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## Isolation, characterization and evaluation of antimicrobial and cytotoxic activity of estragole, obtained from the essential oil of *croton zehntneri* (euphorbiaceae)

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### ABSTRACT

*Croton zehntneri* (Euphorbiaceae) is a native aromatic plant from Northeast region of Brazil. The monoterpenoid estragole (ESL) has been isolated by classical chromatographic methods from the essential oil (EO) of *C. zehntneri* leaves and characterized by GC-FID and GC-MS, its antimicrobial and cytotoxic potentials being assessed. The analysis of the EO enabled the identification of 100% of the integrated constituents, of which yield was about 1.8%. The main components identified were: eucalyptol, estragole (84.7%) and spathulenol. The dosage of 50 µg/disk of ESL presented fairly significant zones of inhibition against Gram-positive bacteria and fungi. The ESL presented toxicity against *Artemia salina* with LC<sub>50</sub> and LC<sub>90</sub> of 4,54 and 8,47 µg mL<sup>-1</sup>. However, in tumor inhibition assays (human cells), there were no rewarding inhibition in any of the human cancer cell lines (MCF-7, HEP-2 and NCI-H292).

**Key words:** Antimicrobial, *Croton zehntneri*, cytotoxic, estragole.

### INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths (Emori and Gaynes 1993). Cancer is the cause of more than six million deaths in the world each year. In 2001, about 1,268,000 new cancer cases and 553,400 deaths were reported in the United States (Izevbigie 2003).

Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Substantial investment and research in the field of anti-infectives are now desperately needed if a public health crisis is to be averted. Structural modification of antimicrobial drugs to which resistance has developed has proven to be an effective means of extending the life-span of antifungal agents such as the azoles (Jeu et al. 2003),

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antiviral agents such as the non-nucleoside reverse transcriptase inhibitors (De Clercq 2001), and various antibacterial agents including  $\beta$ -lactams and quinolones (Poole 2001).

It is not surprising then, that in response to antimicrobial resistance, major pharmaceutical companies have tended to concentrate their efforts on improving antimicrobial agents in established classes (Taylor et al. 2002).

Rational drug design does not always yield effective antimicrobials. In the past, potent enzyme inhibitors have been successfully designed and synthesized but they had only modest antibacterial activity, probably owing to the complex issue of drug uptake by cells. Broad empirical screening of chemical entities for antimicrobial activity represents an alternative strategy for the development of novel drugs. Natural products have been a particularly rich source of anti-infective agents, yielding, for example, the penicillins in 1940, the tetracyclines in 1948 and the glycopeptides in 1955 (Silver and Bostian 1990).

The impact of infectious diseases is dodgy to the developing countries due to relative unavailability of medicines and the emergence of widespread drugs resistance (Zampini et al. 2009).

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has lead to the search of new antimicrobial agents with the goal to discover new chemical structures, which overcome the above disadvantage (Okemo et al. 2003, Bounamama et al. 2006).

Observing that microorganisms resistant to antimicrobials agents represent a challenge in the treatment of infections, it is notorious the need for finding new substances with antimicrobial features to be used in future formulations of commercial products. *Croton zehntneri* (Figure 1) is an aromatic plant native to Northeastern Brazil, and widely distributed in the municipality of Simões, State of Piauí, Brazil, where it is popularly called “Canelinha” and used as a sedative, as an antiseptic, and for gastrointestinal disturbances.

Because of the potential activities of the essential oil of *Croton zehntneri* collected in different regions of Brazil (Morais et al. 2006), and possible carcinogenic and genotoxic activity documented in the literature, we decided to determine the chemical composition of the essential oil from *C. zehntneri* from Simões city - Brazil, and evaluate the antimicrobial and cytotoxic activities of its main component: estragole (iv).



**Figure 1** - Photo of a specimen of *Croton zehntneri* (Euphorbiaceae), showing branches and inflorescence, and structure of estragole.

