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# Comparative Analysis of Antibacterial Properties of Clove and Ginger Extracts Using Gram Negative Bacteria *E.coli*

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## ABSTRACT

Spices are predominantly used for their flavour, and aroma in foods and beverages. Since decades they are known to have diversified uses. The nutritional, anti-oxidant, anti-microbial and medicinal properties of spices have far-reaching implications. Clove is aromatic flower buds of a tree Syzygium aromaticum a commonly used spice. All over the world Clove has been used successfully since ages as it is one of the most effective antibacterial agent .Ginger (Zingeiber officinale) a member of family zingerbeceae is also known for its medicinal use. It is prescribed for treating headache, nausea, rheumatism and cold etc. It also has a capacity to eliminate harmful bacteria responsible for diarrhoea. Growth curve and well diffusion were the methods used for the study. In comparative study of clove and ginger it was found that both are equally effective in inhibiting the growth of E.coli. Escherichia coli commonly known as E.coli are the most common bacteria in human intestine, which helps in preventing the entry of pathogenic microorganisms. Thus it serves as a better experimental model for bacteriological and anti-microbial studies. Growth curve was studied with the help of optical density with the help of Elico CL63 Photometer. Growth curve method indicated that the sterile aqueous extract of clove was more effective than the sterile aqueous extract of ginger, whereas well-diffusion method also indicated that crude extract of clove is more effective than wet ginger extract.

**Keywords :** Syzygium aromaticum, Zingeiber officinale, Escherichia coli, Inhibition zone.

## I. INTRODUCTION

Microbiome plays a major role in healthy living of an organism [1]. A healthy, balanced and proper diet is one of the key factors for healthy living.Gut encloses several types of bacteria including *E.coli*. Contaminated food and water are the main sources of this microflora that enters our gut. Few *E.coli* species help in preventing diseases as well as building up the immune response of living organisms, while others can be a causative agent for various diseases [1]. Healthy and proper diet comprises of the food rich in nutritional value as well as taste. Taste being the key factor of raised appetite is brought about by the addition of spices to the food. Spices not only adds flavor to food but also gives it a rich aroma that enhances the craving for food [2]. Spices not only hold a vital place in food industry but also in pharmacology. Spices such as cardamom, cinnamon, black pepper, asafoetida, bay leaves, clove, cumin seeds garlic, ginger, onions etc are been used in every kitchen since ages. Apart from its use in kitchen they are been widely used for various medicinal properties [7,8].

Clove is aromatic flower buds of a tree *Syzygium aromaticum* has been used for its antiseptic and analgesic effects and has been studied for use as an anticoagulant and anti-inflammatory effects [6]. Clove has a long history of culinary and medicinal use. The oil was used as an expectorant and antiemetic with inconsistent clinical results [7,8]. Clove tea was used to relieve nausea. Use of the oil in dentistry as an analgesic and local antiseptic continues today.

Clove buds yield approximately 15% to 20% of a volatile oil that is responsible for the characteristic smell and flavor. The bud also contains a tannin complex, a gum and resin, and a number of glucosides of sterols. The principal constituent of distilled clove bud oil (60% to 90%) is eugenol (4-allyl-2-methoxyphenol). Clove oil is applied for the symptomatic treatment of toothaches and is used for the treatment of dry socket (post extraction alveolitis) [7,8,9].Clove oil is reported to have antihistaminic and antispasmotic properties, most likely due to the presence of eugenyl acetate. Cloves are also said to have a positive effect on healing stomach ulcers. A 15% tincture of cloves is effective in treating topical fungal, ringworm infections. As with many other volatile oils, clove oil inhibits gram-positive and gram-negative bacteria [10].

Ginger scientifically known as *Zingeiber officinale*) a flowering plant, is widely used as a spice or a folk medicine. It has been used for its antibacterial, antifungal, pain-relieving, anti-ulcer, anti-tumor and other properties. Ginger is originated in the tropical rainforests from the Indian subcontinent to southern Asia where ginger plants show considerable genetic variation.it is well advised to use as it is cheap and have large effective cure [15].

## II. METHODS AND MATERIAL

**Collection of Sample**: clove was collected from the local grocery shop and fresh ginger was also collected from local market Kalyan.

**Preparation of Extracts**:

**Aqueous soxhlet extract:**

The powdered form of clove was used for the experiment. The aqueous extract of clove was prepared using the soxhlet apparatus. 25 Gms of clove powder was used for preparing 100 ml of extract using distilled water. This extract was further concentrated to 25 ml by evaporation. The extract was then autoclaved and stored in sterile condition at 4$^{o}$C.

