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Phytochemistry and pharmacological aspects of *Syzygium aromaticum*: A review

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Abstract
Syzygium aromaticum (Family Myrtaceace) commonly called clove is most important and second valuable spice in world trade and is widely cultivated in North Maluku Islands in Indonesia. Gas Chromatography-Mass Spectrometery (GC-MS) studies of essential oil revealed the presence of eugenol as major compound. Phytochemical analysis of essential oil showed the presence of saponins, alkaloids, flavanoids, glycosides, tannins and steroids. The essential oil of *S. aromaticum* possess various biological activities such as antibacterial, antifungal, herbicidal, nematicidal, antitumor and anti-inflammatory. This review covers the phytochemistry and pharmacological activities of cloves, it's essential oil and various extracts.

Keywords: *Syzygium aromaticum*, essential oil, phytochemical, pharmacological activities

1. Introduction
The role of plants in human life has been increasing day by day due to advancement in the nutritional and medicinal disciplines. Spices are dried root, seed, bark fruit or flowers of plants which served several functions including flavoring agents, food additives, coloring agents, preservatives and medicines. During prehistoric times the discoveries of spices have been a period of joy as they are used as flavoring agents (Osuntogun *et al.* 2004) [65]. For aeons now, spices are irreplaceable part of cuisions all over the world. Beginning from the Ayurveda, these spices are used to cure several aliments due to their medicinal properties. Several phytochemicals have been isolated from spices responsible for their medicinal properties (Parthasarathy *et al.* 2008) [66]. They also possessed several pharmaceutical and phytochemical properties and hence, helpful in preparation of many medicines.

2. Distribution
Syzygium aromaticum (Clove) belongs to family *Myrtaceace*, a taxon of dicotyledon plants is one of most valuable and second most important spice in the world trade. Various synonymes used for the clove are *Caryophyllus aromaticus*, *Caryophyllus silvestris*, *Eugenia caryophyllus*, *Jambosa caryophyllus* and *Myrtus caryophyllus* (Soh and Parnell 2015) [77]. Clove is commonly used in cultivation and indigenous to North Maluku Islands in Indonasia. Major cultivator countries of clove are Pemba, Zanzibar, Indonasia, Madagascar and some of wild clove varieties are found in Bacan, Ternate, Motir, Tidore, Makian and Western parts of Irian Jaya. In India cultivation of clove is restricted to three states Karnataka, Tamil Nadu and Kerala. India becomes second largest consumer of clove after the Indonesia (Board 2010) [12]. Cloves are available throughout the year due to different harvest seasons in different countries. The different varieties of clove tree vary in canopy shape from pyramidal to cylindrical. The clove tree can live upto 100 years and above. The tree prefers to grow in well drained soil with sufficient soil moisture. Clove tree requires heavy sunlight with high atmospheric temperature (25 to 35°C), well-distributed rainfall above 150 cm and high humidity above 70% (Danthu *et al.* 2014) [18]. The crop cannot withstand water logged conditions. In India clove grows well in deep black loamy soil of humid tropics and successfully grows in the red soils of midlands of Kerala and in the hilly terrain of Western Ghats in Karnataka and Tamil Nadu (Byng 2016) [13].

3. Morphology and taxonomy
Clove is an aromatic spice tree. The term clove is taken from French word 'clove' and 'clou' which means 'nail'. Clove is conical myrtle, medium sized tree with straight trunk which grows up to 10 to 12 m in height. The branches are semi erect, grayish in color and dense. Leaves are large oblong to elliptic, simple obovate opposite, glabrous and possess plenty of oil glands on the lower surface. Tree begins flowering in about 7 years and continues flowering for 80 years or more.

Flowers are small, crimson in color and are hermaphrodite (bisexual) borne at the terminal ends of small branches. Each peduncle carries 3 to 4 stalked flowers and inflorescence length remains between 4 to 5 cm. Initially flower buds are pale yellow in color with glossy appearance and turn green to bright red at maturity. These are 1-2 cm long with cylindrical thick ovary consisting of four fleshy sepals. Buds are divided into elongated stem and a globose bulbous head which stimulates into nail. Commercially cloves used are air-dried unopened flower buds, 2.5 cm in length and 1.25 cm wide. Fruit matures nine months after flowering and the red ovary gradually turns to reddish purple. The fruit nearly contains one or two seeds known as 'mother of clove'. The cultivated trees are rarely allowed to reach fruit stage. These are harvested when they develop dark red ellipsoid berry (Kamatou *et al.* 2012, Ortes-Rojas *et al.* 2014) [40, 64].

