

Synergism of Crude Extracts and Essential Oils from Medicinal Plants with Antimicrobial Drugs

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Abbreviations:

Ext.: Extract, EO: Essential oil, MIC: Minimal Inhibitory Concentration, MIC₉₀%, Minimal Inhibitory Concentration 90%, GC-MS: Gas Chromatography-Mass Spectrometry, ATCC: American Type Culture Collection, CLSI/NCCLS: Clinical and Laboratory Standards Institute/ National Committee for Clinical Laboratory Standards.

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Abstract

The emergence of resistant strains to conventional antimicrobial drugs has been constant as well as research aimed new alternatives of antibacterial agents. Therefore, considering that natural products have been an important potential source of new antimicrobial drugs, aim to verify the synergism by disk and time kill curve method between antimicrobials (extracts-Ext. and essential oils-EO) from four plant and eight antimicrobial drugs against *Staphylococcus aureus* and *Escherichia coli* strains from human specimens. The *S. aureus* strains were highly susceptible with all plant antimicrobials (eg., 1.24 mg/ml with *Vernonia polyanthes* Ext. and 2.21 mg/ml with *Eugenia uniflora* EO for the Minimal Inhibitory Concentration-MIC). According disk method, the *Bacharis dracunculifolia* and *V. polyanthes* EO had synergism with all eight tested drugs while only *Matricaria chamomilla* Ext. showed synergism against *S. aureus*. The synergism was found with *V. polyanthes* and *E. uniflora* Ext. while *M. chamomilla* Ext. had antagonism against *E. coli* strains. By time kill curve, the bacterial growth inhibition was superior when drugs were tested alone and the synergism effect also was verified. The antagonism effect was detected only for *E. coli* strains and only with Ext. Results indicated the potential use of these products as coadjutants during treatment of infectious diseases.

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1. Introduction

The popular tradition of plants and diseases has basic aspects that are essential to modern pharmacology, and several drugs currently used by conventional medicine directly or indirectly originated from medicinal plants. However, some authors reported that such drugs do not often have the same efficacy since they are compounds isolated from plants which were synthesized or purified (Silver and

Bostian, 1993). On the other hand, inventory of biologically active has gained importance in recent years. This involves the process such as extraction, separation purification and characterization (Gomathi et al., 2013).

A significant number of plants are used as crude extracts to treat common infections, although there is scarce scientific evidence to

prove their efficacy. Based on these aspects, phytotherapy has grown in Brazil, becoming an important economic sector due to its popularity as an alternative for health care (Lima et al., 2006).

In the search for new antimicrobials those of plant origin have received interest, and Brazil has been an important center for the study of new antimicrobials since it has the greatest biodiversity in the world; besides, several of its plants have been widely used and tested for hundreds of years for many different purposes. There are also reports indicating that the largest part of the Brazilian population (80%) consumes only 37% of the available medicines, depending almost exclusively on drugs of natural origin (Funari and Ferro, 2005).

Studies on the antimicrobial action of plants have been constant in literature (Candan et al., 2003; Gallucci et al., 2006; Rosato et al., 2007; Aguiar et al., 2008; Costa et al., 2009). (Bertucci et al., 2009) tested the *in vitro* antimicrobial action of 3 species from genus *Eugenia* and found an important inhibitory effect against *S. aureus*, *Mycobacterium*, *Candida* and *Aspergillus* strains. (Ferronato et al., 2007) reported that 10 μ l of *B. dracunculifolia* essential oil diluted in 15 ml of Mueller Hinton Agar was capable of inhibiting the development of *E. coli*, *S. aureus* and *P. aeruginosa*. (Asolini et al., 2006) reported the *S. aureus* growth inhibition with *M. chamomilla*, and (Oliveira et al., 2007) reported that hydroalcoholic extracts of *V. polyanthes* had significant antimicrobacterial activity, decreasing the bacterial growth in 2 log₁₀ CFU/ml in an exposure time from 30 minutes to 1 hour.