The ginger were washed and dived in two parts. One part of the ginger was used for the aqueous

extract preparation and the other part was kept at 40ºC for drying. The aqueous extract of fresh as well as dried ginger was prepared using the soxhlet apparatus. 25 Gms of fresh ginger and dried ginger powder was used for preparing 100 ml of extract separately using distilled water. The extract was then autoclaved and stored in sterile condition at 4ºC.

**Crude extract preparation:**

Crude extract was prepared using 25 gms of clove in 50 ml of distilled water and was further evaporated to 25 ml separately cooled and preserved at 4ºC till further use.

Crude extract of ginger was prepared using 25gms of wet ginger and dried ginger in 50ml distilled water separately and was evaporated to 25ml, cooled and preserved at 4ºC till further use.

**Preparation of bacterial suspension**:

Pure isolated colonies of the *E.coli* were obtained from the Department of Microbiology Birla College Kalyan. Colonies of *E.coli* were sub-cultured on a nutrient agar slant and was further preserved in refrigerator.

**Preparation of nutrient broth**:

25 ml of nutrient broth was prepared using distilled water, autoclaved and stored at 4ºC.

**Preparation of nutrient agar plates**:

20 ml of nutrient agar was poured on sterile plates

**ANTIMICROBIAL ASSAY BY GROWTH CURVE METHOD:**

**Growth curve analysis using sterile aqueous extract:**

24 ml of sterile autoclaved nutrient broth was inoculated with 1ml of soxhelated clove extract and 1ml of *E.coli* culture. It was then incubated in shaker incubator at 37ºC for 11 hrs. , and the growth was analyzed at regular intervals of 1 hr. similarly growth analysis of *E.coli* was studied using ginger extract. All the studies were performed in triplicates. Control was also maintained similarly using sterile distilled water.

**Growth curve analysis using crude extract:**

24 ml of sterile autoclaved nutrient broth was inoculated 1ml of *E.coli* culture and 1ml of crude clove extract separately and was incubated in shaker incubator at 37ºC for 3hrs. , and the growth was analyzed at regular intervals of 10 min. similarly growth curve analysis of *E.coli* was studied using 1ml of *E.coli* culture and 24 ml of clove and ginger extract individually, was also All the studies were performed in triplicates. Control was also maintained similarly using sterile distilled water.

**ANTIMICROBIAL ASSAY BY WELL DIFFUSION ASSAY:**

Nutrient agar plates were used as medium for screening antibacterial activity.100µl of *E.coli* culture was spread on culture plates uniformly using a sterile spreader. Wells of 1cm were dug out using a sterile cork borer in solidified agar medium. The plates were divided into three quadrants I, II, III. Dilutions of 1%, 10% and 100% solutions of crude extracts were used. The plates were incubated at 37°C for 24hrs and observed for inhibition zone of growth around the wells.

