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CLOVE (*SZYGIUM AROMATICUM*) AND ITS DERIVATIVES IN RUMINANT FEED

MUZAMMIL I[*1], NASEER MA[2], BAKHT P[1], ATTA K[3], SHOUKAT S[4], AZAM M[5]

[1]Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan
[2]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad-38000, Pakistan
[3]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan
[4]Department of Chemistry, University of Agriculture, Faisalabad-38000, Pakistan
[5]Department of Food Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100, Pakistan

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ABSTRACT

Livestock, more prominent of which are ruminants, has a significant contribution to methane emission, a potential greenhouse gas as a good portion of their ingested energy is wasted in the form of methane (2–15%) and major of the ingested nitrogen as ammonia (75–95%). Microbial fermentation in ruminants result in loss of energy in methanogenesis and protein by ammonia nitrogen excretion which causes decreased animal optimal production and also act as environmental pollutants. Previously antibiotics were used to decrease these losses in the rumen, but this approach was restricted due to presence of antibiotic residues in animal products. Some plants or their bioactive extracts/metabolites such as organo-sulphur compounds, saponins, essential oils, flavonoids, tannins and many other metabolites at higher concentration exhibited the potential to limit the methanogenesis by altering the rumen microflora. To overcome this problem, plant extracts including clove bud oil (Syzygiumaromaticum) was introduced as an alternative for manipulating rumen fermentation. Clove bud oil possesses the capability to interact with bacterial cells and inhibits the growth multiplication of methanogenic and deaminating bacteria. This results in reduction in ammonia nitrogen, methane and acetate concentration, while higher propionate and butyrate concentrations were noted. Eugenol is one of the bioactive constituents of clove which has the ability to manipulate rumen fermentation by increasing propionate production, decreasing acetate and methane production, and altering pattern of proteolysis, peptidolysis and amino acid deamination in the rumen. Current review will focus on the use of clove for manipulation of rumen fermentation for inhibition of methanogenesis and energy loss in ammonia – nitrogen waste.

1. INTRODUCTION

Dietary protein is broken down to ammonia to increase nitrogenous waste material instead of becoming part of animal body.

*Corresponding Author: iqramuzammil25@gmail.com

Almost 75 to 85% of nitrogen taken by lactating animals is excreted out in the urine and feces (Goel and Makkar, 2012) and it badly affect the environment by increasing discharge of nitrous oxide in the air (Patra and Saxena, 2010). To overcome this, ruminal microbial ecosystem is manipulated by adding herbal extracts and spices as feed additives which can eradicate or decrease rumen ciliate protozoa, decrease protein degradation and methane production.

Although, previously antibiotics were recommended as feed additives to manipulate rumen fermentation by decreasing methane and ammonia nitrogen production (McGuffey *et al.*, 2001). But this practice was eliminated due to higher antibiotic residues in animal products(Russell and Houlihan, 2003). So, FDA has recommended the use of natural antimicrobials like plant extracts which are normally considered safe for human consumption by modifying microbial fermentation in the rumen. Plant extracts possess antimicrobial activity due to presence of secondary plant metabolites including phenyl propanoids (e.g.,eugenol, cinnamaldehyde, saponins, anethol, tannins) possessed by the oil extracted from various plants. Main constituent of clove oil are eugenol and b-caryophylene which is a phenolic non-nutrient constituent which have effect on all in vitro rumen fermentation products like volatile fatty acids, N-ammonia and ruminal microorganisms (Busquet *et al.*, 2006a). Essential oils are naturally occurring volatile components responsible for giving plants and spices their characteristic essence and color (Patra and Yu, 2014).

