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ESSENTIAL OIL EUGENIA ASTRINGENS CAMBESS. QUANTITATIVE ANALYSIS AND POTENTIAL ASSESSMENT ANTIMICROBIAL, CYTOTOXIC AND GENOTOXIC.

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ABSTRACT: *The essential oil from the leaves of Eugenia astringens Cambess (OE-2) was obtained by hydrodistillation in a Clevenger apparatus modified. The essential oil chemical composition (0.17% yield) was analyzed in GC-MS. The main component in the leaves was the α - pinene. The result of the quantification of the OE-2 sample, the calibration curve showed that the percentage of α-pinene in the essential oil is 2.5% ± 3.9. Cytotoxic potential of the essential oil of Eugenia astringens Cambess was assessed by indirect diffusion in agarose gel in a strain of Staphylococcus aureus. The essential oil obtained in vitro antibacterial activity relevant for high dosage (25 µL). It has been found that the use of essential oil (12.5mL) along with the antibiotic (amoxicillin, 12,5 µL volume) did not potentiate the drug action, this remains invariable. The linearity of the calibration curve was proven in the evaluated concentration range, but through the linear model obtained by Ordinary Least Squares Method.*

KEYWORDS: Eugenia Astringens Cambess, Essential Oil, GC-MS, Linearity, Evaluation of Homocedasticity, Cytotoxicity Test, Staphylococcus Aureus, ATCC8096.

INTRODUCTION

The use of essential oils as active drugs or as adjuvants in drug preparation, in the food industry or in cosmetology has forced scientists to enhancing awareness about the potential adverse including toxicity and interactions with other drugs. The complexity of the compositions is not negligible the possibility of the existence of substances with high toxicity, capable of interfering with physiological systems or interact on the pharmacokinetics and pharmacodynamics or other xenobiotics administered concomitantly (FIGUEIREDO *et al*, 2007; OLIVEIRA *et al*, 2005; STIEVEN *et al*, 2009). The ANVISA (2003) recommends the toxicological knowledge of each ingredient used in the manufacture of medicines, foods or in cosmetology and their characteristics. The adoption of these measures avoid problems during the development of the final product and even after it is placed on the market (ANVISA, 2003). Preclinical tests are performed in vitro and in vivo. In vitro studies are considered experimental models of preliminary. These require additional studies for accuracy of the results. The main vitro cytotoxicity tests are tests genotoxicity and phototoxicity (ANVISA, 2003).

The Myrtaceae family has 500 species and about 80 genera, one of the most important families due to its wide distribution throughout the Brazilian ecosystem. One of the greatest representatives of Myrtaceae family are the species of the genus *Eugenia*. It features a variety of biological activities, among which we highlight anti-inflammatory activities, antibacterial, cytotoxic, antitumor, hypoglycemic, among others (MACHADO, 2005; OLIVEIRA et al, 2005; STIEVEN et al., 2009). *Eugenia astringens* (Synonymy: *E. rotundifolia*, *E. umbellilora*, O. Berg, *E. cassinoides* O. Berg) is another important species of this genus. The main components found in this species are: The α and β- pinene, α-copaene, β-elemene, alloaromadendrene, δ-cadineno, spathulenol, globulol, epiglobulol, β-caryophyllene and α-humulene (DEFAVERI *et al*, 2011 ; DE RAMOS *et al*, 2010). According to studies conducted by Celedonio and colleagues (2008), α-pinene has antimicrobial and neuroprotective activity

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in human neuroblastoma, also affecting the energy metabolism of isolated mitochondria by uncoupling of oxidative phosphorylation or inhibition of electron transport chain.

The choice of *Eugenia astringens* Cambess *(Myrtaceae)* was based on the high oil yield and easy plant location in the state of Rio de Janeiro, which facilitates the collection and transport reduce the cost of extraction process. According to the data obtained from the literature review, have not yet been carried out cytotoxicity tests, genotoxicity, skin sensitivity and irritation, for the essential oil obtained from species Eugenia astringens Cambess nor were assessed evaluation of linearity and through homocedasticity the preparation of three calibration curves in three days different to verify possible differences of linear behavior with daily variation of the analysis, one of the minimum requirements for the validation of bioanalytical methods (BRAZIL, 2012b). The absence of this information also corroborated with the choice and increased scientific interest in it. Therefore, the present study evaluated the antimicrobial, cytotoxic and genotoxic in vitro of essential oil in bacterial culture of *Staphylococcus aureus* ATCC 8096. The essential oil cytotoxicity (different essential oil concentrations, as well as its major constituent (α-pinene) was evaluated by disk diffusion method, using an antibiotic clinical use, described in the National List of essential Drugs (RENAME) within the Unified Health System (BRAZIL, 2012a). In addition, the quantitative and qualitative evaluations were performed the chemical constituents present in the essential oil from the leaves of *Eugenia astringens* Cambess by GC / FID and GC-MS and evaluation of linearity and homocedasticity by preparing three calibration curves in three different days for verification of possible differences of linear behavior with the change of the day of analysis (BRAZIL, 2012b).

