WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 5, 1589-1606. Review Article ISSN 2277– 7105

PROFILE OF *EUGENIA CARYOPHYLLUM* ONE OF THE MOST USEFUL TRADITIONAL HERBAL DRUG IN INDIA

D. S. Patil*, M. B. Jograna and S. V. Kotwal

Pune District Education Association's Shankarrao Ursal College of Pharmacy, Kharadi, Pune -411014 (MH).

Article Received on
15 March 2020,
Revised on 05 April 2020,
Accepted on 26 April 2020
DOI: 10.20959/wjpr20205-17409

***Corresponding Author**
D. S. Patil
Pune District Education Association's Shankarrao Ursal College of Pharmacy, Kharadi, Pune -411014 (MH).

ABSTRACT

Clove is one of the potent traditional crude drug having mark onset of action. Clove may be looked upon as a champion herbal drug known till date. The oxygen radical absorption capacity test is a scale developed by U.S. Department of agriculture for comparing anti-oxidant activity. Health benefits from the use of clove have been known over the centuries. It is beneficial as a home remedy in curing several aliments/diseases. In addition to its culinary uses, the clove buds have an abusance of medicinal and recreational uses. The major part of the world's consumption of the clove spice is in the home kitchens. However, commercial use of the clove is for the production of clove oil that contain active constituents, which possess antioxidant, anti-fungal, anti-viral, anti-microbial, anti-diabetic, anti-inflammatory, antithrombotic, anesthetic pain reliving and insect repellent responsible for the medicinal properties. Eugenol is the main constituent responsible for the medicinal properties of the clove bud. In the light of above, we thought it worthwhile to compile an up-to-date review article on clove covering its, synonyms, chemical constituents, psychopharmacology and medicinal uses.

KEYWORDS: Clove bud, *Eugenia Caryophyllum*.

INTRODUCTON

Clove

Synonym: Clove flower, Clove bud, Caryophyllum.

Biological Source: Clove consists of dried flowers of buds of *Eugenia Caryophyllum*.

Family-Myrtaceae.

Geographical Distribution

In India it grows in Nilgiri and Kanyakumari and is indigenous to Mollusca Island. It is cultivated in Zanzibar, Sumatra and South America.

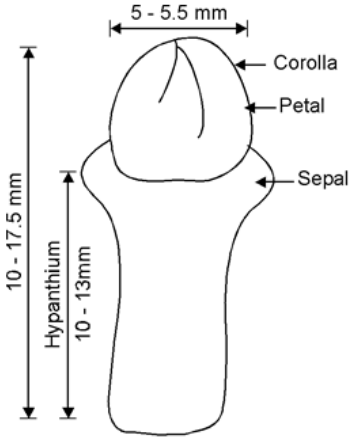
History *Eugenia* is Latin term and *Caryophyllus* in Greek means nut-leaf, which refers to nut-like flower bud. Clove is from Latin word *clavus* meaning a nail, which refers to the shape of the whole spice.[1]

Cultivation collection

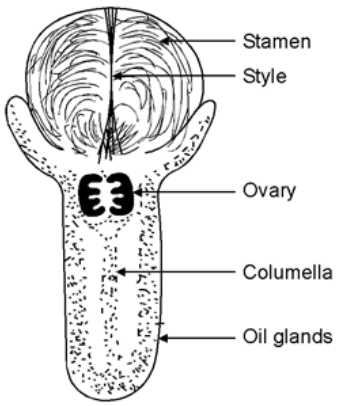
Clove is an evergreen tree that grows 15 m in height. Seeds are sown in well-drained, rich loamy soil, with high humus content. It needs warm humid climate and grows well in vicinity of the sea.

Seeds are sown from August to October. Seeds are placed in nursery beds at a distance of 10 cm. They take 4-5 weeks to germinate. The seedlings of clove are slender and delicate. For the first two to three years, the clove is allowed to grow in the shade by planting banana plants in between. Clove is collected from 6 years old plants, till 70 years old. The buds are gathered when they change from green to crimson and are carefully dried in the sun. Collection of cloves is done by picking the buds with stalk or beaten by bamboos.[2]

Clove plant



Entire clove bud



L.S. of Bud

Morphology

Size : 10-17.5 mm long

Shape : Sub-cylindrical slightly flattened.

Color : Dark brown

Taste : Pungent and aromated, followed by numbness of the tongue.

Microscopy

T. S. of clove through hypanthium shows:

1) Epidermis: It consists of heavy cuticularised epidermal cells made up of small straight-walled tubular cells. Many anomocytic stomata interrupt the epidermal cells.

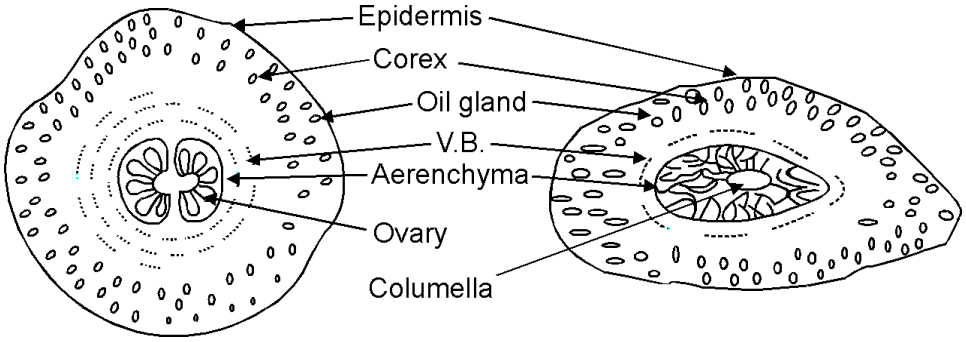


Fig. T.S of clove passing through ovary **T.S of clove passing through columella**

2) Cortex: Cortex consists of three zones.

a) Roughly, radially arranged parenchymatous cells with two to three layers of big ellipsoidal, schizolysigenous oil glands.

