfural and vanillin (Politeo *et al*,2010). Clove oil is well known around the world for its potential medical uses. It is getting used within the cookery of many places like Asia, Africa, and many middle east countries as a flavoring agent to curries, meats, and marinades (Alma *et al*., 2007). Few vital properties of clove like its antimicrobial and inhibitor properties enhance the medical uses of the oil i.e., it has the capability to resist and kill the microorganism that cause the illness like respiratory disease like influenza and pneumonia and few respiratory conditions usually cough and respiratory illness (Chaieb *et al*., 2007). It is widely used as an analgesic as eugenol is that the main constituent of the oil that is an effective good pain killer and thus

the oil is getting used to treat the toothache and other types of pain (Muruganandan *et al*,2001)

Thus, the aim of the research is to make a comparison and scientifically validate the concentration of different chemical components in clove ball and calyx to check the main ingredients and quantify the eugenol concentration in different parts of clove. The studies can further be used for specific extraction and herbal formulations.

RESEARCH METHODS AND DESIGNS:

Collection of Sample material:

Sample material clove was collected from local market in Malviya Nagar, Jaipur. The sample was authenticated and was provided with identification number and submitted the sample to Ethno-medicinal Herbarium, Centre with Potentials for Excellence in Biotechnology funded by DST, to JECRC, Jaipur, India.

MATERIAL AND METHODS:

Processing of clove material:

Clove buds were collected and screened for foreign material. Collected sample was shed dried and then separation of ball and calyx part was done. Further, both the materials were weighed and crushed in mixer grinder. Powder was used for extraction purpose (Saini *et al*,2017).

Extraction of clove sample:

10 gm of each powder was refluxed on water bath with submerged in methanol for ten to twelve hours on water bath. Methanol extract of dried clove material was prepared by using hot extraction in vitro technique with powder plant material. The crude extract of clove was prepared using methanol as a solvent with continuous stirring at 10°C-20°C. After that a homogenous solution was formed. This homogenous solution was mixed gently till it gets cooled and then this solution is get filtered and then concentrated by vacuum distillation method using rotavap. The concentrated clove extracted was further used for experiment procedure (Saini *et al*,2017).

GC-MS analysis of clove ball and calyx extract:

The clove extracted was screened for GC-MS analysis so that comparison of chemicals and validation and quantification can be done. The sample was analyzed in GCMS-QP2010 Plus from Ayushraj Enterprises Pvt. Ltd. Jaipur, India. Concentrated methanol extract was dissolved in methanol solution and a 2µL aliquot of sample was injected into the column where the temperature of the injector was 260°C with the split ratio of 10:0 ,the initial temperature of oven was 100 °C and then 250 °C for 5 min, 30 min for 280 °C and hold it for 70.00 min. ACQ Mode Scan: 40 to 600 *m/z*, Column flow is 1.21 mL/min and total flow is 16.3 mL/min. Flow control with linear velocity which 40.9 cm/s (Saini *et al*,2017).

Identification of bioactive compounds:

Identification of compounds was based on NIST (National Institute Standard and Technology) database. The in-built library of NIST in GC-MS was used for interpretation of the data. The library possesses more than 65000 patterns in its repository. The detail of the chemical compounds was noted from the library with molecular weight, structure of the compound. GC-MS also reveals the concentration of all the components. Thus, quantification of the compounds in plant parts can be measured.

GC-MS spectrum of methanolic extract of Clove (ball and calyx) confirmed the presence of different compounds in different parts.

