

RESEARCH ARTICLE

Evaluation of antioxidant and antimicrobial activity of the Indian clove *Syzygium aromaticum* L. Merr. & Perr.

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Manuscript Details	ABSTRACT
<p>Received : 24.06.2015 Accepted: 21.08.2015 Online Published: 30.08.2015</p>	<p><i>Syzygium aromaticum</i> (Synonym; <i>Eugenia aromatica</i> Kuntz.) belongs to family Myrtaceae is a small medium evergreen tree, 2-6 m tall. Generally, plant found medium sized, crown base low, branches semi erect and numerous. Leaves glabrous, with numerous oil glands on lower surface. Flowers small, in terminal cymose cluster, each peduncle bear three - four stalked flowers at the end while sepals minute with triangular projection. Fruits found typically olive shaped, one seeded popularly referred to as mother of clove with distinct aroma. The brown, dried, unopened flower buds called cloves, a name coming from French word "clou" meaning nail. Plant distributed in south-west and central Indian subcontinent and under cultivation in large since ancient time. Mostly, the aromatic or spices plants contain compounds that possess confirmed strong or potent anti-oxidative properties. Antioxidants are very important to human health, including lowering the risk of cancer. Antioxidant combats the effect of free radicals. More danger of free radicals, plants produces more antioxidant. Antioxidant system prevents these reactive oxygen species from being formed at optimum level. They act as scavengers to neutralize effect of free radicals. In present investigation, evaluation of few antioxidants carried out from the fresh plant material and studied. Considering the potent medicinal value of the plant its antimicrobial sensitivity test also carried out against few human pathogenic bacteria like gram positive <i>Staphylococcus aureus</i> MTCC -96, gram negative, <i>Salmonella typhi</i> MTCC- 98, <i>Klebsiella pneumoniae</i> MTCC -661, <i>Proteus vulgar</i> MTCC - 744, and <i>Shigella flexneri</i> MTCC- 1457 along with fungus <i>Candida albicans</i>-183, were used with disc diffusion method. The results of antimicrobial sensitivity compared with the standard antibiotic like Ampicillin and Nystatin (10 µg/ml)</p>
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<p>Acknowledgement The author is thankful to Dr. N.A. Ghanwate, Department of Microbiology, Sant Gadge Baba Amravati University, Amravati for providing the laboratory facility.</p>	<p>Keywords: Antioxidants, Antimicrobial sensitivity, Phytochemistry</p>
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INTRODUCTION

Since time immemorial to humankind, clove i.e. *Syzygium aromaticum* is used as medicine in Ayurveda, Chinese, Unani and Western countryside against many diseases like oral diseases or dental complaints (Cai and Wu, 1996). The essential oil derived from aromatic plants not only serves as fragrance and flavor agents but also as dietary antioxidant expected to prevent several diseases caused by free radicals (Halliwell, 1999). The medicinal properties of plants have been investigated in the recent scientific development, throughout the world, due to their antioxidant activities, no side effect and economic viability (Anndy *et al.*, 2003). Antioxidant prevent the propagation of free radicals in all cell membrane in the human body and capable of neutralizing free radicals or their action, act of different stages. They act at the level of prevention, interception and repair preventive antioxidant attempt to stop the formation of Reactive Oxygen Species (ROS). Interception of free radicals is mainly by radicals scavenging and at the repair and reconstitution level, mainly repair enzymes involve (Rhee, 2006). The antioxidant properties of the aromatic clove or eugenia oil extensively reviewed by Chaieb *et al.* (2007a). This aromatic plant buds, cloves and leaves used as a carminative, to increase hydrochloric acid in stomach and to improve peristalsis. In dentine problems, clove oil is an important ingredient in the treatment possessing anti-inflammatory, antioxidant, antiulcerogenic and antithrombic properties Chaieb *et al.* (2007b) and (Saeed and Tariq, 2008). Clove oil also used in food and beverages as preservative against bacteria or fungi and also flavoring agent (Pundir *et al.*, 2010). Its noteworthy that Pande and Singh, 2011 reported the compound Eugenol, having mold and bacterial inhibiting activity in bakery food items. Ground beef i.e. cattle meat generally spoiled by psychrotropic bacteria but can be inhibited by the use of clove oil (Oliveira *et al.*, 2013). Recently, the antibacterial activity of *Syzygium aromaticum* reported against many microorganisms like *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* (Kumar *et al.*, 2014) and antifungal activity