Parenchymatous cells contain tannin; hence show dark color when stained with ferric chloride.

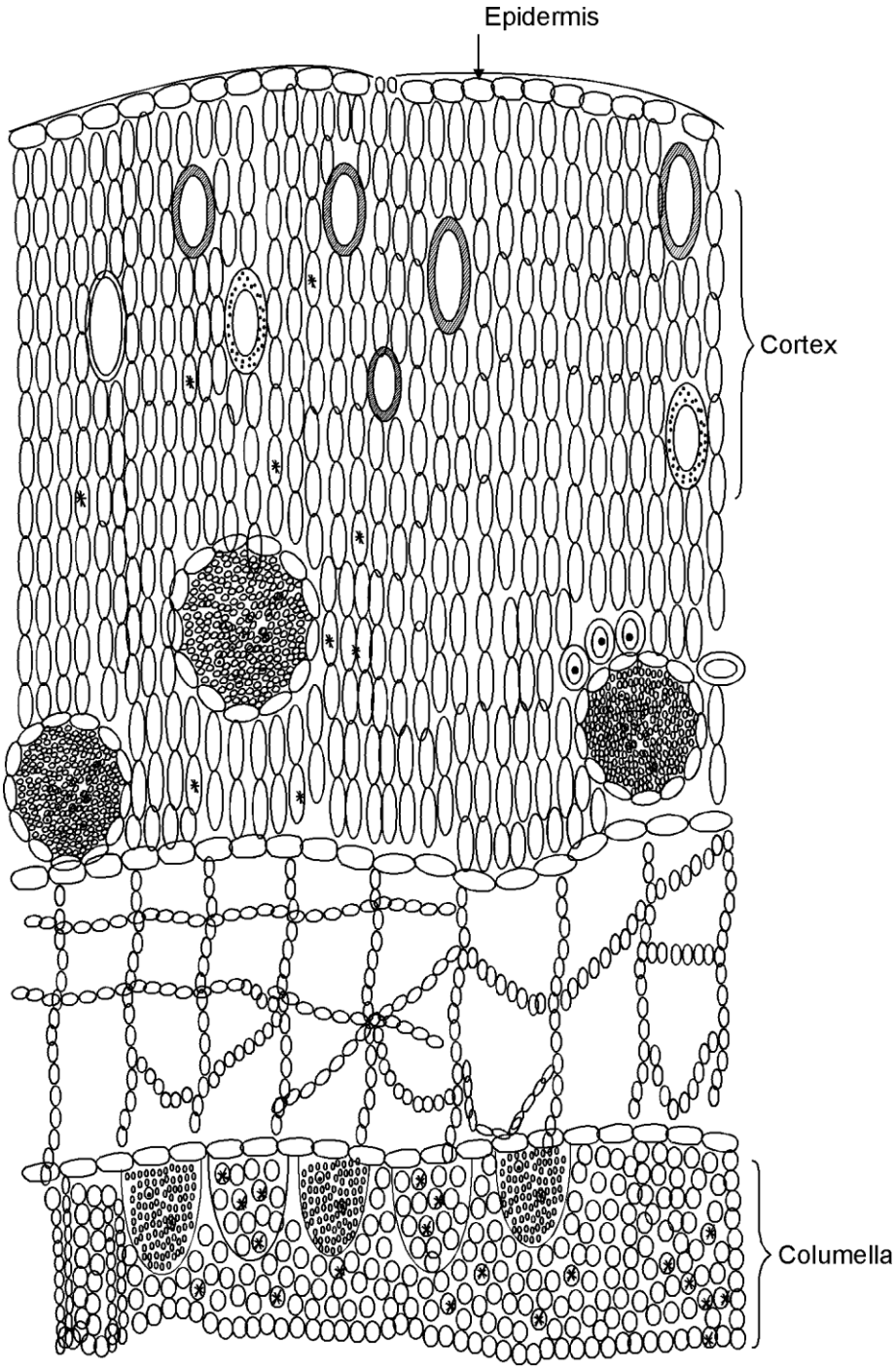
b) Middle layer consists of thickened wall cells embedding a ring of bicollateral vascular bundles. A few thick-walled lignified pericyclic fibers are found to be associated with vascular bundles. Xylem is composed of 3-5 lignified spiral vessels. About 15 vascular bundles are present in the ring.

c) Within the ring of vascular bundles is a zone of parenchyma composed of oil spaces separated by one-cell thick lamellae, which supports the central columella.

3) Columella: The columella consists of parenchymatous tissues. The outer region of columella shows the presence of ring of about 25-30 small vascular bundles. Clusters of calcium oxalate crystals occur throughout the tissues. Starch grains trichomes and stone cells are absent.

When the T. S. of clove is taken through its ovary, it shows the epidermis, zones of oil glands and bicollateral vascular bundles. A zone of ovary is present instead of parenchymal tissues. It consists of cells of very strongly thickened cellulose walls limited from the inner side by an inner epidermis flooring the wall of the ovary. The dissepiment is parenchymatous and the placenta shows the presence of numerous cluster crystals of calcium oxalate.

The central collumella possesses a ring of small vascular bundles.[2]



T.S. of clove bud

Chemical constituents

Clove contains 15-20% of volatile oil and 10-13% of tannins, (gallotannic acid) volatile oil contains 70-90% eugenol, which is colorless liquid. It contains eugenol acetate (3%) and 5-8% β-caryophyllene. The characteristic aroma of clove is due to the presence of methyl amyl ketone, which is in trace amounts.