Investigations on plant derivatives antimicrobial activities and possible synergisms with conventional antimicrobial drugs have been frequent (Betoni et al., 2006), and the synergistic interaction between antibiotics and medicinal plant extracts against resistant microbial strains may be a new strategy to treat infections, allowing the use of antimicrobial drugs when alone they are not effective against bacterial strains (Kumar et al., 2009). Betoni et al., (2006) found synergism between 8 plant extracts and 13 antimicrobial drugs, of which 2 had synergism with ginger extract and 11 with lemongrass and clove when tested against *S. aureus* strains.

Aqueous extract from *Cuminum cyminum* (cumin) seeds led to a 35% increase in

rifampicin levels in the plasma of rats, and this activity was attributed to the presence of a flavonoid glycoside in the extract (Sachin, et al., 2007).

2. Objectives of Research

We consider an important advance for the clinical microbiology, the numerous investigations that aim to determine the antimicrobial properties of natural products and its ability to potentiate the effect of drugs that are already in use in the treatment of infectious diseases. Thus, we aimed to study *in vitro* antibacterial activities of essential oils (EO) and extracts (Ext.) from samples of *M. chamomilla* L, *V. polyanthes* Less, *B. dracunculifolia* D.C. and *E. uniflora* L and interactions of these natural products with antimicrobial drugs such as chloramphenicol, ciprofloxacin, tetracycline, cefepime, sulfazotrim, rifampicin, cephalothin and gentamicin against *S. aureus* and *E. coli* strains isolated from clinical human specimens.

3. Materials and Methods

Samples were collected from four plants species and their essential oils and methanol extract were prepared and assayed for their antibacterial activities and also found the synergism of these products with antibacterial drugs of conventional use in the treatment of infectious diseases using the disc (Kirby & Bauer) and time kill curve methodology. All experimental was developed in the Laboratory of Bacteriology, Department of Microbiology and Immunology, Institute of Biosciences, São Paulo State University in 2010.

3.1 Plants and chemical characteristics of plant antimicrobials

B. dracunculifolia D.C. and *V. polyanthes* leaves were harvested from individuals naturally growing in areas around the Botucatu campus, São Paulo State, Brazil (22° 57' 13, 67" S and 48° 30' 23, 19" W). The *E. uniflora* L sample consisted of dehydrated leaves obtained from the EO producer (Fazenda Alpina-Ivo Gregori Ltda, Santa Bárbara Municipality, São Paulo State (23° 01' 58".5 S and 49° 09' 52".7 W); *M. chamomilla* L flowers, also dehydrated, were supplied by a company of the medicinal plant sector (Centro Flora-Anidro, Botucatu, São Paulo State; 22° 57' 30.72" S and 48° 31' 27.13" W). All plant samples were identified and the respective specimens were deposited in the Herbarium "Profa. Dra. Irina Delanova Gemtchujnicov" at

the Department of Botany, IBB, UNESP, Botucatu, receiving the deposit numbers: BOTU 25794 (*M. chamomilla*), BOTU 25795 (*B. dracunculifolia* D.C.), BOTU 25796 (*E. uniflora*) and BOTU 25797 (*V. polyanthes* Less).

Dehydrated and powdered plants materials were mixed with 70% methanol solution, followed by 48 hours/±4° C for extraction and filtration using common filter paper during extracts preparation. Then, the solvent (methanol) was evaporated from the filtrate in a rotary evaporator (Brand Phoenix), at ±45° C. One-ml aliquots of the extracts were transferred to containers made of common aluminum foil, previously weighed, and placed over a heated plate for solvent evaporation; the extracts were successively weighed until a constant weight and had their dry weight calculated (mg/ml) (Betoni et al., 2006).