MATERIAL AND METHODS

Plant Material

Eugenia astringens Cambess samples were collected in the morning (7: 00 a.m.) in Guaratiba Island, Rio de Janeiro, Brazil, in July 2012. The voucher specimen of the plant material is deposited in the Herbarium of the UFRJ National Museum identified by Dr. Marcelo da Costa Souza, the Department of Botany of the National Museum of the same University.

Extraction of Essential Oil and GC-FID analysis and GC-MS

Fresh leaves of *Eugenia astringens* Cambess (~704.46g) were submitted to hydrodistillation for 4 hours, distillation apparatus of the modified Clevenger type (Brazilian Pharmacopeia, 1988). The essential oil obtained in triplicate was dried over anhydrous Na_2SO_4, obtaining a yield of 1.2mL. Subsequently, the pure essential oil of *E. astringens* Cambess (OE-2) was placed in sealed glass vials wrapped in foil and stored in a freezer at -20ºC. The yield obtained for the oil *Eugenia astringens* Cambess was calculated based on the weight of the biomass used (EMBRAPA, 2004; CASTRO *et al*., 2006).

The analysis by gas chromatography with flame ionization detector in and automatic injection system (GC-FID) and gas chromatography-mass spectrometer (GC-MS) were performed in triplicate in the Analytical Methods Platform (AMP) of the Oswaldo Cruz Foundation - Farmaguinhos. We used the GC-FID Schimadzu the brand and model GC-6890 fused silica capillary column DB-5 (30cm x 0.25mm ID, film thickness of 0.25μm). Helium was used as

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carrier gas with a flow rate of 1.0ml/min. The oven temperature was programmed from 40°C (10 minutes) to 260 °C at 3°C/min. The temperatures of the injector and detector was 270°C and 280°C respectively. Used a GC-MS of Schimadzu brand, QP-5000- model Quadrupole and MS operating at 70 eV ionization energy. We used a fused silica capillary column DB-5 (30mm x 0.25 mm ID, film thickness of 0.25μM. Helium was used as carrier gas at a flow rate of 1mL/min split. The temperatures of injector and detector was 270 ° C and 280 ° C, respectively the temperature of the oven, injector and detector were the same as used in GC-DIC.

The components were identified based on retention index (RI) determined by using a calibration curve of a homologous series of n-alkanes (C_7-C_{30}) injected under the same chromatographic conditions and sample spectra of the fragmentation models mass, both compared with literature data. The concentration of the components were calculated through the full area of the respective peaks related to the total area of all the constituents of the sample obtained by chromatographic analysis of the gas phase system (VIEGAS & BASSOLI, 2007).

Linearity and Assessing homocedasticity Calibration Curve

To assess the linearity of the method was taken in quadriplicata analysis of five different concentrations of α-pinene (100, 200, 300, 400 and 500μg / ml). Three calibration curves were prepared in three days different to verify possible differences of linear behavior with variation of the day of analysis (ICH, 1995; LEITE, 2002; RIBANI *et al*., 2004). The statistical data was performed by Statistica software.

The standard solutions used for the verification were prepared using a standard solution containing the α-pinene (Sigma Aldrich, Lot 80796DJV) in concentration 5000.00mg/mL. The α-pinene OE-2 standard and were weighed on an analytical balance Sartorius model CP225D, standard solutions were prepared in volumetric flasks (5, 10, 25, 50 and 100mL) and volumetric pipettes (1, 2, 3, 4 and 5mL) calibrated. The solvent dilution of the solutions was dichloromethane (Tedia, Lot 912167R, validity: 01/22/2015). The concentrations of standard solutions for injection were 100, 200, 300, 400 and 500μg / ml α-pinene. The preparation of the samples for measurement was done with an OE-2 dichloromethane dilution. The concentration of the sample solution for injection was approximately 10mg/mL.