against *Fusarium oxysporum* (Hamini *et al.*, 2014), (Shrivastava *et al.*, 2014). The synergistic antimicrobial activity of ethanol and acetone extract of clove plant positively evaluated against cultures of *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. (Reji and Rajshekharan, 2015).

MATERIALS AND METHODS

I. Evaluation of antioxidants

In present investigation, plant *Syzygium aromaticum* collected from botanical garden of the college. Fresh plant parts like leaves, young buds and stem are chosen for the experimental purpose as more metabolically active. Quantitative estimation of antioxidants like (Vitamin C) Ascorbic acid, Anthocyanin, Lycopene, Chlorophyll carried out as per the protocols of Thimmaiah, (1999).

II. Phytochemical Tests

Phytochemical tests carried out as per Harborne, 1998.

A) Tests for alkaloids

Dragendroff's Test: About 0.2 g of the extracts (or 2-3 ml of filtrate) was warmed with 2% H₂SO₄ for two minutes. It was filtered and few drops of Dragendroff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

B) Tests for flavonoids

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. The yellow solution that turns colourless indicates the presence of flavonoids.

C) Tests for Tannins

Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride added to the filtrate. A dark green or blue-black solution indicates the presence of tannins.

D) Test for Saponin

About 0.2 g of the extract shaken with 5ml of distilled water and then heated up to the boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins by forming 1cm layer of foam.

E) Test for steroids

2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of acid i.e. H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

F) Test for Glycosides

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

G) Test for Terpenoids

(Salkowski test): 0.2 g of the extract of the whole plant sample was mixed with 2 ml of chloroform and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration was formed to indicate positive results for the presence of Terpenoids.

III. Antimicrobial sensitivity test

The plant parts cut in small pieces, cleaned carefully and washed under tap water to remove impurities followed by shade drying. Dried plant parts crushed in blender, powdered and preserved in airtight bottles. Soxhlet extraction process followed in water, ethanol, methanol, and acetone and different solvent fractions obtained. Dried extracts were stored in labeled sterile wide mouthed screw capped bottles at 4°C and used for further study (Parekh and Chanda, 2008). The standard pathogenic cultured microbial strains obtained from Microbial Type Culture Collection and Gene Bank (IMTECH), Chandigarh, India. The bacteria rejuvenated in Nutrient broth (Hi-media laboratories, Mumbai, India) at 37°C for 18 hrs and then stored at 4°C on Nutrient agar. The fungal organisms were sub cultured on Sabaroud's dextrose agar. Few human pathogenic bacteria like gram positive *Staphylococcus aureus* MTCC - 96, gram negative, *Salmonella typhi* MTCC- 98,

Klebsiella pneumoniae MTCC -661, *Proteus vulgaris* MTCC - 744, and *Shigella flexneri* MTCC- 1457 along with fungus *Candida albicans*-183, were chosen for the experiment. Disc diffusion method used for performing the antibacterial sensitivity test by following the standard methods (NCCLS, 1990). The results were compared with the standard antibiotics Hi-Media discs like (10 µg/ml) Ampicillin and Nystatin for fungi.

