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Phytochemical screening and GC-MS analysis of ethanol extract of *Syzygium aromaticum* L.

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Abstract
Syzygium aromaticum L. (Clove) is used in Indian traditional medicine to cure various diseases. In the present study, phytochemical and its chemical constituents were analyzed by GC-MS. The phytochemical analysis of the three extracts showed the presence of alkaloids, flavonoids, steroids, terpenoids, protein, carbohydrates and phenolic compounds. The GC-MS analysis of the ethanol extract of *S. aromaticum* revealed the presence of fourteen compounds. The study revealed that higher percentage of phenol, 2-methoxy-3-(2-propenyl) (64.44%) followed by eugenol (14.97%). Eugenol is the major component present in ethanol extract of *S. aromaticum* and also could be used for the advancement in developing different drugs in Pharmaceutical industries.

Keywords: *Syzygium aromaticum*, phytochemical screening, GC-MS, eugenol

1. Introduction
S. aromaticum commonly known as clove belongs to the family Myrtaceae and it has numerous medicinal properties. The flower buds are carminative, stimulant and antimalarial and it is used in dyspepsia, gastric trouble, nausea and vomiting. Its oil is a strong germicide, antiseptic, analgesic, local anaesthetic, antioxidant, emetic and spasmolytic. It contains eugenol which is an effective local anaesthetic and has long been used in dentistry [1-3]. Cloves are also said to be a natural anthelmintic and also applied to a decayed tooth cavity and it relieves a toothache reported [4].
Traditional medicine is an important source of potentially useful compounds for the development of chemotherapeutic agents. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are organic substances and could be obtained in both primary and secondary metabolic process; they also provide a source of medicine since the earliest time. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the World's pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development [5].

2. Material and Methods
2.1 Collection of plant material
S. aromaticum was collected from the nearby market of Saibaba colony, Coimbatore Tamil Nadu, India. The authenticity of the plant confirmed in Botanical Survey of India (BSI/SRI/5/23/2017/TECH/3447), Tamil Nadu Agricultural University, Tamil Nadu, Coimbatore.

2.2 Preparation of extracts
The *S. aromaticum* were washed with water to remove the dirt and shade drying for four weeks. The shade dried samples were powdered separately using an electric grinder. The powder was stored in screw cap bottles until further analysis. 10 g each *S. aromaticum* powder was weighed using an electronic balance (Denver XS-210) and made into packets using zero haze filter paper (A Grade, SD's). These powders subjected to extraction with 500 ml of the solvents for 8 h using a Soxhlet apparatus [6, 7]. Petroleum ether (60-800C) extraction was followed by chloroform extraction and ethanol extraction so that the powders subjected to extraction with solvents of increasing polarity (Fig 1 & Fig 2). The *S. aromaticum* extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 400C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use.

Fig 1: *S. aromaticum*

Fig 2: Powder of *S. aromaticum*

2.3 Phytochemical screening
2.3.1 Qualitative analysis
The phytochemicals screening of *S. aromaticum* extracts were carried out to determine the alkaloids, phenols, flavonoids, terpenoids, steroids, anthraquinones, proteins, quinines and carbohydrate using standard methods [8].

2.4 GC-MS analysis
Mass experiments were performed on GC (T8000 Top CE) combined with Mass Spectrometer (Md 800 FIS ONS). The sample was dissolved in methanol and introduced into the column TR-5-MS capillary standard non-polar by splits injection system. Ultra high purity helium was introduced as the buffered collision gas with the flow rate of 1.0 ml/min. The source temperature for ionization was set at 250 ^{0}C. All the experiments were performed on the positive ion mode.

3. Result and Discussion
3.1 Phytochemical screening
In the present study, preliminary phytochemical screening of *S. aromaticum* extracts showed the presence of phytochemical constituents. The phytochemicals screening of petroleum ether extract of *S. aromaticum* revealed the presence of alkaloids, flavonoids, steroids, terpenoids, proteins, phenols and carbohydrates. Alkaloids, steroids and proteins were observed in chloroform extract. Presence of alkaloids, flavonoids, steroids, proteins, phenols and carbohydrates were in ethanol extract of *S. aromaticum* (Table 1). Similar observations were made by [9, 10] as stated that alkaloids, flavonoids, protein, steroids, phenols, carbohydrates and quinine were present in *Brassica oleracea* leaf and stem extracts. Biologically active components have always been of huge interest to scientists working on infectious diseases [11]. Chemical constituents from natural sources have contributed significantly to the development of new drugs from medicinal plants [12, 13]. Medicinal plants have therapeutic properties due to the presence of various complex chemical substances of different composition which are found as secondary metabolites.

