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RESEARCH ARTICLE

Investigating and Exploiting the Antibacterial Potential of Clove (*Eugenia caryophyllyum*) Extracts while Utilizing it to the Maximum to Develop Liquid Soap against Drug Resistant Bacteria Causing Skin Diseases

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ABSTRACT

The present study was carried out to investigate the effectiveness of antibacterial potential of decoction and powder extract of Clove against multiple drug resistant bacteria such as *Escherichia coli* (3), *Pseudomonas aeruginosa* (7), *Staphylococcus aureus* (4), *Enterococcus faecalis* (3). The screening using extracts was performed by standard Disc diffusion method, well-diffusion and MIC (Minimal inhibitory concentration) method. This is a comparative study of the two extracts of clove. This comparative study showed that decoction of clove exhibited maximum activity against *Escherichia coli* with 32 mm mean diameter of zone of inhibition, but powder extract was more effective against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* with 34 mm, 30 mm and 33 mm mean diameter of zone of inhibition respectively. Antibacterial activity of both the extracts using macrobroth dilution method was characterized by minimum inhibitory concentration (MIC) ranges of 0.0625-0.25 x10^4 µg/ml for gram negative and 0.0625- 0.25 x 10^4 µg/ml for gram positive for clove decoction and 0.0625- 0.125 x 10^4 µg/ml and 0.0625- 0.125 x 10^4 µg/ml for gram negative and gram positive bacteria respectively, in case of clove powder. The clove oil being used till date is prepared by extracting oil from clove while solid remains of the clove are wasted. Additionally, this idea will make maximum utilization of clove without the need to waste solid remains.

Key words: *Eugenia caryophyllyum*, clove, decoction, antibiotic sensitivity, disc diffusion method.

INTRODUCTION

Infectious diseases remain an important cause of morbidity and mortality in developing and developed nations [1].They account for approximately one half of all deaths in tropical countries of which bacterial infections seems to be the most prevalent [2]. Infectious diseases account for about half of the deaths in tropical countries [3]. Bacterial infections if untreated can lead to serious and life threatening complications such as sepsis, kidney and liver failure, toxic shock and even death. Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents [4]. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs [5]. Although medicinal plants produce slow recovery, the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms [6].

Syzygium aromaticum, belonging to Myrtaceae, is a moderate-sized, conical, evergreen tree that attains a height up to about 12 m. Cloves are aromatic dried unopened dark reddish-brown, 13 – 21mm long floral buds of the evergreen tree, *Syzygium aromaticum* belonging to the family *Myrtaceae* [7,8]. Clove bud oils have various effects such as antibacterial, antifungal, insecticidal, anti-inflammatory, antioxidant [9], and

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antifungal effects [10]. These are used traditionally as flavouring agent and as antimicrobial material in food [11,12]. For example; clove oil was effective against *L. monocytogenes* and *S. Enteritidis* in tryptone soya broth (TSB) and cheese [13]. The high levels of eugenol present in clove essential oil give it strong biological activity and antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability [14,15]. Clove oil also has several therapeutic effects, including antiphlogistic, antivomiting, analgesic, antispasmodic, anticarminative, kidney reinforcement, antiseptic, HCMV extracorporeal restraining effect [16]. In Korea, clove oil has been successfully used for asthma and various allergic disorders by oral administration [17]**.**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 peristalsis [18]. It is also used in dentistry where the essential oil of clove is used as anodyne for dental emergencies [19,20]. Clove buds and their essential oils have been known to possess various antimicrobial properties [21].The present study was therefore conducted to evaluate the antibacterial potential of clove decoction and powder against 17 different isolates belonging to 4 different species of bacteria viz., *Escherichia coli* (3), *Pseudomonas aeruginosa* (7), *Staphylococcus aureus* (4), *Enterococcus faecalis* (3).

MATERIALS AND METHODS

Maintenance of isolates:

A total of 17 gram positive and gram negative bacterial isolates were selected for study. The microbial strains used in this study were obtained from Yes Diagnostic Centre, Jaipur (Rajasthan) and Dhanvantari Hospital, Jaipur (Rajasthan). The microbial strains studied were *E.coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecalis.* The isolates were maintained on Nutrient Agar.

Preparation of decoction:

The aqueous decoction was prepared by boiling 10 gm clove in 100 ml distilled water in a flask for 20 minutes. The contents was removed from heat and allowed to cool. The content of flask was filtered to obtain clear decoction.