## MATERIALS AND METHODS

### PLANT MATERIAL

Leaves and stems of *C. zehntneri* (Euphorbiaceae) were collected in January of 2011 in Simões, Piauí, Brazil. The voucher specimen was collected by Dr. Sidney Lima, and identified by Dr. Roseli Farias Melo Barros (UFPI), and deposited in the Herbarium Graziela Barroso the Federal University of Piauí, Brazil, (number 27.273)

### EXTRACTION AND ANALYSIS

Samples of fresh leaves and stems (about 300 g) of *C. zehntneri* was subjected to hydrodistillation (4h) to obtain the essential oil, and classic column chromatography technique was used as purification of the estragole. The essential oil and pure estragole was analyzed by Shimadzu GC-17A/MS QP5050A-GC-MS system according to Medeiros et al. (2012). The identity of each compound was determined by comparison of its retention index relative to C<sub>8</sub>-C<sub>20</sub> n-alkanes (Fluka Analytical, 1.0 mL Alkane Standard Solution), as well as by its spectra with the database library Wiley 229. The retention data (retention indexes) were compared to those of the literature (Adams 2007). The identification of estragole (1-allyl-4-methoxybenzene) was confirmed by coinjection with standard solution (estragole analytical standard, Sigma-Aldrich).

The analyses were also carried out by Gas Chromatography with Flame Ionization Detection (GC-FID) in an Agilent 5975C instrument using a capillary column coated with DB-5 (30 m x 0.25 mm i.d, 0.25 µm film thickness; J & W Scientific, Folsom, CA, USA), in condition similar to GC-MS above. Hydrogen was used as carrier gas. The GC-FID chromatogram was used to determine the relative concentrations using peak areas.

### MICROORGANISMS

Clinical strains of human pathogenic bacteria comprising 4 Gram positive bacteria: *Bacillus*

*subtilis*, *B. megaterium*, *B. cereus* and *Staphylococcus aureus* and 7 Gram negative bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *S. sonnei*, *Salmonella typhi*, *S. paratyphi* and *Vibrio cholerae*. In addition, 7 pathogenic fungi: *Aspergillus niger*, *Blastomyces dermatitidis*, *Candida albicans*, *Pityrosporum ovale*, *Trichophyton* sp., *Microsporum* sp. and *Cryptococcus neoformans* were used in this study. All the microorganisms were collected from the Microbiology Lab., Department of Pharmacy, BGC Trust University, Chittagong, Bangladesh.

### Preparation of media for antibacterial, antifungal and MIC test

To prepare nutrient agar (NA), saboured dextrose (SD) and nutrient broth (NB) media for antibacterial, antifungal and MIC tests: 24 g NA, 65 g SD and 13 g of NB were dissolved in 1000 mL of distilled water and adjusted to pH 7.4±0.2 and sterilized by autoclaving at 121°C for 15 min at 15 psi pressure (Ananthanarayanan and Paniker 2000). The source of media was Hi-Media Laboratories Ltd., India.

### Antimicrobial Screening

The antimicrobial activity of the ESL was evaluated by the disk diffusion method (Bauer et al. 1966), against 14 pathogens (11 bacteria and 7 fungi) using ciprofloxacin (CFN) and fluconazole (FCN) (Square Pharmaceuticals Ltd., Dhaka, Bangladesh), as standards for bacteria and fungi respectively. Three consecutive doses (5, 25 and 50 µg/disc) of ESL and a single dose (30 µg/disc) for both standards were applied on 5 mm sterile paper disc. The results were read by presence or absence of zone of inhibition. The zone of inhibition (mm) was then measured. Experiments were run in triplicate.

### Minimum inhibitory concentration (MIC)

Micro-dilution method was used to determine the MICs of ESL. In this test, microorganisms are tested for their ability to produce visible growth

on a series in dilution tubes (broth dilution). The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism is known as the MIC (Andrews 2001).

#### *Brine shrimp lethality bioassay*

Brine shrimp lethality bioassay method (Meyer et al. 1982), was applied for the determination of cytotoxic activity of ESL. Dimethyl sulfoxide (DMSO) solutions (serially diluted) of the samples (ESL and VS) were applied against *Artemia salina* in a 1 day *ex vivo* assay. Vincristine sulfate (VS) (Gedeon Richter Ltd. Bangladesh) was used as standard. Experiments were run in triplicate with different concentrations (10.5, 9.0, 7.5, 6.0, 4.4, 3.0, 1.5, 0.75, 0.375 and 0.187  $\mu\text{g mL}^{-1}$ ).