Table 1: Phytochemical constituents of *S. aromaticum*

S. No.	Test		Petroleum ether	Chloroform	Ethanol
1	Alkaloid	Mayers	+	+	+
		Wagners	_	_	_
		Hagers	_	+	+
2	Flavonoids	NaOH	_	_	_
		H_2SO_4	+	_	+
3	Steroids	Libermann-Burchard	+	+	+
4	Terpenoids	Libermann-Burchard	+	_	_
5	Anthraquinone	Borntragers	_	_	_
6	Proteins	Ninhydrin (Aqueous)	+	+	+
		Ninhydrin (Acetone)	_	_	_
		Biuret	+	-	-
7	Phenols	Ferric Chloride	_	_	+
		Libermann	+	_	_
8	Quinones	Conc HCl test	_	_	_
9	Carbohydrates	Molish	+	_	+
		Fehlings	_	_	_

+Detected, – Not detected

3.2 GC-MS analysis of ethanol extract of *S. aromaticum*
The studies on the active principles in the ethanol extract of *S. aromaticum* by GC-MS analysis revealed the presence of fourteen major peaks at retention time 3.23, 5.39, 4.02, 5.46, 7.10, 38.35, 8.50, 7.38, 5.03, 4.49, 11.38, 5.65, 8.19 and 23.82 (Fig 3). The active principles with their retention time (RT), concentration of area %, molecular weight and molecular formula are presented in table 2. The results showed that the Phenol, 2-methoxy-3-(2-propenyl) (66.44%), Eugenol (14.97%), Caryophyllene (2.93%), Naphthalene (2.37%), Ethyl.alpha-d-glucopyranoside(1.39%),Stigmast-5-en-3-Ol(1.28%), 2',3',4', Trimethoxyacetophenone(1.02%), Cedr-9-Ene (0.74%), Alpha-Farnesene (0.69%), 1,4,8-Cycloundecatriene (0.63%), Farnesyl Acetate 3 (0.58%), Naphthalene (0.52%), 3-Methyl-5 and 1,2-Benzenedicarboxylic acid (0.24%).

Table 2: Qualitative constituents of ethanol extract of *S. aromaticum* by GC-MS

S. No.	RT	Area %	Name of the compound	Molecular Weight	Molecular formula
1.	3.23	66.44	Phenol, 2-methoxy-3-(2-propenyl)	164	C10H12O2
2.	5.39	14.97	Eugenol	206	C12H14O3
3.	4.02	2.93	Caryophyllene	204	C15H24
4.	5.46	2.37	Naphthalene	204	C15H24
5.	7.10	1.39	Ethyl.alpha-d-glucopyranoside	208	C8H16O6
6.	38.35	1.28	Stigmas-5-en-3-Ol	414	C29H50O
7.	8.50	1.02	2',3',4', Trimethoxyacetophenone	210	C11H1404
8.	7.38	0.74	Cedr-9-Ene	204	C15H24
9.	5.03	0.69	Alpha-Farnesene	204	C15H24
10.	4.49	0.63	1,4,8-Cycloundecatriene	204	C15H24
11.	11.38	0.58	Farnesyl Acetate 3	264	C17H28O2
12.	5.65	0.52	Naphthalene	204	C15H24
13.	8.19	0.29	3-Methyl-5-(2,6,6-trimethyl-1-Cyclohexen-1-yl)-1-Pentyn-3-OL	205	C15H24O
14.	23.82	0.24	1,2-Benzenedicarboxylic acid	279	C16H22O4

