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Antifungal and Antibacterial Activities of Eugenol and Non-Polar Extract of *Syzygium aromaticum L.*

Coralie Pavesi[1], Lucy A. Banks[2] and Taghread Hudaib[2*]

[1]*Faculty of Pharmacy, University Paris-Sud, 5, rue Jean-Baptiste Clément - 92 296 Châtenay-Malabry CEDEX*
[2]*Joseph Banks Laboratory, University of Lincoln, Brayford Pool, Lincoln LN6 7TS, United Kingdom*

Abstract

The antimicrobial activity of eugenol and the non-polar extract from cloves (*Syzygium aromaticum*), was tested against the *Staphylococcus aureus* strain resistant to cefotaxime, *Escherichia coli* and *Candida albicans* using the disc diffusion antibiotic sensitivity technique. Soxhlet extraction was performed to separate the essential oil and GCMS method was used to identify the eugenol which is the main component of the clove essential oil. The measured growth inhibition zones showed significant activity against the 3 microbes. To conclude, eugenol is the major active ingredient of the clove essential oil extract.

Key words *Eugenol*; *Staphylococcus aureus*; *Escherichia coli*; *Candida albicans*; Soxhlet extraction; growth inhibition zone

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is Gram-positive bacterium from the Staphylococcaceae family and a normal flora in the respiratory tract, nose and on the skin. It can cause a variety of diseases in man according to the intrinsic virulence and the status of host's immune system. Although it is not always pathogenic, it can cause skin infections, respiratory infections and food poisoning when the immune system is depressed [1].

The major problem with *S. aureus* and other types of bacteria is that they have the ability to develop resistance against a wide range of antibiotics, leading to worldwide MRSA (Methicillin Resistance *Staphylococcus aureus*) problem.

MRSA is prevalent in European countries ranges from less than 1% in countries like Norway, to more than 40% in the UK [2] hence, the growing concerns about how to control that threat.

Escherichia coli (*E. coli*) is a Gram-negative and aerobic commensal bacterium from the Enterobacteriaceae family that is showing a worldwide emergence of multidrug resistance. It mainly causes enteric and diarrheal diseases, urinary tract infections and sepsis/meningitis [3].

Candida albicans (*C. albicans*) is a saprophyte and commensal yeast from the Saccharomycetaceae family and a member of the human gut flora. It is also an important human fungal pathogen that causes invasive and difficult to treat fungal infections [4], thus it involves an increase of medical and economic issues related to the high mortality rates, the increased costs of care and of duration of hospitalization [5].

To deal with the antibiotic resistance problem against currently used antibiotics, scientists should look for new alternatives such as plant origin substances that have proved their efficacy against microbial infections. Here we investigated the efficacy of clove oil extracts compared with pure eugenol against the three mentioned microbes.

Cloves, also known as *Eugenia aromatica*, are dried buds harvested when the calyces change from green to yellow pink. They come from the evergreen tree *Syzygium aromaticum* (*S. aromaticum*) from the *Myrtaceae* family, which flowers twice a year, and grows in tropical and subtropical conditions [6]. Cloves have been used in Ayurveda, Chinese medicine and Western herbalism for many medicinal purposes since ancient times [7].

Nowadays, the clove oil from *S. aromaticum* and eugenol are well known to present strong antibacterial and antifungal activities [8, 9] that have been tested in several animal models [10]. In order to improve our knowledge about the microorganisms' behaviour against *S. aromaticum* extracts, it is necessary to carry out more studies like this one.