Preparation of clove powder:

The dried clove was ground in fine powder. 10 gm of dried powder was boiled in 500 mL of distilled water. The cooled filtrate was used.

Screening for the antimicrobial potential:

1) Agar diffusion susceptibility test [22] :

The bacteria cultures were grown in Brain Heart Infusion liquid medium at 37 °C. After 6 h of growth, each microorganism, at a concentration of 10^6 cells /mL, was inoculated on the surface of Muller-Hinton agar plates. Subsequently, filter paper discs (6 mm in diameter) saturated with plant extract (clove and garlic) (50 µL) were placed on surface of each inoculated plate. To evaluate the efficiency of the methodology, each extract was inserted simultaneously in a well (50 µL) in new plates. The plates were incubated at 37 °C for 24 h. After this period, inhibition zone was observed. Overall, cultured bacteria with inhibition zone equal to or greater than 7 mm were considered susceptible to either the tested extract. Antibiotic susceptibility discs of Ciprofloxacin were used as control.

2) MIC (Minimal Inhibitory Concentration) determination using Broth Macro Dilution Method: The extracts were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial sample. Bacterial samples were grown in nutrient broth for 6 h. Then 100 µL of 106 cells/mL was inoculated in tubes with nutrient broth supplemented with different concentrations (1- 0.0625 µg/ ml prepared by 2 fold serial dilution) of the extracts. After incubation for 24 h at 37 °C, the MIC of each sample was determined by measuring the optical density in the spectrophotometer (620 nm), taking non inoculated nutrient broth as a standard.

PREPARATION OF SOAP: (UPCOMING PATENT)

Sterility test:

Sterility of the soaps thus prepared was tested using Fluid Thioglycollate medium and Soybean Caesin Digest medium. The two mediums were prepared, autoclaved and dispensed in boiling tubes. Whatmann filter paper discs impregnated with the liquid soaps were then put into the above tubes. The tubes were then incubated at 37ºC for 15 days. The tubes were observed daily for the increase in turbidity.

Test for the effectiveness of the soap:

To test the effectiveness of the soap following two methods were used:

1). A swab culture from bare hands (exposed to the environment) was taken and was plated onto the nutrient agar plates. Another swab culture from same hands after washing it with the above soaps were taken and plated onto another plates of nutrient agar. These plates were then incubated at 37º C for 24 hrs and observed.

2). A thumb impression of the bare hands (exposed to the environment) was made on one half of the nutrient agar plate. While on the other half of the plate a thumb impression was made after washing hands with above soaps. The plates were then incubated at 37º C for 24 hrs and observed.

Interpretation:

Complete absence or a decrease in number of bacteria after using soap as compared to the no. of colonies before washing hands indicate the effectiveness of the soap.

Observation:

Comparatively lesser growth was observed in the plates or the portions of the plate inoculated after washing hands with the soap which shows the effectiveness of the soap. Slight growth which appeared in these regions may be ascribed to presence of water on the hands after washing, use of saline to keep the swabs wet, and exposure to the environment after washing hands and before taking swab cultures.

RESULTS AND DISCUSSION

The present study was done to investigate the antimicrobial activity of clove (decoction and powder extract) against 17 isolates of 4 species of gram positive and gram negative bacteria. Both the extracts exhibited growth inhibition of both gram positive and gram negative bacteria on MHA (Muller Hinton Agar). The observed zones of inhibition on agar of gram-positive and gram-negative bacteria were comparable to those elicited by ciprofloxacin, showing that the isolates exhibited susceptibility. The observed inhibition zone diameter for different extracts were as follows: Clove decoction-

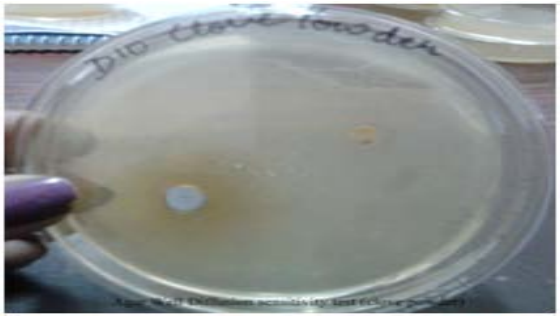

Figure 1: Nutrient agar plate showing well diffusion test by clove powder for gram negative bacteria (*E.coli)* (28 mm)

23-21 mm for gram positive bacteria and 32-26 mm for gram negative bacteria. Clove powder showed more promising results as compared to clove decoction with inhibition zone diameter of 33-30 mm for gram positive and 34-28 mm for gram negative bacteria (**Fig 1, Fig 2 & Table 1**). Inhibition zones having diameter less than 10 mm were considered as negative results.

