



RESEARCH ARTICLE

The antimicrobial activity of alcoholic extracts of leaves and bark from two varieties of "Sangre de Drago" compared with the antimicrobial activity present in the latex of the same species [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: This research was carried out in order to study the antimicrobial effectiveness of crude latex of two varieties of "Sangre de Drago": *Croton lechleri* Muller Arg. and *Croton urucurana* Baill and compare that effectiveness to the antimicrobial activity of the alcoholic extracts of its leaves and bark.

Methods: The activity of the alcoholic extracts and latex were evaluated against bacterial strains of *Staphylococcus epidermidis*, *Bacillus subtilis* and *Escherichia coli*. The extraction of the alcoholic extracts (20% Tincture) of the leaves, bark and latex from the two *Croton* species was carried out by maceration using 70% alcohol as a menstruum, at room temperature, for 2 to 7 days, with shaking at least twice a day. A 20% tincture was obtained, from which the physical and chemical parameters were determined as indicated by the Ecuadorian Quality Control Standard for natural medicinal products.

Results: It was found that both the alcoholic extracts of the plant material and the crude latex indicate antimicrobial activity for *S. epidermidis*, moderate antimicrobial activity for *B. subtilis* and no antimicrobial activity for *E. coli*. The moderate antimicrobial activity against *B. subtilis*, at doses of 125 p.p.m., is in line with the findings of previous studies by other authors.

Conclusions: he antimicrobial activity of the latex of the two species against *S. epidermidis* is not registered in literature and, the negative antimicrobial activity for *E. coli* does not agree with what has been reported by previous studies.

Keywords

Sangre de Drago, Croton lechleri Muller Arg., Croton urucurana Baill, antimicrobial activity

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Introduction

In Ecuador, there is a growing interest in medical plants from medical practitioners^{1,2}. “Sangre de Drago”, the name by which the plant and its medicinal latex are known, are a group of *Croton* (Euphorbiaceae) tree species widely distributed in Latin America. In Ecuador, they are found in the Amazon region and, as in Bolivia, Colombia and Peru, the most commonly identified species of Sangre de Drago is *Croton lechleri* Muller Arg.³. *Croton urucurana* Baill, a species found in the subtropics of South America, which is representative of the north of the country, islands and coasts of the Uruguay River, does not appear in the literature information for the *Croton* genus in Ecuador⁴.

The red latex and bark of Sangre de Drago have been used in popular South American indigenous medicine for different purposes, including wound healing⁵, diarrhoea, influenza, tonsillitis, intestinal disorders, herpes, fertility enhancement, tuberculosis⁶, hepatitis, cancer prevention, anti-inflammatory problems, acne, weight loss, coughs and colds⁷. In Ecuador, *C. lechleri* is used for treating haemorrhages, wounds and tuberculosis². The chemical composition of latex has been widely studied, revealing the presence of different metabolites, amongst which are the alkaloid taspine and its salt, lignans, proanthocyanidins, terpenes and phenolic compounds. These metabolites are responsible for the different activities that this medicinal plant presents. Taspine and lignans have anti-inflammatory activity, taspine hydrochloride has wound healing activity, and phenolic compounds have antimicrobial and antiviral activity⁸. The antimicrobial activity of Sangre de Drago in Gram-positive bacteria has been demonstrated, such as *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228; and with the Gram-negative *Pseudomonas* and *Klebsiella* FDA 602⁹. In this study, the antimicrobial activity of latex and of alcoholic extracts of leaves and bark of two species of Sangre de Drago was evaluated *in vitro* against specific bacterial strains in order to compare their antibacterial properties.