MATERIALS AND METHODS

Extraction and diffusion disk preparation: 92.2 grams of dry and ground clove was added to 200ml of dichloromethane (Fisher Scientific) and was subjected to 2 hours of sonication at room temperature. After sonication, the clove powder was filtered using Whatman® Qualitative cellulose filter paper and the volume of filtrate was reduced using a rotary evaporator. The concentrated filtrate was further extracted using a soxhlet extractor with 500ml dichloromethane boiling at 55°C for 18 hours. The resulting extract was concentrated using a rotary evaporator, yielding 15.4 grams of solvent free essential oil. The non-polar oil extract was further analysed using GC-MS. Analysis was performed using a Thermo scientific column (TG-SQ3 P/N26070-1300, 15m length, I.D. 0.25mm, film thickness 0.25 µm), oven temperature 40 °C for 1 minute, 40 to 200 °C at a rate of 6 °C/min and from 200 to 280 °C at a rate of 30 °C/min. The injector temperature was 250 °C, the used carrier gas was helium at a linear velocity of 1 ml/min. The ion source temperature was 250 °C and the ionisation energy was 70 eV. Mass ranges between 20 and 500 amu (atomic mass unit). Oil extract and pure eugenol were diluted by DCM to 0.1% (v/v) for the extracted oil and 0.05% (v/v) for the eugenol standard before injection.

The standard and the extract were stored at 4 °C before being used to prepare the diffusion disks. Paper disks were soaked in the extracted essential oil, eugenol or corn oil blank for one hour and dried overnight in a plate drier before being used in the growth inhibition test.

Bacterial and yeast culture

S. aureus, *E. coli* and *C. albicans* were used as models based on their medical importance. Stock cultures of *S. aureus*, *E. coli* (prepared from Thermo Scientific™ Culti-Loops™) were inoculated on nutrient agar (Oxoid™ Nutrient Agar) whereas the *C. albicans* was inoculated on Oxoid™ Sabouraud dextrose agar (SDA), cultured plates were incubated for 24 hours at 37°C. The 2 types of bacteria and the yeast were then grown in nutrient broth at 37°C and 28°C respectively for 24 hours.

Determination of growth inhibition zone:

Antibacterial and antifungal activities of eugenol and the clove extract against the 3 organisms were examined using the agar disk diffusion method; the previously prepared disks where placed on freshly inoculated plates from the broth cultures. Replicates of each culture were prepared and *Cefotaxime* antibiotic discs (Oxoid™) were used as test control. Discs loaded with corn oil were used as blank control. After 48 hours of incubation, the

Table 1: The mean dimeters ± standard errors of growth inhibition zones of the 4 different treatments; Cefotaxime, Eugenol, Non polar extract, Corn oil

Disc content ▸ Tested culture ▾	Cefotaxime 30 µg/ disk	Eugenol 57µg/ disk	Non polar extract 35 µg/ disk	Corn oil 57 µg/ disk
C. albicans	0.0 mm	12.1 ± 2.2 mm	9.7 ± 1.9 mm	0.0 mm
E. coli	10.2±1.5 mm	4.2 ± 1.0 mm	3.4 ± 0.7 mm	0.0 mm
S. aureus	0.0±0 mm	3.8 ± 0.7 mm	3.0 ± 0.6 mm	0.0 mm