Other non-volatile compounds of clove include various triterpenic acids and esters. Stigmasterol, stigmasterol and campesterol have also been reported.

Chemical test

1) An alcoholic solution of clove oil, when treated with a ferric chloride solution, gives blue color due to eugenol.
2) A water decoction of clove, when treated with ferric chloride, gives bluish-black color due to presence of tannins.

Uses

1) Clove is used as stimulant aromatic and as spice.
2) Clove oil is antiseptic, counterirritant and carminative.
3) Clove oil is used as ingredient of tooth paste and mouthwash for relieving toothache and as an antiseptic.
4) The oil is used in perfumery and preparing vanillin (oxidation product of eugenol)

Adulterations

1) Exhausted clove: In this drug, volatile oil is partially/entirely removed by distillation, so contains less volatile oil.

1) Exhausted cloves are dark in color, more shrunken and float on water.
2) **Clove stalks:** Clove stalks contain only 5% of volatile oil. Clove stalks can be detected as it contains thick-walled pitted stone cells, prisms of calcium oxalate and small starch grains and reticulate vessels.
3) **Mother clove or clove fruits:** These are ripe fruits of clove, which are ovoid, brown drupe, one seeded. Mother cloves contain less volatile oil, the presence of which can be detected by the starch present in the seed even in powdered form.
4) **Blown cloves** are fully expanded flowers from which usually corolla and stamens get detached. They contain less volatile oil as compared to clove buds.

Cardamom

Synonym: Grains of paradise, Cardamom Fruit

Biological Source

Cardamom consists of dried ripe seeds of *Elettaria Cardamomum* Maton var.

Family: Zingiberaceae

Geographical Distribution: Cardamom is cultivated in India in Karnataka, Mysore and Malabar hills. It is cultivated in Sri Lanka, Thailand and Tanzania.

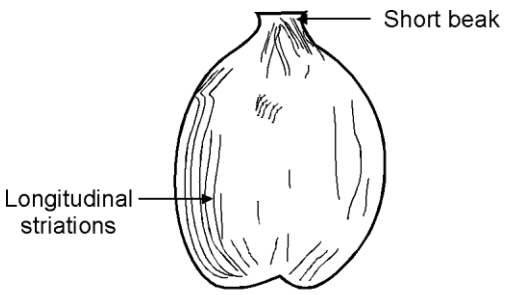
Cultivation Collection

Cardamom plant is perennial herb growing 3-4 meters high. Plant is cultivated by sowing seeds which are hard and take four months to germinate. Oil must be full of moisture and well-drained. Seeds are planted in nurseries. After one year, the young seedlings are transplanted in fields. Fruits are developed on 3-4-year-old plant. Fruits are picked up; just before their ripening so as to prevent capsules from splitting. Fruits are dried in the sun or by placing trays of the fruits over burning surplus. Slow drying of seeds is done to avoid splitting of fruit.

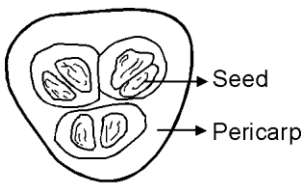
Morphology of fruit and seed

Cardamom fruit is three sided capsule with a fibrous, papery and longitudinal wrinkled pericarp. In each cell two rows of seeds are present in an adherent mass attached to an axile placenta.

Size : 8-15 mm long
Shape : Oblong ovoid
Color : Green to pale buff
Apex : shortly beaked
Size : 4 mm long and 2-3 mm broad
Shape : Irregularly angular, usually tapering to the base
Color : Dark reddish-brown
Odor : Aromatic
Apex : Strongly aromatic



Cardamom Seed



Transverse section of fruit

Chemical constituents

Cardamom contains 2-8% of volatile oil; protein and starch. The volatile oil contains 50% cineole (Eucalyptol), terpineol, borneol and terpinene seeds contain 1-2% of fixed oil starch and protein. Fixed oil contains glycosides of oleic, stearic palmitic and caproic acid.

Uses

1) Cardamom is aromatic, stimulant.
2) It is used as flavoring agent

Substitutes and adulterants

1) Long wild native cardamomums: (*Elettaria cardamomum*)

a) These cardamoms are longer and more elongated.
b) Pericarp is dark greyish-brown.
c) Seeds have somewhat bitter taste and they are less aromatic.

2) Korarima cardamomum

Reddish-brown in color.

3) Loose seeds or fully ripe seeds

Less volatile oil content.

4) Cardamom husk

Husk can be detected from the authentic drug by presence of more ash content.[1-2]

PHARMACOLOGICAL EFFECTS OF CLOVE

Antibacterial activity

Natural remedies have been used for a long time for the treatment of various bacterial infections. In the recent practice of medicine, the misuse of antibiotics has led to the development of bacterial resistance. Therefore, there is a need for identifying novel antibacterial agents against which there is minimal or no bacterial resistance. Studies on clove oil or its extract may contribute to the development of novel antimicrobial agents to offset the effects of resistance to antibiotics. Essential oils have anti-quorum sensing activity which might be important in reducing the virulence and pathogenicity of drug-resistant bacteria.[3-4] (Quorum sensing is a means of bacterial intercellular communication. Anti-quorum sensing interrupts that process). A combination therapy of clove with antibiotics could be another method of overcoming bacterial resistance.