Herbs and spices are most commonly used in feed of ruminants now a day (Chaves *et al.*, 2012). Various anaerobic bacteria, protozoa, fungi and methanogens are present in rumen of animals. Rumen fermentation process is manipulated by using herbs to enhance the efficacy of nutrients digestion and metabolism, reduction of energy loss from unwanted process of formation of methanogens and general increase in the production of animals (Chaves *et al.*, 2011). Previously, some antibiotics and other feed additives were recommended for this purpose, but now trend has been shifted towards the use of herbs and plant extracts to increase the production of animals (Ipharraguerre and Clark, 2003). Herbs and spices mostly exhibit effects on the basis of required dosage, because small dosages show no effect while large amount of dosage can produce toxicity in animals (Goodrich *et al.*, 2018).

2. MECHANISM OF ACTION OF CLOVE AS MANIPULATOR OF RUMEN FERMENTATION

Clove bud (*Syzygiumaromaticum*) contains phenylpropanoids which show antibacterial activity against different bacterial species by interacting with their cell membrane. The activity of clove ows to its unique chemical composition (Fig 4). This interaction produces many changes in the conformation the membrane structure, responsible for its fluidity and expansion (M.Tajodini, P. Moghbeli, H.R. Saeedi, 2014). By disruption of membrane stability, ionic leakage across the cellular membrane occurs and it reduces the ionic gradient across the membrane. Mostly, bacteria equipoise these effects by using ionic pumps and it does not cause cell death but large amounts of energy are distracted to this function and bacterial growth slows down. As a result, this alteration in growth rates causes change in the amount of rumen bacterial populations leading to changes in the profile of rumen fermentation (Benchaar *et al.*, 2008).

Eugenol (4-allyl-2-methoxyphenol; C10H12O2) is a phenolic compound present in clove bud (*S.aromaticum*) and it is responsible for broad spectrum antimicrobial activity against both gram-positive and gram-negative bacteria due to the presence of a hydroxyl group (M.Tajodini, P. Moghbeli, H.R. Saeedi, 2014).It is reported in a continuous culture study that low doses of clove bud oil (2.2 mg/L) decreases production of acetate and branched-chain volatile fatty acidsand increases production of propionate (Busquet *et al.*, 2006a). Clove bud oil has also effects on nitrogenmetabolism by increasing peptide nitrogen and by decreasing ammonia nitrogen concentrations, indicative of reduced peptidolytic activity in the rumen. (Busquet *et al.*, 2006a)reported in an *in vitro* batch culture study that clove bud oil helps in manipulating rumen fermentation, decreasing total volatile fatty acids and ammonia nitrogen concentrations and exhibit a linear increase in the molar proportion of propionate and a quadratic effect on the molar proportions of acetate and butyrate. As a result, clove bud oil helps in improving overall rumen fermentation.

Clove bud oil contains 85% eugenol which is responsible for increase in pH of rumen and reduced total volatile fatty acids and ammonia nitrogen concentrations (M.Tajodini, P. Moghbeli, H.R. Saeedi, 2014).Eugenol causes increase in propionate concentration and clove bud oildecreases acetate concentration (fig. 1). Clove bud oil and eugenol increasedbranched chain volatile fatty acids at 300 mg/L compared with control, but this value decreased in eugenol at dose rate of 3,000 mg/L. So, it is reported that other components of clove bud oil may interact with eugenol and showed additional impacts. Results of*in vitro* continuous culture study revealed that lower inclusion rate of clove bud oil (2.2 mg/L)decreased the molar concentration of acetate and branched chain volatile fatty acids whilean increase in the molar concentration of propionate was noted, however similar dose of eugenol did not show any effects(Busquet *et al.*, 2005a).

