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Original Research

Evaluation of potential use of *Cymbopogon* sp. essential oils, (*R*)-citronellal and *N*-citronellylamine in cancer chemotherapy

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Summary. Essential oil of *Cymbopogon citratus* (lemongrass) and *Cymbopogon nardus* (citronella), as well as the monoterpenic aldehyde citronellal and its chemically modified product, C37A (*N*-citronellylamine), were evaluate for their cytotoxic potential. Two cell lines were used, a breast cancer cell line (MCF-7 - ATCC HTB-22) and a non-tumorigenic cell line (Vero - ATCC CRL-1586). Using the crystal violet assay for evaluate cell viability, we observed that all tested compounds demonstrated cytotoxicity for both cell lines, however with different intensities. Lemongrass oil was cytotoxic in similar way for both cell lines, while the others presented higher cytotoxic response to the tumor cell MCF-7, being citronellal a high selective product. The chemical modification of citronellal, that originated the C37A, promoted higher cytotoxic effects but no selectivity.
Industrial relevance. The *Cymbopogon* genus has demonstrated important health feature. Essential oil of lemongrass, a plant of this genus, shows antitumor properties; however, the essential oil of citronella, a close related plant, does not have similar activity known, as well as its monoterpenic aldehyde component, citronellal. Here we show the potential of these products, especially citronellal, as possible new candidates for further investigation for breast cancer therapy.
Keywords. Breast cancer; citronellal, essential oil; *N*-citronellylamine; medicinal plants

INTRODUCTION

Due the enormous chemical variability from the high diversity of organisms, the nature is an important fount of therapeutic compounds. Among these organisms, plants are considered the source of the most effective and promising drugs (Saklani and Kutty, 2007). The pharmaceutical properties of herbs are partly due to the components of their essential oils (EOs). For this reason, these oils and their components are being studied for use in cancer therapy, as they had demonstrated promising potential as anti-cancer drug (Edris, 2007).

Essential oils (EOs) are volatile compounds with strong-smelling, natural products of the secondary metabolism of aromatic plants. They consist of mixtures of 20 to 60 components in different concentrations, being the most abundant terpenes and terpenoids (Bakkali et al., 2008). The essential oils and their components are hydrophobic, which enables them to interact with cell membranes. By doing so, they have the ability to penetrate the cell and interact with intracellular components or even act in the disruption of cell membranes functions. These can lead to the cell malfunction and eventual cell death (Cristani et al., 2007).

Cymbopogon citratus (DC.) Stapf (Figure 1A), known as lemongrass, is a native Southwestern Asia herb (Castro and Ramos, 2002), used to various purposes ranging from alternative medicine to perfume industry (reviewed by Negrelli and Gomes, 2007). Several researches have demonstrated its important anticancer effect (Manosroi et al., 2006; Puatanachokchai et al., 2002; Suaeyun et al., 1997). On the other hand, *Cymbopogon nardus* (L.) Rendle (Figure 1B), a close related to the former plant, known as citronella (Castro and Ramos, 2002), has been studied as an insect repellent, with very few studies as anticancer drug (Akhila 2010; Castro et al., 2010).

The unique structural features of natural compounds consecrate them as a starting point for development of semi-synthetic and synthetic drugs (Butler, 2008; Lenardão et al., 2007). The use of semi-synthetic drugs allows circumventing the factors that limit the proper action of the active principle, making it more selective, active, and consequently increasing its pharmacological activity (Cechinel Filho and Rosendo, 1998). Therefore, several semi-synthetic drugs are already being used in antitumor chemotherapy (Newman and Cragg, 2007).

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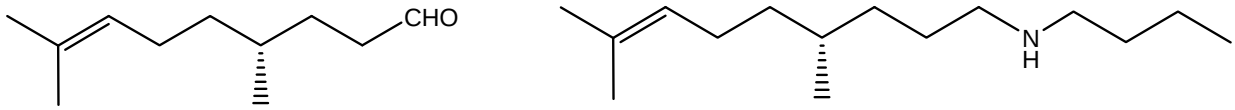
Figure 1. Specimens of *Cymbopogon* genus. **(A)** *Cymbopogon citratus* (DC) Stapf. **(B)** *Cymbopogon nardus* (L.) Rendle (Castro and Ramos, 2002).

