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Full Length Research Paper

Herreria salsaparilha Mart.: A potential source of antimicrobial and cytotoxic compounds

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Herreria salsaparilha **is a plant species popularly used for the treatment of syphilis and cutaneous infections. The aim of this work was to evaluate its potential antimicrobial activity against multi-resistant field strains of *Escherichia coli*, *Staphylococcus aureus,* their respective ATCC cell lines, and strains of *Tricophyton rubrum* H6 and *T. rubrum* mutant Δ*Tru*MDR2. Additionally, the cytotoxic activity against astrocytoma brain tumor cells (U343), human cervical cancer cells (HeLa) and murine melanoma cells (B16) was investigated. The antimicrobial activity was confirmed against both the *S. aureus* multi-resistant strain (MIC: 750 µg) and its ATCC strain (MIC: 50 µg). Moreover, the cytotoxic activity was determined for HeLa (IC_{50}: 2 µg) and B16 (IC_{50}: 10 µg) tumor cells.**

Key words: Cerrado, salsaparilha, anti-tumoral, Herreriaceae.

INTRODUCTION

Hundreds of plants endemic to Brazilian biomes are used as medicinal drugs for treating a variety of diseases like kidney affections, rheumatism, diabetes, arteriosclerosis, ulcers, cancer, venereal disease, urinary system affections and diarrhea (Rodrigues and Carvalho, 2001), though their efficacy have not been validated yet. *Herreria salsaparilha* belongs to these plants, its hydroalcoholic extract roots is popularly used to treat syphilis, eruptions, cutaneous infections, and as depurative, sudorific and stimulant (Peckolt, 1936; Corrêa, 1974).

Due to morphologic similarities, the species is frequently misidentified as belonging to the *Smilax* genus, also referred to as salsaparilha, and are indistinctively used for the same purposes (Cunha, 1940; Stellfeld, 1940).

In Brazil, *H. salsaparilha* is disseminated in diverse biomes such as Mata Atlântica, Cerrado and Pantanal (Brandão et al., 2004; Berg and Silva, 1988). The evaluation of its efficacy as well as the identification of potential chemical compounds is certainly important contributions for the safe use of this plant as therapeutic drug. No phytochemical studies on *H. salsaparilha* have been reported so far. The objective of this study was the evaluation of the antimicrobial and cytotoxic activities of *H. salsaparilha* crude root extracts and fractions; besides, the determination of compounds correlated to the activities displayed by the *H. salsaparilha* phytocomplex.

MATERIALS AND METHODS

H. salsaparilha exsiccates were identified by Rosana Conrado Lopes, Ph.D. (Department of Botany, Federal University of Rio de Janeiro, RJ, Brazil), and deposited at the Herbaria of the Federal University of Rio de Janeiro – UFRJ and at the University of Ribeirao Preto, UNAERP (vouchers, RFA 34467 and HMURP 00396, respectively).

H. salsaparilha roots (970 g) collected from the Medicinal Plant Collection at UNAERP were dried in oven with forced air circulation at 45°C for three days. Dry and powdered material was macerated in 80% ethanol. Hydroalcoholic crude extract (29.39 g) was obtained after evaporation under vacuum and freeze-drying procedures.

The dry crude extract (20 g) was then partitioned in a H_2O: MeOH: $CHCl_3$ mixture (40:10:50). The organic and aqueous fractions were dried and used in bioassays.

Additionally, an aliquot (5 g) of the hydroalcoholic crude extract was chromatographed on Sephadex LH-20 column (62 × 3 cm) with MeOH as eluent. Fractionation was monitored by thin layer chromatography (TLC) developed in $CHCl_3$: MeOH (7:3 v/v), and the chemical profile of extracts was determined using UV light and vanillinsulphuric acid spray reagent. The microbiological activities of four sub-fractions, pooled according to the TLC chemical profile and identified as H1, H2, H3 and H4 were investigated.