3. CLOVE NUTRACEUTICS

Phenolics in herbal oils showed nutritional significance as common antimicrobials and antioxidant and may directly respond with, and extinguish, free radicals to prevent lipid peroxidation, along these lines improving health and preventing certain infections(Botsoglou *et al.*, 2004; Brenes and Roura, 2010; Tariq *et al.*, 2015). 2-grams clove powder possesses 6 calories, 1 gram of carbohydrates and fiber each and small quantity of vitamin K and manganese. Scientifically reported nutraceutical spectrum of clove and clove oil includes cancer prevention, malignancy inhibition, tumor growth suppression, liver health improvement, decrease in oxidative pressure on cell lines, antibacterial action, lowering of blood glucose, enhance bone health and repair, enhance gastric ulcer healing (fig.2) (Shabestari *et al.*, 2011; Morsy *et al.*, 2012a; Roy *et al.*, 2015). Clove is being abundantly produced in some countries and most of its part is of exported (Fig 3)

4. EFFECT OF CLOVE ON METHANE PRODUCTION

Livestock, more prominent of which are ruminants, has a significant contribution to methane emission, a potential greenhouse gas as a good portion of their ingested energy is wasted in the form of methane (2–15%) and major of the ingested nitrogen as ammonia(75–95%)(Bodas *et al.*, 2012; Cobellis *et al.*, 2016a). Ruminant production system is a major point of emission of all the greenhouse gases mainly CH_4(18%) and CO_2(9%). Amongst the greenhouse gases, methane possessessignificant global warming properties (about 23 times) more than carbon dioxide(Ugbogu *et al.*, 2019). Methane production in ruminants mainly depends on forage to be fed and time of harvest of forage (ZHONG *et al.*, 2016). The emission of greenhouse gases from livestock systems is emerged a great global concern asthese emitted gases substantially contribute in global warming.

Some plants or their bioactive extracts/metabolites such as organo-sulphur compounds, saponins, essential oils, flavonoids,tannins and many other metabolites at higher concentration exhibited the potential to limit the methanogenesis by altering the rumen microflora(Patra and Saxena, 2010). The possible mechanisms and effects of these constituents on rumen microbiota are not fully understood yet. The well documented antimicrobial activity of these plant extracts/metabolites has provoked the interest of researchers whether these compounds are worth to use as selective inhibitorsof rumen methanogens(Benchaar *et al.*, 2008). Studies reported plant essential oils as very promising entities for selective reductionin the abundance of methanogens and deamination of protein. Many studies haveinvestigated essential oils as feed additives to reduce enteric methane production. Essential oils derived from oregano, thyme, cinnamon, horse radish, garlic, frangula and rhubarb showed decrease in methane production *in vitro* in a dose dependent manner (Benchaar and Greathead, 2011). However, some reports also stated theessential oils as feed additives can result in decreased feed degradability in rumen and lower amounts of volatile fatty acids due to theirnon-specific and broad antimicrobial activities in the rumen (Cobellis *et al.*, 2016b). The challenge to identify such plant constituents that canpossess selective inhibition of ruminal methanogens at practically applicable feeding rates, with long duration effects and without any negative alteration in the feed digestion and animal productivity (Benchaar and Greathead, 2011). Recent studies showed the adverse effects of high doses of these feed additives on intake and rumen degradation indicating that a single high dose of essential oil may not practically and effectively lower down the methane productionin the ruminants until and unless used in smaller doses and with combination of other anti-methanogenic products(Patra and Yu, 2012). Combinations of these oils in lower doses may be a practical strategy to minimizethe greenhouse gases emission and nitrogen excretion from ruminant without any adverse effect (Cobellis *et al.*, 2016a).

Essential oils showedinhibitory effects against methanogenic microfloraof rumen along with reduced methanogenic protozoal abundance. Although plant secondary metabolites may adversely deteriorate the utilization of nutrients, there are studies depicting that methane production can bemitigated without any negative effect on rumen functioning and feed utilization (Patra and Saxena, 2010). Ruminal microbiota possesses the capability to adopt feed additives in a duration of 7 days. However, some plant extracts/ oils can modify the rumen fermentation patterns by the manipulation of ruminal microbiota(Busquet *et al.*, 2005a). As determined by 16S rRNA sequencing, the α biodiversity of ruminal bacteria was noted similar but relative abundance and methane production level changes depending upon inclusion rate of plant oils as feed additive (Zhou *et al.*, 2020).

