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Research Article

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *SYZYGIUM CARYOPHYLLATUM* ESSENTIAL OIL

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ABSTRACT

Objective: To investigate the phytochemical constituents, TLC bioautography and antioxidants of *Syzygium caryophyllatum* essential oil. The antimicrobial potential was also determined against various multi drug resistant clinical isolates.

Methods: Preliminary phytochemical analysis was performed. The antimicrobial potential of essential oil from *Syzygium caryophyllatum* was evaluated by agar well diffusion method against multi drug resistant clinical isolates. The antibacterial effect was investigated using the TLC-bioautographic method. The antioxidants analyzed include catalase, superoxide dismutase, glutathione-S-transferase and glutathione reductase.

Results: Phytoconstituents analysis demonstrated the presence of alkaloids, cardiac glycosides, flavanoids, steroids, saponins and tannins. *Syzygium caryophyllatum* oil was further investigated for its antimicrobial activity against twelve Multi drug resistant pathogenic bacteria and four fungi respectively. The highest *in vitro* inhibitory activity was observed for *klebsiella sp.*3 with wide inhibition zone diameters (22±0.07 mm) followed by *klebsiella sp.*2 (17±0.12 mm) and *S. aureus* 2 (13±0.11 mm). Among fungal isolates, inhibitory activity was observed for *Aspergillus niger, Aspergillus sp* and *Rhizopus nigricans.* Thin layer chromatography bioautography demonstrated one large inhibitory zone with *Rf* value of 0.91 against the growth of isolate *Klebsiella* sp. 3. The oil was found to be rich in antioxidants such as superoxide dismutase, glutathione reductase and glutathione-S-transferase.

Conclusion: It can be concluded that *Syzygium caryophyllatum* oil can be used as a potential source of natural antimicrobial compound possessing strong antioxidant potential.

Keywords: *Syzygium caryophyllatum,* antibacterial activity, antifungal activity, antioxidant potential, agar well diffusion, TLC Bioautography

INTRODUCTION

Emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure for the treatment of various infectious diseases. Infectious diseases represent an important cause of morbidity and mortality mainly in developing countries. Inappropriate and irrational use of antibiotics provides favorable conditions for selection and spread of antibiotic resistance. Thus there is a continuous need of producing new drugs as resistance has emerged towards all classes of antibiotics. MDR strains are resistant to first line of treatments and also the more expensive second and third-line antibiotics. Thus, there had been a great deal of interest in developing new antimicrobial substances from natural plant products or plant essential oils (EOs), which produce diverse chemical compounds with different biological activities [1]. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries as approximately 80% of the world inhabitants rely on traditional medicine for their primary health care needs.

Essential oils from aromatic plants and spices have a long history of use in folk medicine as natural microbial agents since ancient times. They possess great importance for food, cosmetics and pharmaceutical industries as these oils are known to possess antimicrobial, antiviral and antioxidant properties and they cause fewer side effects as compared to synthetic antimicrobial agents. EOs has been empirically used as antimicrobial agents but little is known about the exact mechanism of action. Spices and condiments exhibit wide range of beneficial, pharmacological, antioxidant, anticarcinogenic and anti-inflammatory effects. Spices are widely used as food adjuncts and cloves constitute one of the major spice species.

Syzygium caryophyllatum (L.) Alston, (syn. *Syzygium aromaticum* (L.) Merr and Perry commonly called clove, is a perennial tropical plant belonging to the family Myrtaceae. Cloves are dried unopened floral buds of an evergreen tree which is native to Moluccas Island Indonesia. It is commercially cultivated in various countries like India, Bangladesh, South of China, Sri Lanka and Madagascar. *Syzygium caryophyllatum* oil is used as a flavouring agent, for flavouring food items. As it has analgesic properties, it is especially used in the medical dental practices [2]. *Syzygium caryophyllatum* buds oil has biological activities, such as antimicrobial, insecticidal and antioxidant properties [3]. The high level of eugenol contained in *Syzygium caryophyllatum* essential oil was found to be responsible for strong antimicrobial activity [4-5]. *Syzygium caryophyllatum* oil also has several therapeutic effects, including anti-phlogistic, anti-vomiting, analgesic, antispasmodic, anti-carminative, kidney reinforcement and antiseptic. Kamel et al. [6] reported the main constituent's flower buds of *Syzygium caryophyllatum* essential oil are phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde.

This current study aimed at evaluating the phytochemical screening, *in vitro* antimicrobial activity and antioxidant potential of *Syzygium caryophyllatum* essential oil against multi drug resistant clinical isolates.

MATERIALS AND METHODS

Syzygium caryophyllatum essential oil

Commercial brands of *Syzygium caryophyllatum* oil was purchased from Delhi, India. As per manufacturer's information, it was prepared by steam distillation. The oil was further distilled by rotary evaporator. The essential oil was dissolved in methanol (0.3 ml oil/2 ml methanol). The oil was transferred into sterile vials and stored at 4°C till further analysis.