The chemical profile of constituents from the $CHCl_3$ root extract was

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determined by gas chromatography-mass spectrometry (GC-MS) using a Varian 3900GC/2100MS/injector CP8410 with capillary column Varian Factor FOUR Vf-5ms (WCOT fused silica) 30 m × 0.25 mm ID, 0.25 µm operating by electron impact (70 eV). Injector: 240°C; detector: 230°C; carrier gas: He; flux: 1.0 mL/min; dilution: 10 µg extract or fraction/1.0 mL $CHCl_3$; injection volume: 1 µl split: 1/20; program: 60 to 165°C, 3°C/min; 165 to 240°C, 10°C/min.

Microbial strains

S. aureus and *E. coli* clinical isolates resistant to antibiotics were obtained from the oropharyngeal and urinary tracts, respectively, of patients treated in the Electro Bonini Clinical Center at the University of Ribeirão Preto; ATCC bacterial strains, a Gram-positive *S. aureus* ATCC 6538 and a Gram-negative *E. coli* ATCC 25922; a field strain MYA-3108 (H6) and a mutant strain with a disrupted resistance gene named *ΔTru*MDR2 of *Trichophyton rubrum* (Fachin et al., 1996).

Determination of antimicrobial activity

The minimum inhibitory concentration (MIC) values of *H. salsaparilha* crude root extracts and fractions were determined against the bacterial and fungal strains following the CLSI M27-A2 (2003) and M38-A (2002) guidelines, respectively (CLSI, 2003, 2002). Stock cultures were submitted to serial dilutions within the range 15.6 to 1000 µg.

Sensitivity assays of tumor cell lines

The human brain tumor cell line U343 MGa (astrocytoma) was provided by Carlos Gilberto Carlotti, Jr., PhD. (Department of Surgery, Faculty of Medicine of Ribeirão Preto, FMRP, USP), and the human cervical cancer as well as the murine melanoma cells by Claudio Miguel da Costa-Neto, PhD (Department of Biochemistry, FMRP, USP).

Cytotoxic effects of extracts against tumoral lineages

Human U343 and HeLa cells were maintained in DMEN Medium (Gibco BRL, Eggenstein, Germany) supplemented with 10% fetal calf serum (Cultilab) in a 5% CO_2 atmosphere at 37°C. All cancer cell lines were plated into 96-well plates at a concentration of 1.0×10^5 cells/well. After 24 h, compounds dissolved in DMSO (range of concentrations: 2 to 100 µg/mL) were added to each plate well and incubated for 48 h in the same conditions described earlier. The cytotoxicity was determined using an XTT colorimetric cell proliferation assay (Roche Molecular Biochemicals). Briefly, the culture medium was removed, and 100 µl of fresh culture medium and a pre-formulated 50 µl XTT mixed reagent (50:1 XTT reagent: electronically coupled reagent) were added. The culture plate was incubated at 37°C for 4 h. The absorbance values (OD490) were determined using an ELISA ELX 800 reader. The final concentration of DMSO in the culture medium was kept constant, below 0.1% (v/v). All cell treatments were carried out in triplicate. Actinomycin D was used as positive control.

RESULTS AND DISCUSSION

Extracts and fractions of *H. salsaparilha* roots were active against *S. aureus* ATCC. Our results showed that the activity exhibited by the $CHCl_3$ fraction was 15 times superior to both the aqueous fraction and the crude hydroalcoholic extract, which in turn was less active (500 µg/mL) than its MeOH fraction H2 (125 µg/mL) (Table 1). According to the criteria established by Eloff, compounds are considered to be active antimicrobial when effective in concentrations below 100 mg/mL (Eloff, 2004).

The antibacterial activity of $CHCl_3$ *H. salsaparilha* root extract is associated to the accumulation of the major compounds linoleic acid ethyl ester and palmitic acid ethyl ester in *H. salsaparrilha* roots. The antimicrobial activity of those compounds against *S. aureus* strains has been reported (Bhattacharjee et al., 2005; Yang et al., 2003; Barbara et al., 2002).

