

Antioxidant and Antibacterial Activity of *Syzigium Aromaticum*, *Zingiber Officinale* and *Cinnamomum Zeylanicum* Essential Oils

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Abstract: The present investigation evaluates the antioxidant and antibacterial activity of essential oils of *Syzigium aromaticum*, *Zingiber officinale* and *Cinnamomum Zeylanicum*. The hydrogen donating ability of two essential oils was measured by reduction of DPPH and potassium ferricyanide. It was observed that antioxidant activities of essential oils increase proportionately with concentration. The antibacterial activity was assessed by measuring zone of inhibition using disc diffusion technique. The antibacterial activity test was performed using gram negative bacteria *Eschericia coli* (MCCB 0016), *Klebsiella pneumonia* (MTCC 2405) and *Pseudomonas Aeruginosa* (ATCC 27853). Standard antibiotic meropenem was used as control. It was observed that cinnamon shows maximum activity and minimum activity was shown by ginger essential oil against the three bacterial isolates used. Clove essential oil shows moderate activity.

Keywords: *Zingiber Officinale*, *Syzigium aromaticum*, *Cinnamomum zeylanicum*, DPPH, Gallic acid, BHT, Antioxidant activity, Antibacterial activity, Zone of inhibition, Disc diffusion technique.

Introduction

Essential oils may have antioxidant properties and their consumption can influence immune cell functions. Also their use in food industry may serve to replace synthetic antioxidant food additives. Natural antioxidants, particularly in spices have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer^{1,2}. The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups; *i.e.* vitamins, phenolics and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while

carotenoids are known as lipophilic antioxidants³. In order to contribute to a better knowledge of their antioxidant properties *Syzygium aromaticum*, *Zingiber officinale* and *Cinnamomum zeylanicum* essential oils were investigated using two different assays.

(a) DPPH method It measures the radical-scavenging activity of antioxidants against free radical like 1,1-diphenyl-2-picrylhydrazyl radical. (b) Reducing power activity method. In this method the antioxidants will reduce Fe^{+3} to Fe^{+2} .

Antibacterial activity

It has been known from ancient times that essential oils from aromatic and medicinal plants possess biological activity, antibacterial, antifungal and antioxidant properties. Spices were used from ancient times for different purposes viz flavouring, keeping away the pests and in perfumery. Infectious diseases remain an important cause of morbidity and mortality in developing and developed nations⁴. Unfortunately, development of effective antibiotics has been accompanied by the emergence of drug-resistant organisms. It diminishes the clinical effectiveness of antibiotics⁵. There is therefore a need for continuous search for new, effective and affordable antimicrobial agents⁶. Due to the growing interest in the use of essential oil in food and both food and the pharmaceutical industries, a systematic study on these plant extracts have become very important.

Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the family Myrtaceae^{7,8}. Cloves are used in ayurveda, Chinese medicine and Western herbalism. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis⁹. It is also used in dentistry where the essential oil of clove is used as anodyne for dental emergencies^{10,11}. Ginger has been used as a spice and medicine in India and China since ancient times. Ginger plants were grown in pots and carried to abroad on sea long voyages to prevent scurvy. Ginger is widely used in ayurvedic medicines and in folklore medicines^{12,13}. Ginger contains 1-2 % oil, which imparts the unique flavour to the spice and it has been studied by many workers. Many reports are available on the chemical composition of fresh ginger oil and the naturally occurring flavoring compounds¹⁴.

Cinnamon has been known from remote antiquity. In medicine it acts like other volatile oils and once had a reputation as a cure for colds. The essential oil of cinnamon also has antimicrobial properties, which can aid in the preservation of certain foods¹⁵⁻¹⁸. Dean *et al.*, has reported fifty essential oils in different concentration for their antibacterial activity against 25 genera of bacteria¹⁹.

Experimental

Extraction of essential oil

The Clove, Ginger and Cinnamon essential oils were extracted separately with the help of Clevenger's apparatus in the department of chemistry, SHIATS. About 250 g of fresh ginger rhizomes were taken in the Clevenger's flask and distilled water was added to it. The temperature of heating mantle was set at 60-70 °C. After some time essential oil layer will be seen above the water layer. It was taken in a separating funnel and 0.5 mL diethyl ether was added. Proper shaking was done. Then essential oil was taken in a vial with the help of separating funnel. Then vial was kept on heating mantle for some time. As the boiling point of diethyl ether is less than the essential oil, it will become volatile and 2 mL Ginger essential oil was obtained. Same procedure was applied for extraction of essential oil from Clove dried buds and cinnamon bark.

Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH), methanol, BHT (Buta hydroxy toluene), ascorbic acid, potassium ferricyanide, FeCl_3 and trichloroacetic acid (TCA). All chemicals used including solvents were of analytical grade.

The antioxidant activity of Clove, Ginger and Cinnamon essential oils was evaluated by DPPH method and reducing power activity method in the department of Biochemistry and Biochemical Technology, SHIATS.

DPPH method

The antioxidant activity of methanolic stock solution of Clove, Ginger and Cinnamon essential oils were measured in terms of hydrogen donating or radical scavenging using the stable radical DPPH. The DPPH solution was prepared in methanol at a concentration of 0.002%. The diluted working solutions of essential oils were prepared in methanol at concentrations (1, 0.5, 0.25, 0.125, 0.0625 mg/mL). BHT was used as standard. Different concentrations of stock solutions (1 mL, 0.5 mL, 0.25 mL, 0.125 mL and 0.0625 mL) were taken in each test tube and volume was made up to 2 mL. Then 2 mL of methanolic solution of DPPH was added to all the samples. A control was taken in which 2 mL of methanol and 2 mL of DPPH was added. All these solutions were kept in dark for 30 min. Absorbance measurements were recorded at 517 nm using UV-Vis spectrophotometer. % Inhibition was calculated using the formula.

$$\% \text{ Inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

Where A=Optical density of control

B= Optical density of sample

Reducing power activity method

In this method a methanolic stock solution (1, 0.5, 0.25, 0.125, 0.0625 $\mu\text{g/mL}$) of Clove, Ginger and Cinnamon essential oils were prepared. Different concentrations of stock solutions (1, 0.5, 0.25, 0.125, 0.0625 $\mu\text{g/mL}$) were taken and distilled water was added and volume was made up to 2 mL. A control was taken in which 2 mL of methanol and 2 mL of distilled water was added. Different concentrations were taken. 2.5 mL Phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%) was added. After 20 min at 50 $^{\circ}\text{C}$. 2.5 mL TCA (trichloroacetic acid 10%.) was added to each sample. Then all the samples were centrifuged for 10 min at 3000 rpm. The upper layer (2.5 mL) was mixed with 0.5 mL FeCl_3 (0.1%). Absorbance measurements were taken at 700 nm against a blank using UV-Vis spectrophotometer. Increased absorbance of reaction mixture indicates increase in reducing power.

Bacterial strains

Three bacterial strains were used in this study. *Eschericia coli* (MCCB 0016), *Klebsiella pneumonia* (MTCC 2405), *Pseudomonas aeruginosa* (ATCC 27853). All the bacterial strains were obtained from C.M.P Degree College, Allahabad, India.

Screening of spice essential oils using disc diffusion technique

A standard disc diffusion method by Baurer *et al.*²⁰ was used. In each experiment micro organisms were cultured at 37 $^{\circ}\text{C}$ for 18 h and prepared to turbidity by Mc Farland standard²¹ no. 0.5. Then 100 μL of the suspension made in normal saline was spread on the Macconkey agar and Mueller Hinton Agar plate. Sterile discs (6 mm diameter) were impregnated with 10 μL of the essential oil and left for 30 mins to avoid excess prediffusion of oil. The discs were placed on the surface of petri plates. Meropenem was used as standard antibiotic (10 $\mu\text{g/disc}$). Two Macconkey agar and Mueller Hinton Agar plates without bacteria were used as media control. Plates were subsequently incubated at 37 $^{\circ}\text{C}$ for 18-24 h and zones of inhibition were calculated by measuring the diameter.

Results and Discussion

DPPH method

In order to determine the effect of concentration on radical scavenging power by DPPH method, five different working solutions (1, 0.5, 0.25, 0.125, 0.0625 mg/mL) were used for Clove, Ginger & Cinnamon essential oils. Results showed (Figure 1, 2 & 3) the percentage inhibition is in increasing order with the increase in concentration. IC_{50} for Clove & Cinnamon essential oil is 0.08 mg/mL and 0.1 mg/mL respectively. IC_{50} for Ginger essential oil is 0.19 mg/mL respectively. So, Clove and Cinnamon has much effective radical scavenging activity as compared to Ginger. Here BHT and gallic acid has been used as reference which exhibited maximum activity at all concentrations.