The EO from *B. dracunculifolia* D.C., *V. polyanthes* and *E. uniflora* L were prepared from recently harvested leaves while the *M. chamomilla* EO was prepared from dehydrated flowers and *E. uniflora* EO was obtained from the producer of essential oils (Fazenda Alpina-Ivo Gregori Ltda). The *M. chamomilla*, *V. polyanthes* and *B. dracunculifolia* EO were produced at the Department of Microbiology and Immunology, IBB, UNESP, according to the classic methodology of steam distillation in Clevenger-type equipment (Marconi, model M480) (Souza et al., 2006) while *E. uniflora* EO was also produced through steam distillation but in equipment for the commercial production of this oil. The density of oils was determined according to the formula below (Fonseca and Librand, 2008), where P_1 , obtained in analytical balance, is the weight of the Eppendorf-type container, and P_2 is the weight of the Eppendorf-type container with 1 ml (V) of the analyzed essential oil.

$$D = \frac{P_2 - P_1}{V} = \frac{mg}{ml}$$

The qualitative phytochemical analysis of crude extracts included steroids, triterpenes, saponins, fixed strong acids, phenolic compounds, quaternary amines and alkaloids (Matos, 1988). EO chemical analysis was performed using gas chromatography-mass spectrum (GC-MS) (Shimazu, model QP5050A) (Adams, 1989).

3.2 Bacterial strains

Staphylococcus aureus (n=15) and *Escherichia coli* (n=15) bacterial strains were isolated from human specimens from patients of the Clinical Hospital of Botucatu Medical

School, UNESP, identified (Konemman et al., 2001) and kept in Nutrient Agar (Difco). We used two standard ATCC strains (*S. aureus* ATCC 33591; *E. coli* ATCC 25922). The use of these bacteria was approved by the Ethics Committee on Research at Botucatu Medical School, UNESP, on April 06, 2009, receiving the protocol ECR number 3.152-2009.

3.3 Minimal Inhibitory Concentration (MIC) for plant products and antimicrobial drugs.

MIC and MIC₉₀% values were found against 32 strains (15- *S. aureus*, 15- *E. coli*, 1- *S. aureus* ATCC 33591, 1-*E. coli* ATCC 25922) by agar dilution methodology (CLSI/NCCLS, 2005) in Agar Mueller Hinton (MHA) plus 0.2% Tween 80 whose concentrations ranged from 2 to 44 %v/v for extracts and from 0.05 to 3 %v/v for oils. These concentrations values were converted into mg/ml by using the data on the Ext. dry weight and the EO density. Control plates were prepared to observe the normal growth of bacteria and assays were carried out in duplicate (Silva et al., 2009). The strains were inoculated by using a Steer's replicator with bacterial suspension standardized according to 0.5 Mc Farland scale and diluted to bacterial concentrations around 10⁵-10⁶ CFU/ml and incubation at 37° C/18-24hours. The reading of assays and the determination of MIC values were based on the formation or not of colonies in the plates (NCCLS, 2003).

MIC values for the antimicrobial drugs were obtained through the method of dilution (CLSI/NCCLS, 2005) of salts of the drugs when concentrations between 0.008µg/ml and 1024µg/ml were prepared from a stock solution in a final volume of 2.5 ml Brain Heart Infusion (BHI). The inocula were standardized according to standard 0.5 McFarland scale, and tubes were inoculated with approximately 105CFU/ml. After 37° C /24 hours, assays were read and MIC values for the antimicrobial drugs were determined as those at which there was no turbidity in the medium due to bacterial growth in the culture tube.

3.4 Synergism between plant oils or extracts and antimicrobial drugs by the disk test (Kirby&Bauer method)

Disk diffusion test was employed according to an adaptation used by Fernandes Júnior et al., (2005) and Betoni et al (2006) based on the protocol recommended by NCCLS (2003) (13, 24, 25). Two types of assays were carried out for a total of 10 *S. aureus* and 10 *E. coli* strains, including one antibiogram control,

according to the disk diffusion assay (NCCLS, 2003) and another antibiogram treatment, also according to the disk methodology but mixing the respective natural products into the culture medium at concentrations equivalent to ¼ of the MIC₉₀% values previously obtained for each Ext. and EO. The used plates, 150x10mm, contained Mueller Hinton Agar (MHA) culture medium plus Tween 80 (0.2%) (Silva et al., 2009). After 37° C/18-24 hours, inhibition zones (millimeters) were read, and the results for control and treatment antibiograms were compared statistically. The antimicrobial drugs tested against plant products were chloramphenicol (30µg), gentamicin (10µg), cefepime (30µg), tetracycline (30µg), sulfazotrin (25µg), cephalothin (30µg), ciprofloxacin (5µg), and rifampicin (5µg). The antibiogram disks of drugs were all from Laborclin Industries.