Several studies have shown the effectiveness of clove against numerous strains of bacteria.[5-14] Clove oil and eucalyptus oil exhibited antibacterial properties against the most common oral pathogen S. Mutans.[6-8] Cai and Wu13 showed preferential growth-inhibition activity of a crude extract of clove against the gram-negative oral pathogens: Porphyromonas gingivalis, Streptococcus mutans, Actinomyces viscosus and Prevotella intermedia. Several other studies have confirmed the in vitro antibacterial activity of clove against gram negative bacteria like Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Campylobacter jejuni, and Salmonella enteritidis, and gram-positive bacteria like Bacillus subtilis and Staphylococcus aureus.[9-14] Duraipandiyan, Ayyanar and Ignacimuthu.[15] observed antibacterial activity against Bacillus subtilis, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Ervinia sp, and Proteus vulgaris. Clove oil exhibited antibacterial activity against five strains of S. epidermidis (reference strains CIP106510, E13, S27, S23 and S38) which is mainly attributed to the presence of eugeno[l,12] extracts of which have shown antibacterial activity against Salmonella typhi.[37] Clove oils protects against bacterial colonization of the lungs, seen in vitro and in mice infected with Klebsiella pneumoniae.[16-18] Mytle, Anderson, Doyle, et al.[19] reported that the growth rates of Listeria monocytogenes strains were significantly reduced by treatment with 1% and 2% clove oil. Furthermore Ogunwande, Olawore, Ekundayo et al.[20] Found that the essential oil of the fruit exhibited antibacterial activity against Staphylococcus aureus, while the leaf oil inhibited the growth of Bacillus cereus, with an MIC of 39mg/ mL. Hospital-acquired infections are important due to presence of methicillin-resistant Staphylococcus aureus (MRSA) and its ability to cause severe soft tissue, bone or implant infections.[21] Clove proved to be beneficial against Staphylococcus strains including MRSA and Streptococcus strains.[22-23] The antibacterial property of clove is due to the damaging effect it has on the bacterial cell membrane.

Several authors have investigated the synergistic interaction of clove oil molecule together with a conventional antibiotic.[24-26] Time - kill studies have been used to evaluatethe effect of interaction between eugenol together with ampicillin and with gentamicin. The hydrophilic antibiotics such as vancomycin and β-lactam antibiotics have a marginal activity on gram negative bacteria but exhibit enhanced antibacterial activity when pre-treated with eugeno.[l5-25] This synergistic effect could be explained by the fact that eugenol is able to damage the membrane of bacteria allowing increased penetration of vancomycin and β-lactam antibiotics and therefore effecting a greater antimicrobial effect.[25] In a recent study Moon, Kim and

Cha.[24] assessed the inhibitory effects of a combination of eugenol and antibiotics on cariogenic and periodonto-pathogenic bacteria. Although eugenol is effective against both grampositive and gram-negative microorganisms, contrasting results were found in other studies.[28-29] Clove was found to be less effective when compared with cinnamon oil.[29]

Anti- inflammatory activity Bacteria are responsible for the initiation of periodontal disease, but it is the host immune-inflammatory response which is responsible for the progression of the disease and destruction of the periodontal attachment. One of the pathways is through the synthesis and release of prostaglandin and other arachidonic acid metabolites locally within the periodontal tissue. Tissue damage leads to the production of free arachidonic acid, which is further metabolized via either the cycloxygenase (COX) pathway to the prostaglandin (PGE2), prostacyclin and thrombaxane or the lipoxygenase (LOX) pathway to the leukotrienes. Therefore it is recognised that potential COX and leukotrienes inhibitors be considered as anti-inflammatory agents.

Few authors have investigated eugenol and acetyl eugenol for potential anti-inflammatory action on COX-2 and LOX enzymes.[29-30] Eugenol appeared to directly inhibit COX-2 enzyme activity, possibly through complete inhibition of PGE2, suppression of the nuclear factor kappa B (NF-κB) pathway and inhibition of interleukin (IL) - 6 production.[31] Bachlega, de Sousa, Bastos et al[32] demonstrated immune-modulatory and anti-inflammatory effects of clove, as it inhibited production of IL-1β, IL-6 and IL-10. Likewise, Thompson and Eling[33] demonstrated that eugenol inhibited prostaglandin H synthase activity. Similarly Raghavendra, Diwakr, Lokesh et al.[34] observed in human polymorphonuclear leucocytes that eugenol effectively inhibited the LOX enzyme in a noncompetitive nature. Naidu[35] demonstrated concentrationdependent inhibition of LOX-catalysed lipid peroxidation (LPO) by eugenol. Though no clinical studies have been done, Koh, Murakami, Tanaka et al.[36] found that eugenol exhibits potent anti-inflammatory effects on cultured human gingival fibroblasts.

Antioxidant property The reactive oxygen species (ROS) produced by our body are responsible for tissue damage and cell death and can inhibit normal function of cellular lipids, proteins, DNA and RNA. This can lead to many chronic diseases such as heart disease, cancer and even periodontitis.[37] Clove has been identified as a potent anti-oxidant. Antioxidant activity of clove might be due to the higher concentration of phenolic compounds such as eugenol (71.56%), eugenol acetate8.99 %) and thymol.,[38-39] The

antioxidant activity of clove extract was comparable to that of the natural antioxidant, α-tocopherol (vitamin E) and butylated hydroxyl toluene (BHT).[39-40] Syzygium aromaticum derivatives can prevent injury by ROS by scavenging free radicals, by chelation of transition metal ions, by inhibition of oxidant enzymes or by regeneration of α-tocopherol from α-tocopheroxyl radical.[39-40]