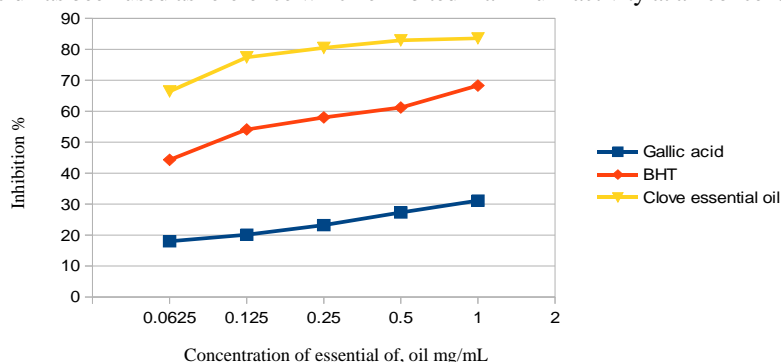


Figure 1. DPPH scavenging activity of BHT, Gallic acid and Clove essential oil

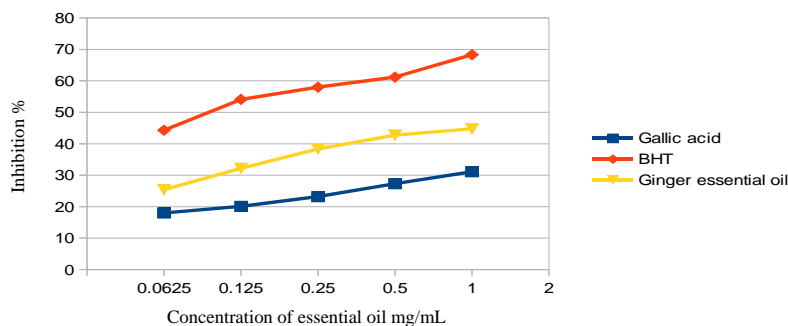


Figure 2. DPPH scavenging activity of BHT, Gallic acid and Ginger essential oil

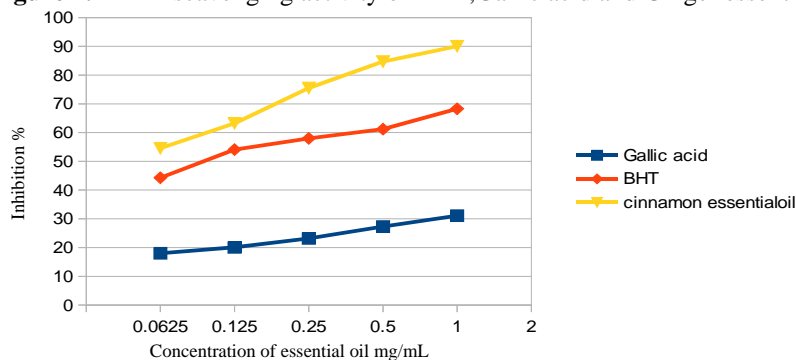


Figure 3. DPPH scavenging activity of BHT, Gallic acid and Cinnamon essential oil

Reducing power activity method

Reducing power characteristic of any compound serves as a significant indicator of its potential as antioxidant. Antioxidants will reduce Fe^{+3} to Fe^{+2} . The concentrations used were (1, 0.5, 0.25, 0.125, 0.0625 $\mu\text{g/mL}$) for Clove, Ginger and Cinnamon essential oils. Absorbance was read at 700 nm. Reducing power was found to be significant ($p < 0.01$). Reducing power of essential oil has been found to be significant ($p < 0.01$) and as good as BHT. The activities were statistically significant (Figure 4, 5 & 6) when compared with control.

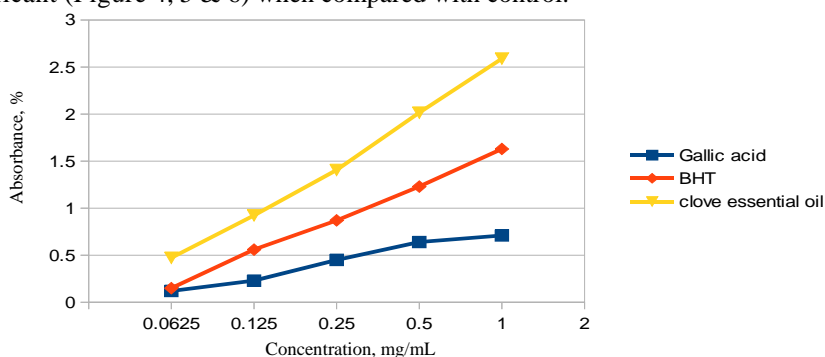


Figure 4. Reducing activity assay with BHT, Gallic acid and Clove essential oil.