Citronellal is a monoterpenic aldehyde, major constituent of several essential oils, including *Cymbopogon nardus* (L.) Rendle, *Cymbopogon winterianus* Jowitt and *Corymbia citriodora* (Hook) K. D. Hill & L. A. S. Johnson (Nhu-Trang et al., 2006). The use of citronellal in the fine chemical industry is well established (Lenardão et al., 2007; Sell, 2003; Swift, 1999) and the pioneer study of Osato (1964), also demonstrated its potential use as chemotherapeutic drug with few side effects.

The literature regarding the use of essential oils of lemongrass and citronella, as well as their constituent citronellal, in the breast cancer therapy, is scarce. Therefore, we propose the study of cytotoxicity and of selective potential of these essential oils and of citronellal in breast tumor cells (MCF-7 - ATCC HTB-22) and in non-tumor cells (Vero - ATCC CRL-1586). We also propose the study of the potential of a new, chemically modified, citronellal compound, named as C37A.

MATERIALS AND METHODS

Essential oils and chemicals. The essential oils of lemongrass (*C. citratus*) and citronella (*C. nardus*) (Pólo Oleoquímico, Três Passos, Brazil) were diluted in absolute ethanol and values were expressed at percentage of oil. Compound (*R*)-citronellal (Figure 1) was obtained by fractioned distillation of citronella essential oil. The chemically modified citronellal was obtained by a reductive amination of (*R*)-citronellal (Jacob *et al*., 2009), being named C37A (Figure 2). The mass of the chemicals were assessed with a precision scale and these were diluted in absolute ethanol. The concentration values were expressed in mM. The percentage of all tested products did not exceed 1% of culture medium.



(*R*)-citronellal (*R*)-*N*-butyl-citronellil amine, C37A

Figure 2. Molecular structure of tested compounds.

Cell Culture. The MCF-7 (ATCC HTB-22) and Vero (ATCC CRL-1586) cells were cultured in Minimum Essential Medium (MEM) (Life Technologies, USA), supplemented with 10% of Fetal Bovine Serum (FBS) (Cultilab, Brazil) and 1% antimicotic-antibiotic solution (Sigma-Aldrich, USA). The cells were cultivated at 37 °C in a humidified atmosphere containing 5% CO_2 in air. Once they reached 80% confluence they were subcultured in 96-well plates at $1.5x10^4$ cell/well for cytotoxicity assay. The plates were incubated for 24 h and after this period, the medium was replaced for 1% FBS medium containing dilutions of the tested products. The cytotoxic effect was evaluated after 24 hours of incubation. Wells with culture media without oils and with oils solvent (ethanol) in the same plate were left as controls. To avoid interference caused by the volatility of the products, the plate's wells were covered with paper wrap.

Cytotoxicity Assay. The cytotoxicity effect was evaluated using the crystal violet assay. Plates were washed with Phosphate Buffered Saline (PBS) and incubated for 10 min at room temperature with 5% formaldehyde (for cell fixation), washed again and incubated for additional 10 min with crystal violet solution (0.05%). The solution was removed and 1% Sodium Dodecyl Sulfate

was added to solubilize the dye. The plate was kept in a rotating platform for 10 min, until obtaining a uniform color. Absorbance was read in a spectrophotometer (TERMOPLATE-TP Reader) at 570 nm.

The percent cytotoxicity was calculated as [1-(optical density of oils-incubated cells/optical density of control cell)] x 100. The Inhibitory Concentration 50% (IC_{50}) values were determined by nonlinear regression using Prism software v5. The selectivity index (SI) was determined by the ratio of the values of IC_{50} for Vero (ATCC CRL-1586) and MCF-7 (ATCC HTB-22) cells. Compounds with SI values $\geq$ 3 were considered selective.

Statistical analysis. Four independent experiments were performed, each one in triplicate. The statistical analysis was conducted by analysis of variance (ANOVA) and T-test. $P<0.05$ was considered statistically significant.

