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PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF COPAIBA OIL: IMPLICATIONS ON PRODUCT QUALITY CONTROL*

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ABSTRACT

Background. The copaiba oil is a common natural product used in cosmetic industry and as a nutraceutical product. However, lack of quality control and scarce knowledge about its antimicrobial activity is a point of concern. The proposal of this study was to investigate the physicochemical properties and the antimicrobial activity of five commercial brands of copaiba oil.

Material and methods. Acidity and ester index, refractory index, solubility in alcohol, and thin layer chromatography were performed to verify the physicochemical properties of five commercial copaiba oils sold in local pharmacies. Ultra performance liquid chromatography coupled with diode-array detection and electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-DAD/ESI-Q-TOF-MS) was used to investigate diterpene acids while the volatile compounds were analysed by gas chromatography-mass spectrometry (GC-MS). Antibacterial and antifungal activities were also evaluated by agar diffusion technique; and minimal inhibitory concentration and maximal bactericidal concentration were defined for each sample and bacteria.

Results. The physical-chemical analysis revealed heterogeneity between all samples analysed. The A1 sample showed characteristics of copaiba oil and was mainly composed by hydrocarbon sesquiterpenes (29.95% β-bisabolene, 25.65% *Z*-α-bergamotene and 10.27% β-cariophyllene). Among diterpene acids, the UPLC-DAD/ESI-Q-TOF-MS data are compatible with presence of copalic and/or kolavenic acid (m/z 305 $[M + H]^+$). *Candida albicans* was sensitive to almost all samples at high concentration and *Saccaromyces. cerevisiae* showed sensitivity to A1 sample at 100 mg/mL. Although variable, all samples showed antibacterial activity. Significant activity was seen for A3 (19.0 ±0 and 15.6 ±0.5 mm), A4 (16.6 ±0.5 and 15.6 ±0 mm), and A5 (17.1 ±0 and 17.1 ±0 mm) on *Staphylococcus saprophyticus* and *S. aureus*, respectively. All samples were active against *Klebsiella pneumoniae* showing ≥15 mm diameter halo inhibition; and only A2 was active against *Eschirichia coli*. Phytopatogens tested revealed resistance of *Ralstonia solanacearum* CGH12 to all samples and susceptibility of Xcv 112 strain of *Xanthomonas campestris* pv *campestris* to almost all samples. MIC and MMC showed bacteriostatic effect against clinical interest bacteria and bactericidal effect against phytopatogens.

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Conclusion. The results from physicochemical analysis reinforce the fact that it is imperative to include simple conventional methods in the analysis of oil products. The analysis of copaiba oil gives safe products and purity which ensure products with quality. Also, since copaiba oil is an over-the-counter product the results indicate that pharmacosurveillance must be improved by the governmental regulation agency to avoid microorganism resistance selection and to achieve better international quality products.

Key words: nutraceutical product, microorganisms, antimicrobial action

INTRODUCTION

The copaiba oil is of great application in the industry to improve life time of food and it is a medicinal product widely used (Pieri et al., 2009; Sartoratto et al., 2004; Tempone et al., 2008; Veiga and Pinto, 2002). Several medicinal properties described for this oil have been scientifically proven, including antimicrobial, anti-inflammatory, and tissue regeneration (Amaral et al., 2005; Correia et al., 2008; Estevão et al., 2009; Santos, 2008; Mendonça and Onofre, 2009; Vieira et al., 2008). However, its great usage, the standardization and quality control of copaiba oil are very difficult. *Copaiba* genus has very homogeneous external characteristics and the environment conditions can lead to different oil composition which contributes to oil variation (Barbosa et al., 2012; Biavatti et al., 2006). Besides, another problem in Brazilian oil production is the adulteration of products sold in the market (Barbosa et al., 2009; Veiga et al., 1997). Thus, although antimicrobial activity of oil has been described in the literature, the relation of this activity with physicochemical analysis of commercial copaiba oil has not been done. Thus, the objective of this study was to investigate the *in vitro* antimicrobial properties of commercial copaiba oils bought in local market associated to their physicochemical analysis.

MATERIAL AND METHODS

Copaiba oil

Five copaiba oils were purchased in local market from Ilheus and Itabuna, Bahia, in June 2013. Samples were within the validity period, labelled and tightly closed. Products were manipulated under aseptic conditions, weighted in 1.5 mL tubes, and dissolved in a dimethyl sulfoxide (DMSO)-sterile distilled water solution (50:50) to obtain biological test concentrations. To perform physicochemical analysis, copaiba oil was used directly from the vessel. Samples were identified by letters and numbers: from A1 to A5.