Microbial cultures and Growth conditions

The microbial cultures included multi-drug resistant isolates of *Escherichia coli, Enterobacter* sp, *Salmonella typhi, Salmonella paratyphi*, *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter* sp. The cultures of bacteria were maintained on nutrient agar slants at 4°C throughout the study. Fungal isolates studied includes *Aspergillus niger, Aspergillus sp, Candida albicans* and *Rhizopus nigricans.* The cultures were maintained on potato dextrose agar at 4°C. These microbial isolates served as test pathogens for studying antimicrobial activity.

Phytochemical analysis

Syzygium caryophyllatum essential oil was dissolved in methanol (0.3 ml oil/2 ml methanol) and was subjected to phytochemical screening. The essential oil was tested for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, reducing sugars, saponins, steroids, tannins and phlobatanins by using wet reactions [7-8].

Antimicrobial activity assay

To determine the antibacterial activities of *Syzygium caryophyllatum* oil in methanol, agar well diffusion method was employed according to National Committee for Clinical Laboratory Standards (NCCLS) [9]. About 25 ml of nutrient agar and potato dextrose agar was poured into each petri plate. Once the agar solidified, the cultures were inoculated on the surface of the plates (1×10^6 cfu/ml). Subsequently, the surface of the agar was punched with a 8 mm diameter wells. Each well was filled with 50 µl of oil in methanol and allowed to diffuse at room temperature for 2 h. Control wells containing the same volume of methanol were made. The plates were then incubated in the upright position at 37°C for 24 h (for bacterial cultures) and 24°C for 3 days (for fungal cultures). After incubation at respective temperatures, the plates were observed for zones of growth inhibition, and the diameter of these zones was measured in millimeters. All tests were performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition.

TLC bioautography assay

Syzygium caryophyllatum oil exhibiting significant antimicrobial potential against *Klebsiella sp.* 3 and *Staphylococcus aureus* 2 as determined by agar well diffusion method (Table 2) was analyzed using TLC bioautography assay [10].

Antioxidant analysis

Syzygium caryophyllatum oil was evaluated for the presence of various antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and reduced glutathione (GSH). Superoxide dismutase activity was measured by the NBT reduction [11]. Catalase activity was estimated by measuring the rate of decomposition of hydrogen peroxide at 240nm [12]. Assay of glutathione-s-transferase was done according to Habig et al. [13]. Glutathione activity (GSH) was assayed based on the reaction with DTNB [14]. Data was expressed as mean± standard deviation (SD).

RESULTS

Preliminary phytochemical analysis of *Syzygium caryophyllatum* essential oil revealed the presence of flavanoids, steroids, tannins, alkaloids, saponins, and cardiac glycosides. The phytochemicals tested including reducing sugar, anthraquinones and phlobatanins were not observed in *Syzygium caryophyllatum* essential oil as shown in table 1.

Table 1: Phytochemical analysis of *Syzygium caryophyllatum* essential oil

Phytoconstituents	*Syzygium caryophyllatum* oil
Alkaloids	+
Flavonoids	+
Reducing sugar	-
Phlobatanins	-
Tannins	+
Anthraquinone	-
Steroids	+
Saponins	+
Cardiac glycoside	+

+: Present, -: Not present

Syzygium caryophyllatum essential oil was further investigated for its antimicrobial activity against multi drug resistant clinical bacteria and four fungi respectively (Table 2). Agar well diffusion method revealed that the oil tested showed significant to moderate antimicrobial activity toward tested isolates except *Pseudomonas aeruginosa, Klebsiella sp.* 1 and *Candida albicans.* The highest *in vitro* inhibitory activity was observed for *Klebsiella sp.*3 with wide inhibition zone diameters (22±0.07 mm) followed by *Klebsiella sp.*2 (17±0.12) mm and *S. aureus* 2 (13±0.11). The control plate did not exhibit inhibition on the tested bacteria. The growth of *S. aureus* 3, *Acinetobacter* sp. and *Rhizopus nigricans* was only slightly inhibited by *Syzygium caryophyllatum* essential oil.

Table 2: **Antimicrobial potential of *Syzygium caryophyllatum* essential oil**

Test Microorganisms	Zone of Inhibition (in mm)
Bacterial isolates	
Acinetobacter sp.	7.1±0.11
E.coli 1	9±0.05
Enterobacter aerogenes	10.3±0.17
Klebsiella sp. 1	-
Klebsiella sp. 2	17±0.12
Klebsiella sp. 3	22±0.07
Salmonella typhi	9.8±0.13
Salmonella paratyphi	10±0.07
S. aureus 1	12.7±0.09
S. aureus 2	13±0.11
S. aureus 3	7.3±0.085
P. aeruginosa	-
Fungal isolates	
Aspergillus sp.	8.2±0.12
Aspergillus niger	8.2±0.08
Candida albicans	-
Rhizopus nigricans	7±0.07

Zone of inhibition is the mean of three readings, -: no inhibition

TLC Bioautographic assay are usually used to screen the antimicrobial potential by separating components onto the surface of chromatographic plates and overlaying the TLC plate with molten agar. TLC bioautography was performed for *Syzygium caryophyllatum* essential oil against *Klebsiella* sp. 3 isolate. One large inhibitory zone with *Rf* value 0.91 was observed against the growth of isolates *Klebsiella* sp. 3 on the TLC plates A and B as white spot on pink background when sprayed with aqueous solution of 2, 3, 5 triphenyl tetrazolium chloride. TLC analysis revealed the presence of alkaloids in the essential oil tested when sprayed with Dragondorff's reagent. Spot with R_f values of 0.91 corresponds to the spots representing alkaloid on spraying with on plate C (Data not shown).