RESULTS

Cytotoxicity of different compounds to MCF-7 and Vero cells. The cytotoxicity mediated by both essential oils of lemongrass and citronella resulted in decreased cell viability when compared to control. The control exhibit expected growth, and the solvent alone showed no significant changes in cell viability. The two tested oils showed dose-dependent effect on MCF-7 (ATCC HTB-22) cell, except at higher concentrations of lemon grass oil, $10x10^{-2}$% and $5x10^{-2}$%, which resulted in decreased cytotoxic effect. The minimum concentration to cause 100% inhibition of cells occurred in $2.5x10^{-2}$% for both oils (Figure 3). In non-tumor cells, Vero (ATCC CRL-1586), there was also a dose-dependent response for the two oils. However, the response for increasing dose did not occurred in the same way when the concentrations were doubled. The minimum concentration to cause lethality of all cells was $1.25x10^{-2}$% for lemon grass oil and $2.5x10^{-2}$% for citronella oil (Figure 3). The terpenoid compounds, citronellal and C37A, also presented cytotoxicity in a dose dependent manner against MCF-7 cells, although C37A showed greater effect. The minimal concentration to cause lethality in all cells was 4 Mm. for citronellal and 1 mM for C37A (Figure 3). As observed with lemongrass oil, when these cells were exposed at higher concentrations of C37A, there was a tendency in decrease its cytotoxic effect. These findings assume that these tumor cells are inhibited at some concentration range. It is believed that at higher concentrations, these products (lemongrass oil and C37A) did not present sufficient hydrophobic characteristic, preventing its entrance into the cell membrane. However, the reason for this phenomenon should be clarified with more detailed analysis.

The Vero cell, when exposed to natural, unmodified citronellal, shown little decrease of viability, and a linear effect. On the other hand, C37A resulted in greater cytotoxic effect to Vero cells, showing dose depend effect. Both products presented cytotoxicity in a dose dependent manner against MCF-7 cells, although C37A showed greater effect. The minimal concentration to cause lethality in all cells was 4 mM for citronellal and 1 mM for C37A (Figure 3).

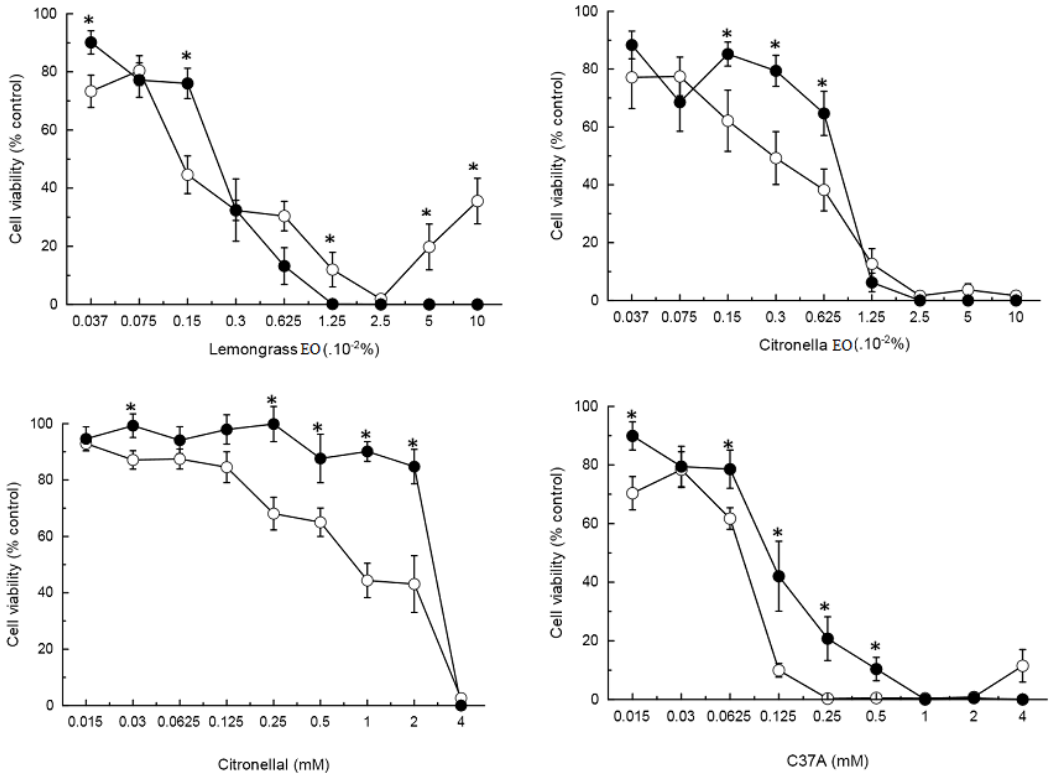


Figure 3. Viability of MCF-7 and Vero cells treated with different compounds. Black dots: Vero; White dots: MCF-7. Values presented as mean +/- SEM, * $p < 0.05$.