3.5 Synergisms by time kill curves method

To confirm only the positive cases of synergism detected through the disk test, assays were done in another step of the study to obtain time kill curves in Mueller Hinton Broth (MHC) plus 0.2% Tween 80 (for one strain of each bacterial species; mixtures of antimicrobial drugs and the respective natural products were used at proportions of ¼ MIC values obtained for the antimicrobial drugs and at proportions of ¼ MIC₉₀% values obtained for the plant products).

These assays were performed in a 24h-incubation period, using initial inocula of approximately 10⁵ CFU/ml, and at the times 0,

1,5, 3, 6, 9 and 12 at 37° C aliquots were collected from the cultures tubes and the subculture (Pour Plate method) in Mueller Hinton Agar medium (MHA Difco) for viable cell count was performed. The values of viable cell count were recorded after 37° C/24 hours and count in a Phoenix digital colony counter. The values of CFU/ml were transformed to log of UFC/ml to establish the bactericidal (when there was a 3-log reduction in the count) or bacteriostatic effects (when the number of viable cells kept close to the initial value of the inoculum). Besides the assays containing the mixtures of antimicrobial products at the defined combinations, curves for natural products and antimicrobial drugs at the respective MIC₉₀% and MIC values were obtained, as well as control growth curve for each bacterial species.

3.6 Statistical analysis

For synergism assays through the disk method, Mann-Whitney Rank Sum Test and T test were adopted, and the result was considered significant if p<0.05 (SAS for Windows version 9.1.3).

4. Results

4.1 Analysis of natural products (dry weight and yield) and Physico-chemical parameters of crude extracts and EO

The values from crude extracts dry weight (mg/ml) and essential oils (mg/ml) density, essential oils yield and Ext. and EO phytochemical analysis are presented in Table 1.

Table 1: Values of extracts (Ext.) dry weight, essential oils (EO) density (mg/ml) and yield (%) and physico-chemical characterization of plant antimicrobials assayed

Vegetal source	Density (mg/ml) (EO)	Dry weight (mg/ml) (Ext.)	Yield (%) (essential oils)	Phytochemical of Ext.	GC-MSa of EO
<i>E. uniflora</i>	924.0	145.0	0.19	Phenols, tannins, chalcones, aurones, flavones, catechins, flavonoids, saponins, fixed strong acids, quaternary compounds, free steroids and quinones	Selina 1, 3, 7(11) trien-8-one (30.1%), Selina 1, 3, 7(11) trien-8-one-epoxide (21.89%), β cariofilene (6.51%)
<i>V. polyanthes</i>	856.0	62.5	0.15	Phenols, tannins, chalcones, aurones, flavonoids, fixed strong acids, saponins, free steroids, quinones and flavananois	Germacrene D (27.79%), ε-Cariofilene (16. 2%), Germacrene B (15.01%)
<i>B. dracunculifolia</i>	857.0	76.0	0.20	Phenols, tannins, flavones, catechins, flavonoids, saponins, fixed strong acids, quaternary bases and xanthones	Nerolidol (18.77%), Germacrene D (10.45%), limoneme (8.75%)

<i>M. chamomilla</i>	940.0	100.0	0.17	Phenols, flavones, flavonoids, fixed strong acids, quaternary compounds, quinones, xanthenes, free triterpenes	Chamazulene (31.48%) α -bisabolol and Bisabolone oxide (15.71%)
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According to mg/ml values of plant products, the Ext. showed around 10% of those obtained in the EO samples and were used for processing the MIC at mg/ml during antibacterial assays. The yields of EO were around 0.15%, which characterizes a very low yield.

