

# Assessment of Potential Duo Out of *Syzygium aromaticum* L., *Zingiber officinale* and *Ocimum basilicum* L. Leaves Extract for Extermination of Clinical Pathogens

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## Abstract

In last three decades numbers of new antibiotics have produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug resistant pathogens. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. Scope of this study is to evaluate the potential duo of *Syzygium aromaticum* L., *Zingiber Officinale* and *Ocimum basilicum* L against clinical pathogens; *E. coli*, *S. aureus*, *B. cereus* and *P. vulgaris*. Well diffusion method was adopted to study antimicrobial properties. The maximum zone of inhibition is produced by *Syzygium aromaticum* than other extracts *S. aureus* (17 mm) followed by *B. cereus* and *P. vulgaris*. Duo of *Syzygium aromaticum* and *Zingiber officinale* shown to have maximum antimicrobial property against *E. coli*, *S. aureus*, *B. cereus* and *P. vulgaris* which is expressed to be a best duo of extract and it is more potent (25 mm) for *B. cereus*. Duo of *Zingiber Officinale* and *Ocimum basilicum* and *Ocimum basilicum* and *Syzygium aromaticum* was least effective but in conclusion combined action of these extract is more enhanced than the individual extract. Our study indicates that phytoconstituents in combination can act better than individual. Therefore, they can be used for preserving various foodstuffs against microbial spoilage and it can be incorporated into medications for topical antifungal or antibacterial therapy.

**Keywords:** *Syzygium aromaticum* L., *Zingiber officinale* and *Ocimum basilicum*

## Introduction

Infectious diseases caused by pathogens; bacteria, fungi, viruses and parasites are still a major threat to public health in spite of tremendous progress in human medicines [1]. Plant kingdom represents an extraordinary reservoir of novel molecules [2]. Nature has itself provided an important source of remedies to cure various ailments of mankind. Plants have been a rich source of bioactive compounds to treat many diseases. Medicinal plants produce a variety of compounds with known therapeutic properties which is been traditionally used [3]. In recent years, all the medicines used were from natural source, especially from plants [4]. Plants contain hundreds or thousands of metabolites. For bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal, cosmetic, agricultural, food industries and cosmetic applications essential oils have widely been used. In recent times plants also have been exploited for pharmaceutical and sanitary purpose. Extraction by hydrodistillation from aromatic plants, they contain a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components, and aliphatic components [5]. Medicinal and aromatic plants are gift of nature, which is being used against various infectious diseases and other curative purpose. Very small of total percentage of plants species have been exploited and investigated for their curative potential and very few fraction is screened for phytochemistry [6]. Nutraceuticals are plants which contribute their phytonutrients and other compounds for maintaining a good and healthy life. There is strong correlation between diet and the incidence of diseases [7]. Study over toxicity profile of most medicinal plants has not been evaluated totally but a strong belief of ayurveda accepted that plant derived medicines far safer than their synthetic medicine [8].

Public health is tremendously threatened by infectious diseases caused by bacteria, fungi, viruses and parasites although antibiotics

have made a strong influence for control of disease caused by pathogens and emergence of widespread drug (antibiotic) resistance for such pathogens turned the path of pharmacological companies to search for potent drug target [9].

Plants have been widely used for the treatment of various ailments in animals. In the study of McGaw and Eloff reported that near about 200 plant species used as ethnoveterinary medicine in South Africa. For the benefits of humans and animals again more species need to be evaluated in depth [10].

The plant species used in this study were chosen based on documented traditional use. Because some commercial antibiotic are not responding to bacterial pathogens and emergence of multi drug resistance, we decided to investigate the possible solution to overtake such pathogens by investigating potential duo of plant extract. Ethyl acetate was selected as the only solvent based on its ability to extract compounds with a wide range of polarities, its low toxicity in antimicrobial bioassays and because it is easily removed from the extract at low temperature. The present study aims to review traditional ethno medicinal knowledge of the local people in local spices and focusing specifically on the medicinal uses of plants. These types of studies are

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urgent considering the loss of traditional knowledge accompanying alteration of the physical and biological environment.

