



In vitro screening of *Eugenia caryophyllata*, local medicinal plant for antimicrobial activity by using disc diffusion method

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ABSTRACT

The achievement of therapy lies in the constant search for novel medications to counter the challenges posed by resistant strains of the microorganisms. Traditionally *Eugenia Caryophyllata* is used for the treatment of countless diseases. In the present study '*Eugenia Caryophyllata*' belongs to the family of Myrtaceae was investigated for in vitro antimicrobial activity against the different types of bacteria, stains namely, *Staphylococcus aureus*, *Bacillus Pumilus*, *Pseudomonas aeruginosa* and *Escherichia coli* by applying disc diffusion method. *Eugenia Caryophyllata* was found to have shown inhibitory effect against tested bacteria. Among the types of tested bacteria *Eugenia Caryophyllata* was more effective against the gram positive bacteria and is less effective against gram negative bacteria. The current study provides a scientific background for the remarkable use of this plant for the treatment of various pathological diseases.

Keywords: *Eugenia Caryophyllata*, antimicrobial, disc diffusion

I. INTRODUCTION

It is unbearable to conceive the existence of human survival, if the earth had no plants. The credibility of human on plants is from the very beginning. Plants are the accustomed authority for the medicines. The specifically desired aim for the use of herbs was to achieve confident collaboration

with body chemistry [1]. The use of medicinal plants for the treatment and prevention of various diseases world widely, because of its efficiency and have low toxicity [2]. The medicinal plants are usually used in those situations mostly which are not chronic and not lethal as associated to the Allopathic medicine in each culture [3]. In

Pakistan, the medicinal plants are frequently used in rural areas as compare to urban. The use of such alternate medicinal plants occurs in several forms for the treatment of different types of diseases [4]. *Eugenia Caryophyllata* (clove) belongs to family Myrtaceae [5]. During the 14th century the clove trade act as a stimulating agent in the grounds of commerce at ports, especially in Asia and Europe, where it was traded for sufficient income. The important clove trade price vigorous analysis, trips in search for new sources of this extremely praised specie and the launch of new sea avenues [6]. In different centuries, its trade went through different phases such as extraordinary trade prices, struggle over control on industries, low trade prices and even trafficking of seedlings for cultivation [7]. Traditionally, numerous part of the plant such as buds and leaves are consumed in pharmacy, cosmetic, perfumes and in cooking etc. Clove has been recycled in antique china as a fragrance and spice etc for more than 1800 years [8].

Clove oil were used for the treatment of tooth pain for the first time in 1640 in 'Practice of Physic' in France [9]. The active ingredient of clove oil (eugenol) is derived from the species name *Eugenia Caryophyllata* which contains a high magnitude of eugenol (45–90%) in addition to acetyl eugenol, acetyl salicylate and chavicol [10]. Clove has antibacterial activities and use In dentistry, eugenol along with zinc oxide for temporary teeth's filling [11]. It has an antiseptic,

bacteriostatic as well as bactericidal effect against several bacteria such *E. coli* and *Staphylococcus aureus* [12]. Its oil is generally used in dentistry to get rid of pain from a dry socket and also utilize in different types of temporary filling materials. In the dental clinics clove oil often leaves a sustained, aromatic smell due to its strong aroma [13]. Clove oil also have an inhibitory action against different microorganisms such as *S. aureus*, *Aspergillus* and *L. monocytogenes* [14].

Synergistic activities of clove oil along with other oils of coriander, dill, eucalyptus and cilantro showed a greater degree of inhibition on various types of gram negative bacteria. Thus proof that the synergism exaggerates the antimicrobial efficacy of clove oil [15]. Different scientific studies have been carried out on *Eugenia Caryophyllata* oil and its main primary constituent eugenol; disclose pharmacological efficiency such as analgesic and anesthetic properties. In addition, anti-inflammatory, anti-oxidant anticonvulsant, [16], anti-mutagenic [17], anti-carcinogenic [18], repellent and ant-fumigant activities have been described [19]. Clove is also usually applied in the food industry as a natural antiseptic to enhance shelf life due to the active antimicrobial actions against some food produced microorganisms [20]. Conventionally clove oil has been used in Chinese as carminative, antispasmodic, antibacterial and anti-parasitic agents, while, the buds of clove were

used for the remedy of dyspepsia, also, in acute and chronic gastritis and diarrhoea [21].



Figure 1. Dried flower buds of *Eugenia Caryophyllata*

II. MATERIAL AND METHODS

Instruments and apparatus

Analytical Balance, Autoclave, Memmert Incubator, Heating Plate, Stability Chamber Petri dishes, Agar media and Laminar flow hood.

Bacteria and reagents

1. The bacteria (*Staphylococcus aureus*, *Bacillus Pumilus*, *Pseudomonas aeruginosa* and *Escherichia coli*) and the reagents were provided by Talha Medical laboratory MKD and were identified by the microbiologist, Mehboob ur Rahman, supervisor of the said Lab.