## III. RESULTS AND DISCUSSION

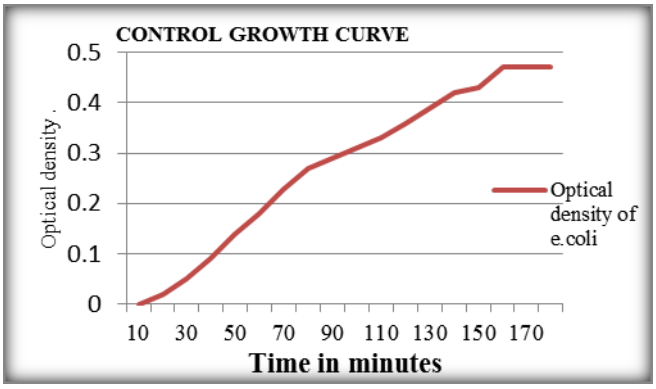


**Figure 1**

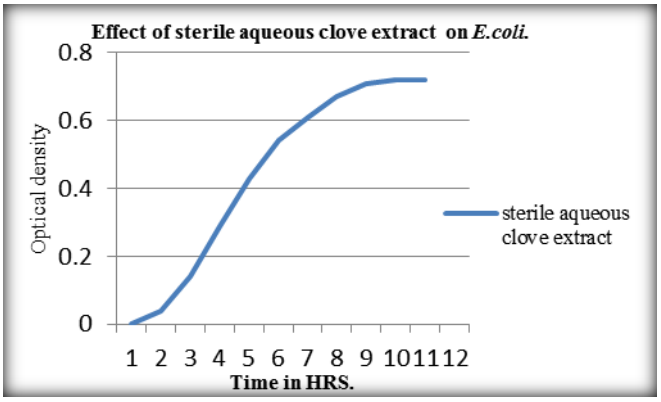


Figure 2

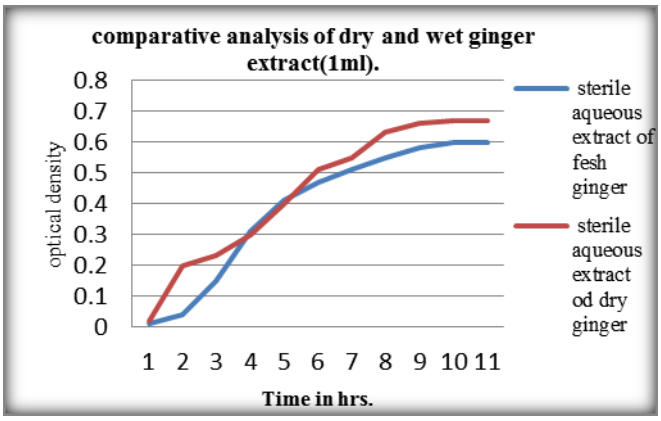


Figure 3

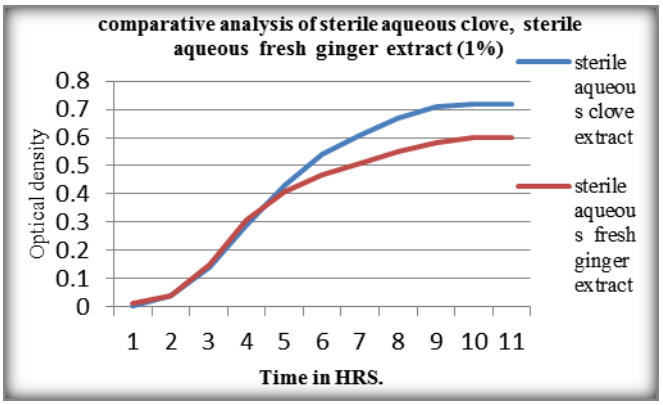


Figure 4

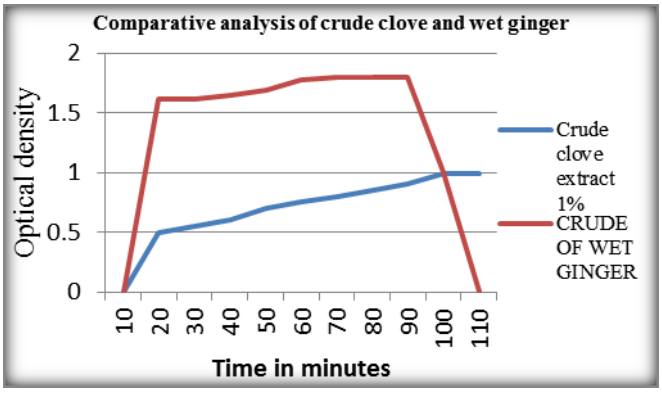


Figure 5


**Figure 6.** Inhibition zone of control sample.


**Figure 7.** Inhibition zone of crude clove extract at different concentrations.

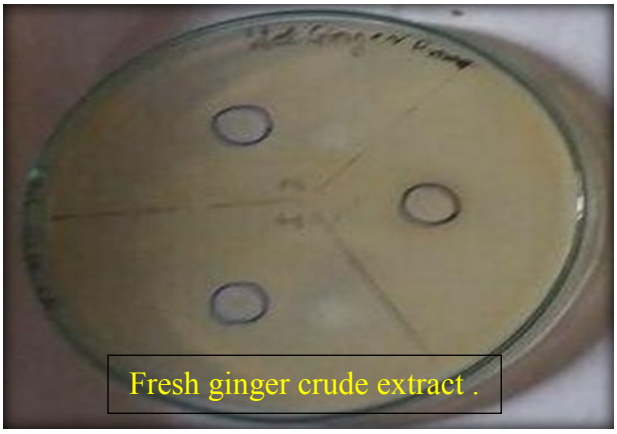


**Figure 8.** Inhibition zone of crude fresh ginger extract at different concentrations.

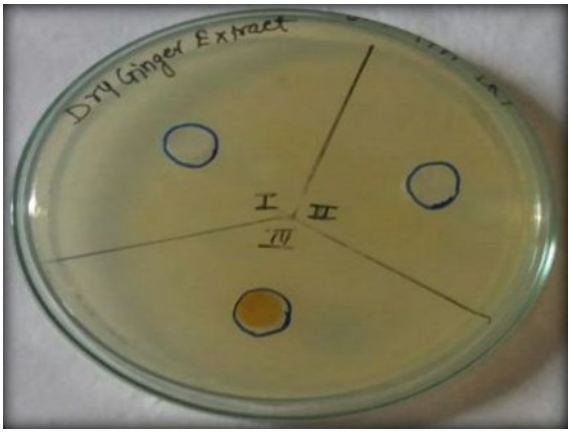


**Figure 9.** Inhibition zone of crude dry ginger extract at different concentrations.

**Table 1.** Showing the inhibition zone of crude extract of clove, crude extract of fresh ginger and crude extract of dry ginger at three different concentrations.

| | Crude extract of clove. | | | Crude extract of fresh ginger. | | | Crude extract of dry ginger | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|
| **Concentration.** | 1% | 10% | 100 % | 1% | 10% | 100% | 1% | 10% | 100% | 100µl *E.coli.* |
| **Inhibition zone.(cm)** | 1.6cm | 1.8cm | 2.2cm | 1.2cm | 1.4cm | 1.6cm | 1.2cm | 1.4cm | 1.4cm | 0 cm |

Figure 1 represents the growth curve of control sample which was not exposed to any herbal extract while the Figure 2 represents the growth curve of E. coli where the sterile aqueous extract of clove was incorporated with the culture medium at the concentration of 1 ml/25 ml of nutrient broth. It was found that sterile aqueous extract of clove was more effective in inhibiting the growth of *E.coli* as compared to control. Figure 3 represents the comparative analysis of sterile aqueous extract of dry and fresh ginger which proved that fresh ginger was more effective than dry ginger extract. Fig 4 represents that in comparative analysis of sterile aqueous extract of clove, 1ml extract / 25 ml of nutrient broth is more effective than fresh ginger extract. From Fig 5 it is evident that crude aqueous extract of clove is effective than fresh ginger extract. Figure 6 indicates the control growth of E.coli on nutrient agar plate.

The agar ditch method primarily focus on the inhibition zones formed using three different concentrations of crude extracts of clove and ginger extracts respectively, as seen in fig .7,8, and 9. no inhibition zone is seen in control agar ditch filled with sterile distilled water. Whereas the inhibition zones formed around the crude extracts of clove and ginger increases with an increase in concentration used (fig 7, 8, 9, table 1).