Physicochemical analysis

The methods described in Vasconcelos and Goldinho (2002) were followed with some adaptation for all physicochemical analysis related to acidity index (*AI*), ether index (*EI*), and solubility in ethanol. Briefly, for *AI*, 2 g of oil were weighted in 125 mL Becker and added of 25 mL an ethyl ether : ethyl alcohol (2:1) mixture. The *AI* value was obtained by the equation $V \times N \times 56.1/m$, where V is NaOH volume in mL used for titration, N is the normality of NaOH solution, and m is the mass of each sample (g). For *EI* determination, the methodology described by Vasconcelos and Godinho (2002) with adaptations was also followed up. Briefly, 2 g of oil were weighed into a 125 mL flask where then 5 ml of ethanol and 2 drops of phenolphthalein alcohol solution for performing titration with 0.1 N NaOH were added. Then, to the titration of the resulting solution 20 mL of 4% KOH were added under heating. After cooling, the solution received 2 more drops of alcoholic phenolphthalein solution. Then, new titration was carried out using 0.5 N HCl solution. A titration without the oil sample was tested for comparison (blank). The *EI* values were obtained also using the equation $V \times N \times 56.1/m$, where V is the difference between the volume of HCl solution spent in titration of blank and sample in milliliters, N is the normality of the HCl solution and m is weight of each sample (g). The solubility in ethanol was performed using 1 mL of each oil sample to which were added 2 mL of ethanol analytical grade under agitation for 5 seconds. Samples were classified as soluble, insoluble, and partially soluble. The refractive index was

performed for each sample using *Abbé* (Optronics Inc., Precision Instruments, Germany).

Thin layer chromatography was performed with 20×20 cm aluminum plates covered with silica gel 60 containing fluorescent indicator Alugram Sil G/UV 254 (Macherey-Nagel, Germany). Elution was done with *n*-hexane:ethyl acetate (9:1) and visualization was done by spraying with 20% sulfuric acid solution and heating. Color aspect of spots and retention factor (R_f) was measured to compare with soybean oil, a common oil found in adulteration of copaiba oil (Barbosa et al., 2009; Vasconcelos and Goldinho, 2002).

The presence of diterpene acids was investigated by ultra performance liquid chromatography coupled with diode-array detection (UPLC-DAD) and electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS) using Waters Acquity UPLC system connected to Waters Q-TOF Premier mass spectrometer (Waters, Milford, CA, USA). A slightly modified method of high performance liquid chromatography (HPLC) (Souza et al., 2013) was successfully transferred to UPLC with aid of ACQUITY UPLC® Columns calculator (Waters, Milford, CA, USA). Separations were carried out on a Hypersyl Gold C18 column (1.9 µm, 2.1 mm × 100 mm; Themo Scientific, USA). The mobile phase consisted of 90% acetonitrile (Merck, Germany), 10% water (Milli-Q) and 1% (v/v) formic acid (96%, Tedia, USA). The analyses were performed at 25°C and at a flow rate of 0.47 mL/min over 5 min. The oils were dissolved in methanol (500 µg/mL) and an aliquot of 8 µL of each sample was injected. Mass detection (m/z = 50 to 1000) was carried out in positive mode and data were processed with MassLynx V4.1 software. Nitrogen and Argon were used as the nebulizer and collision gases, respectively. Other conditions: DAD, 200 to 400 nm; ESI capillary voltage, +3000V; source and desolvation temperatures, 100 and 250°C, respectively; desolvation and cone gas flow, 380 and 50 L/h, respectively; sample cone voltage and collision energy, 30V and 4 eV, respectively.

Additionally, the A1 sample was diluted in ethyl ether (2:100 v/v) and submitted to analysis by gas chromatography-mass spectrometry (GC–MS; Shimadzu QP5000), using a capillary column of fused silica Durabond-DB5 (30 m × 0.25 mm × 0.25 µm). Temperature program was 60 to 300°C at 3°C/min. The injector and detector temperature was set at 220 and 250°C, respectively. Carrier gas was helium at a flow rate of 1 mL/min. The compounds were identified by comparison of retention index, determined by linear interpolation relative to retention times of a homologous series of *n*-alkanes, and mass spectra (electronic impact, 70 eV) with the literature data (Adams, 2009) or National Institute of Technology and Standards database (NIST 62 and NIST 12). The relative percentage of each component was calculated from GC peak areas.