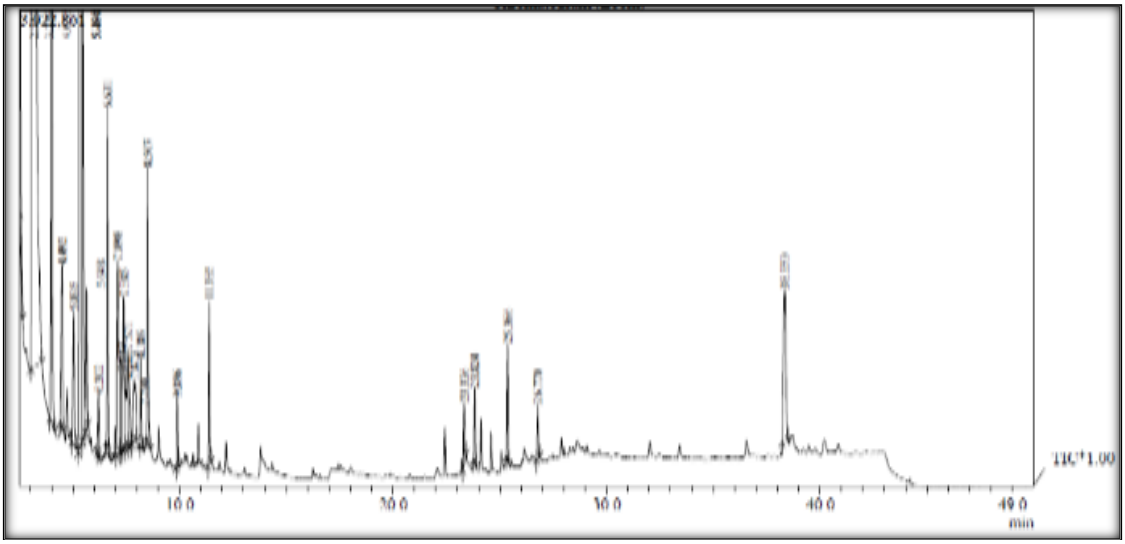
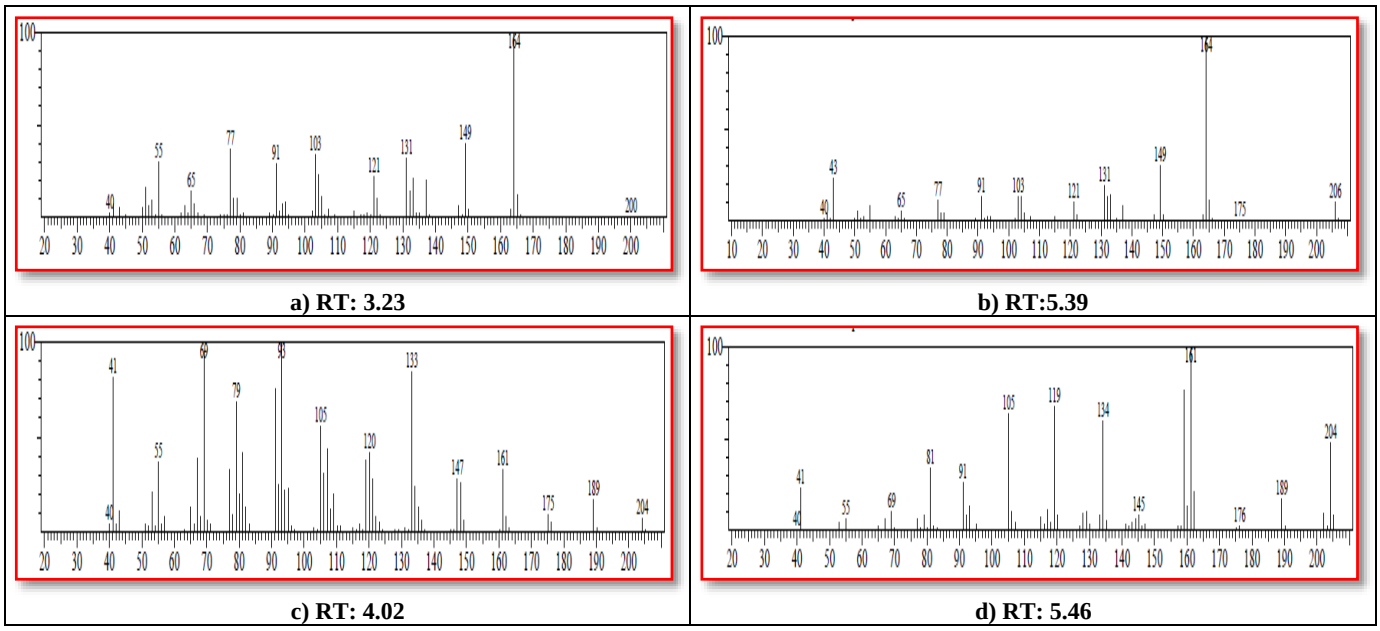