Taxonomically classification of *Syzygium aromaticum* (L.) from kingdom Plantae down to Species

Kingdom – Plantae
Sub kingdom – Tracheobionta
Super division – Spermatophyta
Division – Magnoliphyta
Class – Magnoliopsida
Subclass – Rosidae
Order – Myrtales
Family – Myrtaceae
Genus – *Syzygium*
Species – *aromaticum* (L.)

4. Chemical constituents of clove essential oil

From clove species three essential oils are available: clove stem oil, clove bud oil and clove leaf oil. Each clove essential oil differs in the chemical composition, flavour and color. In clove essential oil amount of secondary metabolites are affected by the nature of soil, climate, cultivation techniques and genetic factors (Vezar-Petri *et al.* 1985, Arslan *et al.* 2004) [83, 5].

4.1 The clove bud essential oil: The clove bud essential oil is yellow in color and denser than water. Alma *et al.* (2007) [4] reported the presence of 18 components in clove bud essential oil. The main components characterized were eugenol (I, 87%), chavibetal (II, 19.7%), β-caryophyllene (III, 13%), eugenol acetate (IV, 8.01%), trisiloxane1, 1, 1, 5, 5, 5-hexa-methyl-3, 3-bis [(trimethylsilyl) oxy] (V, 1.7%) etc. Further studies by (Khan *et al.* 2009, Matta 2010, Marya *et al.* 2012 and Kasai *et al.* 2016) [43, 57, 55, 42] reported eugenol (I, 74.32%) followed by the β-caryophyllene (III, 15.94%) and eugenol acetate (IV, 5.8%) as major compounds of clove bud essential oil. Pruthi (2001) [72] reported that methyl-n-amyl ketone (VI) was responsible for the characteristic fruity and fresh odor of clove bud essential oil. Xu *et al.* (2016) [88] also studied the chemical composition of clove bud essential oil through Gas Chromatography-Mass Spectrometery (GC-MS) and reported the presence of eugenol (I), β-caryophyllene (III), caryophyllene oxide (VII), eugenol acetate (IV), α-selinene (VIII), cadinene (IX), 2-pinene (X) etc. Lee *et al.* (2009) [53] detected total 9 components in clove bud essential oil among them eugenol (I, 49.0%), 3-phenylprop-2-enal (XI, 14.32%) and β-caryophyllene (III, 7.5%) were major compounds. Fankem *et al.* (2017) [28] showed the presence of oxygenated monoterpenes (89.06%), monoterpenes (0.04%), sesquiterpenes (10.6%) and linear components (0.03%) in clove bud essential oil and eugenol (I, 87.62%) as major compound. Recent study by Mohamed *et al.* (2018) [60] reported that monoterpenes were dominant components of clove bud essential oil and major compound was found to be eugenol (I, 76%).

4.2 The clove leaf essential oil: Clove leaf essential oil has characteristic pleasant odor and faint yellow color. Jirovetz *et al.* (2006) [39] reported the presence of 23 compounds with eugenol (I, 76.8%), β-caryophyllene (III, 17.4%), eugenol acetate (IV, 1.2%), α-humulene (XII, 2.1%) as major compounds. Kapahi and Thappa (1989) [41] also determined the presence of eugenol (I, 87.8%), β-caryophyllene (III, 13.0%) and α-humulene (XII, 1.5%) in clove leaf essential oil. Srivastava *et al.* (2005) [79] analysed 22 compounds representing 99.9% of oil with eugenol (I), isoeugenol (XIII), β-caryophyllene (III), α-humulene (XII) and γ-cadinene (XIV) as major compounds.

4.3 The clove stem oil: The clove stem oil is not commercially used as the clove bud oil as the constituents responsible for fruity odor of clove oil are present in lesser amount and results in the flatter odor of clove stem oil but free eugenol (I) was present in much higher quantity in stem oil than the bud oil. (Patil and Dhale 2013) [68].