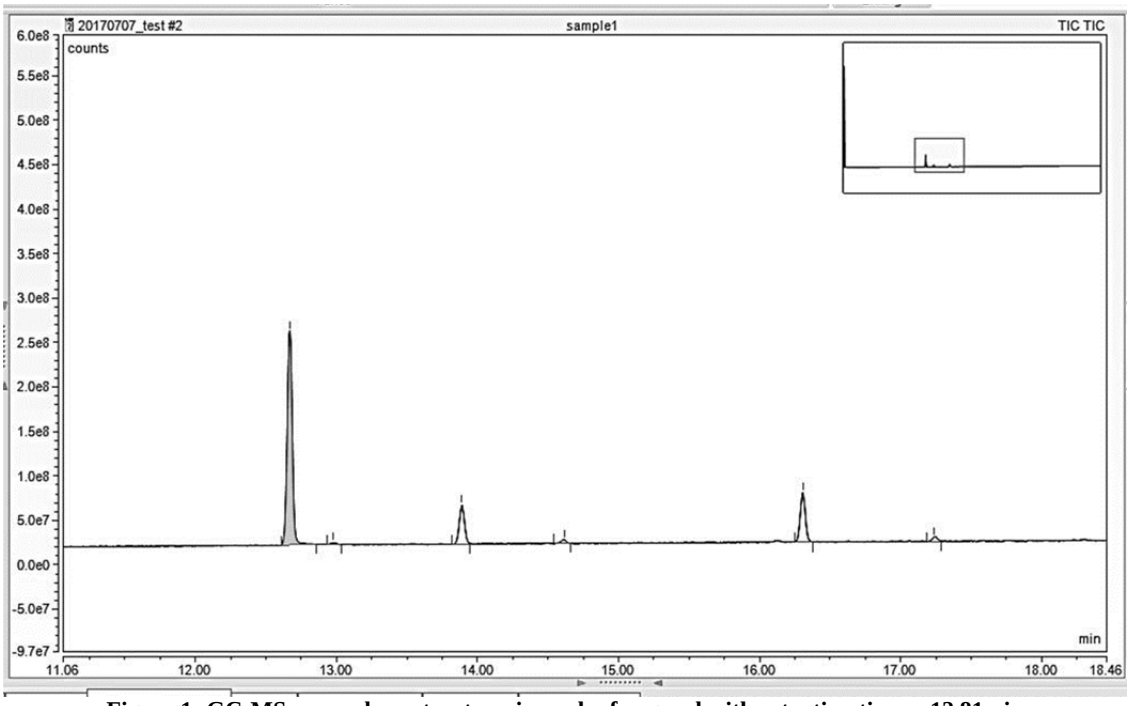

Figure 1: GC-MS non-polar extract, main peak of eugenol with retention time = 12.81min

diameters of the growth inhibition zones around the disks were measured and the resistance to the antibiotic, standard and extracts was determined. The one-way analysis of variance (ANOVA) was used to determine whether the differences between the different treatments were statistically significant using IBM SPSS Statistics.

RESULTS

Eugenol was detected in the oil extract chromatogram as the major peak at retention time of 12.81min. It was identified using eugenol standard which turned up at retention time of 12.67min when the same GC method was used (Figure1 and 2).

Compared with the standard and the wide range antibiotic, the antibacterial and antifungal activities of the clove non-polar extract were significant. The results showed that *C. albicans* was more sensitive to the non-polar extract and the eugenol standard than the *E. coli* or the *S. aureus* when the diameters of the growth inhibition zones around the relevant discs were measured. The activity against both the Gram-negative and the Gram-positive bacteria showed a similar pattern of growth inhibition (Table 1). With approximately half the concentration of the eugenol standard, the non-polar clove extract has similarly and significantly inhibited the growth rate of *E. coli* (Table 1). The known antibiotic *Cefotaxime* showed a significantly greater inhibition zone of the *E. coli* growth than that of the clove extract at the same concentration: *ANOVA* $F(3,46) = 229.00$, $P < 0.001$ (Figure 2A). Both eugenol standard and the clove non-polar extract showed highly significant inhibition in the growth of the gram positive bacteria *S. aureus* which is highly resistant to the Cefotaxime antibiotic at approximately the same concentration: *ANOVA* $F(3,46) = 179.22$, $P < 0.001$ (Figure 2B). The antifungal activity of the clove non-polar extract was highly significant against the yeast C. *albicans*: *ANOVA* $F(2,37) = 143.60$, $P < 0.001$ (Figure 2C).

DISCUSSION

Although, the concentration of eugenol standard was approximately two times more than that of the clove extract, the growth inhibition zones around the eugenol discs were slightly larger than those of the extract which may reflect the contribution of other components of that extract in the yeast growth inhibition. In this short study, we examined the efficacy of clove (*Syzygium aromaticum*) essential oil against resistant microbes. Non-polar extracts of clove exhibited antimicrobial activity against three pathogenic organisms in vitro. The yeast (*C. albicans*) was the most sensitive to the extract and the standard eugenol, which indicates the need for further studies to profile the active components of the oil extract and to understand the exact mechanism of action in prokaryotic and eukaryotic microorganisms.

