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The interest of Sygyzium aromaticum as a cosmetic ingredient: assessment of antimicrobial, anti-oxidant and cytotoxic activities

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Introduction

- *Sygyzium aromaticum,* commonly known as clove, is an aromatic plant belonging to the *Myrtaceae* family. Clove has been used as a medicinal plant, mostly because of its antimicrobial and antioxidant activities [2], that have been associated with clove essential oil main component, eugenol [1].
- Following the rise of the general interest worldwide in natural products, the biological activities of clove have been studied in greater detail, revealing its anti-carcinogenic, anti-inflammatory and anti-diabetic potential [1].

Materials & Methods

Clove essential oil Purchased from a Portuguese producer, committed to biological farming.

- *Phytochemical analyses (GC-MS)*
- *Antimicrobial activity (The minimum inhibitory concentration- MIC-was determined according with Clinical and Laboratory Standards Institute [3])*
- *Cellular biocompatibility (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide-MTT-assay, as previously described [4, 5] using 3T3 cell line -skin murine fibroblasts)*
- *Anti-oxidant capacity (2,2-diphenyl-1-picryl hydrazyl-DPPH- assay)[5]*

<u>OBJECTIVES:</u> to explore the potential role of clove as a cosmetic ingredient by focusing on its antimicrobial, anti-oxidant and cytotoxic activities.

Results & Discussion

Table 1. Major components of clove essential oil determined by GC-MS (gas chromatography coupled to mass spectrometry) analysis. The relative proportion of each compound is also showed (%).

Components	Chemical formula	Relative proportion (%)
Eugenol	$C_{10}H_{12}O_2$	66.12
Eugenol acetate	$C_{12}H_{14}O_3$	24.66
Caryophyllene	$C_{15}H_{24}$	8.34

Table 2. Minimum inhibitory concentration (MIC) of clove essential oil (%, V/V) against the tested microorganisms.

Microorganisms	Minimum inhibitory concentration (MIC)
Yeast	
Candida albicans	0.03%
Gram-Negative Bacteria	
Escherichia coli	0.25%
Pseudomonas aeruginosa	0.25%
Gram-Positive Bacteria	
Staphylococcus aureus	0.5%
Staphylococcus epidermidis	0.5%
Corynebacterium amycolatum	0.5%

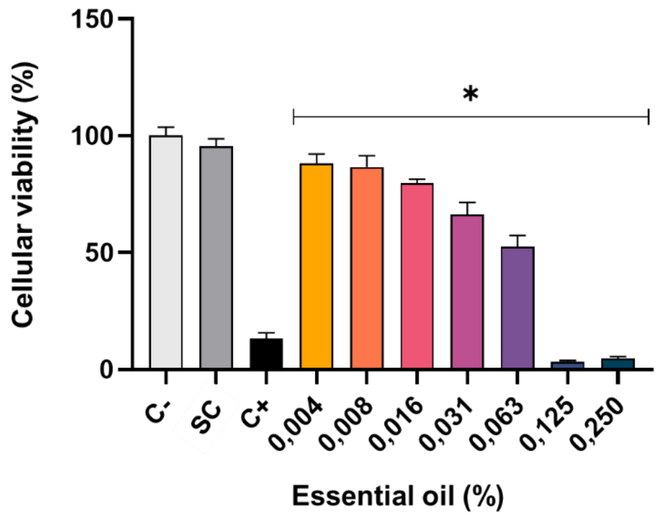

Figure 1. Cellular viability of 3T3 fibroblasts cell line, after 24h exposure to increasing concentrations of clove essential oil (%, V/V) as determined by the MTT reduction assay.

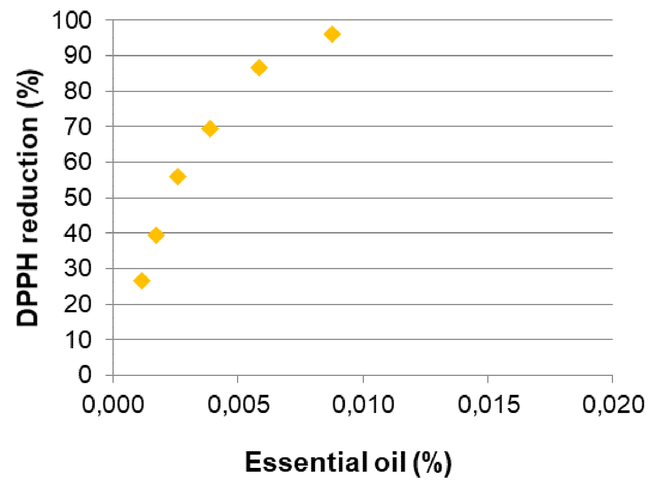

Figure 2. DPPH reduction (%) by clove essential oil (%, V/V)

- The major component of clove essential oil was eugenol, accounting for 66% of all identified components (Table 1).
- Regarding antimicrobial activity (Table 2), clove essential oil was highly active against yeast (MIC, 0.03%) and also showed anti-bacterial activity against Gram-positives (MIC 0.5%) and Gram-negatives (MIC 0.25%).
- Clove essential oil was cytotoxic to skin fibroblasts (3T3 cell line) in a dose-dependent manner (Figure 1). The EC50 (concentration toxic to 50% of the cells in test) was 0.1% (V/V).
- Clove essential oil was able to reduce the DPPH molecules in test at very low concentrations (Figure 2). The EC50 (efficient concentration; ability of the essential oil to reduce 50% of DPPH molecules in test) was 0.002% and the correspondent AAI (antioxidant activity index) was more than 2.0

Conclusions

Clove essential oil, probably due to the high concentration of eugenol in its composition, has relevant antimicrobial activity, specifically against fungi. Due to its high antioxidant and cytotoxic abilities, it should be used with care in skin formulations, though these potent activities should be interesting for anti-aging products.

References

1.Batiha, G.E.-S., et al., Biomolecules, 2020. 10(2): p. 202.
2.DF, C.-R., d.S. CR, and O. WP, Asian Pacific journal of tropical biomedicine, 2014. 4(2).
3.CLSI, 2009.
4.Oliveira, A.S., et al.. International journal of biometeorology, 2020. 64(11).
5.Palmeira de Oliveira, A., et al. Toxicology Letters, 2018. 295: p. S264.

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