4.4 The clove root oil: The clove root oil was obtained by steam distillation with yield of about 6%. Freshly distilled root oil was bright yellow in color and having 85-95% of eugenol (I) (Pruthi 2001) [72].

5. Proximate and Phytochemical composition of clove

Various researches have reported the nutritional value of clove through proximate and phytochemical analysis as Sulieman *et al.* (2007) [80] determined the presence of (%) moisture (10±0.006), ash (5.2±0.01), crude fat (12.1±0.45), crude fibre (20±0.1), carbohydrates (51.5±0.02) and crude protein (1.2±0.02) content in clove bud powder. Bello and Jimoh (2012) [11] also revealed the presence of (%) moisture (23.35±0.02), carbohydrates (30.95±0.17), crude fat (18.90±0.04), crude fibre (10.65±0.03), ash (9.10±0.05) and crude protein (7.00±0.01) content in clove bud and mineral composition in mg/kg as magnesium (1259.86±10.65), calcium (782.54±0.62), iron (710±12.45), potassium (2.69±0.02) and sodium (2.56±0.01) in clove seed powder. Ereifej *et al.* (2015) [24] also showed the presence of (%) dry matter (83.6), ash (7.8), crude fat (4.3), crude protein (9.3), crude fibre (31.2) and carbohydrate (31) content and presence (mg/100g) of 9 minerals namely magnesium (196.8), calcium (117.5), potassium (111.6), sodium (61.6), manganese (20.9), iron (8.3), phosphorus (1.6), zinc (1.4) and copper (0.4) in cloves. Kumar *et al.* (2010) [52] analysed the phtyochemical composition of dichloromethane extract of clove bud oil which showed the presence of carbohydrates, terpenoids, glycosides, steroids, sterols, tannins and phenolic compounds. Soni and Dahiya (2014) [78] revealed the presence of saponins, alkaloids, flavanoids, cardiac glycosides, tannins and steroids in clove essential oil. Jimoh *et al.* (2017) [38] also revealed the presence of tannins, alkaloids, terpenoids, carbohydrates, glycosides, ketones, aldehydes and forty-six phenolic compounds in methanol extract of clove. Various phenolic compounds identified by using Gas Chromatography-Flame Ionization Detector (GCFID) were gallic acid, kaempferol, rhametin, myricetin, salicyclic acid, syringic acid, eugenin, caffeic acid, eugenitin, isohamnetin, quercetin, phenylacetic acid, isohamnetin, protocatechuric acid and p-hydroxybenzoic acid.