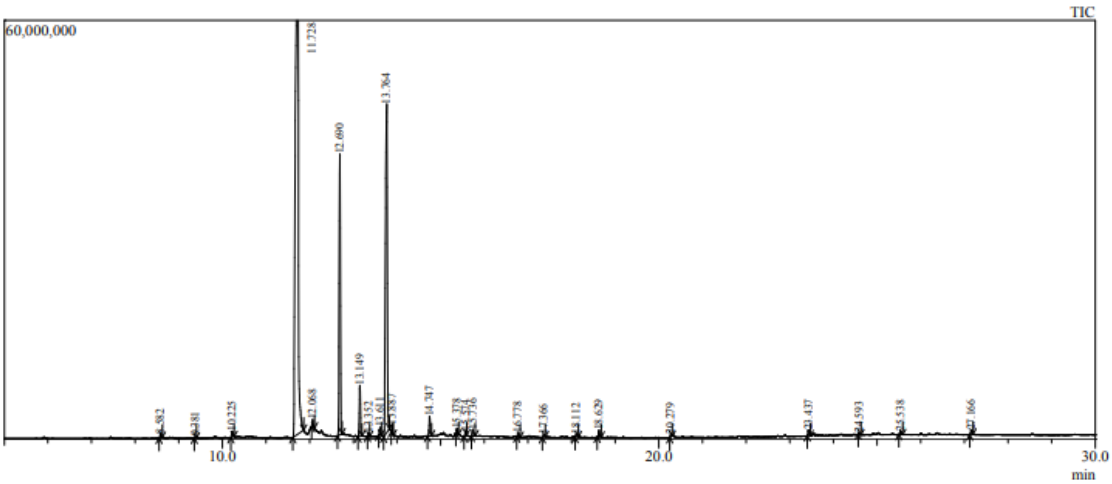

Figure 1: Chromatogram of *S. aromaticum* (clove) methanol extract Ball

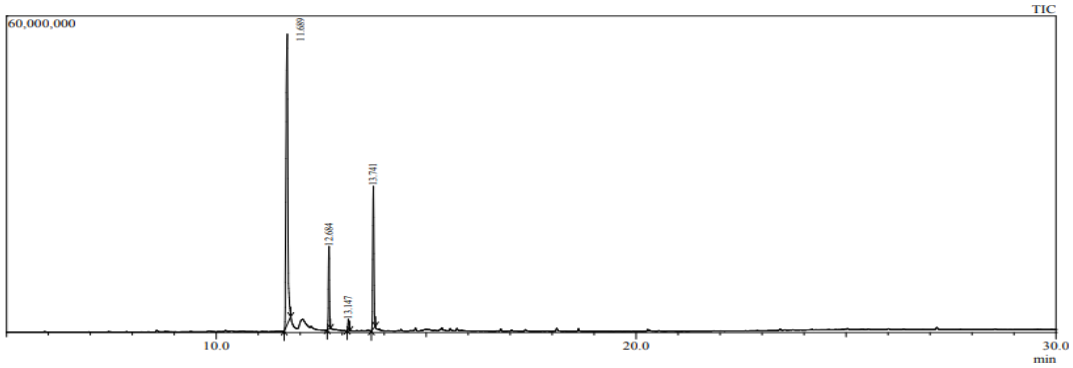

Figure 2: Chromatogram of *S. aromaticum* (clove) methanol extract calyx

Table 1: List of compounds identified from GCMS screening of *S. aromaticum* methanol extract calyx.

Peak	R.Time	F.Time	Area	Area%	A/H	Name
1	11.689	11.775	168999560	67.19	3.07	Eugenol
2	12.684	12.720	26264060	10.44	1.68	Caryophyllene
3	13.147	13.180	3399038	1.35	1.61	1,4,7, -Cycloundecatriene,1,5,9,9-tetramethyl-
4	13.741	13.790	52865702	21.02	1.96	Phenol,2-methoxy-4-(2-propenyl)-, acetate
			251528360	100.00		

Table 2: List of compounds identified from GC-MS screening of *S. aromaticum* methanol extract ball.