However, the high content of eugenol in the non-polar extract appears to be responsible for its strong antimicrobial activity. The variation in the rate of inhibition could be due to the quality of the used discs (ability of paper to absorb and release the oil). It may also result from the synergistic effect of other minor components in the extract such as carvacrol and thymol [11].

The modes of action of eugenol's antimicrobial activity were discussed in previous studies; the hydrophobicity of the oil enables it to partition the lipids and disrupt the outer membrane of Gram- positive and Gram- negative bacteria [12]. Eugenol also found to enhance protein leakage of cell membranes in both types of bacteria [13].

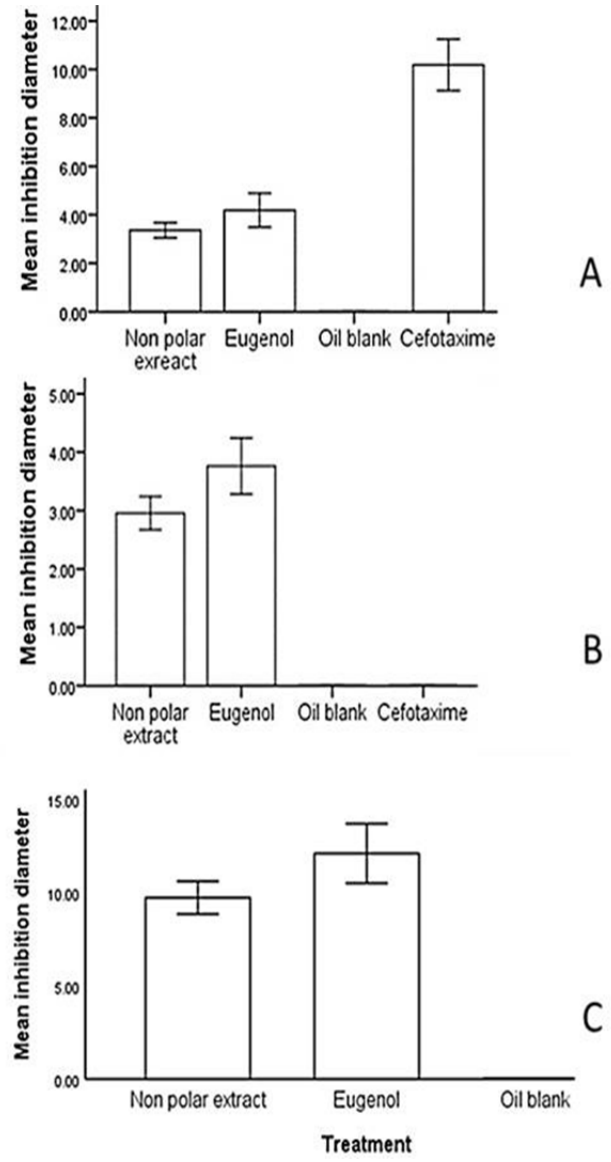

Figure 2: Mean inhibition zone diameter ± SE, A) *E. coli*, B) *S. aureus* C) *C. albicans*

CONCLUSION

Clove contains bioactive compounds which can be potentially used for medicinal purposes. Eugenol is the major component of the clove essential oil and it is the main responsible compound for the antimicrobial activity of that extract. This study showed no sufficient evidence to support the synergistic effect of other compounds in the clove oil as both the extract and the eugenol standard showed similar pattern of antimicrobial activity. Further studies are required, before the incorporation of eugenol into a drug formulation. The exact mechanism of action must be fully understood and a pharmacological evaluation is needed to determine the effective dosage and minimise any potential side effects [14].

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