The anti-oxidant capacity of clove was present when measured by the metal chelating activity, by bleomycin dependant DNA oxidation, by diphenyl-p-picryl hydrazyl (DPPH) radical scavenging activity and by the ferric reducing antioxidant power (FRAP).[38-39] Syzygium aromaticum showed the highest antioxidant activity among 19 different extracts from Thai medicinal plants.[63] Cloves showed the highest DPPH radical scavenging activity (90%), highest FRAP values, high metal chelation ability and DNA oxidation among different spices.[38-39] Clove also showed increased ability to inhibit metalion induced LPO.[38-39] Some of the earlier in vitro studies have demonstrated that flavonoids can scavenge O2, OH and peroxyl radicals, and inhibit LPO in different systems.[43-44] High DPPH scavenging activity as well as O2 radical scavenging activity and metal chelating activity may be responsible for the marked antioxidant action of cloves. Thus the antioxidant properties of spices may be attributed to various mechanisms, which include prevention of chain initiation, chelation of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging activity.[38]

The effect of eugenol is concentration dependant. At low concentrations it has anti-oxidant and anti-inflammatory effects, whereas at high concentrations it acts as a prooxidant, leading to tissue damage resulting from the enhanced generation of free radicals.[39] Clove exhibited a higher bleomycin-dependent DNA oxidation activity indicating a prooxidant effect.[39] Clove oil thus shows a powerful antioxidant activity, and can be used as an easily accessible source of natural antioxidants in pharmaceutical applications. No direct studies on periodontal cells or markers have been done to elucidate the effect of clove extracts as an anti-oxidant.

Antifungal activity Clove possesses fungicidal characteristics in vitro and in vivo due to its phenolic components, carvacrol and eugenol.[45-46] The potential drawback in the treatment of fungal diseases is the possibility of the development of antimicrobial resistance. Combination therapy with clove could form an alternative treatment method especially in treating fluconazole-resistant or multi-drug resistant fungal diseases. Studies have shown synergistic

interaction with the use of eugenol and/or methyl-eugenol or either in combination with fluconazole or amphotericin B.[47]

Antifungal activity has been seen against Candida albicans and Trichophyton mentagrophytes,[48-49] Onychomycosis,[50] Saccharomyces cerevisiae,[51] E. Caryophyllata[52] and Aspergillus niger.[53] The antifungal activity is due to considerable reduction in the quantity of ergosterol, a specific fungal cell membrane component.[55] Eugenol displayed in vitro activity against C. albicans cells within biofilms.[56] Garg and Singh[57] conducted an experiment using eugenol-loaded lipid nanoparticles in immunosuppressed rats and showed significant improvements in the eugenoltreated site. Similarly eugenol exhibited the minimum inhibitory concentration (MIC) ranging from 0.06 to 0.25% (v/v) and minimum concentration of drug that inhibited 50% of the isolates (defined as MIC50) ranging from 0.06 to 0.12% (v/v) when tested against 38 strains of Candida species from denture wearers and 10 collection strains.[58]

Antiviral activity Viruses are highly sensitive to the components of essential oils. The antiviral activity of eugenol has been tested against the herpes simplex-1 (HSV-1) and HSV-2 viruses.[59-60] Hussein, Miyashiro, Nakamura et.al.[61] found that Syzygium aromaticum extract was highly active at inhibiting replication of the hepatitis C virus. Synergistic interaction between acyclovir and eugenol combination has been seen.

Analgesic activity For many ages eugenol has been used as a natural remedy for relieving tooth pain. Similarly this technique in modern dental practice has been adopted by many clinicians, in which eugenol can act as an analgesic agent. Eugenol exhibited an analgesic effect in different experimental pain models in mice.[62-63] Kurian and co-workers[84] studied the anti-nociceptive ability of eugenol (100mg/kg) in several mouse models and found that the effect was more pronounced in the inflammatory phase than the neurogenic phase. Eugenol can, however, alleviate neuropathic pain.[84] Guenette, Beaudry, Marier et al.[64] in their study in male Sprague-Dawley rats, showed that eugenol, at a dose of 40mg/kg, was capable of prolonging reaction time to thermal stimuli. All these results suggested the possible use of eugenol as an analgesic agent.

Anaesthetic activity Eugenol is cheap and is an easily available topical anaesthetic. It is relatively user-friendly and can be used effectively in lower concentrations than other local

anaesthetics.[86] It is rapidly metabolized and excreted, thus requiring no withdrawal period.87 Eugenol shows good anaesthetic effects on inflamed pulpal tissues.[67]

Potential of Clove For Treatment Of Periodontal Disease

Periodontal disease initiation and progression occurs as a consequence of the host response to microorganisms present in dental biofilm.[1,2] The pathogens stimulate the host response resulting in the release of harmful by-products such as cytokines and prostaglandins by leukocytes, fibroblasts or other host tissue-derived cells and enzymes. These break down extracellular matrix components, such as collagen, as well as host cell membranes, consequently leading to periodontal attachment loss and bone resorption.[2]

Host modulation therapeutic strategies are aimed at inhibition of the progression of inflammatory bone loss associated with periodontitis.[5] Although a range of biological and pharmacological activities of clove have been reported, there has been a lack of research into its therapeutic potential for destructive periodontal disease. Table 2 describes the therapeutic use of clove for dental application.