Clove also well known as *Syzygium aromaticum* has various phytochemicals identified and extracted using various extraction solvents. Sixteen volatile compounds were identified from the n-hexane extract of the buds of Syzygium aromaticum by using gas chromatography-mass spectroscopy (GC-MS).

The major components were eugenol (71.56 %) and eugenol acetate (8.99 %). The dichloromethane extract of the buds yielded limonin and ferulic aldehyde, along with eugenol. The flavonoids tamarixetin 3-O-b-D-glucopyranoside, ombuin 3-O-b-D-glucopyranoside and quercetin were isolated from the ethanol extract [4]. In past the antimicrobial properties of these componds are been studied using various extraction solvents [4]. As stated above in this investigation water was used as an solvent .when compared with the earlier literature it was found that clove possess antibacterial properties and thus was able to inhibit the growth of *E.coli.* [5]

Ginger, the rhizome of the *Zingeiber officinale*, plays an important role in prevention of diseases. Numerous active ingredients are present in ginger including terpenes and oleoresin which called ginger oil. Ginger also constitutes volatile oils approximately 1% to 3% and non-volatile pungent components oleoresin [18]. The major identified components from terpene are sesquiterpene hydrocarbons and phenolic compounds which are gingerol and shogaol [18] and lipophilic rhizome extracts, yielded potentially active gingerols, which can be converted to shogaols, zingerone, and paradol. It is reported that ginger extract has antimicrobial properties these extracts are been prepared using numerous extraction solvents. The current investigation primarily focuses on the detection of antibacterial properties using aqueous extracts. It was found that fresh ginger extracts were more effective in inhibiting the E.coli growth than dry ginger extracts but in comparison to clove, clove was found to me more effective.

## IV. CONCLUSION

The antimicrobial studies on Clove and fresh Ginger indicated that fresh Ginger was more effect than the dry Ginger. Whereas in further investigation using sterile aqueous extracts it was observed that fresh Ginger was more effective than Clove. In agar ditch method it was observed that crude extract of clove was more effect than Ginger in inhibiting the growth of *E.coli*.

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