Effects of essential oils on methanogens mainly dependent on the oil source and its dose. Clove oil can reduce methanogenesis without any negative impact on rumen fermentation at the inclusion rates of 125, 250, and 500 mg/L (Günal *et al.*, 2017). Clove oil supplementation has the ability to reduce methane production by 34.4%, higher than eucalyptus oil (17.6%) and peppermint oil (25.7%), when used at 1.0 gram per liter without affecting total VFAs production (Patra and Yu, 2012). Clove oil usage at 600 ppm dose results in reduction ($p<0.05$) of total gas production without any alteration intotal VFA,feed degradability and acetate to propionate ratio. Methanogenesis was noted to be significantly lowered ($p<0.05$) when clove oil and peppermint oil was used at 300 and 600 ppm, respectively(Roy *et al.*, 2015). Clove bud oil and clove leaf oil can also reduce the methanogenesis by 12.9% and 8.0% respectively as compared to lemongrass and turmeric oil (7.3% andno effect, respectively) (Pawar *et al.*, 2014). (Patra *et al.*, 2006)evaluated that ethanolic and methanolic extracts of clove can inhibit methanogens*in vitro*, but feed digestibility was also reduced.Inhibition of methane production occurred at higher doses (i.e., >300mg/L of culture fluid) and mostly associated with a decrease in total VFA concentrations and feed degradation(Benchaar and Greathead, 2011). According to the results of in vitro study, a significant decrease on methane production was observed by administration 0.5mL clove methanol extract at 16, 24, 48, 72 and 96 h post incubation ($p <0.05$). Additionally, a significant descending procedure on methane production was observed using 1mL clove methanol extract during the study except 4 and 24 h after injection ($p <0.05$)(Shabestari *et al.*, 2011). *Invitro* investigation of (Patra *et al.*, 2010) showed that methanogenesis was not affected by clove water extract. However, methanolic and ethanolic extracts of clove inhibited CH_4 production by 35 and 83% respectively. The spices do not alter the ruminal pH and total VFA concentration. However, the presence of clove can reduce acetic acid concentrationsignificantly (Chaudhry and Khan, 2012).(Patra and Yu, 2012)showed that use of essential oil mixture(1.0 g/L) from clove, oregano, eucalyptus,peppermint and garlic can reduce methane production without altering dry matter and NDF degradability.

5. EFFECT OF CLOVE ON PROTEIN METABOLISM

Ruminant nutritionistsand microbiologists are continuously exploring the alternative waysfor the manipulation of rumen fermentation for improvement of feed efficiency and animal performance. Recent studies revealed that plants secondary metabolite and their derivatives possess the capabilities to improve dietary energy and nitrogen in animals. These metabolic improvements are more likely to be carried out by lowering the abundance of hyper-ammonia producing ruminal microflora which results in reduction in amino acids deamination and ammonia nitrogen production (Busquet *et al.*, 2006b; Patra *et al.*, 2006; Calsamiglia *et al.*, 2007). However, these improvements are noted only at higher concentrations of these additives but such higher inclusions adversely affect microbial fermentation and decrease total VFAs concentration(Benchaar *et al.*, 2007). Essential oils in combination are reported to have promisingresults as feed additives to improve feed efficiency, nutrients utilization and pathogenic control livestock (Benchaar *et al.*, 2008). Unlikely, essential oils supplementation are also reported to have limited effects on apparent ruminal microbial fermentation,nutrient digestibility, metabolites, blood cells and protozoa and bacteria count (Khateri *et al.*, 2017).Same observations of plant oils additives on nutrient utilizationand milk performance inlactating cows has also been reported (Benchaar *et al.*, 2007).

