



IN SITU EVALUATION OF BASIL OIL ESSENTIAL OIL CYTOTOXICITY (OCIMUM BASILICUM L.)

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ABSTRACT

Essential oils derived from plants used as condiments represent a large group of natural antimicrobials. Extracts and essential oils from plants have been shown to be efficient in controlling the growth of a wide variety of microorganisms, including filamentous fungi, yeasts and bacteria. Practical uses of these activities are suggested in humans and animals, as well as in the food industry. This study aimed to evaluate the basal basil essential oil (*Ocimum basilicum* L.) cytotoxicity in NCTC Clone 929 cell line, mouse connective tissue cells (CCIAL 020), which were seeded in Petri dishes and incubated for 48 hours to form the cell monolayer. For the this test accomplishment of this test, we counted with the collaboration of the Nucleus of Cell Cultures - NCC of the Adolfo Lutz Institute, of the city of São Paulo-SP. The culture medium used is the solid coating medium, and fragments of the samples fragments are were placed on this coating medium and the plates were again incubated for 24 hours. The Biological Reactivity Degree (GR) index, which is the area not stained by the vital dye, is were observed macro and microscopically. Samples were tested in quadruplicates on separate plates. ThusThereunto, the test result showed that the essential oil was cytotoxic in the cell line studied, and that the future work in pharmacology and toxicology needs to be performed to better standardize the therapeutic dose, which is not cytotoxic as the pure oil behaved.o assess the attitude and practice of dental professionals towards using of advance Radiographic technique.

KEYWORDS: *Ocimum basilicum* L., *Coriandrum sativum* L., cytotoxicity

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INTRODUCTION

Emergence and dissemination of microorganisms resistant to antimicrobials available in the Market has been reported for decades. Among agents used for bacterial biofilm chemical control called Chlorhexidine digluconate is considered as Golden

standard due to the antimicrobial good effects and its high substantivity, what enables this substance action for longer than other solutions. However, despite its benefit effects, Chlorhexidine digluconate prolonged use may bring side effects, such as staining in teeth, restorations, prosthesis and tongue,

desquamation of the buccal mucosa, reduction of taste sensitivity and formation of supragingival calculus (Maekawa et al., 2010). It leads us to detach the importance of possibility to use a product with similar action, which presents less aggression power to the buccal cavity. Therefore, there is an

incentive on the search for new sources of substances with antimicrobial activity, like plants used in traditional medicine (Mendes et al., 2011). The World Health Organization stimulates the study of regional medicinal plants for medicine as a way to reduce costs on public health programs. The use of vegetal species as drugs results in low cost products and less production time, consequently being more accessible for population (Mendes et al., 2011; Furlam, 1998). Antioxidant, antimicrobial and antifungal activities of basil (*Ocimum basilicum* L.) has been reported on literature in several areas (Lee et al., 2005; Opalchenova & Obreshkova, 2003; Alves et al., 2009; Cavalcanti et al., 2012; Araújo da Silva, et al., 2008), what shows effective antimicrobial results.

According to Ostrosky et al., (2008), to evaluate antimicrobial activity of vegetal extracts is necessary determinate the lower amount of substance to inhibit the microorganism test growth. This value is known as Minimal Inhibitory Concentration (MIC). A very relevant aspect to determine vegetal extracts MIC is the concern regarding to antimicrobial, toxicological and legal aspects pertinent to the use of natural composites or their combination in living beings. For the same authors, MIC determination may suffer variations depending on the microorganism or strain used for test. Then, MIC test must be applied according to the primary etiologic agent and the type of pathology in which the medicine will be proposed as therapy. In the study performed by Lourenço et al. (2015), in which MIC of *Ocimum basilicum* L. and *Coriandrum sativum* L. oil extracts were evaluated, the extracts presented inhibitory activity before *S. mutans* strain. Basil in 1:4 concentration showed bacteriostatic, and in 1:3 concentration, bactericidal.

However, to use a product in the oral cavity, it must be effective against oral pathogens and cannot cause lesions. This study had as aim to evaluate basil (*Ocimum basilicum* L.) essential oil cytotoxicity in cells NCTC Clone 929 lineage, mouse's connective tissue (CCIAL 020).

MATERIAL AND METHODS

CELL CULTURE

To perform this test, we count on Nucleus of Cell Culture – NCC of the Adolfo Lutz Institute collaboration, of city of São Paulo-SP.

To carry out the basil essential oil cytotoxicity test, NCTC clone 929 (CCIAL 020) cell lineage was used, mice's connective tissue from the cell culture section of the Adolfo Lutz Institute.

NCTC clone 929 lineage cell was cultivated in minimal middle Eagle (MME) supplemented with 0.1 nM non-essential amino acid, 1,0 mM Sodium pyruvate and 10% de fetal bovine serum (SFB), no antibiotics (MME 10% SFB).

