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Chemical composition and antibacterial activity of Brazilian propolis essential oil

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ABSTRACT: The present study aimed at investigating the chemical composition of essential oil extracted from Brazilian propolis and the susceptibility of *Staphylococcus aureus*, *Staphylococcus epidermides*, *Streptococcus pyogenes* and *Escherichia coli* to this substance. The essential oil was obtained by steam distillation of propolis and examined by gas chromatography/mass spectrometry (GC/MS). In addition, the agar diffusion method using filter paper disks was employed. Antibacterial activity was measured as equivalent diameters of inhibition zones (in millimeters) after incubation at 37°C for 24 hours. From the 26 identified constituents, β-caryophyllene (12.7%), acetophenone (12.3%) and β-farnesene (9.2%) were found to be major components. New components, namely linalool, methyl hydrocinnamate, ethyl hydrocinnamate, α-ylangene, γ-elemene and valencene, are reported for the first time to be present in propolis essential oil. This oil also exhibited antibacterial activity.

KEY WORDS: *Apis mellifera,* propolis essential oil, terpenoids, antibacterial activity.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Propolis is a resinous substance collected, transformed and used by bees to seal holes in their honeycombs, smooth out the internal walls and protect the hive entrance against intruders. Honeybees (*Apis mellifera* L.) collect the resin from tree bark and leaf buds. Resin is masticated, salivary enzymes are added and the partially digested material is mixed with beeswax and used in the hive (1, 2).

In addition, propolis is known to be a health food that presents antibacterial, antioxidative, immunomodulatory, antifungal, antiviral, antiprotozoal, anti-inflammatory and antitumor activities (3-12). Bosio *et al.* (13) observed *in vitro* activity of propolis extracts and supported the efficaciousness of this natural drug against *Streptcoccus pyogenes*, a microorganism responsible for several otorhinolaryngological infections.

Many essential oils from propolis have been analyzed and chemical substances such as terpenoids, alcohols, aldehydes, hydrocarbons and aliphatic ketones have been reported (14-20). The chemical composition of the analyzed essential oils showed qualitative and quantitative variations due to the influence of local soil conditions and seasonal harvest periods (15).

Though the antibacterial activity of propolis essential oil from Paraná and Piauí states has been reported, the literature on the antimicrobial activity of this oil from Rio de Janeiro State is scarce (17, 21). In this study, we report the chemical composition of propolis essential oil and its capacity to inhibit the *in vitro* growth of *Staphylococcus aureus*, *Staphylococcus epidermides*, *Streptcoccus pyogenes* and *Escherichia coli*.

MATERIALS E METHODS

Propolis Samples

Propolis samples were collected by *Apis mellifera* bees in Rio de Janeiro, during Brazilian autumn time (July) and was supplied by a beekeeper co-operative (COAPI-RJ).

Extraction of Essential Oil

The sample was submitted for six hours to steam distillation using a Clevenger apparatus to produce 0.9 mL of a rich essential oil extract from 1.373 g of sample, yielding 0.06% (v/w) (22). Oil was stored at –4°C until tested and analyzed.

Gas Chromatography/Mass Spectrometry Analysis

Essential oil constituents were analyzed by a GC/MS QP-5000® (Shimadzu, Japan) gas chromatograph equipped with an electron ionization mass spectrometer. The gas-chromatographic (GC) conditions were as follows: injector temperature, 260°C; carrier gas, helium; flow rate, 1 mL/min; and split injection with split ratio, 1:40. Oven temperature was initially 60°C and then raised to 240°C at a rate of 3°C/ minute. One microliter of the sample, dissolved in CH_2Cl_2 (1:100 mg/µL), was injected into a ZB-5 MS column (i.d. = 0.25 mm, length = 30 m, film thickness = 0.25 mm) that was directly coupled to the mass spectrometer. The mass spectrometry (MS) conditions were ionization voltage, 70 eV, and scan rate, 1 scan/second.

The linear retention indices for all the compounds were determined by co-injection of the sample with a solution containing a homologous series of C_7-C_{26} n-alkanes (23). The individual constituents were identified by their identical retention indices referent to the compounds known from the literature data and also by comparing their mass spectra with those stored in the NIST mass spectral libraries (24).

Microorganisms

Staphylococcus aureus ATCC25923, *Staphylococcus epidermides* 25/04, *Staphylococcus epidermides* 194/02 and *Escherichia coli* ATCC36298 obtained from the culture collections of the Laboratory of Microbiological Control, School of Pharmacy, Fluminense Federal University, were used for the antibacterial activity experiments. The *Streptococcus pyogenes* ATCC75194 and 93007 used in this experiment were provided by the *Streptococcus* Laboratory, Microbial Institute, Federal University of Rio de Janeiro, courtesy of Dr. Leslie Claude Benchetrit.

Disc Diffusion Method

Antimicrobial tests were then carried out by the disc diffusion method (25). Briefly, bacterial inocula were prepared by diluting overnight cultures in saline to obtain approximately 10^8 CFU/mL. This suspension was spread onto Mueller-Hinton agar solid plates for *S. aureus, S. epidermides* and *E. coli*. In the case of *S. pyogenes*, 5% sheep blood was added to Mueller-Hinton agar. Paper discs (6 mm in diameter), previously impregnated and saturated with the propolis essential oil were placed on the agar plates. Standards discs of vancomicin (30 µg/disc), penicillin (10 U/disc) and rifampicin (5 µg/disc) were used as positive controls for *Staphylococcus* strains, *S. pyogenes* and *E. coli*, respectively. The agar plates were incubated at 37°C for 24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. All tests were performed in triplicate.