Peak	R.Time Initial	F.Time Final	Area	Area%	A/H	Name
1	8.582	8.625	1174115	0.20	2.12	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
2	9.381	9.405	315751	0.05	1.52	Methyl salicylate
3	10.225	10.270	1820417	0.31	2.21	Phenol, 4-(2-propenyl)-
4	11.728	11.860	341776147	57.85	4.64	Phenol, 2-methoxy-3-(2-propenyl)-
5	12.068	12.110	1771379	0.30	1.67	alfa. -Copaene
6	12.690	12.740	75278588	12.74	1.87	Caryophyllene
7	13.149	13.200	12380571	2.10	1.69	1,4,7, -Cycloundecatriene, 1,5,9,9-tetramethyl-, Z, Z, Z-
8	13.352	13.375	720886	0.12	1.43	gamma. -Muurolene
9	13.611	13.640	1423024	0.24	1.31	alpha. -Farnesene
10	13.764	13.825	133119652	22.53	2.83	3-Allyl-6-methoxyphenol
11	13.887	13.920	1700866	0.29	1.38	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-
12	14.747	14.795	5081576	0.86	1.83	Caryophyllene oxide
13	15.378	15.410	1240234	0.21	1.67	10,10-Dimethyl-2,6-dimethylenebicyclo [7.2.0] undecan-5. beta. -ol
14	15.574	15.600	1262075	0.21	1.79	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl) prop-2-en-1-ol
15	15.736	15.795	2065635	0.35	2.09	(1R,7S, E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol
16	16.778	16.825	1108591	0.19	1.72	Benzyl Benzoate
17	17.366	17.400	743064	0.13	1.91	3.alpha.,7. beta. -Dihydroxy-5. beta.,6. beta. -epoxycholestane
18	18.112	18.155	1023897	0.17	2.15	(3S,3aS,6R,7R,9aS)-1,1,7-Trimethyldecahydro-3a,7-methanocyclopenta [8] annulene-3,6-diol
19	18.629	18.680	1722025	0.29	1.69	l-(+)-Ascorbic acid 2,6-dihexadecanoate
20	20.279	20.305	762661	0.13	1.64	Linoelaidic acid
21	23.437	23.470	1463852	0.25	1.80	Anethole
22	24.593	24.630	482598	0.08	1.60	3-Allyl-6-methoxyphenol
23	25.538	25.590	993894	0.17	2.16	Phenol, 2-methoxy-4-(1-propenyl)-, acetate
24	27.166	27.205	1347980	0.23	2.39	13-Methylheptacosane
			590779478	100.00		

Results:

While performing GC-MS analysis of ball, the presence of 4 dominant components at different retention time. i.e., Methyl salicylate, Caryophyllene, Ascorbic acid, Anethole was observed. In GC-MS analysis of calyx presence of 2 dominant components i.e., Eugenol and Caryophyllene was noticed. Clove essential oil and eugenol derived from *S. aromaticum* have been documented to possess useful analgesic, aesthetic, and antiseptic effects and are therefore commonly used in dentistry (Chaieb *et al*,2007). In addition to that, they showed an anti-inflammatory efficacy against murine macrophages by suppressing the pro-inflammatory cytokines production (Rodrigues *et al*, 2009). The chemical structure of the compounds following are drawn with the help of Chemdraw.

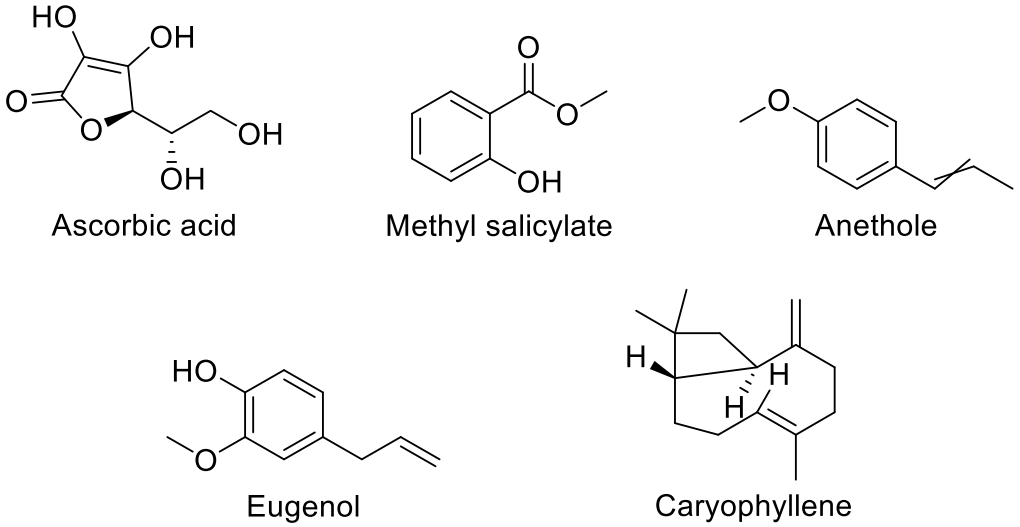

Figure 3: Chemical constituents observed in Clove ball and calyx.