The cytotoxic activity of *H. salsaparilha* crude extract and the $CHCl_3$ fraction against B16 (IC_{50}: 100 µg and 10 µg, respectively) and HeLa (IC_{50}: 2 µg) cells was confirmed. The efficacy of the $CHCl_3$ fraction was compared to Actinomycin D and considered just as efficient as that reference drug (Table 2).

GCMS chromatograms from the $CHCl_3$ fraction (Figure 1) and fraction H2 (Figure 2) showed the predominance of two peaks with retention times at 29.95 and 31.53 min, respectively. Peaks were identified by comparing their mass spectra (Figures 1A and 1B) with those in a data system library (NIST). Furthermore, co-injection with standard compounds allowed the identification of major compounds (Figure 3). In the $CHCl_3$ fraction, the major components found were the linoleic acid ethyl ester and palmitic acid ethyl ester in the proportion of 3:2 respectively, while the fraction H2 contains the same major components in the proportion of 2:3 respectively. Both compounds seem to play a role in the antimicrobial and cytotoxic activity displayed by the fractions, but the reduced efficiency of fraction H2 in relation to the $CHCl_3$ fraction suggests that the relative concentration of those fatty acids derivatives is modulating such activities.

The effect of saturated fatty acids, particularly linoleic acid, on tumoral cells is controversial. Though there is confirmation that this compound promotes the multiplication of cancer cells (Bartsch et al., 1999), several authors reported that there is no clinical evidence of that (Zock and Katanm, 1998; Erickson, 1998). In addition, some studies showed that it is the correlation between saturated and polyunsatured fatty acids that determine the beneficial or damaging effect (Chajes et al., 1999; Nkondjock et al., 2003). Moreover, the cytotoxic activity of palmitic acid ethyl ester against human leukemia cells was reported by Harada et al. (2002).

The activity of *H. salsaparilha* on the HeLa cells was significant when compared to other plant extracts suggested as anti-tumoral, as those from *Euphorbia arenaria* (IC_{50}: 74.6 µg) reported by Betancur-Galvis or to isolated compounds such as triterpenes saponins and alkaloids from *Ixeris sanchifolia* and *Guiera senegalensis* (IC_{50}: 9 µM) (Betancur-Calvis et al., 2002; Fiot et al., 2006).

The $CHCl_3$ fraction of the *H. salsaparilha* hydroalcoholic root extract, rich in palmitic and linoleic acid derivatives,

Table 1. MIC determinations of extracts and fractions obtained from roots of *Herreria salsaparilha* against *Escherichia coli, Staphylococcus aureus* and *Trichophyton rubrum.*

Sample name	*Escherichia coli* – ATCC (µg/mL)	*Escherichia coli* – Clinical (µg/mL)	*Staphylococcus aureus* – ATCC (µg/mL)	*Staphylococcus aureus* – Clinical (µg/mL)	*Trichophyton rubrum* H6 (µg/mL)	*Trichophyton rubrum* ΔTruMDR2 (µg/mL)
Crude extract	*	*	500	*	*	*
Aqueous fraction	*	*	750	*	*	*
Organic fraction ($CHCl_3$)	*	*	50	750	*	2500
H1**	*	*	*	*	*	*
H2**	*	*	125	*	*	*
H3**	*	*	1000	*	*	*
H4**	*	*	*	*	*	*
Antibiotics						
Sulfamethoxazole	1250	5000	1250	2500	n.t.	n.t.
Ampicillin	20	156	20	78	n.t.	n.t.
Chloramphenicol	20	20	20	78	250	250
Silver sulfadiazine	78	78	156	625	n.t.	n.t.
Gentamycin	20	20	30	90	n.t.	n.t.
Streptomycin	156	187	156	312	n.t.	n.t.
Tetracycline	500	1000	250	250	n.t.	n.t.
Griseofulvin	n.t.	n.t.	n.t.	n.t.	2.50	1.25
Fluconazole	n.t.	n.t.	n.t.	n.t.	36	36
Itraconazole	n.t.	n.t.	n.t.	n.t.	2.50	1.00
Amphotericin B	n.t.	n.t.	n.t.	n.t.	20	12

** H1 to H4 = fractions of the crude hydroalcoholic extract; * MICs over 2.5 mg/mL; n.t. = not tested.

Table 2. Percentage of growth inhibition of tumoral cells (B16, U343, HeLa) treated with the crude hydroalcoholic root extract of *Herreria salsaparilha* and its fractions.

