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In vitro and *in silico* evaluation of the immunomodulatory effects of *Laurus nobilis* L. essential oil and eucalyptol on polymorphonuclear neutrophils

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INTRODUCTION & AIM

Inflammation is a vital response to harmful stimuli, primarily involving polymorphonuclear neutrophils (PMNs) which combat infections through phagocytosis, degranulation, and oxidative burst. These processes, stimulated by chemoattractants, enable PMNs to eliminate pathogens and signal other immune cells [1]. Platelets also play a significant role in inflammation through pro-inflammatory mediators and interactions with leukocytes. However, excessive immune responses can lead to inflammatory diseases [2]. Medicinal plants, rich in anti-inflammatory phytochemicals like polyphenols and flavonoids, are gaining attention for modulating PMNs activity, reducing reactive oxygen species, and preventing abnormal platelet aggregation, offering a natural alternative to traditional anti-inflammatory drugs [3].

Laurus nobilis L., also known as bay laurel, is an evergreen shrub native to the Mediterranean region. It is commonly used in cooking and traditional medicine. Laurel essential oil (LEO), containing eucalyptol as its main compound, is utilized for treating various ailments due to its potential therapeutic properties, including anti-inflammatory, analgesic, and antimicrobial effects [4].

The aim of this study evaluate the immunomodulatory effects of LEO and its main compound eucalyptol on fMLP-stimulated PMNs, in particular on their inflammation-related functions, such as degranulation, oxidative burst, platelet aggregation, and free radical-induced hemolysis.

METHODS

Laurel essential oil (LEO) was isolated by hydrodistillation for 3h from 100 g of dry powder by Clevenger-type apparatus and then characterized using the GC-MS technique [5].

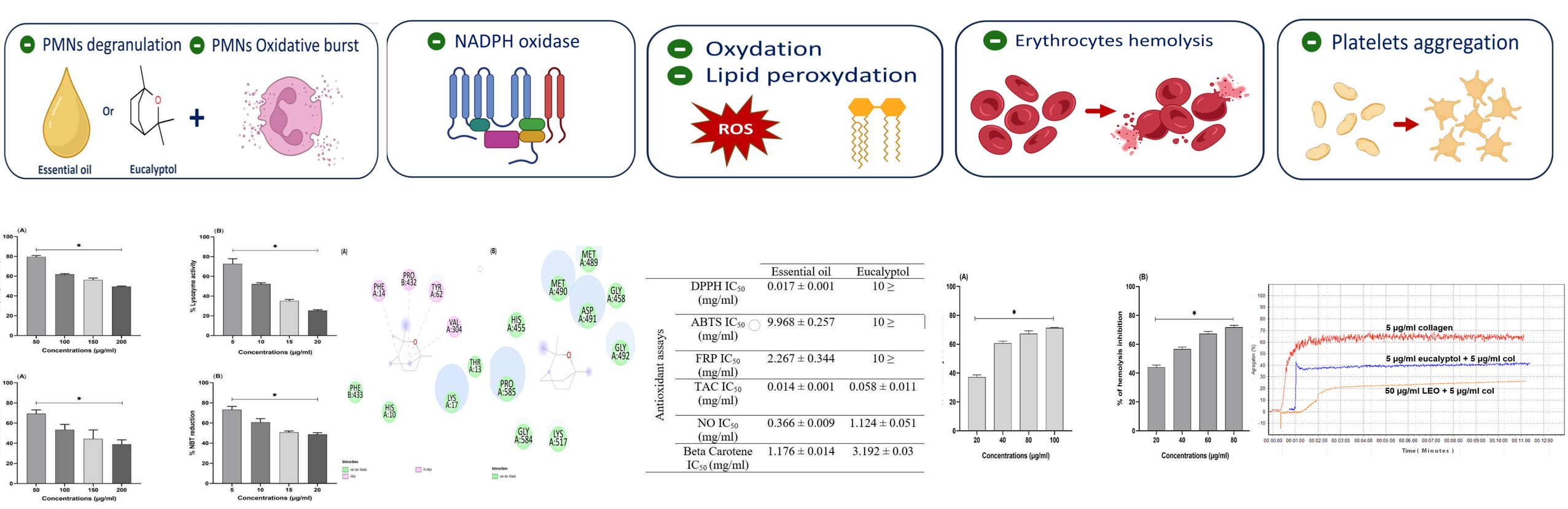
The immunomodulation effects of LEO and eucalyptol on isolated PMNs were studied on both, fMLP-PMNs degranulation measured by the release of lysozyme, an enzyme that is located in all granule subsets, and oxidative burst measured by superoxide anion production using the reduction of nitroblue tetrazolium (NBT).