Maintenance of these lineages was performed at 36°C in bottles 75cm² or 250 mL and peal at 72 hours medium interval. Cell monolayer dispersion was carried out using an association of trypsin 0.20% and versene 0.02% (ATV). After dispersion, the cells were suspended again in their respective culture medium and distributed on Petri dish, which were used during the tests performing.

AGAR DIFFUSION METHOD

Cells were seeded in 5mL volume in Petri dishes (60X15 mm), at concentration 3.0X10⁵cells/mL for NCTC – clone 020 lineages. Inoculation was performed during 48 hours at 37°C in humid atmosphere containing 5% CO₂. After this period with monolayer cells

formed, culture medium was discarded and added 5 mL overlay medium in each Petri dish. This medium is composed by equal parts of MEM concentrated twice and agar at 1.8% containing 0.01% neutral red as vital coloring. In the moment of use, agar was melted and mixed to MEM, both at 44 ± 1°C temperature.

Fragments of latex were used as positive controls, and filter paper dish as negatives ones, respecting 0.5 dimensions for both.

Basil essential oil aliquots were placed directly on this cover middle, the dishes were incubated for 24 hours, and this procedure was performed in quadruplicate.

Dishes were analyzed macroscopically and microscopically, and cytotoxicity was noticed out by the presence clear halo under or around the sample tested. From toxicity measurement, zone indexes were graduated according to the table below.

Table 1. Zone Indexes classification (ZI) reference values.

GR	DESCRIPTION	CLASSIFICATION
0	No zone under or around the sample	None
1	Some change or cell degeneration under the sample	Weak
2	Limited zone under the sample	Light
3	Zone between 0.5-1.0 cm around the sample	Moderate
4	Zone bigger than 1.0 cm around the sample	Severe

GR – Biological reactivity degree; Positive control: Toxic latex fragment 0.5 cm x 0.5 cm; Negative control: Nontoxic filter paper discs 0.5 cm diameter.

Macro and microscopically we observed the index Biological Reactivity degree (GR) which is the non-colored area by vital coloring. The samples are tested in quadruplicate in dishes separated.

PHARMACOLOGICAL AGENT EVALUATED

Basil essential oil (*Ocimum basilicum* L.) used was obtained from LASZLO enterprise.

Crude vegetal extracts are normally complex mixture constituted almost always divided for several natural products classes containing different functional groups. Separation process of these bioactive natural products correspond to three main stages: extraction from plant matter,

fractionation of extract or oil and purification of active principle.

Ocimum basilicum L. essential oil (basil) used in this research is from Brazil, and the extraction method used was steamed herb, its cultivation was organic non-certified, and obtained at Laszlo Aromaterapia Ltda.

ESSENTIAL OIL CHEMICAL COMPOSITION

Phytochemistry prospection of samples was obtained through Chromatographic analysis performed by the Federal University of Minas Gerias, solicited by LAZLO AROMATERAPIA LTDA (Table 2).

Table 2. Green Plant Basil essential oil chemical constitution.

Constituent ID	%
α -pinene	0.1
Sabinene	0.2
β -pinene	0.3
Mircene	0.5
1.8-cineol	3.1
Linalol	82.8
Canphor	0.3
terpinen-4-ol	0.1
α -terpineol	0.4
methylcavicol	0.4
Geraniol	0.3
Eugenol	1.8
β -elemene	0.7
β -caryophyllene	0.1
α -bergamotene	2.4
γ -cadinene	1.1
17 δ -cadinene	1.0
α -muurolol	0.9

BASIL ESSENTIAL OIL ANTIBACTERIAL ACTIVITY

Basil essential oil antibacterial activity was conducted in another research work developed by supervisors and students in Streptococcus mutans strains and the results showed were satisfactory.

To perform this activity verification, microdilution test was used to find out the Minimum Inhibitory Concentration (MIC).

Determination of Minimum Inhibitory Concentration (MIC) by microdilution in broth showed Ocimum basilicum L. essential oil presented

inhibitory activity before S. mutans strain. We observed basil extract with better activity was the crude one until dilution -2, which did not show bacterial growth (Table 3).

RESULTS

The sample presented severe toxic effect for NCTC Clone 929 (CCIAL 020) cell lineage.

Unsatisfactory conclusion on test means the result obtained within reference parameters adopted by Brazilian Pharmacopeia and or ISO

10993-5 standard, whose recommend the sample with reactivity greater than 2 is considered cytotoxic.

Table 3. Basil extract dilution in which there were bacterial growth.

	Crude	-1	-2	-3	-4	-5
	Extract					
-1	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
-2	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.
-3	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.
-4	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.
-5	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Source: LOURENÇO (2015).