Sample name	IC_{50}/(µg mL^{-1})			
	B16 (µg)	HeLa (µg)	U343 (µg)	3T3 (µg)
Crude extract	100	2	> 100	> 100
Organic chloroform fraction	10	2	> 100	> 100
Aqueous fraction	> 100	> 100	> 100	> 100
H1	> 100	> 100	> 100	> 100
H2	> 100	> 100	> 100	> 100
H3	> 100	> 100	> 100	> 100
H4	> 100	> 100	> 100	> 100
Actinomycin D	2	2	10	2

The highest concentration tested was 100 µg /mL^{-1}; IC_{50} = concentration that inhibits cell growth by 50%. U343, brain astrocytoma; HeLa, uterine cervix; B16, melanoma. H1 to H4 = fractions from the crude extract.

exhibited cytotoxic activity against B16 (murine melanoma) cells with an IC_{50} of 10 µg. Regular cells were not affected. Similar results were obtained with triterpene saponins from *Ixeris sansifolia* with an IC_{50} between 8 and 50 µM against melanoma A375 cells (Feng et al., 2003). Other reports on steroidal saponins from *Hedea helix* and phenolic compounds from *Mallotus japonicus* showed an IC_{50} of $\leq$ 5 µg against the same lineage (Danloy et al., 1994; Arisawa et al., 1990).

The biological activities and correlated chemical constituents of *H. salsaparilha* have never been reported before. Obtained results confirm the antimicrobial and