The *in silico* analysis was performed by AutoDock Tools (ADT) to study the interaction of eucalyptol, α-terpinyl acetate, and β-phellandrene with NADPH oxidase (NOX) and NOX subunit receptor.

The antioxidant activity was evaluated using six distinct analytical methods. These included the 2,2-diphenyl 1-picrylhydrazyl ($DPPH^{\bullet}$), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid ($ABTS^{\bullet+}$), and nitric oxide (NO) scavenging tests, alongside the ferric reducing power (FRP), total antioxidant capacity (TAC), and β-carotene bleaching inhibition assays.

The anti-hemolytic activity of LEO and eucalyptol was assessed using H_2O_2-induced hemolysis of Human red blood cells, and the platelets aggregation assay was carried out with the aggregometry testing.

RESULTS & DISCUSSION



Antioxidant assays	Essential oil	Eucalyptol
DPPH IC_{50} (mg/ml)	0.017 ± 0.001	10 ≥
ABTS IC_{50} (mg/ml)	9.968 ± 0.257	10 ≥
FRP IC_{50} (mg/ml)	2.267 ± 0.344	10 ≥
TAC IC_{50} (mg/ml)	0.014 ± 0.001	0.058 ± 0.011
NO IC_{50} (mg/ml)	0.366 ± 0.009	1.124 ± 0.051
Beta Carotene IC_{50} (mg/ml)	1.176 ± 0.014	3.192 ± 0.03

LEO and eucalyptol have immunomodulatory activities on PMNs functions. Thus, LEO and eucalyptol inhibits in a dose-dependent manner both fMLP-induced degranulation of PMNs with maximal percentages of inhibition of 50.44 % and 74.69 %, respectively and in a same manner, they reduce oxidative burst with maximal percentages of inhibition of 61.07 % and 51.29 %, respectively ($p < 0.001$). Moreover, *in silico* docking of LEO studies showed that its selected major compounds (eucalyptol, α-terpinyl acetate, and β-phellandrene) have an energy change ranging between – 4.2 and – 7.4 kcal/mol on both NADPH oxidase, its subunits and PKC. These results suggest that eucalyptol, α-terpinyl acetate, and β-phellandrene found in LEO, may have an effect on the intracellular signaling pathways within PMNs.

LEO and eucalyptol were found to possess a significant antioxidant activity with various IC_{50}, protective capabilities against H_2O_2-induced hemolysis in erythrocytes in a dose-dependent manner ($p < 0.001$), and also inhibited collagen-induced platelet aggregation. These multifaceted properties of LEO, primarily attributed to the presence of eucalyptol and possibly other active compounds, indicate its potential as a therapeutic agent. Its effectiveness in reducing oxidative damage and preventing thrombosis makes it a promising candidate for the treatment of various inflammatory diseases. The antioxidant and antiplatelet activities of LEO could be particularly beneficial in conditions where oxidative stress and blood clotting are contributing factors, offering a natural and potentially safer alternative to conventional treatments.

CONCLUSION

In conclusion, this study provides valuable insights into the potential immunomodulatory effects of *Laurus nobilis* essential oil and eucalyptol by inhibiting PMNs functions, highlighting the therapeutic potential of these natural compounds in conditions where PMNs activity needs to be regulated. However, more research is needed to fully understand these mechanisms and to validate these findings in more complex biological systems and clinical settings, specifically in the context of arthritis-related inflammation and LPS-induced lung inflammation.

FUTURE WORK / REFERENCES

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