Essential oilssupplementation significantly reduced ($p<0.05$) the rate of amino acids deamination by affecting ammonia-hyperproducing bacteria and anaerobic fungi(McIntosh *et al.*, 2003). Ruminal ammonia production was notedlowest($p<0.01$) for clove when compared to cumin, coriander and turmeric supplementation in ruminal feed. The addition of spices does not alter total VFAsand the pH of ruminal fluid. However, the supplementation of clove in feed can significantly reduce($p<0.05$) acetic acid concentration(Chaudhry and Khan, 2012).

Right combination mixture of essential oils may practically prove to boost up the animal productivity by reducing methanogenesis and mitigating protein degradation by ruminal microflora. Clove oil usage at 600 ppm dose results in reduction ($p<0.05$) of total gas production without any alteration in total VFA, feed degradability and acetate to propionate ratio(Roy *et al.*, 2015). Addition of essential oils showed inhibitory effects on proteolytic bacteria of rumen which results in reduction of ammonia production (Patra and Yu, 2014).(Lin *et al.*, 2013)reported that the inclusion of essential oils mixture (1 g/day) from clove, cinnamon, lemon andoreganocan inhibit ammonia production in sheep. A study on the investigation of effects of essential oils mixture (from clove, peppermintand oregano) supplementation on proteolytic bacteria of rumen revealed that this mixture effectivelyreduced the number of *S. ruminantium*, *P. ruminicola*and *P. bryantii*(Patra and Yu, 2014). Eugenol Inclusion (chief bioactive component of clove) was noted to enhance protein utilization and energy efficiency in the ruminants (Calsamiglia *et al.*, 2007; Lin *et al.*, 2013; Abo-EL-Sooud, 2018).

Essential oils mixturehas the potential to inhibit the multiplication of methanogenic speciesprominent of which are*Fibrobacter succinogenes* and*Butyrivibriofibrisolvens*.(Busquet *et al.*, 2006b)described that mixture of clove bud oil, anise oil, capsicum oil, oregano oil,cinnamon oil, ginger oil, dill oil, garlic oil, cade oil and tea tree oil and their bioactive components (*i.e.*, eugenol,anethol, carvacrol,cinnamaldehyde, carvone andbenzyl salicylate) significantly decreased the ammonia – nitrogen amounts at higherinclusion rates (*i.e.* 3000 mg/L), marginal effects atmoderate concentrations (*i.e.* 300 mg/L) and non-existent effects at low concentrations (*i.e.* 3 mg/L). This reduction was also accompanied by total VFAs reduction, main source of energy in ruminants, revealing the negative impact on microbial fermentation in the rumen and nutritional consequences in the animals.

(Busquet *et al.*, 2005b)demonstrated that clove bud oil addition (at 2.2 mg/L) to a continuous culture fermenter can reduce (80%) large peptides concentration without any alteration on ammonia – nitrogen amount, revealing that clove bud oil can alter microbial proteolytic patterns in the rumen. However, eugenol addition (chief bioactive component of clove oil) at the same concentration showed no effect on nitrogen metabolism, suggesting that anti-peptidolytic properties of clove oil is due to some unidentified oil fractions.Clove oil supplementation has no significant effect on milk yield and milk composition in lactating animals, while milk non-protein nitrogen and milk fat contents decreased and an increase in milk protein was noted compared to control(Morsy *et al.*, 2012b). The mixture of both clove and cinnamon essential oils have a potent use in animal feed, favoring a greater sarcomere length, enhancing meat tenderness, without any negative change in the chemical composition or fatty acid profile of meat(Monteschio *et al.*, 2019).

Overall, considering that body size is a master trait driving fundamental characteristics of organisms, its study along altitudinal gradients under different bioclimates may allow better understanding of the factors driving elevational patterns in the populations structure of Carabidae and ecogeographical rules as well. The proposition that Carabidae generally follow Bergmann rule or any common pattern is clearly challenged by available studies. The results suggest that to improve understanding of the drivers of the observed patterns further investigations on changes in ground beetles communities along altitudinal gradients should consider different species and bioclimatic contexts and use similar sampling designs.