Microorganisms

For this study, clinical interest bacteria and yeast and phytopathogens were used. Bacteria were kindly provided by Pedro Costa Campos from the Biological Sciences Department from Bahia State University and yeasts were kindly provided by Dr. Sydney Hartz Alves from Mycological Research Laboratory (LAPEME) from Federal University of Santa Maria. The microorganisms used were of clinical interest (*Enterococcus faecalis*, ATCC31299; *Escherichia coli*, ATCC35218; *Klebsiella pneumonia*, ATCC700603; *Pseudomonas aeruginosa*, ATCC27853; *Staphylococcus aureus*, ATCC25921; *Staphylococcus saprophyticus*, ATCC35552; *Candida albicans*, ATCC 14057; *C. glabrata*, ATCC 2301; *C. parapsilosis*, ATCC 22018; *C. krusei*, ATCC 6258; *Saccaromyces cerevisiae*, ATCC 2601) and phytopathogens (*Acidovorax citrulli*, Aac. l.12; *Pectobacterium caratovorum* subsp. *caratovorum*, Pcc23; *Ralstonia solanacearum*, CGH12; *Ralstonia solanacearum*, B19; *Xanthomonas campestris* pv *campestres*, Xcv112; *Xanthomonas campestris* pv *campestris*, Xcc56).

Agar gel diffusion technique

For qualitative analysis, the well agar gel diffusion technique was used. For that, bacterial and yeast inoculums were prepared at an equivalent of 0.5 of McFarland scale (approx. cell density of $1.5 \cdot 10^8$ CFU/mL) and spread with sterile swab all over the Petri dishes containing nutrient or Saboureaud dextrose agar. The oils were prepared immediately before the test at 50, 100, and 1,000 mg/mL. After bacterial and yeast culture in Petri dishes, holes with 6 mm diameter were done in the agar where 50 µL of samples and controls were applied. Controls consisted of chloramphenicol (30 µg/mL), ketoconazole (50 µg/mL), chlorexidine (1%), sterile distilled water and DMSO (50:50). The inverted plates were incubated overnight at 37°C in a humidified

chamber to avoid evaporation of the medium. All tests were done in triplicate and repeated at least twice.

To analyse the antibacterial activity, inhibition halo ≥14 mm at ≤100 mg/mL were used as criteria to consider a sample as antibacterial. Following criteria established by Pedroso et al. (2014), the inhibition halo of ≥10 mm was used as the criterion to consider a sample as antifungal.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC)

The MIC and MMC were performed only for bacterial samples. The determination of MIC was done using a micro-dilution assay. Briefly, after filtration in 0.22 μm pore membrane, oils from 3.9 to 500 mg/mL were prepared in Müeller-Hinton broth and 90 μL of samples were distributed in plate wells. Additionally, 10 μL of bacterial suspension standardized at 0.5 of McFarland scale, was added to each well except in broth sterility controls. Controls consisted of chloramphenicol (30 μg/mL) and DMSO (50:50). After 24 h culture at 37°C, 30 μL of Rezasurin (Sigma-Aldrich, Brazil) were applied given 0.01% final concentration. Pink colour wells were considered positive and purple colour wells were considered negative. The MMC were then performed by taking 5 μL of CIM positive samples and inoculating Petri dishes containing agar Müller-Hinton. The plates were incubated at 37°C in humidified chamber. After 24 h, bacterial colonies were counted. The bacterial growth at lowest concentration was considered the MMC of the oil.

RESULTS

Physicochemical analyses

The physicochemical analysis revealed heterogeneity between all samples analysed. The A1 showed *in natura* copaiba oil characteristics and A5 showed strongly characteristics of soybean oil adulteration (Table 1) confirmed by the presence of an orange spot at R_f 4.2, equal to soybean oil (Fig. 1).

According to UPLC-DAD/ESI-Q-TOF-MS all samples presented one peak at 1.74 ±0.01 min (Fig. 2) consistent with m/z 305 $[M + H]^+$ (Fig. 3) of copalic acid and/or kolavenic acid (Messiano et al., 2008; Marchesini et al., 2009). The A1 sample was selected to GC-MS analysis and presented β-bisabolene (29.95%), *Z*-α-bergamotene (25.65%) and β-cariophyllene (10.27%) as major compound (Table 2).