Statistical Analysis

The mean, standard deviation and coefficient of variation (CV) of the three experiments were determined. CV values of at least 15.0 signify significant differences in inhibition zones. The CV values were calculated using the Microsoft Excel® program.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil

Propolis hydrodistillation produced a yellow volatile oil. The 26 components identified comprised 67.5% of the total oil. The qualitative and quantitative compositions of the essential oil are presented in Table 1, where the compounds are listed in the order of their elution in the ZB-5 MS column. The most abundant components were β-caryophyllene (12.7%), acetophenone (12.3%), and linalool (6.47%), followed by γ-elemene (6.25%), γ-cadinene (5.86%) and γ-muurolene (3.61%). Our results corroborate those of Clair and Peyron (14) that also found β-caryophyllene as the major constituent of essential oil from French propolis. The oxygenated monoterpenes represent 7.96% of the oil, with linalool as the main part. Sesquiterpene hydrocarbons comprised 37.58% of the oil, while β-caryophyllene, γ-elemene and γ-cadinene were also in appreciable percentages, respectively, 12.7%, 6.25% and 5.86%. The oxygenated sesquiterpene fraction consisted of 2.89% of the

total oil, with spathulenol found to be the principal compound. The chemical composition reported in other studies for propolis volatile oil showed some similarities with our analyses, except for the presence of linalool, methyl hydrocinnamate, ethyl hydrocinnamate, α-ylangene, γ-elemene and valencene, which were observed for the first time in this study as components of propolis essential oil (14-20, 26).

Table 1. Chemical composition of propolis essential oil (%)

	Compounds[a]	Rt (min)[b]	KI[c]	%
1	Benzaldehyde	7.59	964	1.97
2	β-pinene	8.16	979	0.47
3	Limonene	10.19	1030	0.44
4	Acetophenone	11.80	1066	12.26
5	Linalool	13.35	1097	6.47
6	Nonanal	13.56	1101	0.51
7	α-terpineol	17.77	1193	1.49
8	Methyl hydrocinnamate	21.45	1273	0.86
9	Ethyl hydrocinnamate	24.86	1347	2.11
10	α-ylangene	25.77	1367	1.00
15	α-copaene	26.08	1373	3.03
16	β-caryophyllene	28.04	1416	12.69
17	γ-elemene	28.83	1435	6.25
18	Aromadendrene	29.09	1442	0.40
19	α-caryophyllene	29.57	1453	1.92
20	γ-muurolene	30.43	1472	3.61
21	Valencene	31.31	1492	1,25
22	α-muurolene	31.43	1494	1.47
23	γ-cadinene	32.26	1515	5.86
24	δ-cadinene	32.41	1519	0.50
26	Spathulenol	34.66	1573	2.89
	Total			**67.45**

[a]Compounds listed in order of elution from a ZB-5 MS column.

[b]Retention time (minutes).

[c]Kovats Index on ZB-5 MS column in reference to *n*-alkanes (22).

Antibacterial Activity

Propolis essential oil exhibited antibacterial activity against *S. aureus*, *S. epidermides, S. pyogenes* and *E. coli* (Table 2). The differences in inhibition-zone diameters of *S. aureus* ATCC25923 (CV = 4.2%)*, S. epidermides* 25/04 (CV = 6.0%), *S. epidermides* 194/02 (CV = 6.0%), *S. pyogenes* 93007 (CV = 3.6%) and 75194 (CV = 5.3%) and *E. coli* (CV = 5.9%) were not statistically significant. The antibacterial effect of propolis essential oil on *S. aureus* was reported by other authors (17, 20). On the other hand, the present study is the first report of antibacterial activity of propolis essential oil against *E. coli* and *S. pyogenes*. Only ethanolic extract of propolis showed antibacterial activity against these two bacteria (13, 27, 28). However, ethanolic extract and essential oil of propolis present different chemical compositions (20). Probably, diverse extraction procedures result in dissimilar chemical compounds, and ultimately, could contribute to differences in the antibacterial activities (29, 30). It is not so clear whether the antibacterial effect may be caused by a single active component or by the synergy of many active constituents found in the essential oil. Terpenoids (48.4%) were found to be the major compounds of total oil. These substances are active against bacteria, but their action mechanism is not fully understood. It is has been hypothesized to involve membrane disruption by lipophilic compounds (31). The antibacterial activity could be attributed to terpenoids, according to Sacchetti *et al.* (32), who found that essential oil with high terpenoid percentages is probably more effective.

The present study is the first to report the presence of linalool, methyl hydrocinnamate, ethyl hydrocinnamate, α-ylangene and γ-elemene in propolis essential oil. In addition, the study has also demonstrated the antibacterial activity of propolis essential oil against gram-negative (*E. coli*) and gram-positive (*S. aureus, S. epidermides, S. pyogenes* 93007 and 75194) bacteria. Thus, it appears that chemical properties of propolis are not only advantageous to bees, but present pharmacological value both as a natural mixture and as a natural antibacterial agent.

Table 2. Antibacterial activity of propolis essential oil

Microorganisms	Essential oil	Antibiotics		
	Inhibition zone (mm)	Vancomicin[a]	Penicilin[b]	Rifampicin[c]
Staphylococcus aureus	14	29	NT	NT
Staphylococcus epidermides 25/04	10	21	NT	NT
Staphylococcus epidermides 194/02	10	20	NT	NT
Streptococcus pyogenes 93007	14	NT	46	NT
Streptococcus pyogenes 75194	18	NT	46	NT
Escherichia coli	17	NT	NT	23

NT: not tested.

[a]Vancomicin 30 (µg/disc), [b]penicilin (10 U), [c]rifampicin 5 (µg/disc).

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