#### *Statistics*

Data obtained are reported as the mean  $\pm$  SD (standard deviation) and were followed by *t*-Student- Newman-Keuls as *post hoc* test. Differences were considered to be statistically significant when  $p < 0.01$ .

#### CYTOTOXICITY AGAINST TUMOR CELL LINES

The samples used for estragole in cytotoxicity tests were diluted in sterile pure DMSO and tested at a concentration of 25  $\mu\text{g mL}^{-1}$ . Tumor cell lines used were MCF7 (breast carcinoma), NCI H 292 (lung carcinoma), and HEP-2 (laryngeal carcinoma) obtained from Rio de Janeiro Cell Bank (RJ-Brazil). All cancer cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U  $\text{mL}^{-1}$  penicillin, 100 mg  $\text{mL}^{-1}$  streptomycin at 37°C with 5%  $\text{CO}_2$ . For all experiments, 190  $\mu\text{L}$  of tumor cells were plated in 96-well plates ( $10^5$  cells/ml for adherent cells or  $3 \times 10^5$  cells/mL for leukemias). Tested compounds (0.1–25 mg  $\text{mL}^{-1}$ ) dissolved in DMSO 1% were added to each well and incubated for 72h. Control groups received the same amount of DMSO. After 69 h of treatment 25 mL of MTT (5mg  $\text{mL}^{-1}$ ) was added, three hours later, the MTT

formazan product was dissolved in 100 mL of DMSO, and absorbance was measured at 595 nm in plate spectrophotometer (Berridge 1996). The  $\text{IC}_{50}$  values and their 95% confidence intervals for two different experiments were obtained by nonlinear regression using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, California USA). Doxorubicin was used as positive control for the tested cell lines.