Antimicrobial activity tests

The antifungal activity revealed that however at high concentration (1,000 mg/mL), *C. albicans* was sensitive to almost all samples with exception of sample A1. It is worth to note that the best response was for *S. cerevisiae* which showed sensitivity to A4 sample at 100 mg/mL (Table 3).

Table 1. Physicochemical analysis of commercial samples (A1 to A5) of copaiba oil and soybean oil

Oil sample	AI	EI mg KOH/g	RI	SE
A1	80.04	23	1.5020	soluble
A2	42.82	20	1.5034	partially soluble
A3	24.90	16	1.4986	partially soluble
A4	38.93	41	1.4920	partially soluble
A5	10.39	105	1.4851	insoluble
Soybean oil	–	181	1.4747	insoluble

AI – acidity index. EI – ether index. RI – refractive index. SE – solubility in ethanol. Standard values for *in natura* copaiba oil: AI – 80 mg KOH/g, EI – 23 mg KOH/g, RI – 1.50284 to 1.50786, SE – soluble.

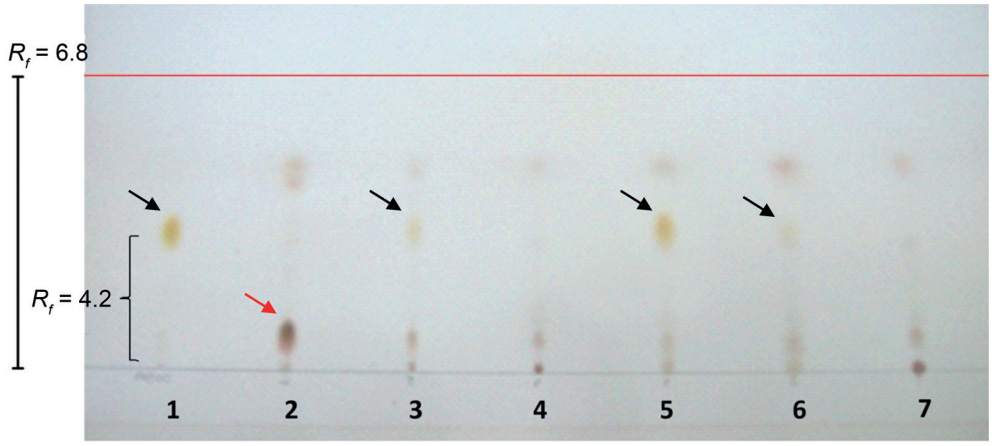

Fig. 1. Thin layer chromatography profile of oil-resin commercial samples and soybean oil: 1 – soybean oil, 2 – A1, 3 – Af, 4 – A2, 5 – A5, 6 – A3, 7 – A4. Red arrow – characteristic of copaiba oil-resin spot; black arrow – characteristic of copaiba oil-resin spot, red line – upper limit of mobile phase, R_f – retention factor

