

Review Article

PHYTOCHEMICAL EVALUATION AND PHARMACOLOGICAL ACTIVITY OF *SYZYGIUM AROMATICUM*: A COMPREHENSIVE REVIEW

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ABSTRACT

Medicinal plants are generating an ever-increasing amount of interest due to the effectiveness, low cost and minimal side-effects associated with drugs derived from them. Clove (*Syzygium aromaticum* (L.) (Family *Myrtaceae*) is one of the most important herbs in traditional medicine, having a wide spectrum of biological activity. Phytoconstituents of clove comprise of various classes and groups of chemical compounds such as monoterpenes, sesquiterpenes, phenolics and hydrocarbon compounds. The major phytochemicals found in clove oil is mainly eugenol (70-85%) followed by eugenyl acetate (15%) and  $\beta$ -caryophyllene (5-12%). Their derivatives result in biological benefits such as antibacterial, antifungal, insecticidal, antioxidant, anticarcinogenic capacities. In addition to clove oil's worldwide use as a food flavoring agent, it has also been employed for centuries as a topical analgesic in dentistry. This review presents an overview and details of the phytochemical and pharmacological investigations on the *S. aromaticum*.

**Keywords:** Clove, *Syzygium aromaticum*, Phytoconstituents, Pharmacological activity.

INTRODUCTION

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. Natural products have been an integral part of the ancient traditional medicine systems like Ayurveda, Chinese and Egyptian. It is estimated that 40% of the world population depends directly on plant based medicine for their health care. India has rich medicinal plant flora of some 25,000 species, out of which 150 species are commercially used for extracting medicines or drug formulation. Over the last few years, researchers have aimed at identifying and validating plants derived substances for the treatment of various diseases. Interestingly, it is estimated that more than 25% of modern medicines are directly or indirectly derived from plants. In this context, it is worth mentioning that Indian plants are considered as vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments. The focus of this review is to provide information on the phytochemicals, ethno medicinal uses and pharmacological activities of *Syzygium aromaticum* commonly known as clove.

Clove (*Syzygium aromaticum* (L.) Merril. & Perry, syn. *Eugenia aromaticum* or *E. caryophyllata*) is one of the most ancient and valuable spices of the Orient. It is a member of the family *Myrtaceae*. The clove of commerce is its dried unopened flower buds. Whole and ground cloves are used to enhance the flavor of meat and rice dishes and used widely in curry powders and masalas. They are highly valued in medicine as a carminative and stimulant and are said to be a natural anthelmintic. It is used throughout Europe and Asia and is smoked in a type of cigarette, known locally as kretek in Indonesia and in occasional coffee bars in the West, mixed with marijuana to create marijuana spliffs. In the last several years, it has been recognized as an effective anesthetic for sedating fish for a number of invasive and noninvasive fisheries management and research procedures [1,2]. Oil of clove is used extensively for flavoring all kinds of food products, such as meats, sausages, baked goods, confectionery, candies, table sauces, pickles, etc. It is used in medicine for its antibacterial, antiseptic and antibiotic properties. It has also been successfully used for asthma and various allergic disorders by oral administration [3]. Sesquiterpenes, found in clove were also investigated as potential anti-carcinogenic agents [4]. The oil has many industrial applications and is used extensively in perfumes, soaps and as a clearing agent in histological work. In addition, the cloves are anti-mutagenic, [5] anti-inflammatory, [3] antioxidant, [6] antiviral, [7] anti-thrombotic [8] and anti-parasitic [9].

PHYTOCHEMICAL CONSTITUENTS OF CLOVE

Various studies have been carried out to find various constituents of *S. aromaticum* [10-12]. Clove buds contain 15-20% essential oil, which is dominated by eugenol (70-85%), eugenyl acetate (15%) and  $\beta$ -caryophyllene (5-12%). Other essential oil ingredients of clove oil are vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, flavonoids eugenin, kaempferol, rhamnetin, eugenitin and triterpenoids like oleanolic acid. The constituents of the oil also include methyl amyl ketone, methyl salicylate,  $\alpha$  and  $\beta$ -humulene, benzaldehyde,  $\beta$ -ylangene and chavicol. The minor constituents like methyl amyl ketone, methyl salicylate etc., are responsible for the characteristic pleasant odour of cloves. Gopalakrishnan *et al.* (1984) characterized six sesquiterpenes, namely:  $\alpha$ -cubebene (1.3%),  $\alpha$ -copaene (0.4%),  $\beta$ -humulene (9.1%),  $\beta$ -caryophyllene (64.5%),  $\gamma$ -cadinene (2.6%) and  $\delta$ -cadinene (2.6%) in the hydrocarbon fraction of the freshly distilled Indian clove bud oil [13]. The pharmacological activities of different phytoconstituents of *S. aromaticum* are presented in **Table 1**.