6. WHAT INFLUENCE WILL CLIMATE CHANGE HAVE ON THE PRODUCTION OF CLOVES?

The climatic conditions have largely influenced the cloves production on worldwide scale in past few years. The yield recorded in Madagascar in 2018, due to unexpected moisture conditions, was an imperceptible disaster. The production in Malagasy appeared nearly 10 to 20% of a mean year. A similar observation was made in Brazil, Comoros and Zanzibar, where the yield was reduced from 7000 tonnes in 2017 to 1300 tonnes in 2108. Meanwhile, the production in Indonesia was high because of favorable climatic conditions. This raises a concerning question for the stakeholders that, whether these are primarily important indications of the impact of climate change?

7. WHAT IS THE POTENTIAL IMPACT OF THE COVID-19 PANDEMIC?

This last but prospective section aims to evaluate the aftermath of health crisis related to COVID-19 and its spread on a global scale. Although it affects the horticultural sectors as a whole, depending upon the considered sector, this effect will be uncommon and unusually linked to the span of current pandemic. Regarding clove sector, it is not easily possible to forecast the effect of the financial crisis following the COVID-19 on domestic consumption in main consumer countries.

Furthermore, it is still not known that to what extent this pandemic will affect the economic conditions of two major cloves purchasing countries, namely India and Indonesia. Industrial purchaser, distributors, banks, freight forwarders and transporters, all are deeply concerned with this.

However, the question for the cloves will arise at the end of 2020, at the time of harvesting. We can expect for the improvement of COVID-19 situation by the end of this year along with the liberation euphorbia resulting in revised interest on markets.

Regarding clove oil, the eugenol market seems to remain unaffected by this pandemic as there is no decrease in demand of eugenol based vanillin which is the main outlet and acknowledged as a natural product by the USA.

Additionally, the major applications of eugenol are found in food, livestock, pharmaceutical and cosmetic industries which are relatively less affected by the COVID-19 unlike perfumery, which is adversely impacted by the abandoned tourism in 2020.

Resultantly, the raw materials sector for perfumery will have to face the economic consequences in next few months in the form of a noticeable decline in the prices of the raw materials.
The producer countries can take the advantage of this economic setback by adapting to the market demands, regarding the time of delivery and quality of product as well as assuring the competitive prices of products. These pre-COVID-19 issues seem to become even worse by this pandemic.

8. CONCLUSION

Livestock systems are major contributors of greenhouse gases globally. Plant originated metabolites are choice of time to mitigate this challenge. Clove and its derivatives are considered as an important feed additive for boosting up the milk yield and growth rate in ruminants by reducing methanogens is and deamination of dietary amino acids. Its chief bioactive component is eugenol which is responsible to enhance animal productivity by reducing methane producing microflora and inhibiting protein degradation in rumen. Now a days, clove bud oil is being used as essential oil to add in the feed of ruminants for better milk and meat production.

9. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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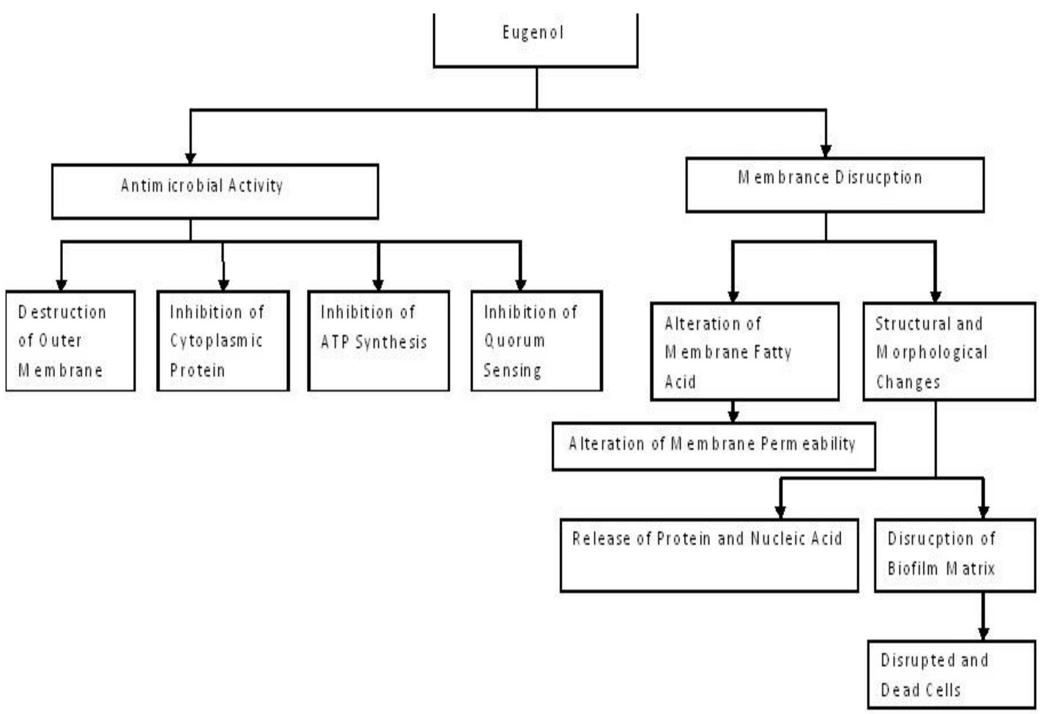