RESULTS AND DISCUSSION

Antioxidant analysis of *Syzygium aromaticum*

In plant *Syzygium aromaticum*, leaves, buds, or inflorescence, possess antioxidants like ascorbic acid, lycopene, anthocyanin, chlorophyll etc. and its quantitative analysis showed the presence of ascorbic acid more in buds with 6660 µgm while less in leaves with 1350 µgm. The concentration of lycopene found more in buds with 1.96 µgm and found less in leaves with 0.84 µgm. Meanwhile, about 80 µgm content of anthocyanin found in buds of plant and 50 µgm in the leaves. Interestingly, the amount of chlorophyll content found in the leaves parts was more with 1.123 mg while in buds it found about 0.900 mg. In human body ascorbic acid essential for the normal regulation of the colloidal condition of connective tissue, in the hydroxylation of proline and hydroxyproline, dentine and the intercellular cement substance of the capillaries (Smirnoff, 2005). Lycopene is a powerful antioxidant that categorically retards damage caused to DNA and protein (Xianquan *et al.*, 2005). The antioxidant activity (Scavenging free radicals metal chelation; protein binding) of anthocyanin including the protection of LDL against oxidation has been demonstrated in a number of *In vitro* systems (Aviram, 2000). Chlorophyll possess inflammatory, antioxidant and wound healing properties. Chlorophyll also removes carbon dioxide and carbon monoxide, and has been found to reduce fecal, urinary, and body odor. Chlorophyll may reduce the binding of carcinogens to DNA in the liver and other organs (Hsu *et al.*, 2013). Table-I.

Table-I: Evaluation of antioxidants from plant *Syzygium aromaticum*

Sr. No	Name of the compound	Plant part taken for analysis	Weight of plant part	Vol. of extract	Vol. of extract taken for analysis	Absorbance (525nm)	Value found in µgm
1.	Ascorbic acid	Leaves	1 g	10 ml	1ml	0.199	1350 µgm
		Buds	1 g	10 ml	1ml	1.268	6600 µgm
2.	Lycopene	Leaves	1g	10 ml	1ml	0.027	0.84µgm
		Buds	1g	10 ml	1ml	0.063	1.96µgm
3.	Anthocyanin	Leaves	1g	10 ml	1ml	0.200	50µgm
		Buds	1g	10 ml	1ml	0.320	80 µgm
4.	Chlorophyll	Leaves*	1g	Chl_a at 645nm = 0.332		Chl_b at 663nm = 0.563	1.123 mg
		Buds**	1g	Chl_a at 645nm = 0.254		Chl_b at 663nm = 0.483	0.900 mg

Calculations * Total chlorophyll 1.123 mg = Chl_a - 0.625mg + Chl_b- 0.496mg

** Total chlorophyll 0.900mg = Chl_a 0.545mg + Chl_b- 0.555mg

Table-II: Preliminary phytochemical analysis of *Syzygium aromaticum*

Sr. No.	NAME OF THE PLANT	Phytochemical Tests						
		Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Glycosides	Terpenoids
1.	<i>Syzygium aromaticum</i>	+	+	+	+	+	+	+

Table-III: Antimicrobial sensitivity test of the plant *Syzygium aromaticum*

Sr. No.	Solvent Extract	Zone of Inhibition [mm]				
		<i>Proteus vulgaris</i> [MTCC-744]	<i>Shigella flexneri</i> [MTCC-1457]	<i>Staphylococcus aureus</i> [MTCC-96]	<i>Salmonella typhimurium</i> [MTCC-98]	<i>Aspergillus niger</i> [MTCC-281]
1.	Aqueous	09	08	09	11	16
2.	Petroleum ether	12	11	10	15	17
3.	Methanol	11	07	12	14	14
4.	Ethanol	12	14	13	16	17
5.	Ampicillin [control]	29	27	30	32	-
6.	Nystatin [control]	-	-	-	-	31