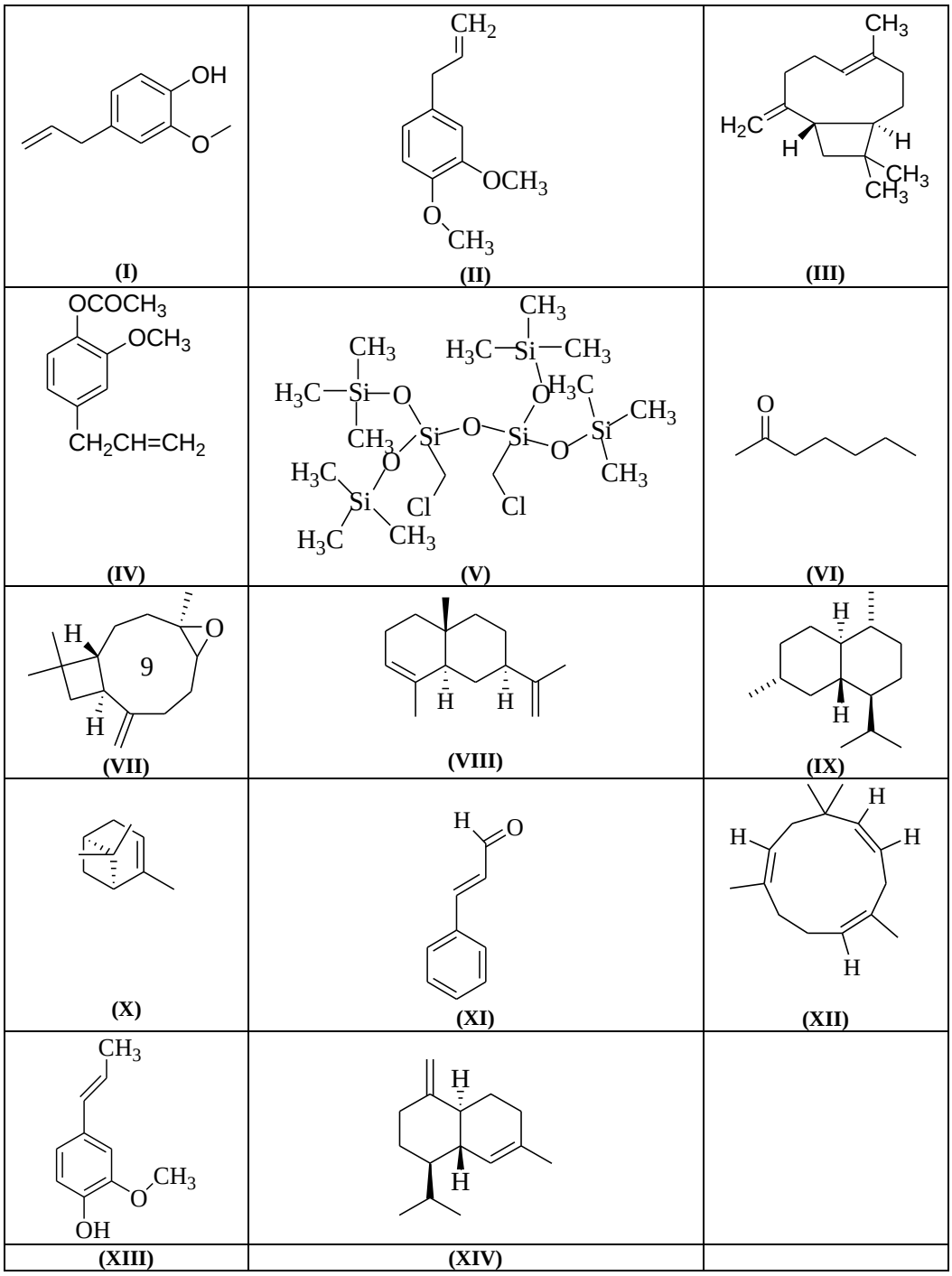

Fig 1: Structure of compounds present in *Syzygium aromaticum*

6. Pharmacological Activities of Clove

6.1 Antibacterial activity: Antibacterial activity of clove essential oil has been reported against *Staphylococcus aureus* (Mishra and Sharma 2014) [59] and *Listeria monocytogenes* in pasteurized milk (Cava *et al.* 2007) [14]. Matan (2012) [56] reported that clove oil showed strong antimicrobial resistance against *Penicillium* sp., *Aspergillus flavus* and *Staphylococcus aureus* found on dried fish (*Decapterus maruadsi*). Zengin and Baysal (2014) [91] reported the antimicrobial activity of clove oil against three gram-positive bacteria (*Listeria innocua, Carnobacterium divergens* and *Staphyloccous aureus*) and four gram-negative bacteria (*Salmonella typhimurium, Escherichia coli, Serratia liquefaciens* and *Shewanella putrefacians*) by broth microdilution method. The result showed that clove essential oil inhibited the growth of all bacteria while *Shewanella* and *Listeria* were found to be resistant to oil. Gupta *et al.* (2013) [34] also found clove oil to be effective against food borne gram negative bacteria (*Penicillium aerugenosa, Escherichia coli, Staphylococcus chloeraesius* and *Yersinia enterocolitica*) and gram positive bacteria (*Bacillus cereus, Listeria monocytogenes, Staphyloccous aureus* and *Enterococcus faecalis*). Mytle *et al.* (2006) [61] reported decreased growth rate of *Listeria monocytogenes* when treated with 1 and 2% clove oil. Fu *et al.* (2007), Yang *et al.* (2003) and Chaieb *et al.* (2007) [30, 90, 15] reported that antimicrobial activity of clove oil was due to presence of eugenol, 2-heptanone, methyl salicylate, kaempferol, gallic acid, isoeugenol and oleanolic acid. These compounds generally denatured proteins that reacted with cell membrane and changed their permeability. Warnke *et al.* (2009) [86] reported antimicrobial activity of different essential oils including clove bud oil against six *Staphylococcus* strains including methicillin-resistant *Staphylococcus aureus* (MRSA), three Candida strains and four Streptococcus strains