4.2 MIC₉₀% values from bacterial strains

MIC₉₀% values for natural products are shown in Table 2, and the MIC values obtained for *S. aureus* and *E. coli* strains against the tested antimicrobial drugs are shown in Table 3.

Table 2: Values of Minimal Inhibitory Concentration (MIC - mg/ml) for 90% (MIC₉₀%) of *S. aureus* and *E. coli* strains against extracts (Ext.) and essential oils (EO) from studied plants

Microorganisms	<i>E. uniflora</i>	<i>V. polyanthes</i>	<i>B. dracunculifolia</i>	<i>M. chamomilla</i>
<i>S. aureus</i> (n=15 + ATCC 33591)				
Ext.	2.90	1.24	5.47	3.70
EO	2.21	24.08	3.35	2.82
<i>E. coli</i> (n=15 + ATCC 25922)				
Ext.	15.95	26.91	32.53	43.40
EO	27.60	25.80	25.80	28.20

The MIC₉₀% values obtained for the natural products are essential for the assays on interactions between natural products and antimicrobial drugs; although the aim of this study is not to verify the antimicrobial potential

of each of these products, it is possible to infer that all of them were effective to control the growth of the studied bacteria, according to the obtained MIC₉₀% values.

Table 3: Values of minimal inhibitory concentration (MIC - μ g/ml) for the tested drugs against *S. aureus* and *E. coli* strains

Microorganisms	CIP	GEN	TET	CLO	RIF	CPM	SUT
<i>E. coli</i>	1.024	256	0.128	1.024	0.064	64	1024
<i>S. aureus</i>	128	32	128	16	64	0.256	1024

CIP: Ciprofloxacin, GEN: Gentamicin, TET: Tetracycline, CLO: Chloramphenicol, RIF: Rifampicin, CPM: Cefepime, SUT: Sulfazotrin.

4.3 Synergism between antimicrobial products by disk test

In the assays performed to verify synergism between plant products and antimicrobial drugs using the disk methodology, 1/4 of the respective MIC₉₀% values were used in the plates that received the plant products, named

treatment, and synergism was established according to the statistical analysis and the comparison of the results obtained for each strain, totaling 10 for *S. aureus* and 10 for *E. coli*, in the antibiograms control and treatment. The obtained results are shown in Tables 4 for *S. aureus* and *E. coli*.

Table 4: Results for synergism of essential oils (EO) and extracts (Ext.) with antimicrobial drugs against *S. aureus* and *E. coli* strains

Drugs	<i>B. dracunculifolia</i>		<i>V. polyanthes</i>		<i>M. chamomilla</i>		<i>E. uniflora</i>	
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
	EO	Ext	EO	Ext	EO	Ext	EO	Ext
Cephalothin	I	I	S	I	I	A	I	I
Gentamicin	I	I	S	I	I	A	I	S
Tetracycline	I	I	S	I	I	I	S	I
Sulfazotrin	I	I	S	I	I	I	S	I
Ciprofloxacin	I	I	S	I	I	I	S	I
Cefepime	I	I	S	I	I	A	I	I

Chloramphenicol	I	A	S	I	I	I	S	I	I	A	I	I	I	I	I
Rifampicin	I	I	S	I	I	I	S	I	I	I	S	S	I	I	I

Synergism and antagonism when $p < 0,05$ (S - Synergism, A – Antagonism, I – Indifferent)

There was a noticeable difference for synergism frequency when both bacterial species were considered, whereas the absolute number of synergism cases between the several natural products and the antimicrobial drugs was 24 for *S. aureus* and 3 for *E. coli*. It must be emphasized that *B. dracunculifolia* and *V. polyanthes* EO had synergism with all drugs tested in the assays for *S. aureus*. On the other hand, there is a superior antagonism frequency (a total of 5 cases) against *E. coli*, *M. chamomilla* Ext. presenting a high incidence of antagonism. In addition, the cases of synergism against *S. aureus* occurred almost exclusively with EO; only two cases of synergism with *M. chamomilla* Ext. were found. For *E. coli*, however, there was 100% indifference between EO and antimicrobial drugs.