Clove exhibited antibacterial activity against gramnegative anaerobic periodontal pathogens, including Porphyromonas gingivalis and Prevotella intermedia. Clove may reduce periodontal inflammation by modulation of the signalling pathway (NF-κB) and suppression of IL6, COX-2 and TNF-α.[31-34] Besides its anti-inflammatory properties, clove also has antioxidant property.[38-41] It has an important property for reducing the oxidative stress which is often seen in periodontal disease. It promotes DPPH scavenging activity, hydroxyl radical scavenging and inhibits lipid peroxidation.[38-41] It exhibits antifungal activity against Candida. and antiviral activity against Herpes Simplex virus (HSV) 1 and 2.80,81 Analgesic.[62-64] and anaesthetic albicans[47-56]properties66of clove could be a natural way of performing painless dental and oral procedures. Additionally, Karmarkar, Choudhury, Das et al.[75] observed that dried clove buds rich in eugenol and eugenol derivative were effective in preventing bone loss and this property would be beneficial for treating periodontal disease. All these studies demonstrated that therapy with clove and its active components like eugenol can be beneficial for the treatment of periodontal disease as a natural antiplaque or anti-gingivitis agent. Research has been particularly lacking in the areas of periodontal disease control. Clove can be effectively incorporated in therapeutic agents formulated against periodontal diseases in the form of mouthwashes, tooth pastes, topical agents and local drug delivery devices.

REFERENCES

1. Kokate CK.Practical pharmacognasy, 4th edition, Nirali Prakashan
2. Abhyankar A.A. Pharmacognasy Tex max publication Pune July 2012 978-93-5077-020-7.
3. Khan MS, Zahin M, Hasan S, Husain FM, Ahmad I. Inhibition of quorum-sensing regulated bacterial functions by plant essential oils with special reference to clove oil. Lett Appl Microbiol, 2009; 49: 354-60.
4. Dorman HJ, Deans SG. Antimicrobial agents from plants: an-25. Antibacterial activity of plant volatile oils. J Appl Microbiol, 2000; 88: 308-16.
5. Prabuseenivasan S, Jayakumar M, Ignacimuthu In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med., 2006; 6: 39-47.
6. Chaudhari LK, Jawale BA, Sharma S, Sharma H, Kumar CD, Kulkarni PA. Antimicrobial activity of commercially available essential oils against Streptococcus mutans. J Contemp Dent Pract, 2012; 13: 71-4.
7. Rahim ZH, Khan HB. Comparative studies on the effect of crude aqueous (CA) and solvent (CM) extracts of clove on the cariogenic properties of Streptococcus mutans. J Oral Sci., 2006; 48: 117-23.
8. Uju DE, Obioma NP. Anticariogenic potentials of clove, tobaco and bitter kola. Asian Pac J Trop Med., 2011; 4: 814-8.
9. Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Lett Appl Microbiol, 2003; 36: 162–7.
10. Feres M, Figueiredo LC, Barreto IM, Coelho MN, Araujo MW, 31. Cortelli SC. In vitro antimicrobial activity of plant extracts and propolis in saliva samples of healthy and periodontally-involved subjects. J Int Acad Periodontol, 2005; 7: 90–6.
11. Larhsini M, Oumoulid L, Lazrek HB, Wataleb S, Bousaid M, 32. Bekkouche K, et al. Antibacterial activity of some Moroccan medicinal plants. Phytother Res., 2001; 15: 250-2.
12. Friedman M, Henika PR, Mandrell RE. Bactericidal activities of 33. BurtSA, Reinders RD. Antibacterial activity of selected plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica. J Food Prot, 2002; 65: 1545–60.
13. Magina MD, Dalmarco EM, Wisniewski A Jr, Simionatto EL Dalmarco JB, Pizzolatti MG, Brighente IM. Chemical composition and antibacterial activity of essential oils of Eugenia species. J Nat Med., 2009; 63: 345-50.

14. Keskin D, Toroglu S. Studies on antimicrobial activities of sol-35. vent extracts of different spices. J Environ Biol., 2011; 32: 251-6.
15. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity 36. of some ethno-medicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med., 2006 17; 6: 35-42.
16. Devi KP, Nisha SA, Sakthivel R, Pandian SK. Eugenol (an es-37. sential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. J Ethnopharmacol, 2010; 130: 107-15.
17. Fabio A, Cermelli C, Fabio G, Nicoletti P, Quaglio P. Screening 38. of the antibacterial effects of a variety of essential oils on microorganisms responsible for respiratory infections. Phytother Res., 2007; 21: 374-7.
18. Saini A, Sharma S, Chhibber S. Induction of resistance to 39. respiratory tract infection with Klebsiella pneumoniae in mice fed on a diet supplemented with tulsi (Ocimum sanctum) and clove (Syzgium aromaticum) oils. J Microbiol Immunol Infect, 2009; 42: 107-13.
19. Mytle N, Anderson GL, Doyle MP, Smith MA. Antimicrobial activity 40. of clove (Syzgium aromaticum) oil in inhibiting Listeria monocytogenes on chicken frankfurters. Food Control, 2006; 17: 102–7.
20. Ogunwande IA, Olawore NO, Ekundayo O, Walker TM, 41.Schmidt JM, Setzer WN. Studies on the essential oils composition, antibacterial and cytotoxicity of Eugenia uniflora L. Int J Aromather, 2005; 15: 147–52.
21. Neely AN, Maley MP. Survival of enterococci and staphylococci 42.on hospital fabrics and plastic. J Clin Microbiol, 2000; 38: 724–6.
22. PH, Becker ST, Podschun R, Sivananthan S, Springer 43.IN, Russo PA, Wiltfang J, Fickenscher H, Sherry E. The battle against multi-resistant strains: Renaissance of antimicrobial essential oils as a promising force to fight hospital-acquired infections. J Craniomaxillofac Surg, 2009; 37: 392-7.
23. Enzo AP, Susan JS. Antibacterial activity of Australian plant 44. extracts against methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE). J Basic Microbiol, 2002; 42: 444–8.
24. Moon SE, Kim HY, Cha JD. Synergistic effect between clove 45. oil and its major compounds and antibiotics against oral bacteria. Arch Oral Biol., 2011; 56: 907-16.
25. Hemaiswarya S, Doble M. Synergistic interaction of eugenol 46. with antibiotics against gram negative bacteria. Phytomedicine, 2009; 16: 997-1005.