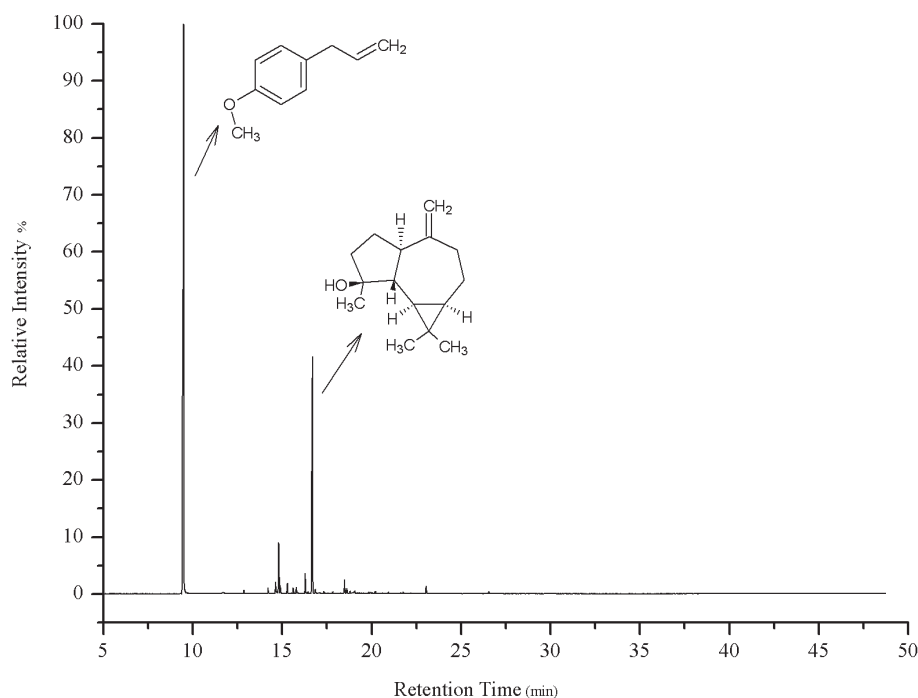
#### *Statistics*

The obtained data was evaluated by one-way analysis of variance (ANOVA) followed by Student Newman Keuls test. In all cases, differences were considered significant if  $p < 0.05$ , the means and respective standard errors were analyzed using the software Graph Pad Prism version 5.0 (GraphPad Software Incorporated, San Diego, USA). An intensity scale was used to assess the cytotoxic potential of the tested samples. Samples without activity (1-20% inhibition), with little activity (cell growth inhibition ranging from 20 to 50%), moderate activity (cell growth inhibition ranging from 50 to 70%) and with a lot of activity (inhibition of growth varying from 70 to 100%).

#### RESULTS AND DISCUSSION

Identification of essential oil components was performed by comparison of their retention indices GC-MS. The spectra were considered coincident if the similarity index was above 95%. The yields of essential oil obtained by steam distillation of the leaves 200 g were 1.8%. Table I shows the chemical composition and retention indices of the compounds identified. The chromatogram of the essential oil of *C. zehntneri* showed that seven peaks were detected and identified a total of seven compounds. Were identified 100% of chemical constituents (Figure 2, Table I) among mono- and sesquiterpenes, being recognized as a major component estragole, representing 84.7% of the essential oil content.



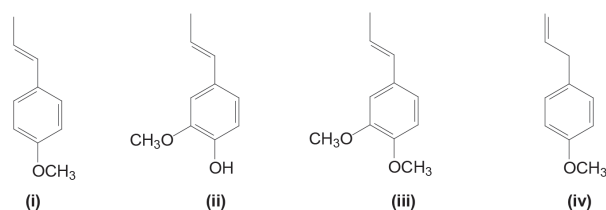


**Figure 2** - Chromatographic profile (GC-MS) of the essential oil of the *Croton zehntneri* leaves.

**TABLE I**  
Main components of the essential oil of the *Croton zehntneri* leaves.

Compound	Retention time /min	Perceptual area %
1,8 cineole	4.53	0.3
Estragole	9.39	84.7
Anisaldehyde	10.58	1.6
3(2H)-Benzofuranone, 2,4-dimethyl-	18.13	3.2
(+) Spathulenol	18.29	5.6
Aromadendrene	18.40	3.1
Methyl farnesoate	22.76	1.5
		<b>100</b>

According to literature (Morais et al. 2006, Santos et al. 2010), there are variations in the concentration of chemical constituents majority of *C. zehntneri* essential oil according to origin. Thus, this species was characterized in four chemical types: anethole (i) - for specimens collected in Fortaleza (CE) and Viçosa Ceara (CE), eugenol (ii) - for those collected in Areia Branca (RN) and Quixadá (EC); methyleugenol (iii) - for specimens collected in the Ipu (CE) and Oeiras (PI); estragole (iv) - for specimens collected in Tianguá (CE),



Salitre (CE) and Granja (CE). When compared to literature data, our study evidenced some differences in the chromatographic profile (GC-MS) as well as in quantitative composition and yield (about 1.8%) of essential oil *C. zehntneri*.

A large variety of commercial antibiotics are used to control infectious diseases. These may cause severe hypersensitivity reactions and lead to resistance to the pathogenic microorganisms. Next to the threat of drug resistance, and other infection related phenomena, there is a growing consumer demand for new chemicals. Furthermore, there is increasing legislation against the use of these, especially of chemical antimicrobials. It is, therefore, necessary to develop alternative agents and safe methods for controlling said diseases.

In the antibacterial and antifungal sensitivity test (Table II), the highest zone of inhibition (15.76 mm) was found against *Microsporium* sp. by the ESL at concentration 50 µg/disc. This was followed by 15.25, 14.81, 14.53, 14.53, 14.53, 14.33, 13.43, 13.27, 13.12, 13.09 and 12.12 mm against *B. megaterium*, *B. subtilis*, *S. aureus*, *S. sonnei*, *S. paratyphi*, *C. neoformans*, *B. cereus*, *P. ovale*, *C. albicans*, *B.*

*dermatitidis* and *V. cholerae* respectively. But it was inactive against *P. aeruginosa*, *Trichophyton* sp., *S. dysenteriae*, *S. typhi*, *A. niger* and *E. Coli*.