Discussion: Documented and approved uses of Eugenol

Interestingly, several reports documented that eugenol isolated from S. *aromaticum* extracts have shown potent trypanocidal as well as leishmanicidal efficacy against *Trypanosoma cruzi*, *Leishmania donovani*, *L. amazonensis, L. major* and *L. tropica* (Ueda-Nakamura *et al*, 2006). Additionally, eugenol showed a potential lethal efficacy against the growth and multiplication of various parasites including *Giardia lamblia*, *Fasciola gigantica*, *Haemonchus contortus*, and *Schistosoma mansoni*. Eugenol has been suggested to possess recovery effects on arthritis and thus can be used in the treatment of arthritis (Grespan *et al*,2012).

CONCLUSION:

The study of clove (*Syzygium aromaticum*) helps us to predict the different chemical components present in the distinct part of the clove (ball, calyx). The study shows us that ball of the clove consists of the dominant component Methyl salicylate, Caryophyllene, Ascorbic acid derivatives, Anethole. Anethole is the aromatic compound used as flavoring agent and is used in essential oil.

The calyx is the part of the clove which consists of two dominant component Eugenol and Caryophyllene. Eugenol is the most essential component of clove as it is used as antiseptic, analgesic, perfumery and as a flavoring agent. As it is absent in the ball. The calyx alone can also be used for the further medicinal use of the clove and its benefits.

Acknowledgements:

The authors want to thank JECRC University STI Hub (DST/SEED /SCSP /STI/2019/100) sanctioned under the auspices of Science for Equity Empowerment and Development Division, Department of Science and Technology, Government of India for providing support for the work done.

Competing Interests:

The authors declare no conflict of interest.

Author's contribution:

R.T. carried out the experiment and wrote the manuscript with support from S.S & E.M. S.S & E.M. helped in the supervision of the project and conceived the original idea.

Disclaimer:

The views expressed in this article are the author's own.

Funding information:

This article received no external research funds.