Disc Diffusion method

In the current activity Disc diffusion scheme for antimicrobial liability was explored according to the reported plan by Bauer et al

(1966). To identify the presence of the antibacterial moieties in the plant extracts, a bacteria culture was attuned to 0.5. The petri dishes were dried out for 15 minutes and then utilized for the antibiotic test. The discs which had been immersed with the *Eugenia Caryophyllata* extracts were placed on the Mueller-Hinton agar externally. Each test plate consists of four discs. One for standard act as positive control (commercial antibiotic) disc, one for negative control and two treated discs, samples under study. The standard antibiotic discs were clarithromycin (30 µg) and Amoxicillin (10 µg) were applied on the mentioned bacteria. The plates were then incubated for 18 to 24 hrs at 37°C, depending on the kinds of bacteria utilized in the test samples. After the incubation, the plates were studied for zone of inhibition. The zone of inhibition was then calculated using callipers and then recorded. The tests were repeated four times to confirm reliability of the activity.

Antimicrobial Activity

Antimicrobial potential of *Eugenia Caryophyllata* was explored against four tested microorganisms (two-gram negative and two gram positive bacteria). Amoxicillin and Clarithromycin were used as a standard antimicrobial reagent. The Mueller Hinton agar medium was formulated by dissolving 38 gm per dm³ of distilled water. The

mixture was heated gently up to boiling. The agar media was sterilized by autoclaving at 15 psi for 15 minutes. At 45-50°C the media was cooled, after cooling it was transferred into sterile Petri dishes (4 mm deep). Sterile swab was immersed in the tube comprising microbial suspension. The Swab was lined on the required surface of the Mueller Hinton agar medium to make a complete lawn of bacterial suspension under the laminar flow hood [22]. Inoculums were incubated at room temperature for 5 minutes to adjust in media. After a 5 minutes' interval empty discs were placed with the help of sterilized forceps aseptically on media. Three ml of VBO 3% was taken [23]. This required quantity of 3% ECO was poured on those empty discs with the help of micro pipette. Petri dishes were incubated for 24 hours at 37°C overnight. It was taken out of the incubator and zone of inhibition made around the discs were calculated in terms of the diameter (mm) with the help of a calliper and was matched with the standard [24].

III. RESULT AND DISCUSSION

To explore the antimicrobial potential of *Eugenia Caryophyllata* two different kinds of gram negative and gram positive bacteria were selected. *Staphylococcus Aureus* and *Bacillus pumilus* were chosen from gram positive and *Pseudomonas aeruginosa* and *E. Coli* were chosen from the gram negative bacteria. Best antibacterial potential was detected against gram positive (*Staphylococcus aureus*, *Bacillus Pumilus*) bacteria and gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria. The Zone of inhibition of *Eugenia Caryophyllata* was related with the standard drug, Amoxicillin and Clarithromycin. The obtained results revealed strongest inhibitory potential against the gram positive bacteria, *Staphylococcus Aureus* and *Bacillus pumilus* while showed less effect against the gram negative bacteria *Pseudomonas aeruginosa* and *E. Coli* shown in table 1 and figure 2.

Microorganisms	Zones of Inhibitions (mm)			
	Negative control	Clarithromycin standard disc	Amoxicillin standard disc	ECO Sample
<i>Staphylococcus aureus</i> (gram positive)	0	34	28	21
<i>Bacillus Pumilus</i> (gram positive)	0	31	33	23
<i>Pseudomonas aeruginosa</i> (gram negative)	0	10	12	7
<i>Escherichia coli</i> (gram negative)	0	6	2	4

