

Chemical Composition and Antibacterial Activity of Essential Oil of *Aniba duckei* Kosterman

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Received: 19 Dec 2017/Revised: 20 Jan 2018/Accepted: 21 Feb 2018

ABSTRACT- This research presented a chemical study of essential oil from *Aniba duckei* Kostermans, known as rosewood, as well as its test against bacteria *Aeromonas hydrophila*, *Bacillus cereus*, *Serratia* sp., and *Vibrio alginolyticus*. For control, we used pipemidic acid, ampicillin, cephalothin, cefoxitin, chloramphenicol, erythromycin, gentamicin, oxacillin, tetracycline, and vancomycin antibiotics. Oil yield was 1.2% (m/m) with linalool being its major component, with 89.34%. The essential oil was more efficient than all antibiotics tested against *A. hydrophila* and *Vibrio alginolyticus*. Linalool was less efficient than the *A. duckei* Kosterman's essential oil but more effective than many antibiotics. The essential oil, when tested against *Bacillus cereus*, was second only to gentamicin, while linalool presented less effectiveness than both gentamicin and tetracycline against the same bacteria. But both oil and linalool were effective against *Serratia*. The *A. duckei* Kosterman's essential oil activity was better than linalool's in all cases due to the oil's minor components action and synergy between them, which hinder resistance developed by bacteria.

Key-words- *Aniba duckei* Kostermans, Antibacterial activity, Essential oil, Rosewood, Linalool,

INTRODUCTION

Indiscriminate use of antibiotics and chemotherapeutics over the years has resulted in the development of resistant species [1-3]. Higher rates of microbial resistance, a decrease in a number of approved antimicrobials and the need for drugs that act by different mechanisms of action compared to drugs in use are reasons that justify the search for new antibiotic agents [4,5]. Many studies have turned their attention to natural sources in the last few years and, in many cases, the antibacterial activity of essential oils has been tested against pathogens [6-9].

Essential oils are defined by the International Standard Organization (ISO) as 'products obtained from parts of plants by steam distillation as well as products obtained by squeezing citrus fruit pericarp (Rutaceae). They are complex mixtures of volatile, lipophilic, odoriferous and liquid substances [10].

Botanical species *Aniba duckei* Kostermans, from Lauraceae family commonly known as rosewood, was discovered in Brazil in 1925 [11,12]. Its exploration for essential oil extraction has been ongoing since 1911 [13]. Threat of extinction for this species has led to an increasing control regarding its manipulation, which is regulated and monitored by government agencies in Brazil [14].

Linalool (3,7-dimethyl-oct-1,6-dien-3-ol) occurs naturally as two stereoisomers and is the major constituent of the essential oil [15,16]. Other minor components are also part of the essential oil composition [17]. In this research *A. duckei* Kostermans essential oil was subjected to analytical studies in order to characterize it physically and chemically and it also had its susceptibility tested against strains of *B. cereus*, *A. hydrophila*, *Serratia* sp., and *Vibrio alginolyticus*.

MATERIALS AND METHODS

The leaves and thin branches used for essential oil extraction were collected on March 2016 from three trees in the Adolpho Ducke Forest Reserve, Manaus, Amazonas, Brazil (03° 00' 02" and 03° 08' 00" south and 59° 58' 00" west). Extraction was carried out by hydro-distillation using an adapted Clevenger apparatus. Carrier gas: He. Column: VF-1ms 30 m x 0.25 mm capillary column, film thickness 0.25 µm 100%

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Quick Response Code	Website: www.ijlssr.com
	 DOI: 10.21276/ijlssr.2018.4.2.7

methylsiloxane. Column flow: 1 mL.min⁻¹. Injection temperature: 270°C. Injection mode: split (50:1). Oven temperature programming: 60°C–220°C with heating rate of 4°C min⁻¹ and from 220°C–260°C with ratio of heating of 1°C min⁻¹, with running time remaining in 100 minutes. Linalool was quantified by the external standard method, using linalool chromatographic standards (Aldrich).