## Material and Methods

### Collection of clinical samples

Clinical samples were collected from regional pathology laboratories Bhandara district. Each sample was then preceded for battery of biochemical, cultural and morphological test for isolation and confirmation of *E. coli*, *S. aureus*, *B. cereus* and *P. vulgaris*. The isolated pure cultures were maintained on nutrient agar till its use at 4°C in refrigerator.

### Collection of plant material

*Syzigium aromaticum* L., *Zingiber officinale* and *Ocimum basilicum* L. was collected from vegetable market of Bhandara district.

### Extract preparation from herb

5.0 g of powder of dried clove was mixed in 50 ml of ethyl acetate. The mixture was kept in dark condition at room temperature for 2 days. After 2 days slurry was filtered through Whatmann filter paper no. 1 and filtrate was concentrated by evaporation in hot air oven and volume is reduced to 10 ml.

For single extract activity direct 100 µl extract is used out of reduced 10 ml extract and for duo action 50 µl extract of each member in combination is used.

### In vitro susceptibility testing of extract

Hi-sensitivity test broth was prepared and sterilized at 15 lbs for 15 min. and inoculated with the previously screened antibiotic resistant bacteria aseptically. Separately, sterile Hi-sensitivity test agar plates were prepared and allowed it to solidify at room temperature. A 0.5 ml of 6-8 hours old test organism was inoculated in solidified sterile Hi-sensitivity test agar plates and spread with sterile spreader. Wells were cut in previously solidified sterile Hi-sensitivity test agar plates with the help of 10 mm cork borer. A 100 µl of previously prepared *Syzigium aromaticum* L., *Zingiber officinale* and *Ocimum basilicum* L. extract was transferred by micropipette per well and for duo action 50 µl extract of each member in combination was used. Separate plate was used for each concentration. Plates were then incubated at 35 ± 0.5°. Zone of inhibition was measured after 24 hrs of incubation.

## Result and Discussion

Total four test organisms isolated from clinical samples are used in the study. Clinical isolates of *E. coli*, *S. aureus*, *B. cereus* and *P. vulgaris* were used in the study. For the first part of experiment were extract (alone) was tested; Extracts of *Syzigium aromaticum* L., *Zingiber officinale* and *Ocimum basilicum* L. against clinical isolates of *E. coli*, *S. aureus*, *B. cereus* and *P. vulgaris*, the maximum zone of inhibition is produced by *Syzigium aromaticum* than other extracts, and *Syzigium aromaticum* showed maximum zone of inhibition for *S. aureus* (17 mm) followed by *B. cereus* and *P. vulgaris* with zone of inhibition of 15 mm and showed least zone of inhibition of 11 mm whereas *Ocimum basilicum* extract have almost same inhibition property against all test organisms and least antimicrobial property exhibited by *Zingiber officinale* (Table 1).

For the second part of experiment were extract in combination (duo) was tested; three duo of extract is prepared and differential results were obtained. Duo of *Syzigium aromaticum* and *Zingiber officinale*

shown to have maximum antimicrobial property against *E. coli*, *S. aureus*, *B. cereus* and *P. vulgaris* which is expressed to be a best duo of extract and it is more potent (25 mm) for *B. cereus*. Duo of *Zingiber officinale* and *Ocimum basilicum* and *Ocimum basilicum* and *Syzigium aromaticum* was least effective but in conclusion combined action of these extract is more enhanced than the individual extract (Table 2).