Syzygium caryophyllatum oil was further analyzed for the presence of antioxidants. The antioxidants studied include SOD, CAT, GSH and GST as reported in Table 3. It was found to possess good amount of GSH (33.54±0.075µg/mg) and SOD (29.5±0.132 U/mg), whereas small amount of GST and CAT was observed.

Table 3: Level of antioxidants in *Syzygium caryophyllatum* essential oil

Species	Reduced Glutathione (µg/mg)	Glutathione -S-transferase (µg/mg)	Catalase (µg/mg)	Super oxide dismutase (U/mg)
Syzygium caryophyllatum	33.54±0.075	11.2±0.095	4.9±0.011	29.5±0.132

DISCUSSION

In the past few decades, the incidence of spread of drug resistant pathogens is found to be one of the most serious threats towards the treatment of various microbial diseases. Constant antibiotic use resulted in bacterial evolution to MDR forms leading to human epidemics. Down the ages, use of plant derived products and essential oils have been studied as diseases control agents. World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare [15]. Plant essential oils have been used as natural microbial agents since long with fewer side effects than synthetic antimicrobial agents in humans. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. Some oils also possess application in cancer treatment. In the present study, medicinally important *Syzygium caryophyllatum* essential oils was screened for the presence of phytoconstituents, antimicrobial potential, TLC bioautography for bioactive compounds and antioxidant potential.

Preliminary phytochemical analysis of *Syzygium caryophyllatum essential* oil revealed that the oil contains flavanoids, steroids, tannins, alkaloids, saponins, and cardiac glycosides. Bhuiyan et al. [16] reported eugenol (74.3%), eucalyptol (5.8%), caryophyllene (3.85%) and cadinol (2.43%) as the main components present in *Syzygium caryophyllatum* essential oil. High levels of eugenol present in *Syzygium caryophyllatum* essential oil is responsible for strong antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability [17].

Syzygium caryophyllatum essential oil was quantitatively assessed for *in vitro* antibacterial and antifungal activity using agar well diffusion method. The oil was found to exhibit varying degree of inhibitory effect against the selected multi drug resistant bacterial and fungal isolates. The results are consistent with the reports of previous investigators. *Syzygium caryophyllatum* oil is reported to have strong antifungal activity against some common fungal pathogens of plants and animals including, *Fusarium moniliforme* NCIM 1100, *Fusarium oxysporum* MTCC 284, *Aspergillus* sp., *Mucor* sp., *Trichophyton rubrum* and *Microsporum gypseum* [18]. The antimicrobial activity of *Syzygium caryophyllatum* oil showed strong antibacterial activity against all bacterial isolates tested with maximum activity against *P. aeruginosa. K. pneumoniae, S. marcescens, S. typhi, S. dysentriae* and *V. cholerae* were found resistant [19]. The antibacterial activity of *Syzygium caryophyllatum* is attributed to eugenol. High tannin content (10-19%) in *Syzygium caryophyllatum* also provides additional antimicrobial activity [20].

The bioactive components of *Syzygium caryophyllatum* oil were separated on TLC followed by TLC bioautography. TLC bioautography were performed for *Syzygium caryophyllatum* oil against *Klebsiella* sp. 3 isolate. Bioautography showed presence of one large inhibitory zone with *Rf* value 0.91 against the growth of isolates *Klebsiella* sp. 3. Spot with R_f values of 0.91 corresponds to the spots representing alkaloid on spraying with Dragondorff's reagent and tannins as confirmed by spraying with 10% $FeCl_3$ spray reagent. The observed inhibition was may be due to more than one active component that overlap possibly due to the solvent system used. These findings corroborated with the observations of Sibi *et al* [21] who reported tannins to be responsible for antibacterial potential.

Syzygium caryophyllatum oil was analyzed for the presence of various antioxidants. It was found to possess high level of GSH and SOD, and small amount of GST and CAT was observed. Low level of GST and CAT are observed in *Syzygium caryophyllatum* oil. GST offers protection against lipid peroxidation by the conjugation of toxic effect with GSH [22]. Catalase is the most significant antioxidant enzyme that protects the plants by scavenging H_2O_2 and free radicals. SOD prevents the formation of.OH and provides essential defence against the potential toxicity of oxygen. Shyamala et al. [23] reported that on co-administration of *Syzygium caryophyllatum* oil, antioxidant enzymes such as SOD, CAT, GPX and GST showed enhanced activities.

CONCLUSIONS

It may be suggested from the present findings that *Syzygium caryophyllatum* oil can be used as a potential source of natural antimicrobial compound possessing strong antioxidant potential. However, further research is needed for the identification of biologically active compounds present and *in vivo* studies using animal model.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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