Determination of microbial activity

The antimicrobial activity was observed in vitro, by the diffusion method in paper disc according to the Health of Ministry protocol ordinance 1480/90. The methodology used in this project realized an adaptation to test for *Staphylococcus aureus*. The adaptation of the methodology involved the use of mannitol agar culture medium in order to isolate colonies and then subjecting them incubated in soybean casein (TSB) to achieve MC Farlland scale (0, 5) with 0.9% NaCl. The microorganism used in this study was *Staphylococcus aureus* ATCC 8096. Initially set up a quantity of TSB in the test tube and then incubated strains *S. aureus* ATCC 8096 was for 24 hours at 35°C in an oven. After checking the growth of the colonies, were planted with the aid of a bacteriological loop on board with mannitol agar and incubated for 24 hours at 35°C. The formation of isolated colonies were observed. The isolated colonies were resuspended in NaCl (0.9%). It was checked for turbidity of the suspension in saline

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according to McFarland coma scale (0.5) corresponds to a concentration of approximately 10^8 colony forming units - (CFU/mL).

Cytotoxicity Test.

The cytotoxic activity was detected in vitro by the method of diffusion in agarose gel using bacteriological paper discs (ANVISA, 2003). In this cytotoxicity test the essential oil application was done on the disc surface in contact with cells of the bacteria *Staphylococcus aureus*. The application of these products on the surface of nutrient agar in contact with the bacteria cells generates a halo. Halo diameter corresponds cytotoxicity of the tested product and its ability to spread on nutrient agar. The culture medium used was Muller-Hinton (MH). Antibiotic amoxicillin was employed (Neo química) the amount of 0,500g, dissolved in 10 ml of distilled water, according to the clinical dose recommended by the manufacturer (Neo química). The plates with MH were seeded with bacteria *S. aureus* with a swab. Bacterial paper discs for cytotoxicity assay was used. Discs were used in different volumes of the following samples: 25µL NaCl at a concentration of 0.9% (control), and 25µL 12.5µL amoxicillin 12.5µL and OE-2 and the mixture 25µL OE-2 both amoxicillin and with the volume of 12.5µL. Using the serial dilution procedure, we obtained the solution with concentration corresponding to minimum inhibitory concentration-MIC of the tested essential oil. Then the plates were numerically identified and placed in an incubator for 24 hours. The cytotoxic test was performed in duplicate.

RESULTS AND DISCUSSION

Components present in the essential oil

The essential oil of fresh leaves of *E. astringens* collected in july had an actual yield of 0.17%. This income corresponds to the volume / moisture free basis (BLU) or dry matter (EMBRAPA, 2004). The literature suggests that this method (BLU) indicates correct concentration of oil contained in the dry biomass is standardized and can be repeated at any time, without significant deviations (EMBRAPA, 2004). Defaveri and colleagues (2011) obtained a yield of 0.4% essential oil from the dried leaves of *E. astringens* in November. (DEFAVERI *et al*.,2007). Different results with respect to yield of essential oil with dry, fresh leaves have been found in other studies. Pereira and colleagues (2008) studied the essential oil yield of dry leaves *Cymbopogon citratus*, obtained the value of 2.16%, on a dry basis (db), higher yield compared the fresh leaves. Carvalho Filho and colleagues (2006) found that during drying of basil plants there is an increase in the linalool content of the essential oil. Already, Silva and colleagues (2004) found that neither moisture nor the particle size influenced the essential oil content extracted from the leaves of *Aloysia triphylla.*

The concentration of α-pinene (77.57%) was higher than that observed observed by Defaveri and colleagues (2007) corresponds to the essential oil α- pinene. 8 compounds (Table 1) were detected. With the exception of α-cubebeno other similar components to those identified in the work Defaveri and colleagues (2007).

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Table 1: Composition of essential oil of *Eugenia astringens*. KIC = Kovats index value calculated (KIC) and literature (KIL). (%) Corresponds to normalized values of the areas.