4.4 Synergism by time kill curve method

The time kill curve assays were performed in order to confirm only the synergism verified in the tested combinations by disk test previously. However, it must be highlighted that although there were cases of synergism with the drug cephalothin, such assays could not be done due to the limitation for obtaining the salt of this drug from suppliers.

For most interactions, the time kill curves showed a similar or even lower profile with the tested drug, and synergism was confirmed for several combinations through the reduction in the bacterial count, including the complete elimination of the bacterium in the culture medium.

According to *S. aureus* assays, the interactions were detected for sulfazotrim with *V. polyanthes* and *B. dracunculifolia* EO, with a clear synergism capable of more efficiently reducing the microbial growth, compared to the drug tested alone. As shown in Figure 2, ciprofloxacin only had synergistic interaction with *M. chamomilla* EO, including a bactericidal effect. When combined with rifampicin, both *M. chamomilla* and *V. polyanthes* EO showed a synergistic effect. There was also synergism for the combinations of cefepime with *V. polyanthes* and *B. dracunculifolia* EO. In interactions with the drugs chloramphenicol, gentamicin and tetracycline, bacterial growth was greater than that presented by the drugs alone.

When tested against *E. coli*, the interactions cefepime and *V. polyanthes* Ext. and cefepime and *E. uniflora* Ext. yielded a curve profile similar to that of the drugs alone, whereas the curve for the interaction gentamicin and *V. polyanthes* Ext. showed a bacterial growth rate higher than that of the drug alone.

In general, all curves for the interaction between natural products and drugs showed a bacterial growth profile inferior to those of control curves, i.e. assays without addition of any bacterial growth inhibitor.

5. Discussion

As indicated by Ext.dry weight and EO density (Table 1), there is a great difference of concentration between EO and Ext., the former being more concentrated. This is a possible justification for the higher activity of oils concerning synergism. However, this comparison is difficult since the extracts dry weight represents the solid portion, in solution or in suspension in the solvent, which in the case of extract is basically constituted of water due to the removal of methanol. Conversely, the essential oil represents almost the whole material, which in the case of density is considered all mass in 1 ml volume, for example.

As regards the MIC of natural products, *E. uniflora* and *V. polyanthes* Ext. were the most effective against *S. aureus*, but only *M. chamomilla* Ext. had some synergism. Considering EO, *M. chamomilla* and *E. uniflora* had higher inhibitory action against *S. aureus*; however, *V. polyanthes* and *B. dracunculifolia* had the highest synergism rate through disk test, indicating that a higher antimicrobial capacity may not be reflected in a higher synergism rate. For combinations against *E. coli*, the synergism rate was low, but there were 2 cases of synergism with *V. polyanthes* Ext. which had high MIC and low antimicrobial action. Betoni et al., (2006) verified a similar result for lemongrass extract, which presented high MIC value; however this extract had the highest synergism rate together with the values obtained for clove extract.

The results from synergism assays by disk

method were promising against *S. aureus* strains and the synergism rate for EO was considerable, on other words, 100% for *B. dracunculifolia* and *V. polyanthes*. On the other hand, Ext. did not show the same potential and there were only two cases of synergism against *S. aureus* although no case of antagonism was detected. As regards *E. coli*, its profile was opposite to that obtained for *S. aureus*, since synergism was not detected for any of the EO tested but was established for *V. polyanthes* and *E. uniflora* Ext. It must be highlighted that *M. chamomilla* had the highest antagonism rate (4 events). Thus, the type of plant derivative (EO or Ext.) influences the behavior of antimicrobial drugs, which shows that in such studies, phytochemical characterization is essential to better understand the phenomena observed in the assays of interaction between natural products and drugs.