DISCUSSION

Basil is largely used by population, mainly in cooking. Its origin is from Asia and it was introduced in Brazil by Italian Colony that uses it as seasoning in pastry, sauce and meat. Some people consider it as a divine essence and it is chosen by Indians to swear over it in court. It was found growing nest to Christ’s sepulcher after resurrection, and it is used for some churches to prepare the holy water.

It presents in essential oil chemical composition: estragol, linalool, lineol, ancanfor, eugenol, cineol, pinene, thymol. It also contains tannins, saponins, flavonoids, caffeic acid and esculoside. With all this profile, it presents therapeutic properties, as in combat to muscular contractions of stomach and intestinal gases; gargle to cure tonsillitis, laryngectitis and cold sore; against anxieties, being used to treat insomnia; through its galactogenic action, it has the property to stimulate

strongly milk secretion, could normalize problems with breastfeeding; it is a central nervous system and of supra renal cortex; stimulates diuresis.

By this plant therapeutic profile observation and by antimicrobial activity results obtained in other researches with aqueous extract and essential oil, we conducted this work in order to know some further characteristics of basil essential oil in cell cultures in order to verify its cytotoxicity profile to develop prophylactic methods with the use of the oil to decrease or eliminate Odontopathogenic microorganisms.

Essential oil antimicrobial activity has formed the basis for several applying, including food conservation, pharmaceutical products, alternative medicine and natural therapies. Basil (*O. basilicum* L.) and its essential oil have received considerable attention because of their medicinal potential properties. Phytotherapeutic action against *S. mutans* is reported successfully in literature.

Systematic research to obtain new substances with therapeutic goals may be executed through several processes. New molecules synthesis, molecular changing on natural or synthetic substances with pharmacological properties defined, and extraction, insulation and purifying of new composites from natural sources, especially from plant are the most used ones. This last one is characterized as an inexhaustible source of substances potentially actives as medicine. On the other hand, medicinal plants must be considered not only as raw material, starting point for new molecules Discovery, but as a natural resource potentially active on standardized and efficient herbal remedy²³.

Meng et al. (2000) Ho et al. (2001) Michelin et al. (2005) and Barbosa-Filho (2007) highlighted the importance to find efficient and less toxic substances to fight against the resistance and emergence of pathogenic

microorganisms. Silva, in 2015, also affirmed that knowing bacteria resistant to multiple drugs means a challenge to treat infections. Then, find new efficient new substances to fight against microorganisms is necessary.

It is common finding reports on scientific literature describing microorganisms which tolerates toxic composites presence. This tolerance is associated to mechanisms which also involve resistance, and it depends on strategies that these microorganisms have and which are known as virulence factors, among them we may mention: a) efflux pump; b) toxic component sequestration; c) bacterial membrane permeability reduction; d) enzymatic changes for a less toxic way; and e) target cell sensitivity reduction¹⁴.

Silva evaluated, in 2015, the chemical composition of *O. basilicum*, and determined antibacterial, cytotoxic and genotoxic activities of essential oil and its major composite. Besides, bacterial death kinetics and the study of composite association to standard antibiotics were also analyzed. Chemical composition was determined by gas chromatography coupled to mass spectrometry, and antibacterial activity of composites was evaluated through determination of minimum inhibitory concentration and minimum bacterial concentration by microdilution technique. Antibacterial activity was characterized as bactericidal for strains studied in composite concentration CIMx 4 and after 8h contact.

Linalool is a monoterpenoid composite monoterpenico commonly found as major component of essential oils in aromatic species, such as lavender (*Lavandula officinalis*), coriander (*Coriandrum sativum*), nolol (*Citrus aurantium*) and in great amount in basil

essential oil (*Ocimum basilicum*), which has been used in reseraches because its antibacterial activity⁶⁰.

More common chemical contents of essential oils are formed by combination among terpenes, phenols, aldehydes alcohols, esters, ketones, nitrogen and sulfur³⁷. Terpenes are formed by isoprene unities containing only hydrogen and carbon in their structures.

Terpene molecules found in essential oils have between 10 and 30 atoms and they are classified according to the number of carbons (Monoterpenes-10 carbon atoms; Sesquiterpenes-15 carbon atoms; Diterpenes-20 carbon atoms; Sesterpenes-25 carbon atoms and Triterpenes-30 carbon atoms).

Because their diversity, terpenes are largely studied and able to produce several pharmacological and biological effects³⁷. Several essential oils and terpene contents present pharmacological actions already proved. Some among them can be detached, like antibacterial activity, pro-inflammatory, pro-nociceptive, anti-inflammatory, anti-nociceptive, analgesic, antioxidant, anti-convulsivant and Skeletal, cardiac and smooth muscles contractile modulator^{61,60}.