Components	KI_c	KI_L	(%)
□-pinene	930	939	77.57
□-pinene	978	980	12.49
myrcene	993	991	0.56
Terpinene	1062	1062	1.67
terpinen-7-al	1288	1287	0.83
Sesquisabinene hydrate (cis)	1544	1545	2.07
Farnesol (Z,Z)	1708	1713	1.60
Farnesol (E,E)	1716	1722	2.78
Total			**99.57**

Linearity and Assessing homocedasticity Calibration Curve

Statistical analysis of the data (Tables 2-6; Figure 1) began assessing the presence of outliers in each set of repetitions on each level of concentration employing the verification of *outliers* through the *Grubbs* test at $\alpha = 0.05$, assuming that the data come from a normal distribution. *Outliers* were observed in two days of preparation of the calibration curves and these observations were taken from the result set. The tests used to evaluate the homogeneity of variances were the *Levene* test (parametric test less sensitive to the normality assumption), when applied to the raw data without deleting comments, and *Brown & Forsythe* (nonparametric test, a modification of *Levene's* test) when the number of observations differ between groups, where results of the evaluation exclusion of outliers (LEVENE, 1960). The *Levene* test indicated dubious data homogeneity on 12/11/12 and constant variances in the days 11/08/2012 and 11/14/2012. However, in the last two days, were observed *outliers* have been removed and re-evaluated by *Brown & Forsythe* test, with the same assessment (BROWN & FORSYTHE, 1974a, 1974b; BUSSAB *et al*. 2003; ALMEIDA *et al.*, 2008). The probability distribution of the residuals of the regression was evaluated for three days and had a normal probability distribution only for the first day of evaluation, using as criteria the test to verify the normality of *Shapiro-Wilk's* (ROYSTON, 1983; BUSSAB *et al*., 2003). Considerable deviations from normality may be due to the presence of aberrant data and the lack of model fit. The options for a non-normal distribution is to effect a transformation of variables (√, log, reverse, etc.) or use the method of generalized least squares. The autocorrelation of the residuals was assessed by d of Durbin-Watson estimate and waste showed no positive or negative autocorrelation (BUSSAB *et al*., 2003). When the waste is autocorrelated ordinary linear regression not is suitable and you can effect either a transformation of variables (√, log, reverse, etc.) and be employed the method of generalized least squares (BUSSAB *et al*., 2003). The evaluation of the significance of the intercept was adequate, as it showed not significant in the three days of preparation of the calibration curves. If the intercept is statistically significant, the hypothesis that it is zero is rejected and the line does not pass through the origin. That is to say that there are statistical evidence to a 0.05 significance level that the true intercept does not fall within the confidence interval limits and that the system has addiction or bias.

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However, the regression was significant for the three day trial. If the slope is significant, the hypothesis that it is equal to zero is rejected, ie the regression is significant (BUSSAB *et al*., 2003). Otherwise, the non-rejection of the null hypothesis comes from two possibilities: the variable x does not explain the changes in the ratio of y or y to x is non-linear. As can be seen in Table 11.1, in two days there was a violation of the premises for fitting the data by Ordinary Least Squares Methods (OLSM), indicating that a more appropriate setting must be obtained by the Weighted Least Squares Method (WLSM), the which in fact could be verified by the smaller standard errors of the estimates obtained by the equations adjusted with weighting (BUSSAB *et al.*, 2003). Table 11.4 also out the violation of the premises when evaluated data on the global equation for OLSM (BUSSAB *et al*., 2003). The overall equation of the line is shown in Table 11.4.

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Table 2: Statistical evaluation of calibration curves for α-pinene (OLSM).

Tests	Curve 1 – 08/11/12	Curve 2 – 12/11/12	Curve 3 – 14/11/12
	Raw data ordinary linear regression (k=5, n=4, N=20	**Raw data ordinary linear regression (k=5, n=4, N=20)**	**Raw data ordinary linear regression (k=5, n=4, N=20)**
Homogeneity assessment of variances – *Levene Test* $p \leq 0,05$ – not constant variances $0,05 < p \leq 0,1$ – doubtful homogeneity $p > 0,1$ – constant variance	p = 0,396489 constant variance	p = 0.050965 doubtful homogeneity	p = 0.279201 variances constant
Straight-line equation	b_0 = 1.021803 b_1 = 0.695474	b_0 = 2.279748 b_1 = 0.682292	b_0 = 2.577304 b_1 = 0.692353
Assessment of significance of the parameter b_0 $p \leq 0,05$ – statistically significant intercept	p = 0.316254 not significant	p = 0.298641 not significant	b_0 = 2.577304 b_1 = 0.692353
Assessment of significance of the parameter b_1 $p \leq 00,05$ – statistically significant regression	p < 0,000001 statistically significant	p < 0,000001 statistically significant	p < 0,000001 statistically significant
standard error b_0	0.991202	2.130073	1.539260
standard error b_1	0.002936	0.006279	0.004539
R^2	0.99967937	0.99847775	0.99922684
R^2 adjusted	0.99966155	0.99839318	0.99918388
Estimated standard error	1.8901	4.0619	2.9353
Waste distribution assessment (normal test)- *Shapiro-Wilk's* $p \leq 0,05$ – non-normal distribution of waste	p = 0.16942 normal distribution	p = 0.03334 Non-normal distribution	p = 0.02860 Non-normal distribution
Outliers assessment by the *Grubbs* test (α=0.05; k=5, n=4, 1.46)	No outliers	2 *outliers* (1 e 17)	1 *outlier* (14)