26. Mayaud L, Carricajo A, Zhiri A, Aubert G. Comparison of bac-47.teriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. Lett Appl Microbiol, 2008; 47: 167-73.
27. Pei RS, Zhou F, Ji BP, Xu J. Evaluation of combined antibacte-48.rial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against E. coli with an improved method. J Food Sci., 2009; 74: M379-383.
28. Gupta C, Kumari A, Garg AP, Catanzaro R, Marotta F. Com-49. parative study of cinnamon oil and clove oil on some oral microbiota. Acta Biomed, 2011; 82: 197-9.
29. d' Avila Farias M, Oliveira PS, Dutra FS, Fernandes TJ, de 50. Pereira CM, de Oliveira SQ, et al. Eugenol derivatives as potential anti-oxidants: is phenolic hydroxyl necessary to obtain an effect? J Pharm Pharmacol, 2014; 66: 733-46.
30. Daniel AN, Sartoretto SM, Schmidt G, Caparroz-Assef SM, 51. Bersani-Amado C.A, Cuman RKN. Anti-inflammatory and antinociceptive activities of eugenol essential oil in experimental animal models. Rev Bras Farmacogn, 2009; 19: 212–7.
31. Lee YY, Hung SL, Pai SF, Lee YH, Yang SF. Eugenol sup-52.pressed the expression of lipopolysaccharide-induced proinflammatory mediators in human macrophages. J Endod, 2007; 33: 698-702.
32. Bachiega TF, de Sousa JP, Bastos JK, Sforcin JM. Clove and 53.eugenol in noncytotoxic concentrations exert immunomodulatory/anti-inflammatory action on cytokine production by murine macrophages. J Pharm Pharmacol, 2012; 64: 610-16.
33. Thompson D, Eling T. Mechanism of inhibition of prostaglan-54.din H synthase by eugenol and other phenolic peroxidase substrates. Mol Pharmacol, 1989; 36: 809-17.
34. Raghavenra H, Diwakr BT, Lokesh BR, Naidu KA. Eugenol--55. the active principle from cloves inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells. Prostaglandins Leukot Essent Fatty Acidsm, 2006; 74: 23-7.
35. Naidu KA. Eugenol - an inhibitor of lipoxygenase-dependent 56.lipid peroxidation. Prostaglandins Leukot Essent Fatty Acids, 1995; 53: 381-3.
36. Koh T, Murakami Y, Tanaka S, Machino M, Sakagami H. 57. Re-evaluation of anti-inflammatory potential of eugenol in IL-1β-stimulated gingival fibroblast and pulp cells. In Vivo, 2013; 27: 269-73.
37. Nibali L, Donos N. Periodontitis and redox status: a review. 58. Curr Pharm Des., 2013; 19: 2687-297.
38. A.S.Yadav and D. Bhatnagar, Modulatory effect of spice ex-59. tracts on iron-induced lipid peroxidation in rat liver. BioFactors, 2007; 29: 147–57.

39. Yadav AS, Bhatnagar D. Free radical scavenging activity, 60. metal chelation and antioxidant power of some of the Indian spices. Biofactors, 2007; 31: 219-27.
40. Yoshimura M, Ito H, Miyashita K, Hatano T, Taniguchi S, 61.Amakura Y, Yoshida T. Flavonol glucuronides and C-glucosidic ellagitannins from Melaleuca squarrosa. Phytochemistry, 2008; 69: 3062-9.
41. Jirovetz L, Buchbauer G, Stoilova I, Stoyanova A, Krastanov A, 62. Schmidt E. Chemical composition and antioxidant properties of clove leaf essential oil. J Agric Food Chem., 2006; 54: 6303–7.
42. Makchuchit S, Itharat A, Tewtrakul S. Antioxidant and nitric 63.oxide inhibition activities of Thai medicinal plants. J Med Assoc Thai., 2010; 93: S227-S235.
43. Gülçin 64. İ. Antioxidant activity of food constituents: an overview. Arch Toxicol, 2012; 86: 345-391.
44. Lotito SB, Fraga CG. (+)-Catechin prevents human plasma 65. oxidation. Free Radic Biol Med., 1998; 24: 435-41.
45. Morel I, Lescoat G, Cillard P, Cillard J. Role of flavonoids and iron 66.chelation in antioxidant action. Methods Enzymol, 1994; 234: 437-43.
46. Chaieb K, Zmantar T, Ksouri R, Hajlaoui H, Mahdouani K, 67.Abdelly C, et al. Antioxidant properties of the essential oil of Eugenia caryophyllata and its antifungal activity against a large number of clinical Candida species. Mycoses, 2007; 50: 403-6.
47. Chami N, Bennis S, Chami F, Aboussekhra A, Remmal A. 68. Study of anticandidal activity of carvacrol and eugenol in vitro and in vivo. Oral Microbiol Immunol, 2005; 20: 106-11.
48. Khan MS, Malik A, Ahmad I. Anti-candidal activity of essential 69. oils alone and in combination with amphotericin B or fluconazole against multi-drug resistant isolates of Candida albicans. Med Mycol, 2012; 50: 33-42.
49. Tampieri MP, Galuppi R, Macchioni F, Carelle MS, Falcioni L, 70. Cioni PL, Morelli I. The inhibition of Candida albicans by selected essential oils and their major components. Mycopathologia, 2005; 159: 339-45.
50. Núñez L, D'Aquino M, Chirife J. Antifungal properties of clove 71.oil (Eugenia caryophylata) in sugar solution. Braz J Microbiol, 2001; 32: 123–6.
51. Gayoso CW, Lima EO, Oliveira VT, Pereira FO, Souza EL, Lima 72.IO, et al. Sensitivity of fungi isolated from onychomycosis to Eugenia cariophyllata essential oil and eugenol. Fitoterapia, 2005; 76: 247-9.