The diameter of inhibition zone is expressed as Mean  $\pm$  SD ( $n = 3$ ); Ni: zone diameter less than 8 mm was considered inactive.

ESL at the dose of 25 µg/disc produced the highest zone of inhibition (13.65 mm) against *C. neoformans*. Then followed by 13.33, 12.65, 12.35 and 12.31 against *B. subtilis*, *S. aureus*, *S. paratyphi* and *B. megaterium* respectively. Test dose was inactive against other test pathogens. Estragole at 5 µg/disc produced no inhibition to the tested organisms (Table II).

In this study, it was found that doses of 25 and 50 µg/disc showed a potential activity against the evaluated gram positive strains. However, against Gram-negative bacteria the inhibitory level was reduced or absent. The reduced inhibitory activity

TABLE II  
Antimicrobial sensitivity of ESL.

Test microorganisms	Inhibition zone diameter /mm			
	ESL per disc			Standards (30 µg/disc) CFN
	(5 µg)	(25 µg)	(50 µg)	
<b>Gram (+) bacteria</b>				
<i>B. subtilis</i>	Ni	13.33 $\pm$ 0.42	14.81 $\pm$ 0.65	18.01 $\pm$ 0.12
<i>B. megaterium</i>	Ni	12.31 $\pm$ 0.62	15.25 $\pm$ 0.37	19.67 $\pm$ 0.33
<i>B. cereus</i>	Ni	Ni	13.43 $\pm$ 0.57	18.21 $\pm$ 0.49
<i>S. aureus</i>	Ni	12.65 $\pm$ 0.25	14.53 $\pm$ 0.25	19.77 $\pm$ 0.44
<b>Gram (-) bacteria</b>				
<i>P. aeruginosa</i>	Ni	Ni	Ni	17.33 $\pm$ 0.63
<i>E. coli</i>	Ni	Ni	Ni	16.91 $\pm$ 0.25
<i>S. dysenteriae</i>	Ni	Ni	Ni	17.67 $\pm$ 0.37
<i>S. sonnei</i>	Ni	Ni	14.53 $\pm$ 0.21	19.17 $\pm$ 0.17
<i>S. typhi</i>	Ni	Ni	Ni	16.71 $\pm$ 0.42
<i>V. cholera</i>	Ni	Ni	12.12 $\pm$ 0.42	18.41 $\pm$ 0.32
<i>S. paratyphi</i>	Ni	12.35 $\pm$ 0.85	14.53 $\pm$ 0.37	18.25 $\pm$ 0.17
<b>Fungi</b>				FCN
<i>A. niger</i>	Ni	Ni	Ni	17.37 $\pm$ 0.37
<i>B. dermatitidis</i>	Ni	Ni	13.09 $\pm$ 0.42	17.01 $\pm$ 0.32
<i>C. albicans</i>	Ni	Ni	13.12 $\pm$ 0.63	17.97 $\pm$ 0.69
<i>P. ovale</i>	Ni	Ni	13.27 $\pm$ 0.17	18.93 $\pm$ 0.37
<i>Trichophyton</i> sp.	Ni	Ni	Ni	17.43 $\pm$ 0.21
<i>Microsporium</i> sp.	Ni	Ni	15.76 $\pm$ 0.21	18.10 $\pm$ 0.53
<i>C. neoformans</i>	Ni	13.65 $\pm$ 0.35	14.33 $\pm$ 0.47	16.25 $\pm$ 0.47

The diameter of inhibition zone is expressed as Mean  $\pm$  SD ( $n = 3$ ); Ni: zone diameter less than 8 mm was considered inactive.

of estragole on the Gram-negative bacteria can be justified because its outer lipopolysaccharide membrane rich, responsible for the hydrophilic character of the surface, hindering the penetration of hydrophobic substances such as estragole (Dorman and Deans 2000).