Fig 3: Gas Chromatogram of ethanol extract of *S. aromaticum*

The mass spectrum of fragmentation pattern showed (M-13), (M-14), (M-15), (M-18), (M-28) and (M-43) indicated the presence of hydrocarbon unit, methyl and methane group, hydroxyl group, carbonyl group and Mclafferty rearrangement in the compound that indicates the presence of organic compound group (Fig 4). Eugenol (82.6%), eugenol (89.2%), eugenyl acetate (8.6%) and eugenol (77.4%), eugenyl acetate (19.5%) and caryophyllene (2.01%) in essential oil contents of *S. aromaticum* was reported by [14-16].



a) RT: 3.23 **b) RT:5.39**

c) RT: 4.02 **d) RT: 5.46**

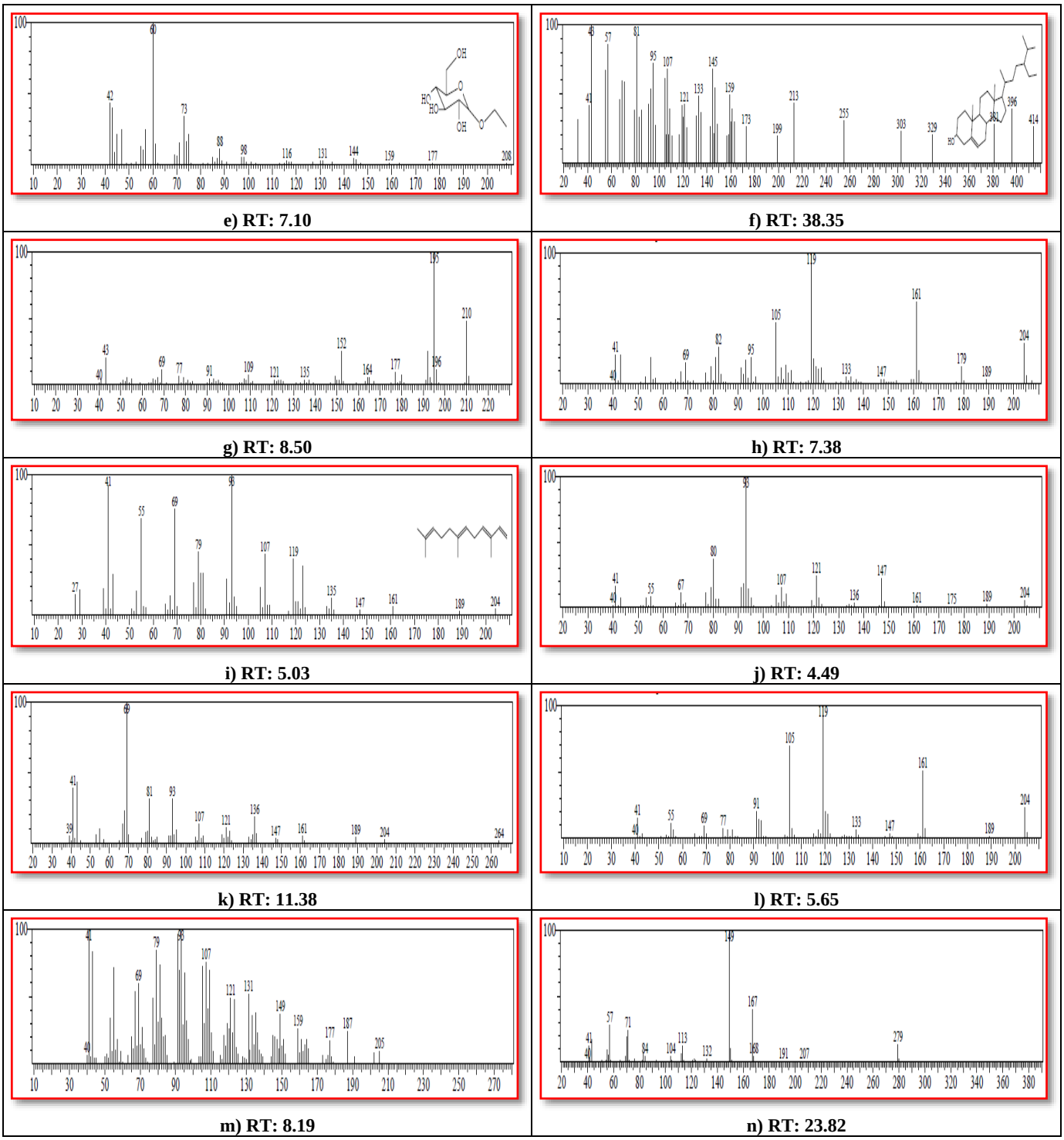


Fig 4: Mass spectrum of ethanol extract of *S. aromaticum*

The major component of eugenol were present in ethanol extract of *S. aromaticum* has been reported to diverse biological properties like antiseptic, antimutagenic, antiulcerogenic, antiviral, antioxidant, anti-inflammatory, antithrombotic, antifungal and antiparasitic [17-27]. *S. aromaticum* a potent chemopreventive agent used to traditional ayurvedic healers of India since ancient times to treat respiratory and digestive ailments [28, 29].

The isolated and identified two components of eugenol and acetyl eugenol which inhibit platelet aggregation induced by arachidonate, adrenaline and collagen [30]. *S. aromaticum* have many therapeutic uses they control nausea, vomiting, cough, diarrhea, flatulence, dyspepsia, stomach distension, gastrointestinal spasm, relive pain, cause uterine contractions and stimulate the nerves [31-35].

4. Conclusion

To conclude *S. aromaticum* as an ayurvedic herbal medicinal product that shares pharmacological properties with anti-inflammatory drugs. Eugenol has many potent activities and can be advised as a plant of phytopharmaceutical importance. This study results may also be of commercial interest to research institute and different pharmaceutical industries in the development of new medicinal and drugs.