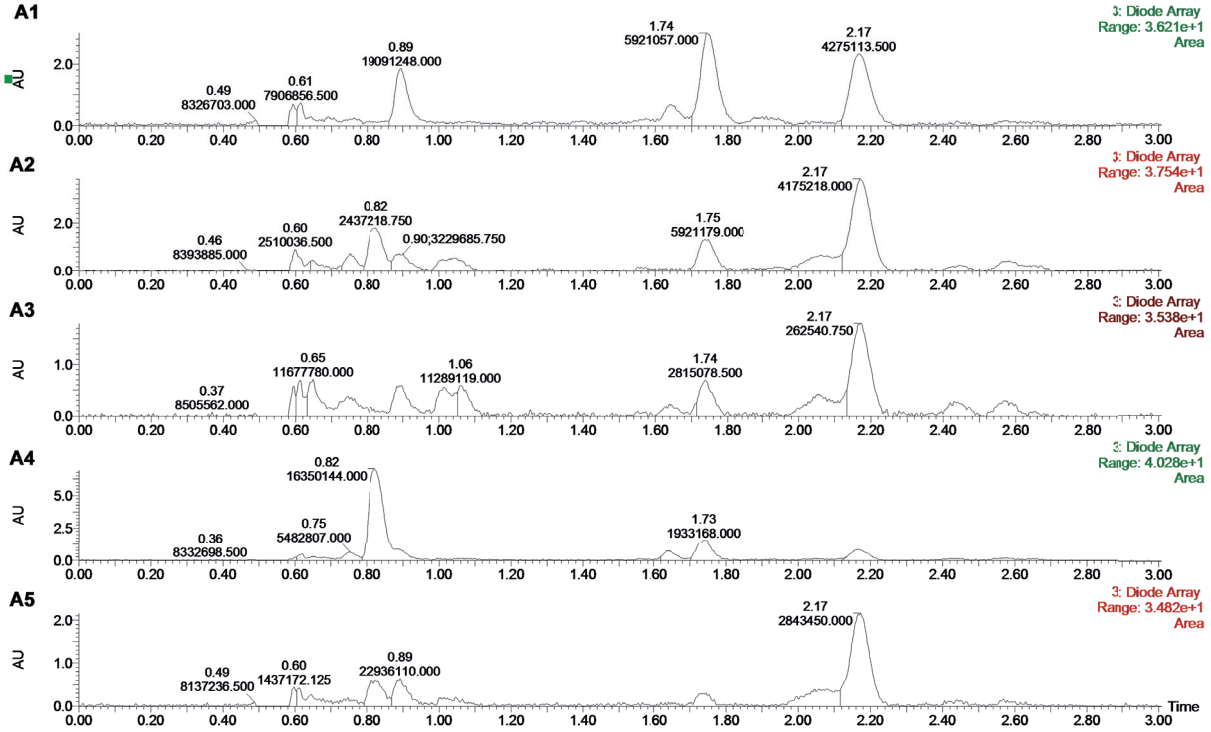

Fig. 2. UPLC-UV (220 nm) profile of copaiba oil commercial samples

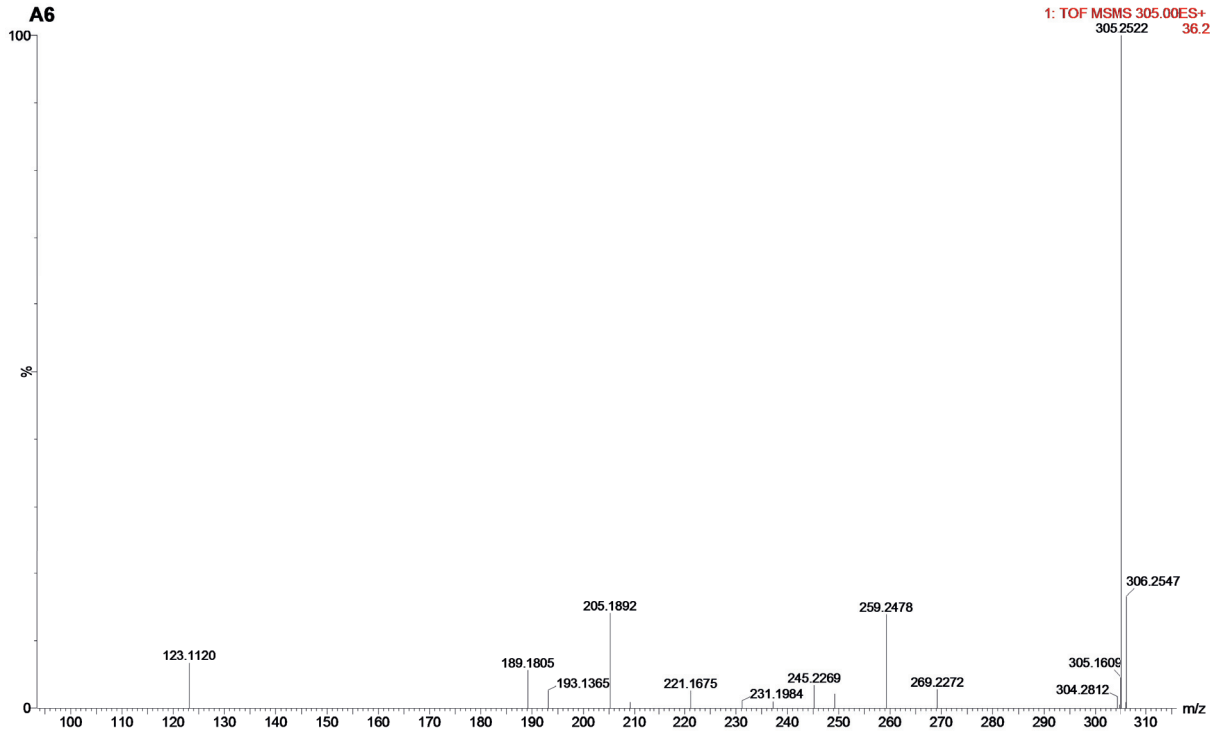

Fig. 3. UPLC-ESI-Q-TOF-MS of A1 sample. MS/MS (ESI: 15 eV) of peak with *m/z* 305 $[M + H]^+$