In this study five species of fungi showed satisfactory zone inhibition (Table II). According to Fontenelle et al. 2008, the essential oil of *C. zenhteneri*, specimen collected in the State of Ceara - Brazil, whose main component was estragole (72.9%), showed activity against some strains of fungi, however, this has not been definitively assigned to estragole. As ESL showed good antifungal activity, and it's a substance from a common aromatic plant in our community, it would the use of this compound in the search for new antifungal that can replace synthetic drugs that cause drawbacks in terms of toxicity, and cost effectiveness.

In MIC by serial tube dilution method, a potent MIC ( $38.52 \mu\text{g mL}^{-1}$ ) was shown by ESL against *S. paratyphi*. The MIC of 58.75, 58.75, 61.54, 63.15, 63.43, 88.51, 131.2 and  $145.0 \mu\text{g mL}^{-1}$  was found against *B. subtilis*, *C. albicans*, *P. ovale*, *B. megaterium*, *S. sonnei*, *C. neoformans*, *B. dermatitidis* and *S. aureus*, respectively. However, there was no inhibition to the other test pathogens (Table III).

**TABLE III**  
**Minimum inhibitory**  
**concentrations (MICs)**  
**by ESL.**

Test pathogens	MICs/ ( $\mu\text{g mL}^{-1}$ )
<i>B. Subtilis</i>	58.75
<i>B. Megaterium</i>	63.15
<i>S. aureus</i>	145.0
<i>S. Sonnei</i>	63.43
<i>S. Paratyphi</i>	38.52
<i>B. Dermatitidis</i>	131.2
<i>C. albicans</i>	58.75
<i>P. Ovale</i>	61.54
<i>C. Neoformans</i>	88.51

The monoterpenes, found in essential oils of citrus fruits, cherry, mint, and herbs, are non-nutritive dietary microconstituents mainly responsible for the distinctive fragrance of many plants. They are used as flavor additives in food, beverages, and perfumes. Recent studies have shown that monoterpenes exert antitumor activities, and suggest that these components can be a new class of chemotherapeutic agents (Elson and Yu 1994, Kellof et al. 1996, Crowell 1999).

Toxicity studies using *A. salina* has been suggest at many compounds with biological activity to determine its potential therapeutic application (Parra et al. 2001).  $\text{LC}_{50}$  and  $\text{LC}_{90}$  greater than  $250 \mu\text{g mL}^{-1}$  are considered to have low toxicity; between 80 and  $250 \mu\text{g mL}^{-1}$  moderately toxic, and  $\text{LC}_{50}$  and  $\text{LC}_{90}$  less than  $80 \mu\text{g mL}^{-1}$  are considered toxic (Carballo et al. 2002). In Table IV, all the  $\text{LC}_{50}$  estragole and Vincristine Sulfate, which is a widely used chemotherapeutic agent. Sulgate are shown the ESL increases the mortality rate of alive brine shrimps with the increasing order of doses. The test sample (estragole) showed potent citotoxicity response when compared to the standard, VS. There is a dose response relationship between the mortality percentage and drug dilutions.

McLaughlin et al. (1998) reported that the brine shrimp assay shows good correlation with cytotoxic activity in certain human solid tumors, suggesting a first step to evaluate the potential anti-tumor agents. Although it has been demonstrated that ESL presents significant results, as shown in Table IV, when compared to Vincristine Sulfate, its inhibition was not satisfactory against human cells (MCF-7, HEP-2, NCIH292) in this study, using other assay.

Aiming to evaluate estragole and/EO as a chemotherapeutic agent, we decided to examine their toxicity on three tumor cell lines MCF7 and NCI H 292 and HEP-2 at a concentration of  $25 \mu\text{g mL}^{-1}$ . The toxicity *A. saline* cannot be directly extrapolated to the toxicity in humans, since humans and other mammals have physiological media to remove toxic substances (Siqueira et al. 1998).



**TABLE IV**  
Percent inhibition of cell growth (IC%) in three tumor cell lines (25 µg mL<sup>-1</sup>).

Samples	LC <sub>50</sub> (µg mL <sup>-1</sup> )	LC <sub>90</sub> (µg mL <sup>-1</sup> )
ESL	4.54 ± 0.21*	8.47 ± 0.42*
VS	0.37 ± 0.02	0.71 ± 0.15

\**p*<0.01; ESL: Estragole; VS: Vincristine sulphate (control positive).