by using agar diffusion test. The clove oil showed considerable antimicrobial activity with diameter of inhibition zone of 12 to 20 mm. Duraipandiyan *et al.* (2006) [22] also investigated antibacterial activity of clove oil against *Enterococcus facalis, Bacillus subtilis, Ervinia sp, Staphylococcus epidermidis* and *Proteus vulgaris* by using paper disc diffusion method at different concentraction of 5, 2.5 and 1.25 mg/disc. Saini *et al.* (2009) [75] reported that clove oil inhibited bacterial colonization of *Klebsiella pneumonia* in lungs of mice.

6.2. Antioxidant activity: High antioxidant activity shown by clove oil was due the presence of phenolic compounds like eugenol, thymol and eugenol acetate (Yadav and Bhatnagar 2007, Dai *et al.* 2013 and Nam and Kim 2013) [89, 17, 62]. Eugenol present in clove oil possessed high antioxidant activity which was comparable with the activities of synthetic antioxidants pyrogallol and BHA (Dorman *et al.* 2000) [21]. Gulcin *et al.* (2012) [33] observed inhibition of (97.3%) lipid peroxidation of linoleic acid emulsion when treated with 15 µg/ml of clove oil. However, standard antioxidant such as trolox, butrylated hydroxyanisole (BHA), α-tocopherol and butylated hydroxytoluene (BHT) showed inhibition of 95.6, 95.4, 84.6 and 99.7% respectively under same conditions. Abdel-Wahhab and Aly (2005) [1] reported that phenolic compounds in clove essential oil resulted in formation of epoxide aflatoxin B_1 by inhibition of CYP450 enzyme and increased the ability of liver microsomes to catalyses aflatoxin-glutathione conjugation. Recent study showed that clove essential oil showed ten times greater antiradical activity than BHT and seven times greater than cocca butter and clove essential oil mixture (Fankem *et al.* 2017) [28].

6.3. Antifungal activity: Several workers have reported antifungal activity of clove oil and eugenol against filamentous fungi, yeast as human pathogenic fungi (Gayoso *et al.* 2005) [32] and food born fungal species (Hammer *et al.*1999 and Eugenia *et al.* 2009) [35, 26]. Pina-vaz *et al.* (2004) [69] found that clove oil killed *Candida albicans* (*C. albicans*) by producing lesions in the plasma membrane due to presence of carvacrol. Antifungal potential of clove oil was studied against five *Candida* species such as *C. gullermondi, C. albicans, C. glabrata, C. tropicalis and C. parapsilosis* isolated from the blood stream infection by disc diffusion method. Results showed that clove oil possessed maximum antifungal activity against *C. tropicalis, C. albicans and C. guilliermondii* (Kumar *et al.* 2012) [50]. Chami *et al.* (2005) [16] reported that antifungal activity of clove oil was due to the presence of phenolic components (eugenol and carvacrol) whereas Pinto *et al.* (2009) [70] reported that inhibitory action of clove oil was due to reduction in the amount of ergosterol, a specific fungal cell membrane component. Viuda-Martos *et al.* (2007) [84] reported antifungal potential of clove oil against food spoilage fungi *Aspergillus flavus* and *A. niger* by agar dilution method. Clove oil was found to be stronger inhibitory against *A. niger* than *A. flavus*. Bansod and Rai (2008) [10] also reported antifungal activity of different essential oils including clove oil against *A. niger* and *A. fumigates* by three methods named as disc diffusion method, broth dilution method and agar dilution method. In disc diffusion method clove oil exhibited zone of inhibition ranging from 10 to 15 mm for *A. fumigates* and 8 to 14 mm for *A. niger* respectively from concentration ranging from 12.5 to 100 mg. Minimum inhibitory concentration (MIC) of clove oil obtained by agar dilution method for both the fungi *A. fumigates* and *A. niger* was found to be 0.12 (% v/v). Similarly, MIC and minimum cidal concentration (MCC) of clove oil for *A. fumigates* by broth micro dilution method were 0.06 and 0.12 (% v/v) respectively and same values for *A. niger* were 0.12 and 0.06 (% v/v) respectively. Tullio *et al.* (2007) [81] demonstrated antifungal activity of clove essential oil against filamentous fungi by vapour contact and broth microdilution assay. In vapour phase inhibiting effect of clove essential oil was higher than broth microdilution assay. Estrada-Cano *et al.* (2017) [25] showed strong antifungal inhibitory action of encapsulated clove oil against *Fusarium oxysporum* by oxford cup method. The result showed that naked clove oil had greatest inhibitary action against *F. oxysporum* in beginning of the test. After 8 hours microcapsulated clove oil resulted in maximum efficiency. Wang *et al.* (2018) [85] tested the antifungal activity of clove oil microcapsule on meat product. Result revealed that clove oil microcapsule showed antiseptic effects on meat products at a concentration above 0.070%. The efficiency of clove oil microcapsule increased to 0.080% by boiling meat for half an hour.

