Chemical composition and anti-acne effect of *Thymus citriodorus* preparations

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Introduction:

Acne vulgaris is the most common human skin disease, affecting quality of life of millions worldwide [1]. Due to its multifactorial pathogenicity, several drugs are used to control disease progression, with antibiotics impending their important role due to development of resistance [2]. Consumers are increasingly searching for “green” cosmetic products, and plant extracts have gained importance as natural alternatives [3]. Therefore we aimed to evaluate the anti-acne potential of essential oil and hydrolate from *Thymus citriodorus* (TC), produced in Portugal, by studying their antimicrobial activity against *Cutibacterium acnes*, their effect against bacterial biofilms and their anti-inflammatory potential by addressing nitric oxide (NO) production.

Materials & Methods:

Chemical characterization

Gas chromatography (GC-FID)
Gas chromatography–mass spectroscopy (GC–MS)

Antimicrobial activity against C. acnes

C. acnes (DSMZ 1897)

Minimum inhibitory concentration
CLSI M11-A6 microdilution method

Minimum lethal concentration

Effect on *C. acnes* biofilms
Crystal violet staining

Biofilm adhesion

Preformed biofilm disruption

Cellular viability and Nitric Oxide (NO) production

Macrophages Culture (RAW 264.7)

LPS (1 µg/mL) + TC preparations, 24h

Medium + Griess Reagent

Spectrophotometric evaluation
Determination of NO stable metabolites

TC preparations, 24h

MTT, 4h

Spectrophotometric evaluation
Cellular viability determination

Effect against C. acnes biofilms

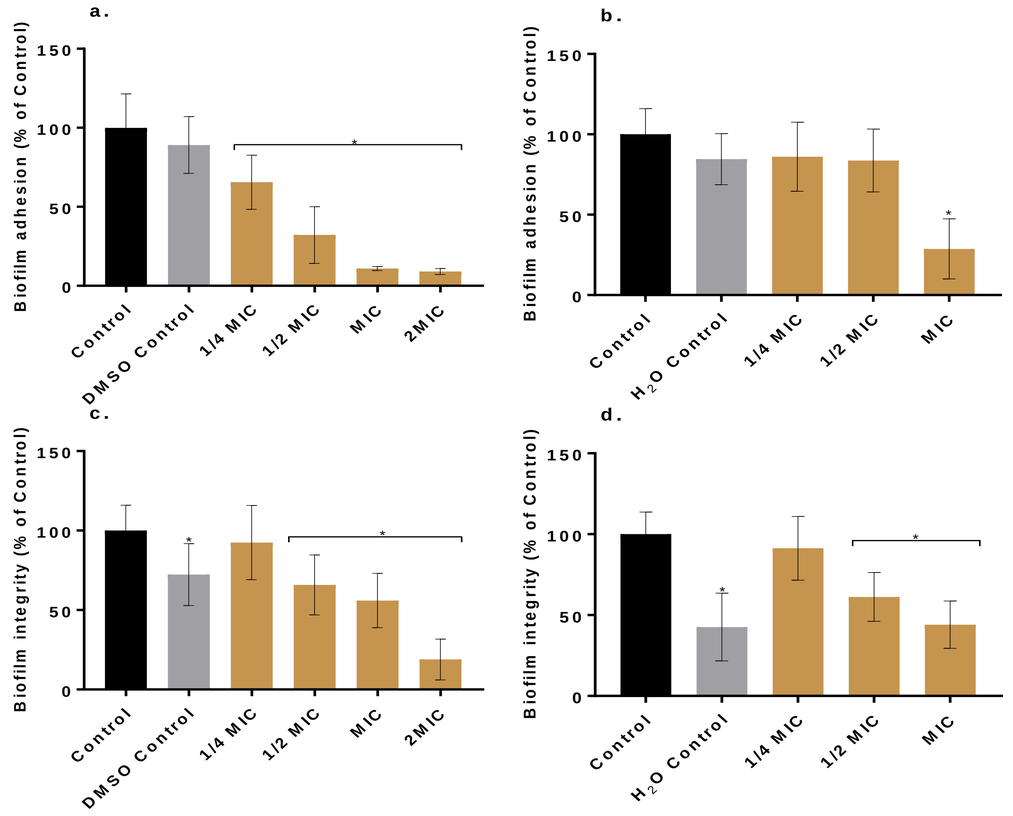

Figure 1 Effect of TC essential oil (a,c) and hydrolate (b,d) on *C. acnes* biofilm adhesion and their effect in disrupting preformed biofilms, represented by biofilm integrity. Results are presented as percentage of Control (in the absence of plant preparations).
* represent statistical significance when compared with the Control, as determined by p value < 0.05.