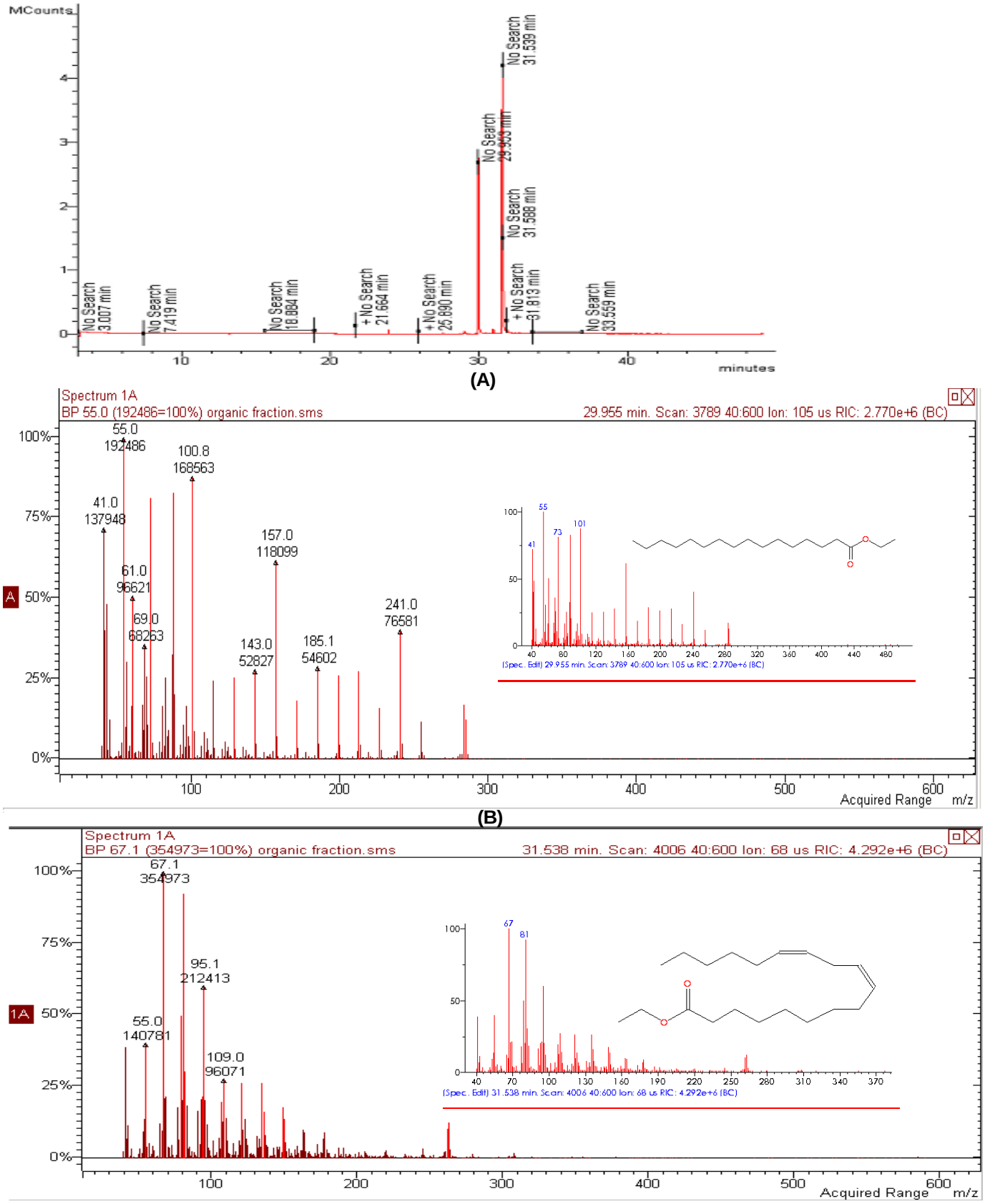

Figure 1. GC chromatogram of organic chloroform fraction from the crude hydroalcoholic extract of *H. salsaparilha* roots. (A) Mass spectrum of palmitic acid ethyl ester. (B) Mass spectrum of Linoleic acid ethyl ester.