6.4. Anti-inflammatory activity: Al-Ameedi *et al.* (2017) [3] evaluated anti-inflammatory action of alcoholic *Syzygium aromaticum* extract (SAE) by using formalin test with twenty four (24) mice divided into four groups. T_1 and T_2 groups were feeded with 100 and 200 mg /kg (SAE) respectively whereas T_3 group was feeded with 0.3 mg/kg of meloxicam and T_4 feeded with distilled water. The results showed significant increase in analgesia time ($p<0.05$) and decrease ($p<0.05$) in licking number in animals exposed to various concentration of SAE. Han and Parker (2017) [36] demonstrated that clove essential oil showed strong antiproliferative effects on human dermal fibroblasts at a concentration of 0.011%. Notably it resisted the production of many proinflammatory biomarkers as interferon-inducible T-cell α-chemoattractant (I-TAC), vascular cell adhesion molecule-1 (VCAM-1) and collagen III expression at both gene and protein levels. Bachiega *et al.* (2012) [6] suggested clove and eugenol exerted anti-inflammatory action on cytokine production *in vitro* by enzyme-linked immunosorbent assay (ELISA). Significantly clove (100µg/ml) inhibited IL-10, IL-1β and IL-6 production and eugenol (50-100µg/ml) inhibited production of IL-10 and IL-6. Koh *et al.* (2013) [48] demonstrated the anti-inflammatory action of eugenol in IL-β-stimilated pulp cells and gingival fibroblasts by ELISA. It was observed that eugenol showed anti-inflammatory action on pulp cells, but not in gingival fibroblasts (HGFs). Rodrigues *et al.* (2009) [74] found that clove oil and eugenol inhibited the macrophage production of IL-1β and IL-6 by ELISA. Nikoui *et al.* (2017) [63] reported the antiflammatory and antipyretic activity of clove oil in dogs. Clove oil treatment showed decrease in band neutrophils, neutrophils and white blood cells as compared to control ($p<0.05$) but no effect on hematocrit and red blood cells. It resulted in decrease in inflammation.

6.5. Anticancer activity: Kumar *et al.* (2014) [51] investigated the anticancer potential of various concentrations of water, ethanol extract and essential oil of clove *in vitro* through MTT and brine shrimp lethality test (BSLT) assay towards MCF-7 human breast cancer cells. In both MTT and BSLT essential oil showed robust cytotoxic effect with LD_{50} value of 37 µg/ml in BSLT after 24 hours. For MTT assay IC_{50} values after 24 and 48 hours were 36.43 and 17.6 µg/ml respectively. Lesgards *et al.* (2014) [54] reported that clove essential oil