Inhibitory concentration 50%. Lemongrass EO demonstrated greater cytotoxic effect for both cell lines when compared with citronella EO. Citronella EO demonstrated higher IC_{50} in MCF-7 cells, when compared with the non-tumor cell, while lemongrass EO showed small decrease IC_{50} in that same cell line, with similar IC_{50} values for both cell lines (Figure 4).

Concerning the chemical compounds, citronellal and C37A, in Vero cells the IC_{50} was greater than the EOs for both compounds; however, difference between cell lines was more evident for citronellal. It was also observed that the modified compound, C37A, was considerably more cytotoxic to both cell lines when compared with the parental citronellal (Figure 4).

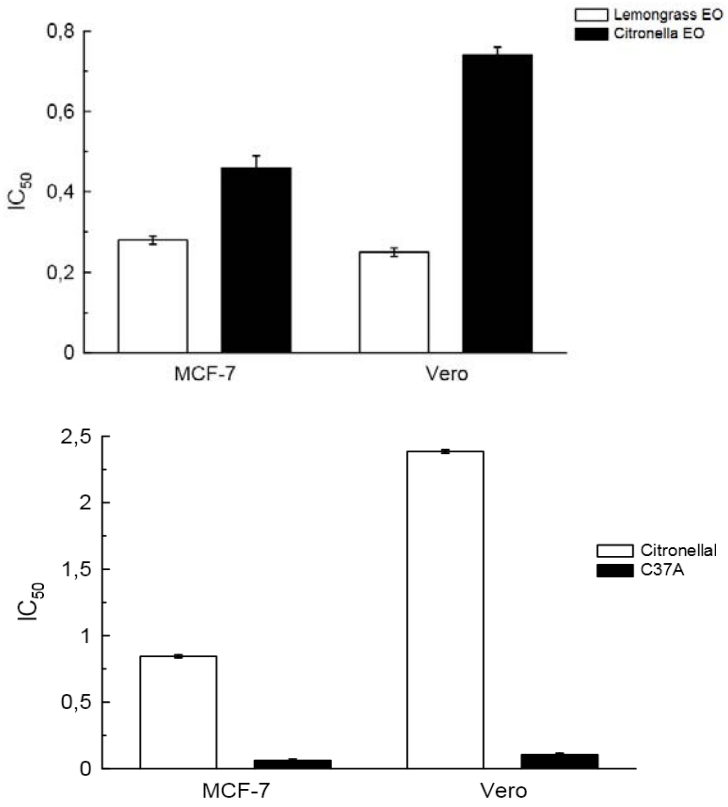


Figure 4. Inhibitory Concentration 50% (IC_{50}) of products. **(A)** Essential oils. MCF-7 –Lemongrass EO: $0.28.10^{-2}$%; Citronella EO: $0.46.10^{-2}$%; Vero – Lemongrass EO: $0.25.10^{-2}$%; Citronella EO: $0.74.10^{-2}$%; **(B)** Chemical compounds. MCF-7 - Citronellal: 0.091 mM; C37A: 0.082 mM; Vero - Citronellal: 2.35 mM; C37A: 0.13 mM. Values presented as mean +/- percentual error.

Selectivity index . To verify if the tested compounds were more cytotoxic to tumor cells than to non-tumor cells, as expect for a good chemotherapy candidate, we evaluated the selectivity index (SI) between the two cell lines used (Table 1). A compound with a selectivity index higher than 3.0 is considered to have high selectivity, whereas compounds with SI less than 3.0 are considered non selective (Prayonga, Barusruxb and Weerapreeyakulc, 2008). Thus, citronellal showed high selectivity (SI = 25.8), while the others tested products did not show selectivity.

Table 1. Selectivity index (SI) of essential oils and chemicals between non-tumor cells (Vero) and tumor cells (MCF-7).

Products	Selectivity index
Lemongrass EO	0.9
Citronella EO	1.6
Citronellal	25.8
C37A	1.6

CONCLUSION

All tested products presented cytotoxic effects in both cell lines, MCF-7 (ATCC HTB-22) and Vero (ATCC CRL-1586). Citronella essential oil, citronellal and its derivative *N*-citronellylamine, presented higher cytotoxic response to tumor cell MCF-7 than to non-tumor cell Vero, being citronellal a high selective compound. Further studies, including *in vivo* studies, should be performed for better determining the mechanism of action of citronellal, suggesting its use as potential antitumor drug.

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