Plants contain several phytochemicals inhibit several pathogens. Selected also contain such compounds that are able to inhibit clinical pathogens among all *Syzigium aromaticum* extract was better in respect of antimicrobial property. The results of Burst and Reinders [11] that clove oil was found effective against strains of *E. coli* O157:H7 [11]. Strains of *S. aureus* are inhibited by clove extract which supports the present study [12]. Similar study of Lopez et al. [13] over clove oil was found active against *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Listeria monocytogenes* and *E. coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis* and *P. aeruginosa* [13]. Lokesh et al. [14], Verma et al. [15] demonstrated that clove extract to have antibacterial properties along different elicitors including sugars and metal ions [14,15]. Phyto-constituents analysis of clove found to have rhamnocitrin, biflorin, ellagic acid, gallic acid, kaempferol, myricetin, and oleanoic acid confers antibacterial activities against *Streptococcus mutans*, *Actinomyces viscosus*, *Porphyromonas*, *Helicobacter pylori* and *Prevotella intermedia* [16-18].

Prasad et al. [19] proved essential oil of *O. basilicum* have the better antimicrobial activity of against Gram-negative than Gram-positive bacteria [19]. Sinha and Gulati, Prasad et al., [19]; Opalchenovaa and Obreshkova [20] demonstrated the effectiveness of the *O. basilicum* against *S. aureus*, *Salmonella paratyphi*, *Proteus vulgaris*, *Salmonella typhi*, *Shigella boydi*, and *E. coli* the results are in harmony with present studies [19,20].

Singh et al. [21] discussed the differences in the effectiveness of antimicrobial potency, this difference in concentration could be due to the nature of solvent and method of extraction [21]. Because of differences in cell wall composition, the resistance of the Gram-negative bacteria could be due to the complexity of the cell wall and the external membrane of Gram-negative bacteria renders highly hydrophilic surfaces whereas the negative charge of the surface of the Gram-positive wall reduces their resistance to antibacterial compounds [22]. A component monoterpen have antibacterial property activity because of the disruption of bacteria or fungal membrane Integrity [23].

Sr. No.	Test organisms	Extracts		
		<i>Syzigium aromaticum</i>	<i>Zingiber officinale</i>	<i>Ocimum basilicum</i>
1	<i>E. coli</i>	11 mm	10 mm	17 mm
2	<i>S. aureus</i>	17 mm	12 mm	18 mm
3	<i>B. cereus</i>	15 mm	11 mm	Nil
4	<i>P. vulgaris</i>	15 mm	14 mm	17 mm

Table 1: Zone of inhibition of extracts (alone) against test organism.

Sr. No.	Test organisms	Duo of extracts		
		<i>Syzigium aromaticum</i> + <i>Zingiber officinale</i>	<i>Zingiber officinale</i> + <i>Ocimum basilicum</i>	<i>Ocimum basilicum</i> + <i>Syzigium aromaticum</i>
1	<i>E. coli</i>	19 mm	13 mm	17 mm
2	<i>S. aureus</i>	21 mm	18 mm	15 mm
3	<i>B. cereus</i>	25 mm	18 mm	21 mm
4	<i>P. vulgaris</i>	20 mm	16 mm	14 mm

Table 2: Zone of inhibition of extracts in different duo against test organism.

Ginger is effective against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella spp.* and *Salmonella spp.* [24]. Hot extract of ginger extract (crude) loss its antimicrobial activity against *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*. Onyeagba et al. [25] demonstrated synergistic effect of ethanol extract of ginger and garlic against *Bacillus spp.* and *Staphylococcus aureus* which supports the present research [25,26].

The result revealed that, *Syzigium aromaticum* L., *Zingiber officinale* and *Ocimum basilicum* L have antimicrobial activity. On the other hand, combination these extract is more potent than alone for extermination of clinical pathogens.

## Conclusion

Our experimental data suggests that *Syzigium aromaticum* L., *Zingiber officinale* and *Ocimum basilicum* L have antimicrobial activity and combination these extract is more potent than alone for extermination of clinical pathogens. This inferred that phytoconstituents in combination can act better than individual. From the obtained results of our present study we can indicate that combined extracts have exhibited wide spectrum of antimicrobial properties. Therefore, they can be used for formulation of pharmaceutical products and preservation of various foodstuffs against microbial spoilage.

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