Table 1. Antimicrobial activity of negative control. Standard and ECO

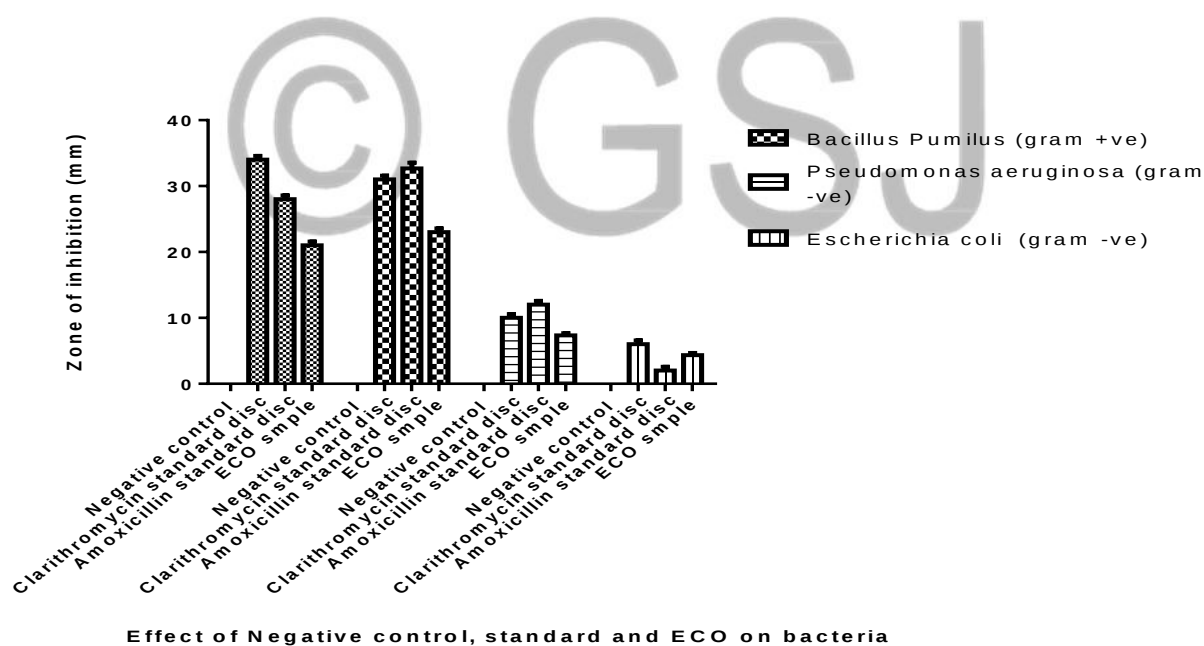


Figure 2. Zone of Inhibition of negative control. Standard and ECO.

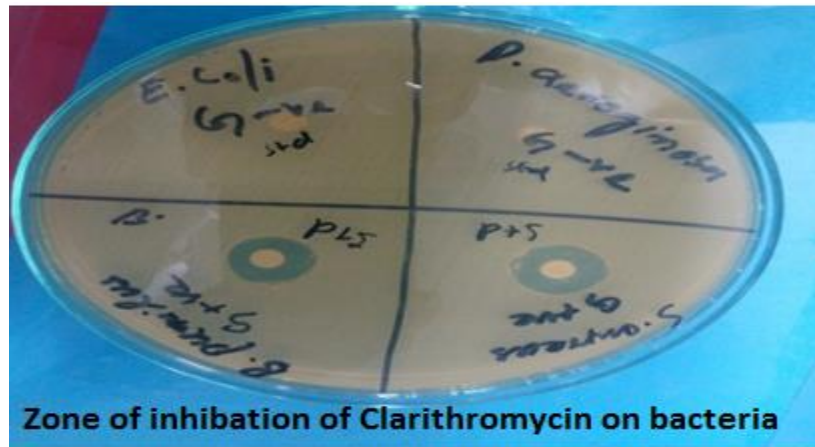


Figure 3. Zone of Inhibition of standard on different bacteria

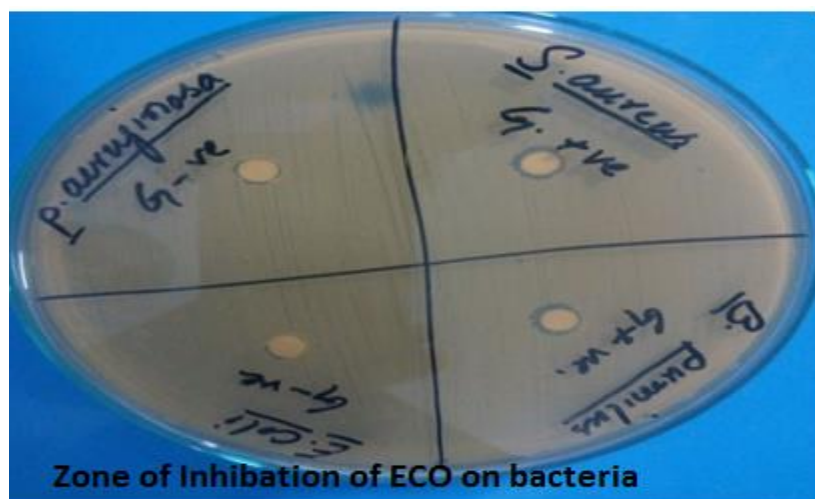


Figure 4. Zone of Inhibition of ECO on different bacteria

IV. CONCLUSION

In the current work, *Eugenia Caryophyllata* oil was studied. Based on the obtained result the following points are concluded.

1. *Eugenia Caryophyllata* showed anti-microbial activity due to the existence of antibacterial moieties.
2. Oil of *Eugenia Caryophyllata* was more potent against the gram positive bacteria (*Staphylococcus aureus*, *Bacillus Pumilus*) and less effective against the gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*).
3. The results provide justification for the usage of the *Eugenia Caryophyllata* in common medicine to treat numerous contagious diseases.

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