Figure 1. Spectral line of Eugenol activities

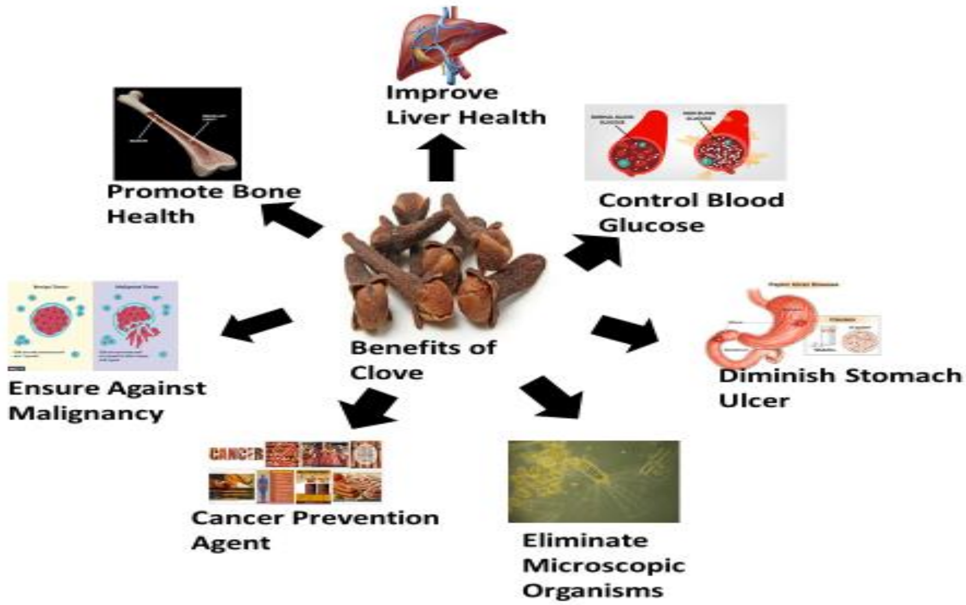

Figure 2. Nutraceutical spectrum of Clove and Clove essential oil

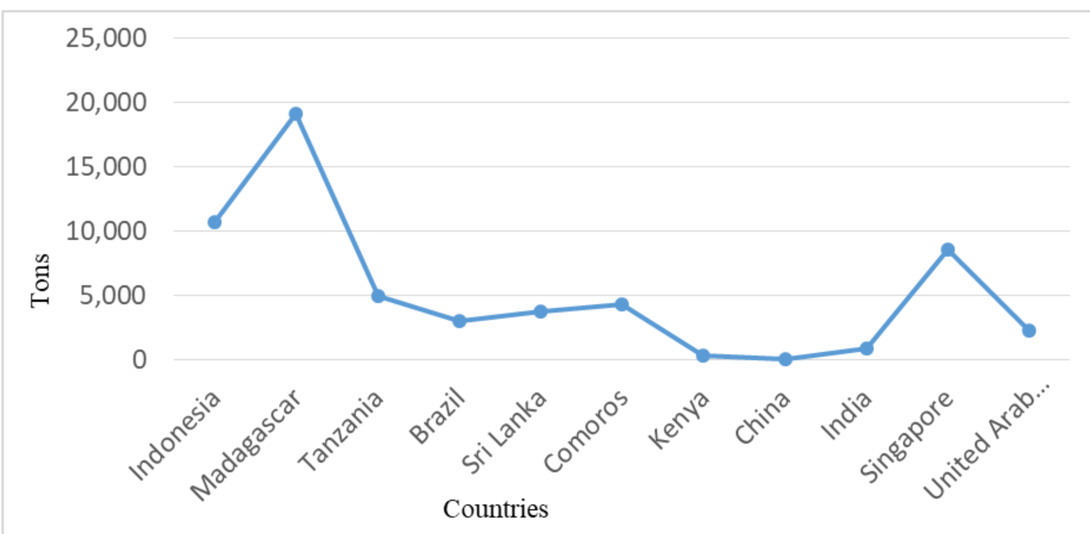

Figure 3. Export potential of Clove observed in different countries (Adapted from Danthu et al., 2020)

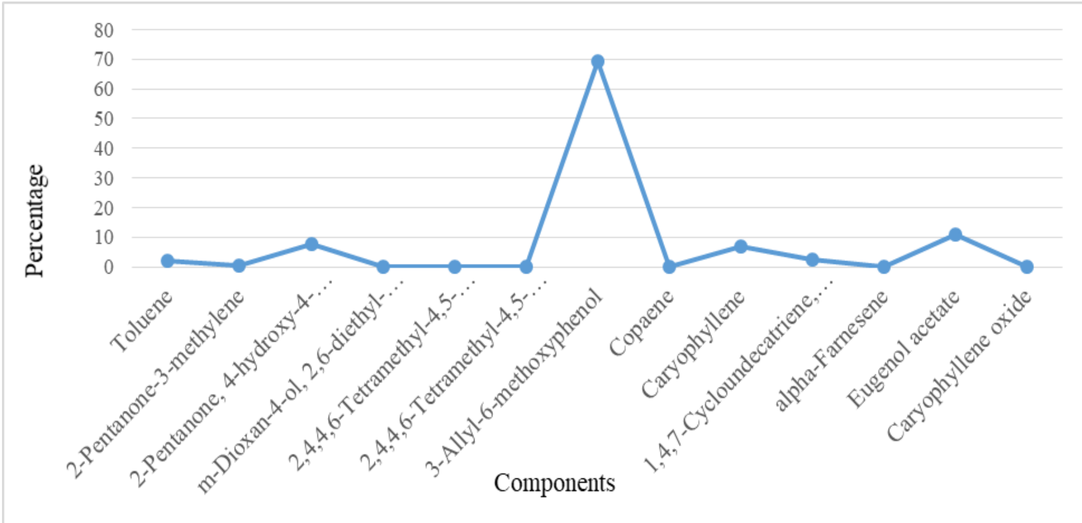

Figure 4. Chemical composition (%) of clove bud oil (Adapted from Uddin et al., 2017)