REFERENCES

1. Joseph, B and Raj, S.J.,2011, 'A comparative study on various properties of five medicinally important plants', International Journal of Pharmacology, *7*(2), 206-211.
2. Mehmood, Z., I. Ahmad, F. Mohammad, and S. Ahmad,1999, 'Indian medicinal plants: A potential source for anticandidal drugs', Pharmaceutical Biology,37(3),237-242.
3. Yates, A.2002, Yates Garden Guide. Harper Collins Publishers, Pymble, Australia.
4. Prasath Kumar, M., Anisha, S., Dhrisya, C., Becky, R. and Sadhasivam, S.,2021, 'Therapeutic and pharmacological efficacy of selective Indian medicinal plants–A review', Phytomedicine Plus, 1(2), 1-28.
5. Panmei, R., Gajurel, P.R. and Singh, B., 2019, 'Ethnobotany of medicinal plants used by the Zeliangrong ethnic group of Manipur, Northeast India', Journal of Ethnopharmacology,235(1),164-182.
6. Hossain, M.A., Harbi, S.R.A., Weli, A.M., Al-Riyami, Q. and Al-Sabahi, J.N., 2014, 'Comparison of chemical constituents and antimicrobial activities of three essential oils from three different brands' clove samples collected from Gulf region', Asian Pacific Journal of Tropical Disease, 4(4), 262-268.
7. Isman, M.B.,2000, 'Plant essential oils for pest and disease management', Crop protection, 19(8-10), 603-608.
8. Calo, J.R., Crandall P.G., Bryan C.A.O, Ricke S.C., 2015, 'Essential oils as antimicrobials in food systems–A review', Food Control, Elsevier ,54 ,111-119.
9. Shaaban, H.A., El-Ghorab, A.H. and Shibamoto, T., 2012, 'Bioactivity of essential oils and their volatile aroma components', Journal of Essential Oil Research, 24(2), 203-212.
10. Zhao, D., Xu, Y.W., Yang, G.L., Husaini, A.M. and Wu, W., 2013, 'Variation of essential oil of *Mentha haplocalyx Briq.* and *Mentha spicata L.* from China', Industrial Crops and Products, 42,251-260.
11. Waseem, R. and Low, K.H., 2015, 'Advanced analytical techniques for the extraction and characterization of plant-derived essential oils by gas chromatography with mass spectrometry', Journal of separation science, 38(3),483-501.
12. Natrajan, D., Srinivasan, S., Sundar, K. and Ravindran, A., 2015, 'Formulation of essential oil-loaded chitosan–alginate nanocapsules, ' Journal of food and drug analysis, 23(3),560-568.
13. Kfoury, M., Auezova, L., Greige-Gerges, H., Larsen, K.L. and Fourmentin, S., 2016, 'Release studies of trans-anethole from β-cyclodextrin solid inclusion complexes by multiple headspace extraction', Carbohydrate polymers, 151,1245-1250.
14. Amelia, B., Saepudin, E., Cahyana, A.H., Rahayu, D.U., Sulistyoningrum, A.S. and Haib, J., 2017, 'GC-MS analysis of clove (Syzygium aromaticum) bud essential oil from Java and Manado. In American Institute of Physics', Conference Proceedings (Vol. 1862, No. 1,1-9. AIP Publishing.
15. Diego, C.R.F. and Wanderley, O.P., 2014, 'Clove (*Syzygium aromaticum*): A precious spice', Asian Pacific Journal of Tropical Biomedicine, 4(2),90-96.
16. Kamatou, G.P., Vermaak, I. and Viljoen, A.M., 2012, 'Eugenol—from the remote Maluku Islands to the international marketplace: A review of a remarkable and versatile molecule', Molecules, 17(6),6953-6981.
17. Guan, W., Li, S., Yan, R., Tang, S. and Quan, C., 2007, 'Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods', Food Chemistry, 101(4), 1558-1564.
18. Rodríguez Luis, O., Sánchez Casas, R.M., Verde Star, M.J., Núñez, A., Ríos, R. and Chávez, A., 2014, 'Obtaining the essential oil of Syzygium aromaticum, identification of eugenol and its effect on Streptococcus mutans', Journal of oral research, *3*(4), .218-224.
19. Politeo, O., Jukic, M. and Milos, M., 2010, 'Comparison of chemical composition and antioxidant activity of glycosidically bound and free volatiles from clove (*Eugenia caryophyllata Thunb.*)', Journal of Food Biochemistry, 34(1), 129-141.
20. Alma, M.H., Ertas, M., Nitz, S. and Kollmannsberger, H., 2007, 'Chemical composition and content of essential oil from the bud of cultivated Turkish clove (Syzygium aromaticum L.)', BioResources, 2(2), 265-269.
21. Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A.B., Rouabhia, M., Mahdouani, K. and Bakhrouf, A., 2007, 'The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae):A short review', Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 21(6), 501-506.
22. Muruganandan, S., Srinivasan, K., Chandra, S., Tandan, S.K., Lal, J. and Raviprakash, V., 2001, 'Anti-inflammatory activity of Syzygium cumini bark' Fitoterapia, 72(4), 369-375.
23. 23.Saini, P., Menghani, E. and Mithal, R., 2017, 'Identification of Phytocomponents in Methanolic Extract of *Cuscuta reflexa* Grown on *Acacia nilotica* Host Plant Through GC-MS Analysis' Asian Journal of Chemistry, 29(7),1637-1638.
24. Bachiega, T.F., de Sousa, J.P.B., Bastos, J.K. and Sforcin, J.M., 2012, 'Clove and eugenol in noncytotoxic concentrations exert immunomodulatory/anti-inflammatory action on cytokine production by murine macrophages. Journal of Pharmacy and Pharmacology, 64(4), 610-616.
25. Rodrigues, T.G.; Fernandes, A., Jr.; Sousa, J.P.; Bastos, J.K.; Sforcin, J.M., 2009, 'In vitro and in vivo effects of clove on pro-inflammatory cytokines production by macrophages. Natural Product Research, 23(4), 319–326.
26. Ueda-Nakamura T., Mendonça-Filho R.R., Morgado-Díaz J.A., Korehisa Maza P., Prado Dias Filho B., Aparício Garcia Cortez D., Alviano D.S., Rosa M. S.,

Lopes A.H., Alviano C.S., *et al*.,2006, 'Antileishmanial activity of Eugenol-rich essential oil from *Ocimum gratissimum*'. Parasitology International,55(2),99–105.

27. Grespan, R., Paludo, M., de Paula Lemos, H., Barbosa, C.P., Bersani-Amado, C.A., de Oliveira Dalalio, M.M., and Cuman, R.K.N., 2012,'Anti-arthritic effect of eugenol on collagen-induced arthritis experimental model', Biological and Pharmaceutical Bulletin, 35(10), 1818-1820.