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Autocorrelation evaluation of Statistics waste *Durbin-Watson* – for K=2 and N=20 d_L tab.=1.201 and d_U tab.=1.411 a 5%. Evaluation of positive autocorrelation: $d<d_L$ – positively autocorrelated waste $d_L \leq d \leq dU$ – inconclusive test $d>dU$ – waste not positively autocorrelated. Evaluation of negative autocorrelation: $(4-d)<d_L$ – negatively autocorrelated waste $d_L \leq (4-d) \leq d_U$ – inconclusive test $(4-d)>d_U$ – waste not negatively autocorrelated.	d = 2.376676 Serial corr. = - 0.194479 Waste not positively or negatively correlated	d = 2.022469 Serial corr. = - 0.026061 Waste not positively or negatively correlated	d = 1.947958 Serial corr. = 0.019916 Waste not positively or negatively correlated

Table 3: Statistical evaluation of calibration curves for α-pinene with outliers exclusion (OLSM).

Tests	**Curve 2 – 12/11/12** **Raw Data ordinary linear regression (k=5, n=4, N=18)**	**Curve 3 – 14/11/12** **Raw data ordinary linear regression (k=5, n=4, N=19)**
Homogeneity assessment of variances – *Brown-Forsythe Test* $p \leq 0,05$ – not constant variances $0,05<p \leq 0,1$ – doubtful homogeneity $p>0,1$ – constant variance	p = 0.058421 uniformity doubtful	p = 0.346426 variances constant
Straight-line equation	b_0 = 1.673071 b_1 = 0.686413	b_0 = 2.545819 b_1 = 0.692661
Assessment of significance of the parameter b_0 $p \leq 0,05$ – statistically significant intercept	p = 0.415947 not Significant	p = 0.125152 not Significant
Assessment of significance of the parameter b_1 $p \leq 0,05$ – statistically significant regression	p < 0,000001 statistically significant	p < 0,000001 statistically significant
standard error b_0	2.003390	1.578311
standard error b_1	0.005966	0.004711

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R^2	0.99879262	0.99921416
R^2 adjusted	0.99871716	0.99916794
Estimated standard error	3.4520	3.0060
Waste distribution assessment (normal test) - *Shapiro-Wilk's* p≤0,05 – non-normal distribution of waste	p = 0.00888 Non-normal distribution	p = 0.02585 Non-normal distribution
Autocorrelation evaluation of Statistics waste *Durbin-Watson* – for K=2 and N=20 d_L tab.=1.201 and d_U tab.=1.411 a 5%. Evaluation of positive autocorrelation: d<d_L – positively autocorrelated waste d_L≤d≤d_U – inconclusive test d>d_U – waste not positively autocorrelated. Evaluation of negative autocorrelation: 4-d)<d_L – negatively autocorrelated waste d_L≤(4-d)≤d_U – inconclusive test (4-d)>d_U – waste not negatively autocorrelated.	d = 1.794170 Serial corr. = 0.099834 Waste not positively or negatively correlated	d = 1.709506 d = 1.709506 Serial corr. = 0.140294 Waste not positively or negatively correlated

Table 4: Statistical evaluation of calibration curves for α-pinene (WLSM).