Table 2. Antifungal activity of five commercial copaiba oil (A1 to A5) by agar diffusion technique – well variable

Copaiba oil mg/mL	*Candida albicans* ATCC 14057			*C. glabrata* ATCC 2301			*C. krusei* ATCC 6258			*C. parapsilosis* ATCC 22018			*Sacharomyces cerevisiae* ATCC 2601		
	50	100	1000	50	100	1000	50	100	1000	50	100	1000	50	100	1000
A1	R	R	R	R	9.0 ±0	R	R	R	R	R	R	R	R	9.0 ±0	R
A2	R	R	10.0 ±0	R	R	R	R	R	R	R	R	R	R	R	R
A3	R	R	10.0 ±0	R	R	R	R	R	R	R	R	R	R	R	R
A4	R	R	10.0 ±0	R	R	R	R	R	R	R	R	R	R	13.0 ±0	R
A5	R	R	10.0 ±0	R	R	R	R	R	R	R	R	R	R	R	R
CHL 1%														21	
KTZ 50 µg/mL		35			30			30			35				
DMSO (50:50)		R			R			R			R			R	

R – Resistant. Agar holes measured 6 mm. The diameter of the inhibition halo is expressed in millimetre. Values represent means and standard deviation of three independent experiments. CHL – chlorhexidine, KTZ – ketoconazole, DMSO – dimethyl sulfoxide.

Table 3. Antibacterial activity of five commercial copaiba oils (A1 to A5) by agar diffusion technique – well variable against clinical importance bacteria

Sample/ Microorganism	A1			A2			A3			A4			A5		
mg/mL	50	100	1000	50	100	1000	50	100	1000	50	100	1000	50	100	1000
E. coli (ATCC 35218)	9.3 ±0	11.0 ±0	11.6 ±0.5	12.0 ±0	14.0 ±0	16.6 ±0.5	R	R	R	R	R	R	R	R	R
E. faecalis (ATCC31299)	11.0 ±0	10.0 ±0	19.0 ±0	10.0 ±0	12.0 ±0	13.3 ±0.5	12.0 ±0	13.6 ±0.5	13.3 ±0	10.0 ±0	10.0 ±0	10 ±0.5	R	R	9.6 ±0.5
K. pneumoniae (ATCC700603)	16.0 ±0	16.0 ±0	19.0 ±0	16.0 ±0	16 ±0	15.0 ±0	9.6 ±0.5	15.9 ±0.5	13.6 ±0.5	13.6 ±0.5	15.0 ±0	15 ±0	15.0 ±0.5	15.5 ±0.5	9.6 ±0.5
P. aeruginosa (ATCC27853)	10.0 ±0	11.0 ±1.1	19.0 ±0	R	12 ±0.5	13.0 ±0	12.0 ±0	13.6 ±0.5	12.0 ±0	13.0 ±0	10.0 ±0	13.0 ±0	11.66 ±0.5	13.0 ±0	9.6 ±0.5
S. aureus (ATCC 25921)	R	10.0 ±0	15.0 ±0	12.0 ±0	14.0 ±0	19.0 ±0	12.6 ±0.5	15.6 ±0.5	16.0 ±0	16.0 ±0	15.6 ±0	15 ±0.5	13.6 ±0.5	17.0 ±0	20.1 ±0
S. saprophyticus (ATCC 35552)	13.0 ±0	19.3 ±1.1	16.6 ±0.5	9.6 ±0.5	9.6 ±0.5	9.6 ±0.5	16.6 ±0.5	19.0 ±0	19.0 ±0	15.6 ±0.5	16.6 ±0.5	19.6 ±0.5	16.6 ±0.5	17.1 ±0	17.0 ±0

R – Resistant. No halo formation.

Although variable, all samples showed antibacterial activity taking into consideration the criteria established for this study. Through agar diffusion technique, significant activity was seen for A3 (19.0 ±0 and 15.6 ±0.5 mm), A4 (16.6 ±0.5 and 15.6 ±0 mm), and A5 (17.1 ±0 and 17.0 ±0 mm) on *S. saprophyticus* and *S. aureus*, respectively. All samples were active against *K. pneumoniae* showing ≥15 mm diameter halo inhibition; and in accordance with the criteria used; only A2 was active against *E. coli* (Table 4).