Effect on cellular viability and NO production

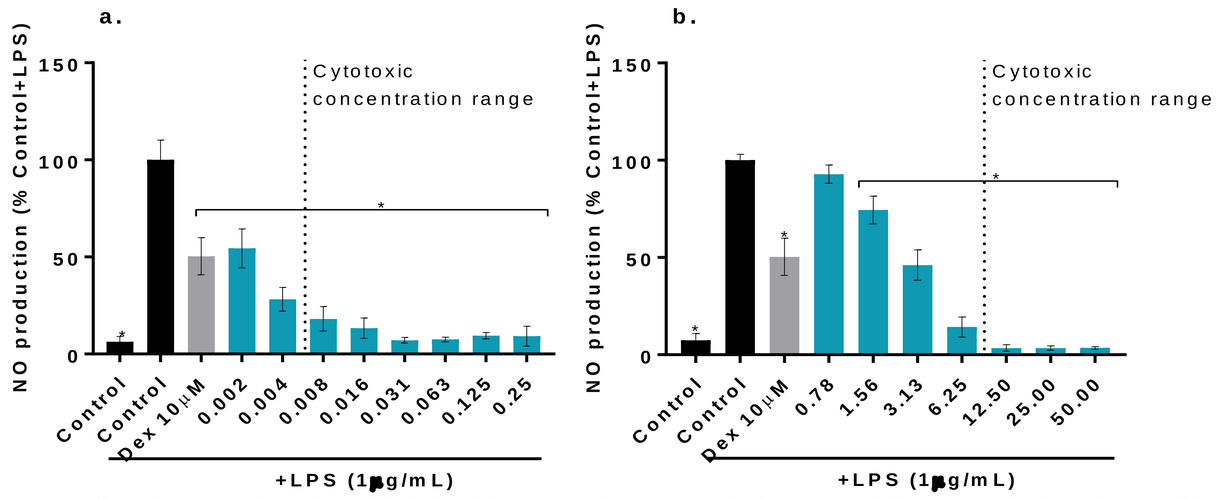

Figure 2 Effect of TC essential oil (a) and hydrolate (b) on macrophage NO production upon an inflammatory stimulus. Biocompatible concentration range is represented at left of the vertical line. Data correspond to the means ± SD and are represented as % of control cells exposed to LPS (Control + LPS). A control without LPS was also included to evaluate basal NO production (Control). Dex – Dexamethasone at 10µM (Positive Control). Statistical analysis: *p < 0.05 was considered a significant reduction.

Results & Discussion:

Chemical composition

Table 1 Partial chemical composition representing the major constituents present on TC samples as determined by gas chromatography (GC-FID) and gas chromatography–mass spectroscopy (GC–MS). The three major compounds in each sample are represented in bold.

Compound	Essential Oil	Hydrolate	
	Percent in Essential Oil	Relative amount in the Hydrolate*	Relative amount in the volatile fraction
1,8-Cineole	**16.3%**	**0.0145%**	**26.3%**
Linalool	1.9%	**0.0134%**	**24.3%**
Geraniol	**27.5%**	**0.0077%**	**13.9%**
Thymol	**9.2%**	0.0050%	9.1%

* Expressed as *n*-dodecane

Antimicrobial activity

Table 2 Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of TC preparations against *C. acnes*. MIC and MLC values are expressed as % (v/v).

Plant Preparation		MIC	MLC
Thymus citriodorus	Essential Oil	0.06%	0.125%
Thymus citriodorus	Hydrolate	50%	ND

ND – No MLC was determined (>50%).

Conclusions:

Our results uncover different applications for TC, highlighting its potential to be used as an active ingredient for skin application, specifically targeting acne vulgaris. TC hydrolate by presenting higher biocompatibility, anti-inflammatory potential and some ability to modulate *C. acnes* virulence may be advantageous to be included in a product for everyday application, acting as a promoter of skin health, in acneic skin. On the other hand, essential oil, by presenting a marked antimicrobial, anti-biofilm and anti-inflammatory activities, still with some cytotoxicity, may be better suited for application in acute flare-ups, for short treatment periods.

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