Tests	**Curve 1 – 08/11/12** **Raw data weighted linear regression (k=5, n=4, N=20)**	**Curve 2 – 12/11/12** **Raw data weighted linear regression raw data linear regression k=5, n=4, N=20)**
Straight-line equation	b_0 = 1.454900 b_1 = 0.694056	b_0 = 2.249921 b_1 = 0.682390
Assessment of significance of the parameter b_0 p≤0,05 – statistically significant intercept	p = 0.051846 not significant	p = 0.106812 not significant
Assessment of significance of the parameter b_1 p≤0,05 – statistically significant regression	p < 0,000001 statistically significant	p < 0,000001 statistically significant
standard error b_0	0.698669	1.325385

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standard error b1	0.002678	0.005056
R^2	0.99973215	0.99901290
R^2 adjusted	0.99971727	0.99895807
Estimated standard error	0.10876	0.20583
Outliers assessment regression (standardized Waste)	No outliers	No outliers

Table 5: Statistical evaluation of the overall calibration curve for α-pinene (OLSM).

Tests	**global curve**
	Raw data ordinary linear regression (k=5, n=12, N=60)
Homogeneity assessment of variances - *Levene Test* $p \leq 0,05$ – not constant variances $0,05<p\leq0,1$ – doubtful homogeneity $p>0,1$ – constant variance	p = 0.098468 doubtful homogeneity
Straight-line equation	b_0 = 1.968206 b_1 = 0.690003
Assessment of significance of the parameter b_0 $p \leq 0,05$ – statistically significant intercept	p = 0.063769 not Significant
Assessment of significance of the parameter b_1 $p \leq 0,05$ – statistically significant regression	p < 0,000001 statistically significant
standard error b_0	1.041420
standard error b_1	0.003075
R^2	0.99884926
R^2 adjusted	0.99882942
Estimated standard error	3.4397
Waste distribution assessment (normal test)- *Shapiro-Wilk's* $p \leq 0,05$ – non-normal distribution of waste	p = 0.00004 Non-normal distribution

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Regression outliers assessment (standardized residuals)	2 *outliers* (41 e 53)
Autocorrelation evaluation of Statistics waste *Durbin-Watson* – for K=2 and N=60 d_L tab.=1.549 and d_U tab.=1.616 a 5% Evaluation of positive autocorrelation: $d<d_L$ – positively autocorrelated waste $d_L \leq d \leq d_U$ – inconclusive test $d>d_U$ – waste not positively autocorrelated Evaluation of negative autocorrelation: $(4-d)<d_L$ – negatively autocorrelated waste $d_L \leq (4-d) \leq d_U$ – inconclusive test $(4-d)>d_U$ – waste not negatively autocorrelated	d = 2.117340 Serial corr. = -0.065232 Waste not positively or negatively correlated

Table 6: Statistical evaluation of the overall calibration curve for □-pinene (OLSM).

Tests	**global curve – OLSM**
	Raw data weighted linear regression (k=5, n=12, N=58)
Straight-line equation	b_0 = 2.001998 b_1 = 0.689893
Assessment of significance of the parameter b_0 $p \leq 0,05$ – statistically significant intercept	p = 0.004008 statistically Significant
Assessment of significance of the parameter b_1 $p \leq 0,05$ – statistically significant regression	p < 0,000001 statistically Significant
standard error b_0	0.667993
standard error b_1	0.002552
R^2	0.99920670
R^2 adjusted	0.99919303
Estimated standard error	0.17983
Outliers assessment regression (standardized Waste)	No outliers