consisted of phenylpropanoids and terpenoids having antitumor activity both on cell line and tumors in animals. An apostasy (cell death) of cancer cells was induced by the protein caspases. Many phenomena seemed to occur as: production of free radicals in cancer cells, over expression and regulation of liver detoxification enzymes and inhibition of angiogenisis. Dwivedi *et al.* (2011) [23] studied comparative anticancer potential of clove oil, its ethanol and water extract against DU-145 prostate cancer, HeLa cervical cancer, TE-13 esophageal cancer, MDA-MB-231 (ER-ve) and MCF-7 (ER+ve) breast cancer along with normal human peripheral blood lymphocytes for antiproliferation by using MTT assay. Maximum cytotoxic activity and maximum cell deaths (80%) were seen in TF-13 cells within 24 hours by clove oil at 300 µl/ml whereas minimal cell death in DU-145 cells but no cytotoxicity was found in human peripheral blood mononuclear cells (PBMC's) with same dose. Banerjee *et al.* (2006) [9] demonstrated chemopreventive potential of aqueous infusion of clove for lung cancer by using western blotting analysis during benzopyrene (BP)-induced lung carcinogenesis in strain A mice. Clove infusion significantly reduced the number of proliferating cells and increased apoptotic cells. Kouidhi *et al.* (2010) [49] investigated the cytotoxic and anticarcinogenic activity of clove essential oil on normal cells (MRC-5) and cancer cells (A549, raw 269.7, HT29 and Hep2) through MTT colorimetric assay with IC_{50} value ranging from 15.75 to 200 µg/ml.

6.6. Nematicidal activity: Wiratno *et al.* (2009) [86] studied the nematicidal activity of clove (*Syzygium aromaticum*) against *Meloidogyne incognita* root-knot nematode by using greenhouse assay and it was found that clove was effective in killing nematodes but EC_{50} value was 5-10 times lower than EC_{50} value of synthetic pesticides chlorpyrifos, deltamethrin and carbosulfan. Bala and Sukul (1987) [8] showed that eugenol as major constituent of clove effectively reduced the root knot nematode infection of *Hibiscus esculentus* and promoted the plant growth at concentration of 0.2 ml/l. Meyer *et al.* (2008) [58] reported the nematicidal suppression of *Meloidogyne incognita* (southern root knot) nematode and its phytotoxicity on vegetable crops *Cucumis melo* (muskmelon), *Solanum lycopersicum* (tomato), *Cumumis sativus* (cucumber) and *Capsicum annuum* (pepper) seedling in green house at concentrations ranging from 0.1 to 0.3%. The result showed that the nematode population was reduced with clove oil and was not phytotoxic to tested vegetable crops

6.7. Acaricidal activity: De Monteiro *et al.* (2012) [19] evaluated the acaricidal potential of eugenol against *Dermacentor nitens* and *Rhipicephalus microplus* larvae which showed 100% mortality from concentrations of 5 to 20µl/ml. Ferreira *et al.* (2018) [29] reported that both clove essential oil and the eugenol showed 100% mortality against cattle tick *Rhipicephalus microplus* at concentration of <0.5mg/ml in larvae and 50mg/ml in female *R. microplus*. Kim *et al.* (2003) [46] studied acaricidal activity of clove bud oil compounds eugenol, acetyleugenol, caryophyllene and humulene against adult *Tyrophagus putrescentiae* (*T. putrescentiae*) through fumigation and impregnated fabric disc method and compared with benzyl benzoate. It was found that all four compounds showed toxicity against *T. putrescentiae*. Kim and Sharma (2011) [47] demonstrated acaricidal activity of clove oil against *Dermatophagoides farina* by using microcapsulation against house dust mites (HDMs) with 94% mortality rate.