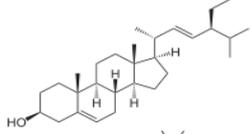
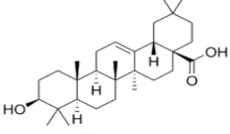
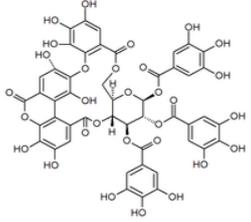
PHARMACOLOGICAL ACTIVITY OF CLOVE

Antibacterial activity

Several studies have demonstrated potent antibacterial effects of clove [47,48]. The inhibitory activity of clove is due to the presence of several constituents, mainly eugenol, eugenyl acetate,  $\beta$ -caryophyllene, 2-heptanone, [6] acetyl-eugenol,  $\alpha$ -humulene, methyl salicylate, iso-eugenol, methyl-eugenol, [9] phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnetin, myricetin, gallic acid, ellagic acid and oleanolic acid [49]. These compounds can denature proteins and react with cell membrane phospholipids, changing their permeability. Burst and Reinders 2003, found clove oil effective against non-toxicigenic strains of *E. coli* O157:H7 [50]. Similarly in another study clove oil was found to be active against food borne gram positive bacteria (*S. aureus*, *B. cereus*, *E. faecalis*, *L. monocytogenes*) and gram negative bacteria (*E. coli*, *Y. enterocolitica*, *S. choleraesuis*, *P. aeruginosa*) [47]. Demirpek *et al.*, 2009 showed that aqueous and ethanolic extracts of clove buds inhibit growth of methicillin resistant clinical isolates at 1000 and 500mg/ml concentration [51]. The isolates were multi drug resistant, mostly against beta-lactams, aminoglycosides, tetracyclines, fluoroquinolones and macrolide antibiotics. In another study eugenol at 2 $\mu$ g/mL inhibited growth of 31 strains of *Helicobacter pylori*, after 9 hours of incubation, which is being more potent than amoxicillin and doesn't develop resistance [52].

Table 1: Pharmacological activities of different phytochemicals isolated from *S. aromaticum*

S. No.	Name of Phytoconstituent	Structure of Phytoconstituent	Biological Activity	Reference
1	Eugenol		Antimicrobial, Analgesic, Antioxidant, Anticancer, Anthelmintic, Antiulcer, Anti-inflammatory, Anti-depressant, Bone preserving, antipyretic, Antithrombotic	[14]
2	$\beta$ -caryophyllene		Antitumor, anti-apoptotic Anesthetic Anti-lishmanial Anti-inflammatory Antioxidant, antibiotic	[15] [16] [17] [18] [19]
3	Vanillin		Antimicrobial Antioxidant Antidepressant	[20] [21] [22]
4	Crategolic acid (Maslinic acid)		Antitumor	[23]
5	Kaempferol		Antimicrobial, Antioxidant Anti-inflammatory Anticancer	[24] [25] [26]
6	Rhamnetin		Anti-inflammatory, Antioxidant Cardio protective Antifungal	[27] [28] [29]
7	Eugenitin		Antifungal	[30]
8	Eugenin		No activity reported	
9	Gallic acid		Antimicrobial, Antioxidant Anti-inflammatory	[31] [32]
10	Biflorin		Antibacterial Antioxidant, Anticancer	[33] [34]
11	Myricetin		Antimicrobial Antioxidant, Anticancer Anti-inflammatory	[35] [36] [37]
12	Campesterol		Antibacterial Antinociceptive Anti-carcinogenic	[38] [39] [40]

13	Stigmasterol		Antimicrobial [38] Antitumor [41] Acaricidal [42] Block cartilage degradation [43]
14	Oleanolic acid		Anti-diabetic [44] Antimicrobial [45] Anticancer [46]
15	Bicornin		No activity reported

### Antifungal activity

Many studies have reported antifungal activity for clove oil and eugenol against yeasts and filamentous fungi, such as several food-borne fungal species [53] and human pathogenic fungi [54]. Clove oil and eugenol have also been tested as antifungal agents in animal models [55]. The phenolic components of clove, carvacrol and eugenol, are known to possess fungicidal characteristics [56] including activity against fungi isolated from onychomycosis. Rana *et al.* determined antifungal activity of clove oil in different strains and reported following scale of sensibility- *Mucor sp.*> *Microsporum gypseum*> *Fusarium moniliforme* NCIM 1100> *Trichophyllum rubrum*> *Aspergillus sp.*> *Fusarium oxysporum* [57]. In chromatographic analysis eugenol was found to be the main compound responsible for the antifungal activity, due to lysis of the spores and micelles. A similar mechanism of action of membrane disruption and deformation of macromolecules produced by eugenol was also reported by Devi *et al* [58]. The large spectrum of fungicidal activity of clove oil and eugenol was reported on *Candida*, *Aspergillus* and dermatophytes and the mechanism of action was attributed to the lesions of the cytoplasmic membrane [53]. Burt proposed that different modes of action can be involved in the antifungal activity of essential oils. The activity may in part be due to their hydrophobicity, which is responsible for their partition into the lipid bilayer of the cell membrane, leading to an alteration of permeability and a consequent leakage of cell contents [50].