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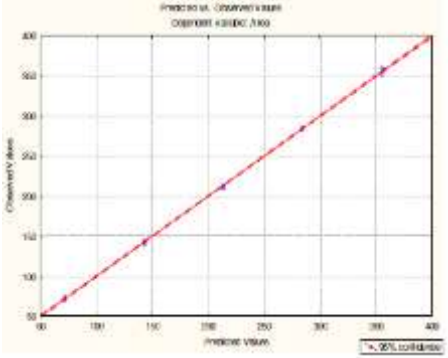
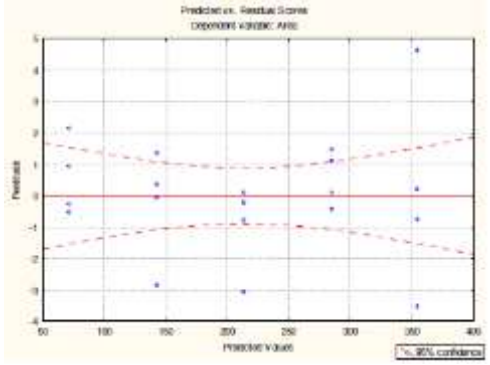
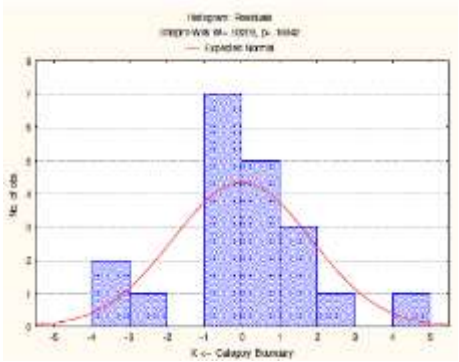
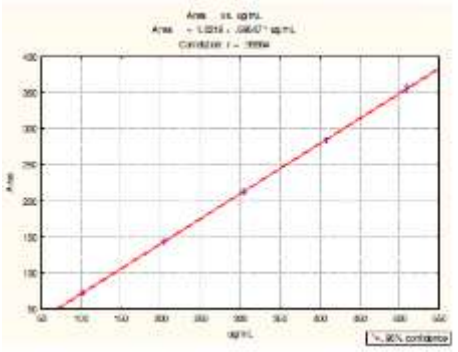
Figure 1. Graphics assessments of calibration functions in three days. 1-4: Graphs of ratings for the day 11/08/12; 5-8: Graphs of ratings for the day 11/12/12; 9-12: Graphics of ratings for the day 11/14/12.

Probability distribution graph for the curve data	Figure calibration function for the data of the curve.	Probability distribution graph for the curve data	Calibration function graph for the curve data
			
1	2	3	4

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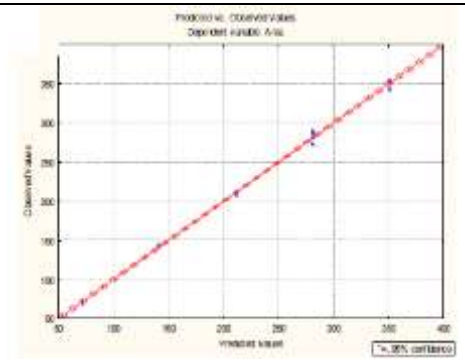

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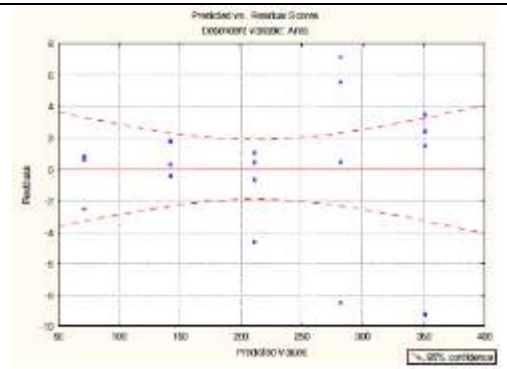

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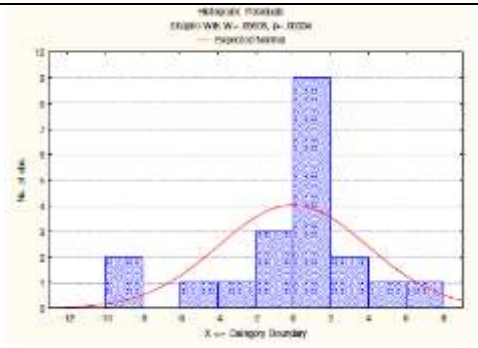

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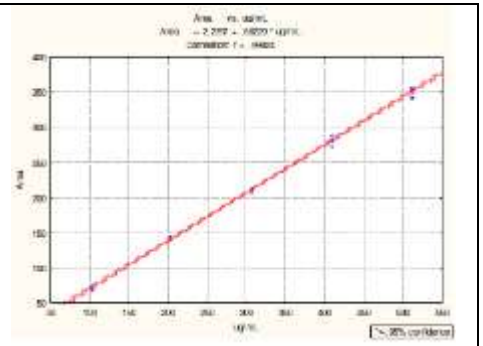

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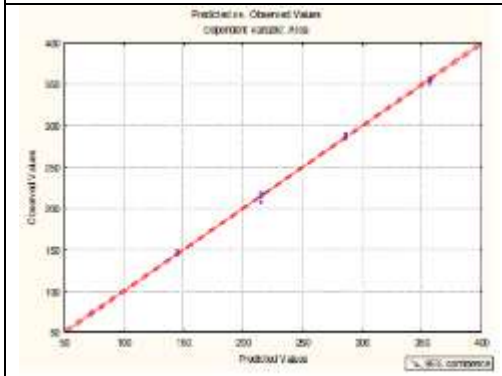