In relation to phytopatogens, *R. solanacearum* CGH12 was resistant to all samples. Conversely, Xcv112 strain of *X. campestris* pv *campestris* was sensible to almost all samples (≥15.0 ±1.0 mm; Table 4).

Minimum Inhibitory Concentration (MIC) and MMC showed bacteriostatic effect against clinical interest bacteria and bacteriostatic and bactericidal effect against phytopatogens (Table 5).

DISCUSSION

The importance of copaiba oil for folk medicine and its standardization and quality control difficulties has been a point of interested. In this work the complemented data concerning copaiba oil confirmed the chemical differences between samples sold in the market and their influence on the antimicrobial property of the products *in vitro*. All samples tested in this work confirmed the antimicrobial potential of copaiba oil. The antifungal activity results showed weak action on *Candida* yeast as shown by Deus et al. (2011) being considered a good antifungal product only for *S. cerevisiae*. In relation to antibacterial activity, the results were a promise regarding therapeutic aspects since the oil proved to inhibit bacterial samples at lower concentrations with attention to the action against *S. saprophyticus* (ATTC35552) by diffusion agar technique. Other reports indicated the *in natura* copaiba oil extract of identified species such as *C. reticulata* (Oliveira et al., 2006; Santos et al., 2008), *C. multijuga* Hayne (Deus et al., 2011; Pieri et al., 2012) antimicrobial potential against Gram positive bacteria, including action against Methicillin-resistant *Staphylococcus aureus* (MRSA). However, these results indicate antimicrobial action, this copaiba oil effect seemed to be Gram positive restrain spectrum, since no action against Gram negative bacteria was reported. Conversely, our results reveal that commercialized oil can also act against Gram negative bacteria. Besides the antimicrobial action, this study revealed physicochemical differences between

Table 4. Antibacterial activity of five commercial copaiba oils (A1 to A5) by agar diffusion technique – well variable against phytopathogens

Sample/ Microorganism	A1			A2			A3			A4			A5		
mg/mL	50	100	1000	50	100	1000	50	100	1000	50	100	1000	50	100	1000
A. citrulli (Aac. l.12)	R	R	10.6 ±0.5	9.6 ±0.5	11.6 ±0.5	16.6 ±1.5	9.6 ±0.5	11.6 ±0.5	16.6 ±1.5	10.6 ±0.5	13.6 ±0.5	18.6 ±0.5	R	R	12.0 ±1.0
P. caratovorum subsp. *caratovorum* (Pcc23)	10.0 ±1.0	10.6 ±0.5	13.0 ±1.0	12.6 ±0.5	11.6 ±0.5	15.6 ±0.5	10.3 ±1.1	10.0 ±1.0	13.6 ±0.5	10.6 ±0.5	14.6 ±0.5	14.6 ±0.5	9.6 ±0.5	14.0 ±1.0	16.6 ±0.5
R. solanacearum (B19)	12.0 ±1.0	14.0 ±1.0	15.0 ±1.0	9.6 ±0.5	9.6 ±0.5	9.6 ±0.5	14.3 ±1.0	14.0 ±1.0	13.6 ±1.0	13.6 ±0.5	20.6 ±0.5	25.6 ±0.5	13.0 ±1.0	18.0 ±1.0	18.6 ±0.5
R. solanacearum (CGH12)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
X. campestris PV *campestris* (Xcc56)	12.6 ±1.1	13.0 ±1.0	13.0 ±1.0	15.6 ±0.5	15.6 ±0.5	15.6 ±0.5	9.3 ±1.1	9.6 ±0.5	9.6 ±0.5	13.6 ±0.5	18.6 ±0.5	13.6 ±0.5	8.0 ±1.0	8.6 ±0.5	9.0 ±1.0
X. campestris PV *campestris* (Xcv112)	15.0 ±1.0	15.3 ±0.5	18.0 ±1.0	13.0 ±1.0	15.0 ±1.0	9.6 ±0.5	17.3 ±1.1	16.0 ±1.0	14.6 ±1.0	13.6 ±0.5	18.6 ±0.5	13.6 ±0.5	16.0 ±1.0	19.0 ±1.0	18.6 ±1.5

R – Resistant. No halo formation.