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6. Reference

1. Shrivastava AK. Medicinal Plants, APH Publishing Corporation. New Delhi, India, 2006, 11.
2. Dhiman AK. Ayurvedic Drug Plants, Daya Publishing House, Delhi India, 2006, 235.
3. Chadha YR. The Wealth of India, Publication and information Directorate, CSIR, New Delhi, India, 1976; 5:93.
4. Alqareer A, Asma A, Lars A. The Effects of Clove and Benzocaine versus Placebo as Topical Anaesthetics. Journal of Dentistry. 2012; 34(10):747-750.
5. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J Biotechnol. 2005; 4(7):658-688.
6. Harbourne JB. Phytochemical methods. Chapman and Hall, London. Inhibitors of fungal growth. Nature. 1973; 324:365-367.
7. Vogel AI. In: Textbook of practical organic chemistry. The English language book society and Longman, London, 1978, 1368.
8. Raman N. Phytochemical techniques. 1st ed, New India Publication, New Delhi, 2006, 19-25.
9. Gowri G, Manimegalai K. Phytochemical and xrd analysis of cauliflower leaf (*Brassica oleracea* Var *Botrytis* L.). World Journal of Pharmacy and Pharmaceutical Sciences. 2017; 6(7):1277-1282.
10. Gowri G, Manimegalai K. Phytochemical analysis and antifungal activity of Cauliflower stem (*Brassica oleraceae* var *botrytis* L.). International Journal of Applied and Pure Science and Agriculture. 2017; 3(9):55-59.
11. Perumal Samy R, Ignacimuthu S. Antibacterial activity of some medicinal plants from the Eastern Ghats, South India. Solai Bull. Ethnopharmacol. 2000; 72:39-41.
12. Cox PA, Balick MJ. The Ethnobotanical Approach to Drug Discovery. Scientific American. 1994; 270(6):82-87.
13. Cox PA. Ethnopharmacology and the search for new drugs. In: Bioactive compounds from plants. (Chadwick D. J, Marsh, J., eds). CIBA Foundation Symposium 154. Wiley, Chichester, 1990, 40-55.
14. Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A *et al*. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils, Food Chemistry. 2005; 89:549-554.
15. Lee KG, Shibamoto T. Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. Et Perry], Food Chemistry. 2001; 74:443-448.
16. Porta GD, Taddeo R, D'Urso E, Reverchon E. Isolation of clove bud and star anise essential oil by supercritical CO2 extraction, Lebensm.-Wiss. u.-Technol. 1998; 31:454-460.
17. Blumenthal M. The Complete German Commission E Monographs*:* Therapeutic Guide to Herbal Medicines, American Botanical Council, Austin, 1998, 685.
18. Miyazawa M, Hisama M. Antimutagenic activity of phenylpropanoides from clove (*Syzygium aromaticum*). J Agriculture and Food Chemistry. 2003; 51:6413-6422.
19. Bae EA, Han MJ, Kim NJ, Kim DH. Anti- *Helicobacter pylori* activity of herbal medicines, Biological and Pharmaceutical Bulletin. 1998; 21:990-992.
20. Li Y, Xu C, Zhang Q, Liu JY, Tan RX. *In vitro* anti-*Helicobacter pylori* action of 30 Chinese herbal medicines used to treat ulcer diseases. J Ethnopharmacology. 2005; 98:329-333.