For antimicrobial activity test, bacterial strains of *B. cereus*, *A. hydrophila*, *Serratia* sp., and *Vibrio alginolyticus* were tested for susceptibility by the Bauer-Kirby method with Ampicillin, 10 µg/L (AMP 10), Cephalothin, 30 µg/L (CFL 30), Chloramphenicol 30 µg/L (CLO 30), Erythromycin, 15 µg/L (ERI 15), Tetracycline, 30 µg/L (TET 30), Gentamicin, 10 µg/L (GEN 10), Cefoxitin, 20µg/L (CFO 20), Pipemidic Acid 20 µg/L (PIP 20), Oxacillin, 0.1 µg/L (OXA 01), Cefotaxime, 20 µg/L (CTX 20), and Vancomycin, 30 µg/L VAN 30) antibiotic disks.

Antibiotic discs were distributed on the plate, 0.02 m from the edge and 0.03 m from each other. Plates were incubated at 37°C for 24 hours. Inhibition halos for each bacterium were then measured and bacteria classified as sensitive, moderately sensitive or resistant according to the National Committee on Clinical Laboratory Standards-NCCLS [18]. The procedure was repeated. Likewise, the antibacterial activity of essential oil and linalool were tested.

RESULTS AND DISCUSSION

The essential oil extracted from *Aniba duckei* Kostermans leaves and thin branches yielded 1.8% (m/m) and presented itself in a yellow color and clear appearance with 860 kg/m³ density, and 1.46 refractive index at 25°C. Absorption spectra in the ultraviolet region, for 60% by volume ethanol/water mixture is shown in Fig 1(a), for the linalool standard in Fig 1(b), and for *Aniba duckei* Kostermans essential oil in Fig 1(c). The ethanol/water mixture did not absorb in the UV region.

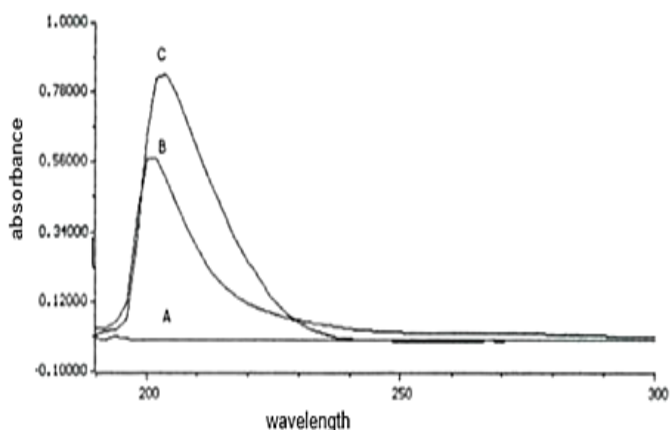


Fig. 1: UV-visible absorption spectra, (a) 60% by volume ethanol/water mixture (b) Linalool standard, (c) Essential oil extracted from *Aniba duckei* Kostermans leaves and thin branches

The λ_{\max} of the essential oil sample and linalool standard were very close, which proved that linalool was the major component of the essential oil. An infrared spectrum for linalool standard is shown in Fig. 2(a) and for essential oil in Fig. 2(b), and they present almost the same frequencies.

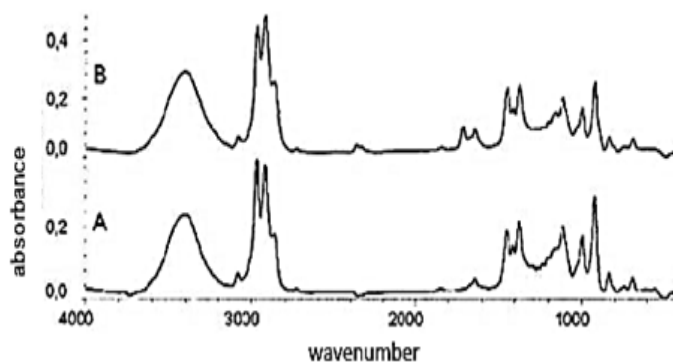


Fig. 2: Infrared absorption spectra, (a) linalool standard, (b) Essential oil extracted from *Aniba duckei* Kostermans leaves and thin branches

Strong absorption bands between 3200 and 3550 cm⁻¹ were attributed to the axial deformation of the hydroxyl group of alcohols in intramolecular hydrogen bonding [19], mainly due to the presence of linalool.