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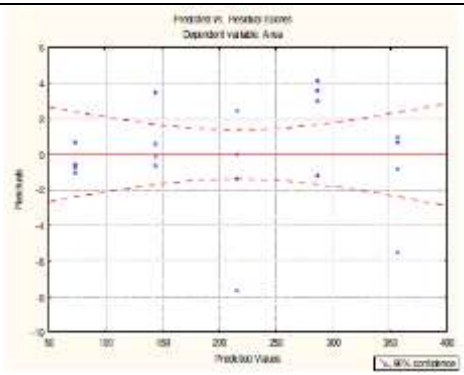

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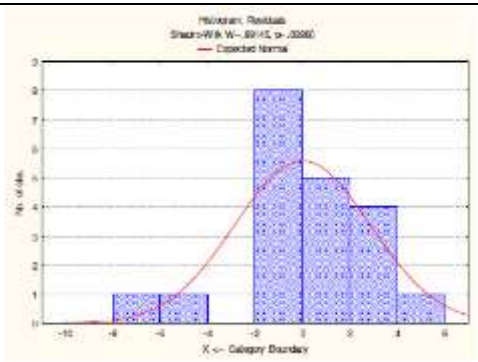

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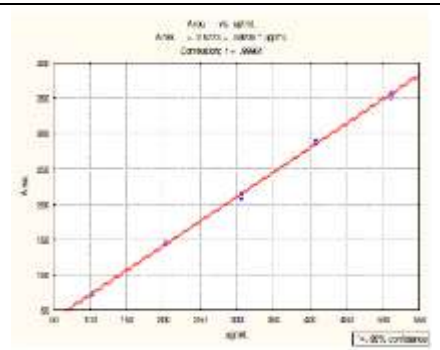

12

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Cytotoxicity assay

The result obtained in the OE-2 cytotoxicity assay was analyzed from the 1480/90 Ordinance data December 31, 1990 of the Health of Ministry (MS, 1480/90) which deals in one of its annexes (Annex 3) on preclinical assays for disposable absorbent products for external use, and determining the percentage of degenerated cells *in vitro* cytotoxic. In the cytotoxic test performed with the antibiotic amoxicillin (Neo química) and OE-2 for the most dose (25µL) were respectively halos of 60 mm and 28 mm. It was observed that the essential oil and the dose of antibiotic in 12.5µL had halos 12mm and 52mm, respectively. The essential oil blend of both antibiotic with volume 12.5µL formed a 50mm halo. Table 2 shows the calculation of the rate of cell lysis in cytotoxic test. The index zone (ZI) defines the diameter of the halo and the lysis index (IL), the percentage of degenerating cells in the cytotoxicity assay (MS, 1480/90).

Tabela 1. Table 7: Contents response (IR) to determine the percentage of degenerated cells

sample	Zone Index (ZI)	Lise Index (LI) *
Amoxicillin 12.5µL	52mm	Greater than 80%
Amoxicillin 25µL	60mm	Greater than 80%
Essential oil 12.5µL	12mm	Less than 80%
Essential oil 25µL	28mm	Greater than 80%
Amoxicillin 12.5µL + Essential oil 12.5µL	50mm	Greater than 80%

** Results obtained from the MS 1480/90 bibliography.*

CONCLUSION

Based on the decree 1480/90 (MS, 1480/90) we found that the essential oil of *Eugenia astringens* Cambes has antibacterial activity in vitro relevant in high dosage (25µL). It has been found that the use of essential oil (12.5µL) along with the antibiotic (amoxicillin, 12.5µL volume) did not potentiate the drug action, this remains invariable.

The result of the quantification of the OE-2 sample, the calibration curve showed that the percentage of α-pinene in the essential oil is 2.5% ± 3.9. In conclusion, the linearity was confirmed in the evaluated concentration range, but through the linear model obtained by WLSM (BUSSAB *et al.*, 2003). However, the results indicate that for each evaluation day is due if the oil, preparing a calibration curve for quantification because the statistical significance of the intercept of one of the three days of evaluation (BUSSAB *et al.*, 2003).

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