### Antioxidant/Free radical scavenging activity

Clove essential oil has the highest antioxidant capability and perhaps one of the best known oil for food or supplement. For this reason, it has been included in some longevity formulae. Clove and eugenol possess strong antioxidant activity, which is comparable to the activities of the synthetic antioxidants, BHA and pyrogallol [59]. Clove oil inhibited 97.3% lipid peroxidation of linoleic acid emulsion at 15 µg/mL concentration. The essential oil demonstrated scavenging activity against the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical at concentrations lower than the concentrations of eugenol, butylated hydroxytoluene (BHT), and butylated hydroxyl anisole (BHA) [60]. Abojid *et al* observed enhanced liver functions, kidney functions, and antioxidant status in clove treated rats and showed that its protective role against H<sub>2</sub>O<sub>2</sub> induced cell damages might be due to the effect of active compounds found in essential oil and plant extract [61]. A recent study by Calleja *et al* reported that β-caryophyllene isolated from clove essential oil protects rat liver from carbon tetrachloride induced fibrosis by inhibiting hepatic stellate cell activation [19].

### Anticarcinogenic activity

Clove essential oil has also been reported to show anticarcinogenic [4] and antimutagenic potential because of its strong free radical scavenging activity [63]. Several Preliminary studies suggested

chemo preventive role of clove oil, particularly in cases of lung, skin and digestive cancers [64]. Ethyl acetate extract of clove inhibits tumor growth and promotes cell cycle arrest and apoptosis. Oleanolic acid one of the components of ethyl acetate extract of clove was found to be responsible for its antitumor activity. Its mechanism was attributed to the promotion of Go/G1 cell cycle arrest and induction of apoptosis in a dose-dependent manner [65]. Eugenol acts as a potential molecule that can interfere with several cell-signaling pathways, specifically the NF-κB. In an another study, eugenol was found to suppress growth of malignant melanoma WM1205Lu of both anchorage-dependent and anchorage independent growth, decreased size of tumors and inhibited melanoma invasion and metastasis by the inhibition of two transition factors of the E2F family [66]. Hussain *et al.* studied the effect of eugenol combined with gemcitabine on cervical carcinoma and found that the combination of eugenol and gemcitabine can inhibit cancer cell growth, even in low concentrations [67]. Studies on related gene also found that eugenol can reduce the possibility of apoptosis of B-cell lymphoma-2 (Bcl-2), Cyclooxygenase-2 (COX-2), and interleukin-1β (IL-1β), reduce inflammation, and increase the treatment efficacy of gemcitabine. Moreover, Eugenol showed better curative effects in skin cancer and melanoma.

### Analgesic activity

Eugenol is a routine analgesic agent widely used in dental clinics due to its ability to alleviate tooth pain. Its anesthetic effects in dental pain as well as analgesic and anti-inflammatory effects in animal models have been well documented [68]. The effects have been attributed to its capability to suppress prostaglandins and other inflammatory mediators such as leukotriene. It is also believed to depress the sensory receptors involved in pain perception, [69] inhibits the conduction of action potential in sciatic nerves [70] and *N*-methyl-D-aspartate (NMDA) receptors but potentiates ionotropic γ-aminobutyric acid (GABA<sub>A</sub>) receptors, which are both involved in pain sensitivity [71].

### Anti-inflammatory activity

Clove oil clear respiratory passages, acting as an expectorant for treating many upper-respiratory conditions including colds, eye sties, bronchitis, sinus conditions, cough and asthma. One of the studies showed that the essential oil possess significant anti-inflammatory effect at doses of 0.05 ml/kg (90.15% inhibition) and 0.200 ml/kg (82.78% inhibition) [72]. Clove has been used in traditional public medicine to relieve nasal obstruction and musculoskeletal pain which implies its anti-inflammatory activity and the activity is due to COX-2 inhibition [73]. The aromatic oil, when inhaled, can help relieve certain respiratory conditions like coughs, colds, asthma, bronchitis and sinusitis. Clove also contains a variety of flavonoids including kaempferol, rhamnetin and β-caryophyllene which also contributed to its anti-inflammatory and antioxidant properties [16]. It has an anti-inflammatory effect

matching to that of etodolac at 0.025 and 0.1 ml/kg and to that of indomethacin at 0.05 and 0.2 ml/kg doses. Eugenol (200 and 400 mg/kg) was also found to reduce the volume of pleural exudates without changing the total blood leukocyte count indicating its anti-inflammatory potential [74].