Vibration around 3090 cm⁻¹ results from axial deformation of the C-H bond of =C-H (vinyl group). In the 2840-3000 cm⁻¹ region absorption results from axial deformation of C-H bonds of aliphatic compounds. The weak band observed at 1625 cm⁻¹ results from C=C double bond (vinyl group) stretching. The intense band near 1416 cm⁻¹ is attributed to the vinyl group, due to the symmetrical angular deformation in terminal methylene plane. Bands in the 1000–1260 cm⁻¹ region are attributed to vibrations resulting from alcohols C-O axial deformation. Vibrations around 990 cm⁻¹ were due to the C-H out-of-plane symmetrical angular deformation [19]. These observations were sufficient to state that linalool was the major component of this essential oil.

Gas chromatography coupled to mass spectrometry (GC-MS)- Through chromatogram for the essential oil, the components listed in Table 1 were identified.

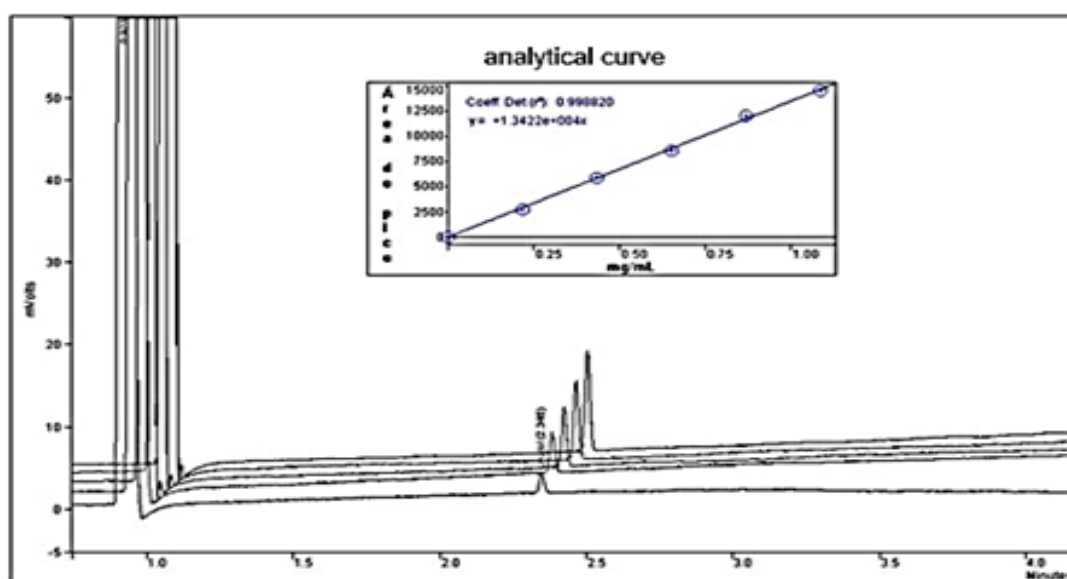
To identify separated and detected compounds in the sample of plant species *A. duckei* Kostermans essential oil, the spectra databases NIST105, NIST21 and WILEY139 and the AMSDIS software (Automated Mass Spectral Deconvolution Mass & Identification System), as well as references available in the previous literatures [19-23]. For linalool, confirmation was also made by the addition of standard. It was observed that the major component was linalool, with 89.34% followed by α -terpineol, with an area percentage of 3.06%.

Table 1: Compounds present in *Aniba duckei* Kostermans essential oil

Peak	t _{RET} ^a	Compound Name	%A ^b
1	15.61	Limonene	0.52
2	15.71	1,8-Cineole	1.07
3	17.43	Cis-linalool oxide	1.94
4	18.06	Trans-linalool oxide	1.86
5	18.60	Linalool	89.34
6	21.88	α-Terpineol	3.06
7	28.26	α-Copaene	0.89
8	31.74	α-Patchoulene	0.77
9	32.02	Caryophyllene	0.55

Fig. 3 shows the quantitative determination of linalool by GC, using the external standard method. The external standard analytic curve is characterized by the correlation coefficient ($r=0.999$). By this quantification, it can be

determined that the content of linalool contained in essential oil was 89.34%, which was in accordance with literature data [17,24].

**Fig. 3:** Analytical curve for determination of linalool in *Aniba duckei* Kostermans essential oil

As for antibacterial tests, *B. cereus* was resistant to the antibiotics pipemidic acid, ampicillin, erythromycin, cephalothin, cefoxitin and cefotaxime, moderately resistant to chloramphenicol and tetracycline, and sensitive to gentamicin and vancomycin.