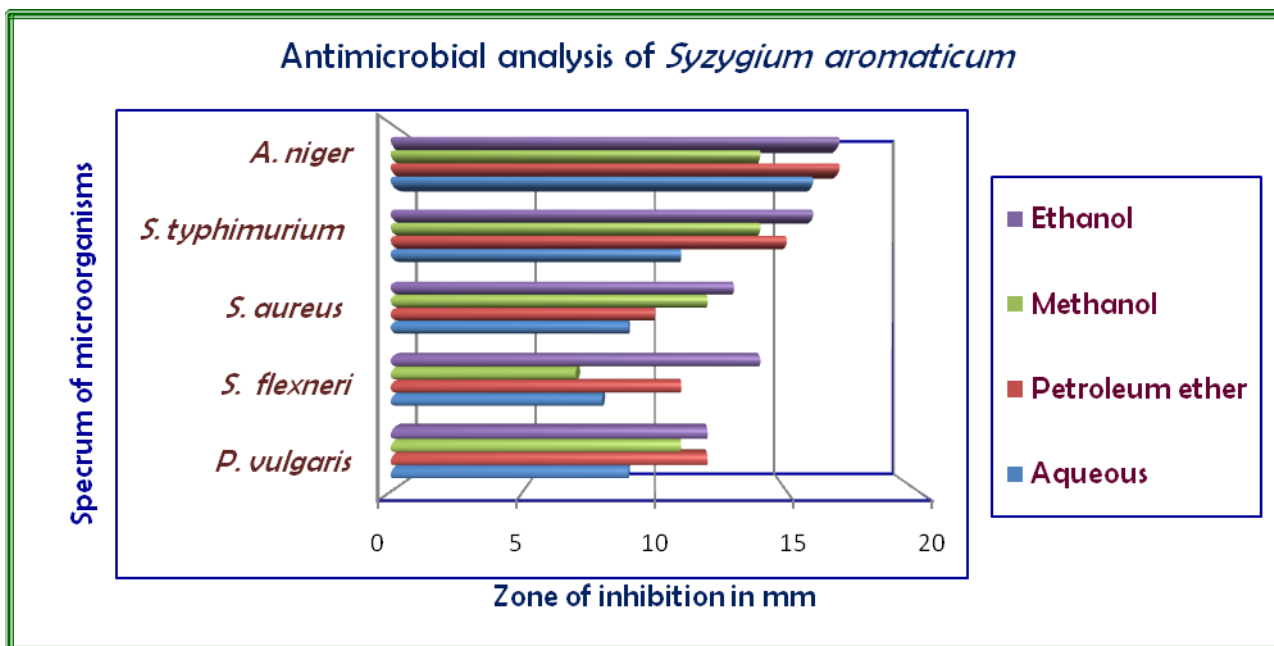


Fig.1: Analysis of antimicrobial sensitivity test of *Syzygium aromaticum*

Phytochemical analysis of *Syzygium aromaticum*

The analysis of the preliminary phytochemical tests of the plant showed the presence of chemical constituents like alkaloids, flavonoids, tannins, saponins, steroids, glycosides and terpenoids. Alkaloids are pharmacologically active as they have physiological effects on human as well as other animals and serves as therapeutic and anti-malarial drugs (Pawar and Arumugam, 2011). It is also noteworthy that saponins, tannins have anti-carcinogenic properties, antioxidant effects, immune modulation and regulation of cell proliferation. Flavonoids, steroids and glycosides reported to be used in treating heart problems (Shrivastava *et al.*, 2015). This data would provide evidence or clues for the probable chemical and antimicrobial activities. Table-2.

Antimicrobial analysis of *Syzygium aromaticum*

Disc diffusion analysis of antimicrobial assay of aqueous extract showed maximum zone in *A. niger* with 16 mm zone and less in *S. flexneri* with 8 mm. The extract of petroleum ether showed highest antimicrobial activity in *S. typhimurium* of 17 mm and less in *S. aureus* (Pande and Singh, 2011). Interestingly, the methanolic extract showed maximum activity in bacteria *S. typhimurium* and

fungus *A. niger* of 14 mm and less in *S. flexneri* with 7 mm. However, the ethanolic extract found profound effect with maximum zone of inhibition against *S. typhimurium* and *A. niger* but less against *P. vulgaris* with 12 mm zone which corresponds complementarily with findings of Kumari *et al.*, 2013.

CONCLUSION

The plant *Syzygium aromaticum* showed synergistic interaction against microbial pathogens when tested and can be formulated for therapeutic uses after further studies. Presence of antioxidants also exhibits its effect as important drugs constituents. This findings conclusive due to tests analysis where various chemical compounds showed their vital presence. Further chemical characterization of these compounds by NMR, GC-MS, HPLC will open new avenue for novel drug discovery.

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