#### Antithrombotic activity

Clove oil inhibited human platelet aggregation induced by arachidonic acid (AA), platelet-activating factor (PAF) or collagen. It was a more effective inhibitor for aggregation induced by AA and PAF (IC<sub>50</sub>: 4 and 6  $\mu$ M respectively) than collagen (IC<sub>50</sub>: 132  $\mu$ M). The *in vivo* experiments in rabbits showed that clove oil (50-100 mg/kg) afforded 100% protection against PAF (11 mg/kg, i.v.) and 70% protection against AA (2.0 mg/kg, i.v.) induced thrombosis and shock due to pulmonary platelet thrombosis. It also inhibited thromboxane-A<sub>2</sub> and 12-HETE production by human platelets incubated with [C<sup>14</sup>] AA [75]. Eugenol inhibits prostaglandin biosynthesis, the formation of thromboxane B<sub>2</sub>, and arachidonic acid-induced platelet aggregation *in vitro* [76]. Eugenol and acetyl eugenol are found to be more potent than aspirin in inhibiting platelet aggregation induced by arachidonate, adrenaline and collagen. In arachidonate induced aggregation eugenol is at par with indomethacin [77].

#### Anesthetic activity

Clove oil is used as a safe anesthetic for aquatic research. It has a mild anesthetic effect in human since antiquity [78] and fish [79]. Eugenol, the active ingredient of clove oil, inhibits the synthesis of prostaglandin H (PHS), which accounts for the analgesic effect of clove oil [80]. Clove oil and eugenol were reported as an acceptable anesthetic for rabbit fish (*Saiganus lineatus*), coral reef fish (*Pomacentrus amboinensis*) and rainbow trout (*Oncorhynchus mykiss*) for use in aquaculture and aquatic research. It was also found to be useful as a crab anesthetic [81]. Doses of 60–100 ppm eugenol produced rapid anesthesia with an acceptably short time for recovery in Zebra fish. These findings suggest that eugenol could be an effective anesthetic for use with aquatic species, and when compared to MS-222, its benefits include a lower cost, lower required dosage, improved safety, and potentially lower mortality rates [82].

#### Other pharmacological activities

Eugenol was found to have myogenic antispasmodic effect on the airway smooth muscle of rats. The mechanisms involved are blockade of voltage and receptor-operated Ca<sup>2+</sup> channels, IP 3-induced Ca<sup>2+</sup> release from sarcoplasmic reticulum and reduction of the sensitivity of contractile proteins to Ca<sup>2+</sup> [83]. It exhibited pronounced antipyretic activity when given intravenously and intragastrically and may decrease fever through a central action that is similar to that of allopathic antipyretic drugs such as acetaminophen [84]. Eugenol and its analogues were tested in CD-1 (ICR) mice using an established antidepressant screening test (forced swim test) and exhibited anti-depressant like effects against monoamine oxidase [85]. In the ovariectomised (OVX) rat model of osteoporosis, the hydroalcoholic extract of dried clove buds showed favorable effects on bone-preserving efficacy. The induced responses on serum alkaline phosphatase, serum tartrate resistant acid phosphatase, and urinary calcium, phosphate and creatinine were significantly restored after supplementation with the extract [14]. Eugenol was also found to be highly repellent to the four beetle species tested with overall repellency in the range of 80–100%. It also inhibited the development of eggs and immature stages inside grain kernels [86].

#### CONCLUSION

This review discusses pharmacological activities of all the phytoconstituents isolated from *S. aromaticum* till date. It has been found that out of all the isolated phytochemicals, 20 constituents are more active. The main constituents of clove essential oil are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde. Eugenol (4-allyl-2-methoxyphenol), the active substance, makes up 90–95% of the clove oil, and as a food additive is classified by the FDA to be a substance that is generally regarded

as safe. The World Health Organization (WHO) Expert Committee on Food additives has established the acceptable daily human intake of clove oil at 2.5 mg/kg body weight for humans. The major pharmacological activities of clove are antimicrobial, anti-inflammatory, anesthetic, analgesic, antioxidant and anticancer. Some other activities are aphrodisiac, mosquito repellent, insecticidal and antipyretic. All the major activities of clove are explained and appreciable results have been reported regarding the various activities discussed in the review. Furthermore considering its versatile medicinal uses, there is an ample scope for future research.

#### CONFLICTS OF INTEREST

All authors have none to declare.

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