Figure 2: Nutrient agar plates showing well diffusion test by clove powder for gram negative bacteria (*pseudomonas aeruginosa*) (34 mm)

The microorganism *E.coli*, which is already known to be multi resistant to drugs, was sensitive to both the extracts. On the other hand, *Pseudomonas aeruginosa*, which is also resistant to different antibiotics had its growth inhibited by the 2 extracts with a maximum sensitivity towards clove powder. In case of gram positive bacteria, both *Enterococci faecalis* and *Staphylococcus aureus* were found susceptible to both the tested extracts showing maximum susceptibility to clove powder.

Further antimicrobial activity testing of these extracts by macrobroth dilution method also revealed growth inhibition in liquid media with MIC's of 0.0625-0.25 x 10^4 µg/ml for gram negative and 0.0625- 0.25 x 10^4 µg/ml for gram positive for clove decoction. 0.0625- 0.125 x 10^4 µg/ml and 0.0625- 0.125 x 10^4 µg/ml for gram negative and gram positive bacteria respectively, in case of clove powder (Table.1).

The present study revealed that all the bacterial isolates tested were sensitive to both the clove decoction and clove powder. Clove powder being more effective as compared to decoction with maximum inhibition zone diameter of 23 mm and 32 mm for gram positive and gram negative bacteria respectively in case of decoction and 33 mm for gram positive and 34 mm for gram negative bacteria for clove powder (**Fig 3**).

Figure 3: Nutrient agar plates showing well diffusion test by clove decoction for gram negative bacteria (*E.coli*)(32mm)

The results of the present study are in harmony with the fact that that clove aqueous infusion and decoction is effective against *E. coli* and *P. Aeruginosa* [23].In one similar study the clove oil was found effective against non-toxigenic strains of *E. coli* O157:H7 [24].

Similarly, in another study clove oil was found active against foodborne Gram positive bacteria (*Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis* and *Listeria monocytogenes*) and Gram-negative bacteria (*E. coli, Yersinia enterocolitica, Salmonella choleraesuis* and *P. aeruginosa*) [25]. Furthermore, active constituents of clove (biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanoic acid) possess antibacterial activities against Gram-negative anaerobic periodontal oral pathogens, including *Streptococcus mutans, Actinomyces viscosus, Porphyromonas* and *Prevotella intermedia* [19]. It has also been reported that the extract of clove potently inhibited the growth of *Helicobacter pylori* [26,-27]. In a study carried out clove extract showed inhibitory effect against *S. Aureus* [28].

Table 1: Table representing results of Screening observed due to Antibacterial Potential of *Eugenia caryophyllyum* (Clove)

S. No	Name of Microorganism and number of isolates	Agar Well Diffusion Test		MIC (Minimal Inhibitory Concentration) In (x 10^4 µg/ ml)			
		Inhibition Zone Diameter (mm)		Decoction		Powder	
		Decoction	Powder	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Gram Negative Bacteria							
1	*Escherichia coli* (3)	32 mm	28 mm	0.0625	0.0625	0.125	0.125
2	*Pseudomonas aeruginosa* (7)	26 mm	34 mm	0.125	0.25	0.0625	0.125
Gram Positive Bacteria							
3	*Enterococci faecalis* (3)	21 mm	33 mm	0.0625	0.0625	0.0625	0.125
4	*Staphylococcus aureus* (3)	23 mm	30 mm	0.25	0.25	0.125	0.125
Fungal Isolates							
5	*Candida albicans* (3)	32 mm	31 mm	0.0625	0.0625	0.125	0.125

Note: Inhibition zone of diameter less than 10 mm were considered as negative results

CONCLUSION

The comparative study of both the clove extracts suggested that Clove powder is more effective as compared to decoction with maximum inhibition zone of diameter 33 mm for gram positive and 34 mm for gram negative bacteria for clove powder while 23 mm and 32 mm for gram positive and gram negative bacteria respectively in case of decoction.

This property of clove was used to prepare a liquid soap which proved to be useful in decrease in number of bacteria. In future this investigation will allow us to utilise clove to the maximum without wasting its solid remains which occurs while preparing clove oil. Thus this finding creates a new scope of research in this area which can further be used to exploit various other properties of clove, which in future could help to curb various other resistant bacteria and microorganisms.

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