6.8. Anesthetic effect: Diyaware *et al.* (2017) [20] demonstrated the anesthetic effect of clove seed extract on *Clarias gariepinus* under semi-arid condition. The study showed that mortality rates were higher in fish which was anesthetized with concentrations (150 and 125mg/l) of clove seed extract. Fujimoto *et al.* (2017) [31] claimed that clove oil induced blunts muscle contraction power and anaesthesia in three Amazon fish species: *Pterophyllum scalare* (angelfish), *Heros severus* (banded cichlid) and *Parachheirodon axelrodi* (cardinal tetra). Clove oil induced deep anaesthesia (< 3min) in cardinal tetra and angelfish at concentration of 90 µl/l whereas in banded cichlid clove oil induced anaesthesia at concentration of 60 µl/l. Kheawfu *et al.* (2017) [44] investigated the anesthetic effect of clove oil loaded nanoemulsion (CLN) on *Oreochromis niloticus* (Nile tilapia). Best CLN was composed of 20% w/w clove oil and 15% w/w polysorbate 20. The result showed that the CLN showed more anesthetic effect than clove ethanolic solution in Nile tilapia. Kheawfu *et al.* (2017) [45] also studied the muscle contraction of silkworm (*Bombyx mori*) under influence of clove oil and eugenol separately. Results showed that eugenol and clove oil at 60 ppm induced anesthesia in fish at stage 4 in comparison to D-glutamic acid which exhibited total loss of equilibrium and muscle tone in fish at stage 4 at 20 and 40 mM respectively. Ribeiro *et al.* (2015) [73] also reported the anesthetic effect of eugenol on larva and juvenile of *Oreochromis niloticus* (Nile tilapia). The result showed that larva having weight (11.64 g) was anesthetized by using concentration between 150 and 175mg/l.

6.9. Herbicidal activity: Park *et al.* (2011) [67] characterized the herbicidal action of clove oil on the cucumber seedlings in light and dark conditions. Clove oil treatment increased superoxide dismutase (SOD) activity and decreased catalase activity whereas SOD and catalase activity decreased in the paraquat treatment. Results showed that the clove oil exerted herbicidal action through a mechanism different from that of paraquat. Evans *et al.* (2009) [27] conducted studies on herbicidal effect of clove oil and vinegar on *Abutilon theopharst* (velvetleaf) and *Amaranthus retroflexus* (redroot pigweed). Results showed that redroot pigweed was easier to control with both products than velvetleaf. Tworkoski (2002) [82] reported the phytotoxicity of twenty five essential oils including clove and cinnamon oil on detached leaves of dandelion. Clove and cinnamon essential oil were applied using dandelion leaf disk assay and whole plant assay with Johnson grass, common lambsquarters and common ragweed in green house. The result showed shoot death occurred from one hour to one day after the application of oil. Bainard *et al.* (2006) [9] reported the herbicidal activity of clove oil and its primary constituent eugenol on *Amaranthus retroflexus* (redroot pigweed), *Chenopodium album* (common lambsquarters) and effect on leaf cell membrane integrity and seedling growth. Result showed that clove oil and its major constituent eugenol caused reduction in cell membrane integrity and inhibition of seedling growth.

6.10. Insecticidal activity: Singh *et al.* (2014) [76] reported the control of bed bug (*Climex lectuarius*) by using direct contact and residual contact bioassay using different essential oil based products. Results showed that clove oil (0.3%) combined peppermint oil (1%) and sodium laruyl sulfate (1.3%) formulation caused more than 90% mortality of bed bugs nymphs in residual and direct contact assay. Akhtar *et al.* (2008) [2] reported insecticidal activity of clove oil against

two nuctuid capterpillars (*Psedudoletia unipuncta* and *Trichoplusia ni*). Result showed that clove oil was more effective when delivered orally than as contact insecticide. The noctuid caterpillars (*Psedudoletia unipuncta* and *Trichoplusia ni*) were more receptive to clove oil when compared with other essential oils. The EC_{50} values were 6900 and 400 ppm respectively for two species. Jairoce *et al.* (2016) [39] demonstrated insecticidal activity of clove oil on *Sitophilus zeamais* (maize weevil) and *Acanthoscelides obtectus* (bean weevil). The result showed that LC_{50} for *Sitophilus zeamais* was 10.15 µl/g and for *Acanthoscelides obtectus* was 9.45 µl/g and caused 100% mortality for both species. Plata-Rueda *et al.* (2018) [71] reported the toxic effect of terpenoids present in clove essential oil against granary weevil (*Sitophilus granarius*). Eugenol showed the strongest toxicity in *S. granaries* than α-humulene, α-pinene and caryophyllene oxide. It reduced the respiratory and mobility rate in the insects.

7. Conclusions

This review attempts to highlight the therapeutic potential of clove oil and its major constituent eugenol in the prevention and cure of various diseases. The results discussed in this study aims to attract researchers' attention for development of new natural drugs of plant origin from clove essential oil and eugenol. Clove essential oil and its major constituent eugenol can be considered as adjuvant to recent medications.

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