21. Saeed S, Tariq P. *In vitro* antibacterial activity of clove against Gram negative bacteria, Pakistan J Botany. 2008; 40(5):2157-2160.
22. Chaieb K, Zmantar T, Ksouri R, Hajlaoui H, Mahdouani K, Abdelly C *et al*. Antioxidant properties of essential oil of *Eugenia caryophyllata* and its antifungal activity against a large number of clinical *Candida* species. Mycoses. 2007b; 50:403-406.
23. Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, Kim SH, Shin TY. Effect of Syzygium aromaticum extract on immediate hypersensitivity in rats, Journal of Ethnopharmacology. 1998; 60:125-131.
24. Srivastava KC, Malhotra N. Acetyl euginol, a component of oil of cloves (*Syzygium aromaticum* L.) inhibits aggregation and alters arachidonic acid metabolism in human blood platelets, *Prostaglandins.* Leukotrienes and Essential Fatty Acids. 1991; 42:73-81.
25. Giordani R, Regli P, Kaloustian J, Mikail C, Abou L, Portugal H. Antifungal effects of various oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris.* Phytotherapy Research. 2004; 18:990-995.
26. Park MJ, Gwak KS, Yang I, Choi WS, Jo HJ, Chang WJ *et al*. Antifungal activities of the essential oils in *Syzygium aromaticum* (L.) Merr. Et Perry and *Leptospermum betersonni* Bailey and their constituents against various dermatophytes. J Microbiology. 2007; 45:460-465.
27. Yang YC, Lee SH, Lee WJ, Choi DH, Ahn YJ. Ovicidal and adulticidal effects of *Eugenia cryophyllata* bud and leaf oil compounds on Pediculus capitis. J Agric. Food Chem. 2003; 51(17):4884-4888.
28. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochemistry and Pharmacology. 2006; 71:1397-1421.
29. Banerjee S1, Panda CK, Das S. Clove (*Syzygium aromaticum* L.), a potential chemopreventive agent for lung cancer. Carcinogenesis. 2006; 27(8):1645-1654.
30. Srivastava KC1. Antiplatelet principles from a food spice clove (*Syzygium aromaticum* L). Prostaglandins Leukot Essent Fatty Acids. 1993; 49(5):885.
31. Elujoba AA, Odelleye OM, Ogunyemi CM. Review: Traditional medicine development for medical and dental primary health care delivery system in Africa, African Journal Traditional, Complementary and Alternative Medicines. 2005; 2:46-61.
32. Gordon L. A Country Herbal. Webb and Bower Publishers Ltd, Devon, England. 1980, 208.
33. Phyllis B, James B. Prescription for Nutritional Healing. 3rd edition, Avery Publishing. 2000, 111.
34. Sulieman AME, Boshra IMO, ElKhalifa EAA. Nutritive value of clove (*Syzygium aromaticum*) and detection of antimicrobial effect of its bud oil. Research J Microbiology. 2007; 2:266-271.
35. Tanko Y, Mohammed A, Okasha MA, Umar AH, Magaji RA. Anti nociceptive and anti inflammatory activities of ethanol extract of Syzygium aromaticum flower bud in wistar rats and mice. African Journal of Traditional, Complementary and Alternative Medicines. 2008; 5:209-212.