Another important factor is the bacterial adherence to material surfaces. It may lead to an infection or contamination and/or destruction (loss and function) of these materials⁷⁷.

Bacterial biofilm produced by some organisms is considered an important virulence factor, what enables the bacterial adherence (by extracellular polysaccharide) to biotic surfaces (alive), which has the aim to increase the survival chance in some middle.

Torres et al. (2000) detached some ideas on antimicrobial: low toxicity, and must be recognized the product interaction with buccal epithelium; low permeability, what do not provoke imbalances which lead to other recurrent diseases; and having good retentivity (substantivity), what may be released slowly.

Sedenho et al. (2014) proved that *Ocimumbasilicum* L. extract has antimicrobial activity in *S. mutans* biofilm over acrylic surface, when used in dilutions until 1:4. These results agree with Opalchenova & Obreshkova findings, who studied, in 2003, basil effect against resistant bacterial lineages of *Pseudomonas*, *Staphylococcus* and *Enterococcus* genera, and found a Strong inhibitory effect from basil over Gram positive bacteria (*Staphylococcus* and *Enterococcus*), also for Gram negative ones (*Pseudomonas*). The literature describes differences in basil extract and essential oil actions before bacteria and fungi. Silva, in 2001, compared antididant and antimicrobial properties from marjoram (*Origanummajorana* L.) and basil (*Ocimumbasilicum* L.) essential oils and hydroalcoholic extracts, and concluded that, despite hydroalcoholic extracts and essential oils from these plants had presented phenolic high content with antioxidant activity, only essential oils demonstrated antibacterial activity, but not much significant. Chemical composition and antimicrobial activity (*S. mutans*) were observed in four species of *Ocimumbasilicum*L, and stronger antimicrobial activity was verified in *O. suave* oil (B); *O. suave* (A), *O. kilimandscharicum* and, *O. lamiifolium* were active moderately, while *O. basilicum* oil was weakly active. However, any oil was active against fungi species.

Millenarian use of medicinal plants showed, along years, that some plants present substances potentially dangerous, like Pyrrolizidine alkaloids, anthraquinones and lactonassespiritoprenes. Then, the balance between biological activities versus toxicity of determined natural product is Paramount parameter to verify its pharmacological application.

Cytotoxic and genotoxic activities of natural composites show themselves important in order to ensure greater safety in use of these products by population. Unfortunately it is unknown the toxic profile of major herbal remedies which are used in self-medication or by medical prescription.

Veiga (2005) asserted, like examples of toxic substances in plants may be cited hepatotoxic effects from apiol, safrole, lignans and alkaloid pyrrolizidine; toxic renal action that can be caused by some vegetal species which contain terpenes and saponins, and some types of dermatitis, caused by species rich in Lactonassesiteraphrenes and natural products type furanocoumarins. Toxic or antinutritional components like oxalic acid, nitrate and erucic acid are in some plants of commercial consumption. Several substances insulated from plants considered medicinal have cytotoxic or genotoxic activity and show relation with tumor incidence. Besides, the same author still mention some main contaminants from plants like heavy metals, which are part of pharmaceutical preparation of several oriental medicine schools, generally used jointly medicinal plants extracts, drugs added in order to increase effects and possibility for microbiological contamination.

Basil essential oil used in this research presented cytotoxicity in NCTC Clone 929 (CCIAL 020) cell lineage in its pure form. We believe that high

concentration of contents may act isolated or jointly presenting this side effect.

Regarding to linalool toxicity, its lethal dose is low, when compared to other contents of essential oils, what becomes target for future therapeutic application. Toxic effects of substances are related with delivery route, time, duration and frequency of dose⁷⁶. Linalool presented low acute toxicity in several experimental models and with different delivery route, what justifies the interest in its content for several areas of basic sciences.

Researches for new experiments are necessary in more advanced levels of preclinical toxicology of medicine plants to evaluate the lethal doses 50% (DL50), which consists in delivery of crescent doses; acute toxicity (single doses), carried out before start pharmacological tests, and represents estimate and preliminary evaluation of toxic properties of a herbal remedy; acute toxicity (repeated doses) is a preliminary and estimative evaluation of toxic properties of a herbal remedy, providing information on health risks from exposition to repeated doses in short time; subchronic toxicity may be performed after obtaining data from DL50 and studies on acute toxicity – single and repeated doses; chronic toxicity has the aim to characterize the toxicological profile of a herbal remedy, used repeatedly in a mammal specie for long period^{15,23}.

Through this study, we observed the basil may be used in its traditional ways without bring damage for human health. However, to use its crude essential oil (pure) in order to develop new products, pharmacological and toxicological tests are necessary.

CONCLUSIONS

We conclude that basil essential oil presented severe toxic effect for NCTC Clone 929 (CCIAL 020) lineage cell, then considered unsatisfactory.

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