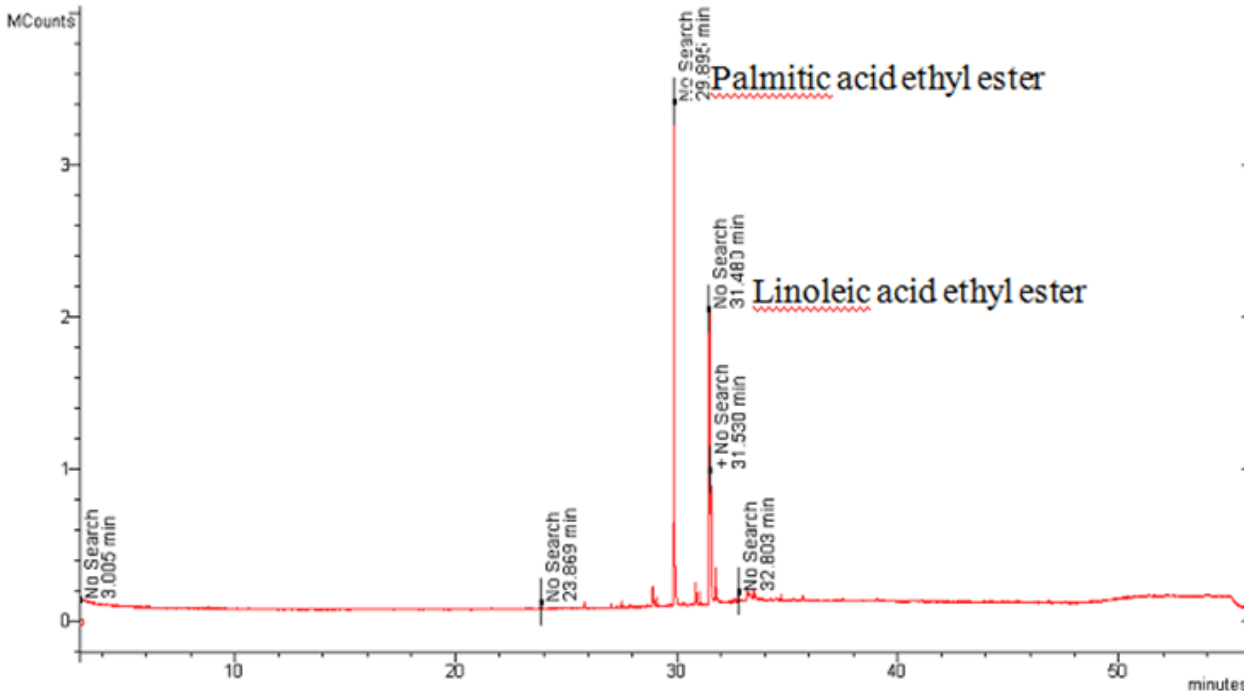

Figure 2. GC chromatogram of H2 fraction from the crude hydroalcoholic extracts of *H. salsaparrilha* roots.

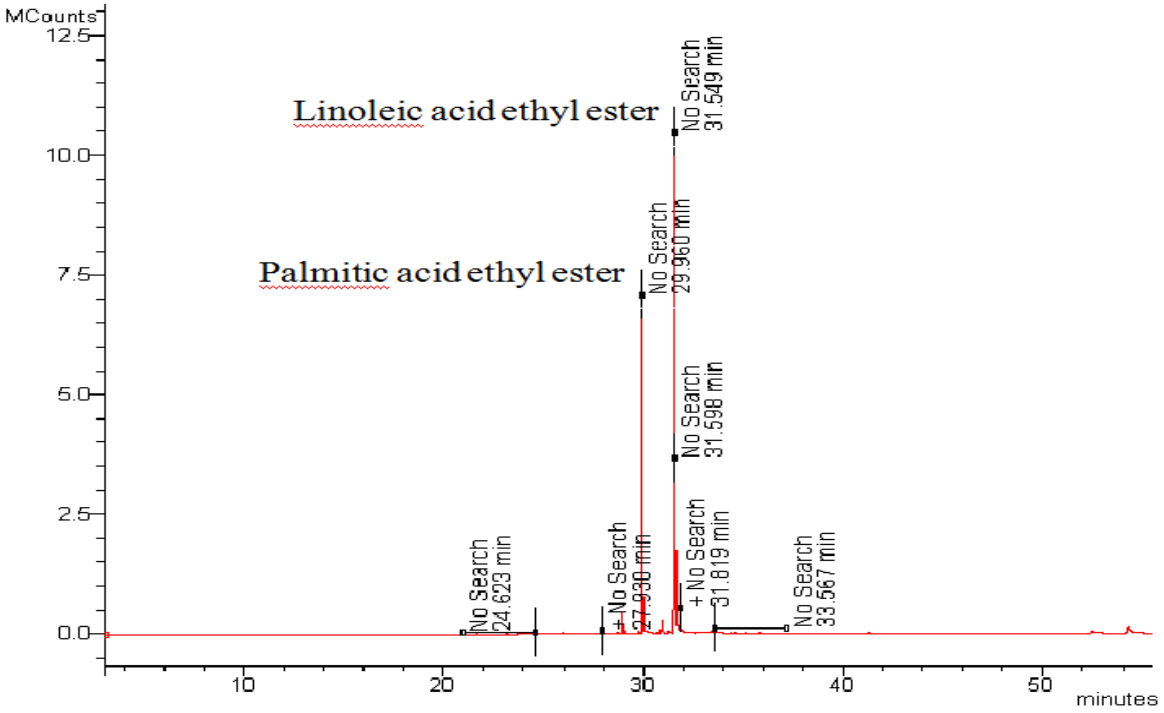

Figure 3. GC chromatogram of organic fraction co-injected with pure standards (hexadecanoic acid ethyl ester and linoleic acid ethyl ester).

cytotoxic activities of this phytocomplex, which are associated to the synergism between the identified compounds produced by that plant. Obtained results corroborate findings reported by Rao et al. (2004),

Slambrouck et al. (2007) and Arulvasu et al. (2010), who found that crude or semi-purified extracts from medicinal plants traditionally used by people all over the world may be, in near future, helpful in the treatment of numerous types of cancer, if associated to the traditional treatments using synthetic chemotherapeutics.

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