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) results of commercial copaiba oil against clinical interest bacteria

	A1	A2	A3	A4	A5
E. coli (ATCC 35218)	≥125.0t	≥125.0t	≥125.0t	≥125.0t	≥250.0t
E. faecalis (ATCC31299)	≥3.90t	≥31.25t	≥3.90t	≥3.90t	≥250.0t
K. pneumoniae (ATCC700603)	≥250.0t	≥250.0t	≥125.0t	≥125.0t	≥250.0t
P. aeruginosa (ATCC27853)	≥125.0t	≥3.90t	≥3.90t	≥3.90t	≥250.0t
S. aureus (ATCC 25921)	≥125.0t	≥3.90t	≥3.90t	≥3.90 t	≥250.0t
S. saprophyticus (ATCC 35552)	≥125.0t	≥125.0t	≥250.0t	≥125.0t	≥250.0t

t – bacteriostactic, f – bactericidal.

commercialized samples which implies adulteration of oil with other oils. Biavatti et al. (2006) discuss that the lack of parameters to characterise and, so far, demand of the quality control certification is an important problem to get registered and to export these products.

The diterpene copalic acid has been considered a marker compound of the *Copaifera* spp. oils. The kolavenic, kaurenoic and hardwickiic acids are also often described (Veiga et al., 1997; Veiga and Pinto, 2002). Differences in the chromatographic profile of

Table 6. Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) results of commercial copaiba oil against phytopathogens bacteria

	A1	A2	A3	A4	A5
Acidovorax citrulli (Aac. l.12)	≥15.62t	≥125.0t	≥250.0f	≥250.0f	≥250.0f
Pectobacterium caratovorum subsp. *caratovorum* (Pcc23)	≥125.0t	≥62.5t	≥125.0t	≥125.0t	≥250.0f
Ralstonia solanacearum (B19)	≥125.0t	≥3.90t	≥125.0t	≥3.90t	≥250.0t
Ralstonia solanacearum (CGH12)	≥250.0f	≥15.62t	≥250.0f	≥250.0f	≥250.0f
Xanthomonas campestris PV campestris (Xcc56)	≥125.0f	≥125.0f	≥250.0t	≥125.0f	≥250.0t
Xanthomonas campestris PV campestris (Xcv112)	≥250.0f	≥15.62t	≥125.0f	≥125.0f	≥250.0t

t – bacteriostactic, f – bactericidal.

Table 7. Volatile content of A1 copaiba oil sample

Retention time	Kovats index	Compound	Percentage
24.03	1365	α-copaene	6.32
24.74	1381	7-*epi*-sesquitujene	3.98
25.88	1406	β-caryophyllene	10.27
26.70	1424	*Z*-α-bergamotene	25.65
27.00	1430	*Z*-β-farnesene	0.71
27.35	1438	α-humulene	0.69
27.65	1445	*E*-β-farnesene	2.04
28.37	1461	γ-muurolene	1.59
28.54	1464	germacrene D	0.64
28.73	1469	NI	2.66
29.54	1486	*Z*-α-bisabolene	1.12
29.83	1493	β-bisabolene	29.95
30.35	1505	δ-amorphene + NI	6.76
31.20	1528	NI	1.46
50.14	2055	NI	0.09
50.19	2056	NI	0.09
53.11	2154	NI	0.29
59.37	2385	copalic acid	0.81
65.69	2626	NI	1.21
65.76	2629	NI	0.13
			96.46

NI – not identified.

the investigated oils (Fig. 1) suggest adulteration, possibly soybean oil in accordance with literature data (Barbosa et al., 2009; Vasconcelos and Goldinho, 2002; Veiga and Pinto, 2002; Veiga et al., 1997). Thus, the results from physicochemical analysis reinforce the fact that it is imperative to include the analysis of copaiba oil which will ensure the quality of commercialized products. On the other hand, the similarity of sample A1 with *in natura* copaiba oil was confirmed by GC-MS analysis with α-copaene, β-caryophyllene, *Z*-α-bergamotene, α-humulene and β-bisabolene (Table 5) being among the main sesquiterpenes of copaiba oleoresins (Leandro et al., 2012).

The antimicrobial findings associated to variable quality of samples representing products from *Copaifera* spp. indicate that public measures of pharmacosurveillance should be the priority of the executive secretariat of the drug market regulation chamber to avoid antimicrobial resistance strain selection as well as to have a suitable product to supply the international market.

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