Another important aspect is the higher sensitivity of Gram-positive bacteria relative to Gram-negative ones, which was somewhat translated into higher synergism rates for *S. aureus*, totaling 24 cases of synergism, compared to 3 cases for *E. coli*. A natural explanation for this phenomenon is the different structure of the bacterial wall of both bacterial groups. However, for essential oils, which are naturally liposoluble, a higher antimicrobial capacity was expected against *E. coli* since it has an external membrane rich in lipids, as well as a thin peptidoglycan, which would lead to a lower restriction for its entrance into the bacterial cell. Therefore, according to these observations, a higher efficacy was expected from natural products in the combined therapy with antimicrobials against infections by *S. aureus*. Studies with other Gram-positive microorganisms are needed to extend the potential recommendation of the combined therapy against other Gram-positive bacteria such as *Streptococcus*, *Bacillus*, etc.

This study also aimed confirms by the time kill curve the synergism detected previously by disk diffusion assays. Thus, similarly to some cases, the survival curves confirmed the synergism results verified through the disk test (*V. polyanthes* EO with sulfazotrim, rifampicin and cefepime; *M. chamomilla* EO with ciprofloxacin and rifampicin; and *B. dracunculifolia* EO with sulfazotrim and cefepime for *S. aureus*); moreover, in some cases the interactions had a growth profile higher than that of the drug alone, not confirming this type of synergism shown by the

disk test, including for example the interactions with chloramphenicol and tetracycline for which the growth curve of the latter was higher than or equal to that of any other drug. Another example of antagonism through the growth curve and synergism through the disk test is the interaction of rifampicin with *M. chamomilla* Ext. and *V. polyanthes* EO. A justification for curves that did not show the expected synergism is that previously obtained MIC values were used in the assays, which were different from those established through the Kirby & Bauer disks assays. However, it must be emphasized that even without an evident synergism all tested interactions showed a bacterial growth profile below that of control assays for bacterial growth in absence of any growth inhibitor.

Thus, similarly to reports about the antimicrobial action of natural products, the chosen methodology is essential to obtain conclusive results; besides, the preparation of plant derivatives, the tested microbial strains and the antimicrobial drugs tested in the synergism assays can also influence the obtained results.

Recent studies have also shown synergism between natural products and antimicrobial drugs; Betoni et al., (2006) verified the synergism between 8 plant extracts and 13 antimicrobial drugs and found an interesting incidence of synergism for the drugs acting on the synthesis of bacterial proteins. In the present study, the preference for this type of antimicrobial drug did not occur, and cases of antagonism were more frequent concerning *E. coli* and the drug chloramphenicol, totaling two cases of antagonism. Similarly, cases of antagonism between natural products and drugs against the Gram-negative bacterium *Klebsiella pneumoniae* strains were reported by Van Vuuren et al., (2009). Thus, our results corroborate those obtained for *E. coli*, demonstrating the more frequent occurrence of cases of antagonism between natural products and antimicrobial drugs besides a higher resistance of Gram-negative bacteria.

Conclusion

According the results and under the present experimental conditions, there is synergism between natural products and the tested drugs, this effect prevailing with EO and especially against *S. aureus* strains. This reinforces conclusion that there is a greater potential for the combined use of these antimicrobial agents against Gram-positive

bacteria, considering the aspects of similarity among species of this bacterial group. However, it must be highlighted that this study was done *in vitro*, indicating the need of future studies using *in vivo* models that will lead to conclusions closer to a real use of these plant products in association with conventional antibacterial drugs to establish potential cytotoxicity of compounds in the plant derivatives during its use as coadjuvant in the treatment of infectious diseases.

Author's Contribution and Competing Interests

Silva, master's degree student, was responsible for preparing the research project under guidance of professor Fernandes Junior, therefore, responsible for the execution and writing of the manuscript of the study. Barbosa, Andrade and Probst are team members in our laboratory has participated in the implementation of microbiological assays, as well as collaborated in collecting plants and chemical characterization. Doyama, was responsible for carrying out the chemical characterization of essential oils by GC-MS

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