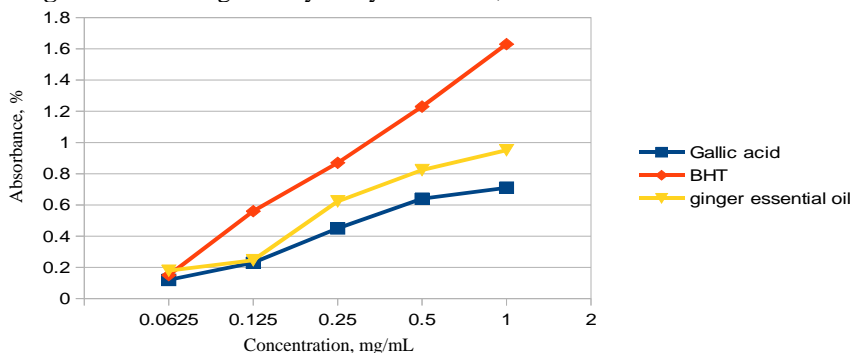


Figure 5. Reducing activity assay with BHT, Gallic acid and Ginger essential oil

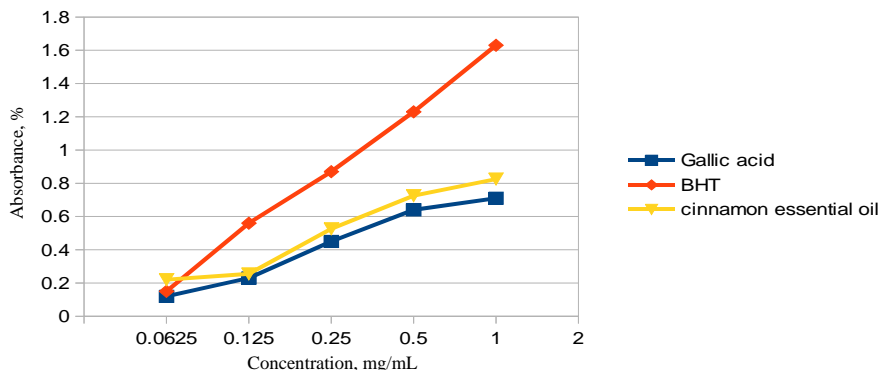


Figure 6. Reducing activity assay with BHT, Gallic acid and Cinnamon essential oil

As indicated in Table 1 among the three essential oils, Cinnamon essential shows maximum activity against *E.coli* (MCCB 0016), *Klebsiella pneumonia* (MTCC 2405) and *Pseudomonas aeruginosa* (ATCC 9027) with zone of inhibition 34, 19 and 21 mm diameter respectively. It is also clear from the table that Ginger essential oil is found resistant to *Klebsiella pneumoniae* and posses minimum activity against *Pseudomonas aeruginosa* (7.7 mm). It shows maximum activity against *E.coli* (MCCB 0016) with zone of inhibition 9 mm diameter respectively. It is also clear from the table that Clove essential oil shows maximum activity against *Pseudomonas aeruginosa* with zone of inhibition 18 mm diameter and minimum activity against *E.coli* with zone of inhibition 15 mm diameter. It shows moderate activity against *Klebsiella pneumoniae* with zone of inhibition 17 mm diameter. Cinnamon essential shows maximum activity against *E.coli* (MCCB 0016) with zone of inhibition 34 mm diameter which is comparable to standard antibiotic with zone of inhibition 33 mm diameter and minimum activity against *Klebsiella pneumonia* (MTCC 2405) with zone of inhibition 19 mm diameter. It shows moderate activity against *Pseudomonas aeruginosa* (ATCC 9027) with zone of inhibition 21 mm diameter. The standard antibiotic Meropenem (10 µg/disc) shows 33 mm zone of inhibition against *E.coli* (MCCB 0016), 31 mm zone of inhibition against *Klebsiella pneumonia* (MTCC 2405) and 34 mm zone of inhibition against *Pseudomonas aeruginosa* (ATCC 9027).

Table 1. Antibacterial activity of Ginger, Clove and Cinnamon essential oils (10 µL/6 mm disc)

Bacteria, Zone of inhibition in mm	Ginger rhizome essential oil	Clove bud essential oil	Cinnamon bark essential oil	Meropenem, Standard antibiotic
<i>Eschericia coli</i>	9	15	34	33
<i>Klebsiella pneumoniae</i>	resistant	17	19	31
<i>Pseudomonas aeruginosa</i>	7.7	18	21	34

Conclusion

From the above studies it can be concluded that the antimicrobial activity of the selected essential oils would be helpful in treating various kinds of diseases as they possess promising antibacterial properties against gram negative bacteria. The bioactive compounds from ginger, clove and cinnamon essential oils can be used as effective antibacterial agents after further studies. With the recent trends in the increase in resistance of microorganisms against various antibiotics it can be suggested that use of Ginger, Clove and cinnamon essential oil would be helpful in the treatment of various diseases.

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