A. hydrophila is moderately sensitive to pipemidic acid and chloramphenicol and resistant to the others. *Serratia* sp. was resistant to tetracycline, chloramphenicol, erythromycin and gentamicin, moderately sensitive to

ampicillin and cefotaxime and sensitive to pipemidic acid.

Vibrio alginolyticus was susceptible to gentamicin, tetracycline and vancomycin, moderately sensitive to cefotaxime and chloramphenicol, and was resistant to oxacillin, ampicillin and erythromycin. Both the essential oil and linalool were able to inhibit the growth of the bacteria *A. hydrophila*, *B. cereus*, *Serratia* sp. and *V. alginolyticus* as shown in Table 2.

Table 2: Microorganisms growth inhibition halos for the antibiotics tested, essential oil and linalool

Antibiotics	Inhibition halos diameter (mm)			
	<i>A. hydrophila</i>	<i>B. cereus</i>	<i>Serratia</i> sp.	<i>V. alginolyticus</i>
Pipemidic Acid	14	12	22	-
Ampicillin	0	10	15	10
Cephalothin	10	12	-	-
Cefotaxime	8	11	20	15
Cefoxitin	12	12	-	-
Chloramphenicol	15	16	11	13
Erythromycin	10	10	8	13
Gentamicin	10	22	13	19
Oxacillin	0	-	-	0
Tetracycline	14	17	0	19
Vancomycin	8	15	-	17
Oil	20	18	18	40
Linalool	14	16	16	18

The *Aniba duckei* Kosterman's essential oil was more effective than all antibiotics against *Aeromonas hydrophila*. Among these antibiotics, chloramphenicol was the most effective inhibiting *Aeromonas hydrophila* bacterium and the only one more effective than linalool.

The essential oil's inhibition halo showed to be more than twice as large as the halo for the most efficient antibiotics when inhibiting *V. alginolyticus*. Linalool proved to be a good antibacterial for this microorganism.

Only gentamicin was more effective than *A. duckei* Kosterman's essential oil inhibiting *Bacillus cereus*. Linalool presented the same efficacy as chloramphenicol but was less effective than gentamicin and tetracycline.

The essential oil presented good antibacterial power for *Serratia* sp., being second only to pipemidic acid and cefotaxime. These drugs were also the only ones more efficient than linalool.

According to the size of the growth inhibition zone (GIZ), in millimeters, the essential oils were classified as antimicrobial as follows: $GIZ \geq 15$ = very active, $10 \leq GIZ < 15$ = moderately active, $GIZ < 10$ = inactive [24].

The essential oil presented good antibacterial activity.

Bacteria tested were more sensitive to oil than to linalool, which may be attributed to the first one's minor components, to the synergism between these components, or between them and linalool, as well as to an antagonistic effect between these substances [24-26].

Records of these compounds having a bactericidal effect, including, in the context of minority groups, can be found in the literature [24-28]. Besides, there were also papers discoursing about the complexity of essential oils' composition and their effects against the integrity of bacteria's cell membrane [26,27].

Particularly, the rupture of these membranes by terpene compounds has been considered for bacteria [29,30], due to the solubility of the oil or the components of the oil [26].

CONCLUSIONS

Aniba duckei Kostermans species provided an essential oil whose yield was 1.24%, which is a good value for extraction by hydro distillation. All bacteria tested were more sensitive to essential oil than linalool. This is due to the contribution of minor components and synergism between components of essential oil. With these results, it may be suggested that this essential oil can be used as antibacterial with important characteristics, such as causing less danger to people and environment, since it is

a natural origin product, and it also presents a greater difficulty for resistance development by pathogenic microorganisms, since it is a complex mixture with different antibacterial activities mechanisms.

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How to cite this article:

Mesquita Teles RD, Mouchrek Filho VE, Mouchrek AN. Chemical Composition and Antibacterial Activity of Essential Oil of *Aniba duckei* Kosterman. *Int. J. Life Sci. Scienti. Res.*, 2018; 4(2): 1657-1662. DOI:10.21276/ijlssr.2018.4.2.7

Source of Financial Support: Nil, **Conflict of interest:** Nil