52. Chami F, Chami N, Bennis S, Bouchikhi T, Remmal A. Oreg-73. ano and clove essential oils induce surface alteration of Saccharomyces cerevisiae. Phytother Res., 2005; 19: 405– 8.
53. Lopez P, Sanchez C, Batlle R, Nerin C. Solid- and vapor-phase 74. antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. J Agric Food Chem., 2005; 53: 6939–46.
54. Pawar VC, Thaker VS. 75. In vitro efficacy of 75 essential oils against Aspergillus niger. Mycoses, 2006; 49: 316 –23.
55. Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal ac-76. activity of the clove essential oil from Syzygium aromaticum on Candida, Aspergillus and dermatophyte species. J Med Microbiol, 2009; 58: 1454-62.
56. He M, Du M, Fan M, Bian Z. 77. In vitro activity of eugenol against Candida albicans biofilms. Mycopathologia, 2007; 163: 137-43.
57. Garg A, Singh S. Enhancement in antifungal activity of eug-78.enol in immunosuppressed rats through lipid nanocarriers. Colloid Surface B, 2011; 87: 280–8.
58. Marcos-Arias C, Eraso E, Madariaga L, Quindós G. 79. In vitro activities of natural products against oral Candida isolates from denture wearers. BMC Complement Altern Med., 2011; 11: 119-21.
59. Benencia F, Courrges MC. 80. In vitro and in vivo activity of eugenol on human herpesvirus. Phytother. Res., 2000; 14: 495–500.
60. Tragoolpua Y, Jatisatienr A. Anti-herpes simplex virus activities 81. of Eugenia caryophyllus (Spreng.) (Bullock & S. G. Harrison) and essential oil, eugenol. Phytother Res., 2007; 21: 1153–8.
61. Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N, 82. Shimotohno K. Inhibitory effects of Sudanese medical plant extracts on hepatitis C virus (HCV) protease. Phytother Res., 2000; 14: 510–16.
62. Daniel AN, Sartoretto SM, Schmidt G, Caparroz-Assef SM, 83. Bersani-Amado CA, Cuman RKN. Anti-inflammatory and antinociceptive activities of eugenol essential oil in experimental animal models. Rev Bras Farmacogn, 2009; 19: 212–7.
63. Kurian R, Arulmozhi DK., Veeranjaneyulu A, Bodhankar SL. Effect of eugenol on animal models of nociception. Indian J. Pharmacol, 2006; 38: 341–5.
64. Guenette SA, Beaudry F, Marier JF, Vachon P. Pharmacokinet-85. ics and anesthetic activity of eugenol in male Sprague-Dawley rats. J Vet Pharmacol Ther., 2006; 29: 265-70.

65. Keene JL, Noakes DLG, Moccia RD, Soto CG. The efficacy of 86.clove oil as an anaestheticfor rainbow trout, Oncorhynchus mykiss (Walbaum). Aquacult Res., 1998; 29: 89–101.
66. Rapp C. Clove oil as effective as topical anesthetic. Herbal 87. Gram., 2007; 74: 26.
67. Markowitz K, Moynihan M, Liu M, Kim S. Biologic properties 88. of eugenol and zinc oxide-eugenol. A clinically oriented review. Oral Surg Oral Med Oral Pathol, 1992; 73: 729– 37.
68. Kaur G, Athar M, Alam MS. Eugenol precludes cutaneous 89.chemical carcinogenesis in mouse by preventing oxidative stress and inflammation and by inducing apoptosis. Molecular Carcinogenesis, 2010; 49: 290-301.
69. Manach C, Mazur A, Scalbert A. Polyphenols and prevention 90.of cardiovascular diseases. Curr Opin Lipidol, 2005; 16: 77-84.
70. Auclair S, Silberberg M, Gueux E, Morand C, Mazur A, Milenk-91.ovic D, Scalbert A.J. Apple polyphenols and fibers attenuate atherosclerosis in apolipoprotein E-deficient mice. Agric Food Chem., 2008; 56: 5558-63.
71. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Di-92.etary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr., 2005; 45: 287-306.
72. Criddle DN, Madeira SV, Soares de Moura R. Endothelium-93. dependent and -independent vasodilator effects of eugenol in the rat mesenteric vascular bed. J Pharm Pharmacol, 003; 55: 359-65.
73. Sensch O, Vierling W, Brandt W, Reiter M. Effects of inhibition 94.of calcium and potassium currents in guinea-pig cardiac contraction: comparison of beta-caryophyllene oxide, eugenol, and nifedipine. Br J Pharmacol, 2000; 131: 1089-96.
74. Damiani CE, Rossoni LV, Vassallo DV. Vasorelaxant effects of eug-95.enol on rat thoracic aorta. Vascul Pharmacol, 2003; 40: 59-66.
75. Karmakar S, Choudhury M, Das AS, Maiti A, Majumdar S, Mi-96.tra C. Clove (Syzygium aromaticum Linn) extract rich in eugenol and eugenol derivatives shows bone-preserving efficacy. Nat Prod Res., 2012; 26: 500-9.