Analyzing samples of essential oil of *C. zehntneri* and estragole (Table V), it can be seen that all strains tested showed inhibition percentage lower than 20%. Given the results presented we inferred that estragole (main component of the essential oil of *C. zehntneri* collected in Simões – State of Piauí) has no inhibitory activity on tumor cell lines at the dose tested.

The test of *A. Saline* is not specific as antitumor guidance or for any physiological action in particular, but can be used in monitoring the fractionation of extracts, as suggested by Meyer et al. (1982). Therefore, substances with LC<sub>50</sub> ≤ 4.54 µg mL<sup>-1</sup> calculated in our experiment may indicate the existence of other biological activities. The dose 25 µg mL<sup>-1</sup> has been used by other authors (Da Silva and Albuquerque 2011) as an average value for assessing the potential of cellular inhibition.

We present a classical purification methodology of estragole from essential oil of *C. zehntneri* (from Simoes - State of Piauí), however more recently (Aguilar et al. 2014) our group has shown that this percentage can reach up to about 100% when leaf

samples were collected in February 2011, flowering period. Therefore, due to the wide application and toxicity the species in study is characterized as an alternative and viable source of estragole.

## CONCLUSION

In summary, when compared to literature data, our study evidenced some differences in the chromatographic profile (GC-MS) as well as in the quantitative composition (1.8% yield) of essential oil *C. zehntneri*. The estragole (ESL) in this study showed significant antimicrobial as well as cytotoxic activities against *A. salina*, however, it does not possess inhibitory activity on tumor cell lines at the dose tested. Due to the importance of that compound, the species *C. zehntneri* may be a potential alternative source of estragole, because of its good yield. The antimicrobial activity of ESL is a new candidate for future studies of synergism, compatibility, and mechanism of action.

It is important to mention that this specie has wide distribution in the “Serra de Simões” community, it is frequently used as tea, and their main component (estragole) has extensive potential therapeutic application, but can to present carcinogenic and genotoxic potential, as indicated by the report of the European Union, Committee on Herbal Medicinal Products.

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**TABLE V**  
Percent inhibition of cell growth (IC%) in three tumor cell lines (25 µg mL<sup>-1</sup>).

Sample	MCF-7		HEP-2		NCIH292	
	Inhibition (%)	Deviation	Inhibition (%)	Deviation	Inhibition (%)	Deviation
Estragole	4.62	1.25	0	0	7.36	2.72
EO	0	0	0	0	16.35	5.5
Doxorrubicina	60.08	3.26	78.12	2.74	87.92	2.84

EO: Essential oil of leaves from *C. Zehntneri*.

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### RESUMO

*Croton zehntneri* (Euphorbiaceae) é uma planta aromática nativa da região Nordeste do Brasil. O monoterpenóide Estragol (ESL) foi isolado por métodos cromatográficos clássicos a partir do óleo essencial (OE) das folhas de *C. zehntneri* e caracterizado por CG-DIC e CG-EM, tendo seu potencial antimicrobiano e citotóxico avaliado. A análise do OE permitiu a identificação de 100% dos constituintes integrados, cujo rendimento foi cerca de 1.8%. Os principais componentes identificados foram: eucaliptol, estragol (84.7%) e espatulenol. A dosagem de 50 µg/disco de ESL apresentou halos de inibição bastante expressivos frente a bactérias gram positiva e fungos. O ESL apresentou toxicidade frente a *Artemia salina* com CL<sub>50</sub> e CL<sub>90</sub> de 4,54 e 8,47 µg mL<sup>-1</sup>. Entretanto, nos testes de inibição tumoral (células humanas), não houve inibição satisfatória em nenhuma das linhagens de células humanas cancerígenas (MCF-7, HEP-2 e NCIH292).

**Palavras-chave:** Antimicrobiana, *Croton zehntneri*, citotóxico, estragol.

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