Methods

Botanical material

The vegetal material used from the two species of Sangre de Drago was collected in the localities of Talag and Canelos belonging to the provinces of Napo and Pastaza, respectively, and located at 1°, 3', 57" south latitude; 77°, 54', 26" west longitude; 40° west longitude; and 1°, 35', 22" south latitude; 77°, 44', 48" west longitude; 40° west longitude, respectively respectively. The sample collection was permitted by the Ministerio del Medio Ambiente, Ecuador (MAE), (registration number: 2015-RO. Nro. 449 and 905-RO.- suplemento 553). Aerial parts (leaves and bark) and latex of the trees aged 3 to 4 years and in a flowering-fructification stage were used. The crude latex for the analysis was obtained from indigenous merchants in the areas. One of the two latex samples corresponding to the Canelos sector was extracted from the same tree from which the plant material was collected. The collecting folders for each species were prepared and sent to the National Herbarium of Ecuador (QCNE) for taxonomic identification. The folder regarding the material collected in the Province of Napo was identified as *C. lechleri*

Muller Arg. and the one corresponding to the province of Pastaza as *C. urucurana* Baill. The aerial parts (leaves and bark) of the two species were dried at 49°C, in a JP SELECTA series 0385081 stove with circulating air for 3 days. The plant material was removed at least once a day for uniform drying until a constant weight was achieved. Next, the parts were crushed in a hammer mill until a moderately thick powder was obtained. The batches of the plant material were identified for the corresponding analyses with the letters “l” and “b” for leaves and bark, respectively, accompanied by the letters T and C to identify provenance from Talag and Canelos, respectively. The latex samples were refrigerated at 4°C for 2 weeks, during which time their analysis began from their acquisition. They were coded with the letters “T” and “C” and the letter “C” was accompanied with numbers “1” and “2” for the latex provided by the indigenous merchants and for the latex obtained from the same tree as the vegetal material was collected, respectively.

Physical-chemical evaluation

The extraction of the alcoholic extracts (20% Tincture) of the leaves, bark and latex from the two *Croton* species was carried out by maceration using 70% alcohol as a menstruum, at room temperature, for 2 to 7 days, with shaking at least twice a day. A 20% tincture was obtained, from which the physical and chemical parameters were determined as indicated by the [Ecuadorian Quality Control Standard for natural medicinal products](#). Under the same conditions, the physical-chemical constants of the crude latex of Sangre de Drago samples were determined. For the determination of the refractive index, a Clean-Prison series 003021 ABBE refractometer was used. pH determination was carried out in a Metrohm potentiometer, type 1.744.0010.

Chromatography analysis

The fractionation of the analysed alcoholic extracts was achieved by thin-layer chromatography, tapping 0.1 ml of the alcoholic extract by means of a glass capillary 1 cm from the bottom edge of 60GF254 silica gel plates. It was allowed to dry for a moment before each application and at the end of it. Then the plates were placed in saturated glass chambers for at least 30 minutes with steam from the components of each of the mobile phases used, which are listed below as ratios:

- Butanol-acetic acid-water (BAW): 4-1-5
- Chloroform-acetone-diethylamine: 7-3-1
- Toluene-ethyl acetate: 70-30

The following were used as developers: 254 nm and 366 nm ultraviolet light, Dragendorff reagent, ammonia fumes. Each chromatography experiment was repeated twice.

Microbiological quality

The microbiological quality of the alcoholic extracts of leaves and barks, as with crude latex, was verified as outlined by the World Health Organization (WHO)¹⁰. The tally of bacteria, fungi and yeast was read after 5 days of incubation as indicated by the WHO. Regarding the reading for the determination of

pathogenic bacteria, this was performed at 24 and 48 h of incubation with two repeats. The results of bacterial growth were compared with that described by Porter and Kaplan¹¹ for each culture medium used, for the purpose of bacterial recognition.

Evaluation of antibacterial and antifungal Activity

The evaluation of the antimicrobial activity was carried out against six bacterial strains (*Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa*, *S. epidermidis*, *Bacillus subtilis* and *Streptococcus sp.*) maintained in nutritious agar at 4°C according to Garcia and Uruburu¹². The antifungal activity was not assessed since growth problems were encountered for the strain *Candida albicans* ATCC10231. The method used was described in the “CYTED” Research Techniques Manual⁹. The extracts were solubilized using dimethylsulfoxide (DMSO) to their respective dilutions (5000, 2500, 1250, 1000, 500, 250 and 125 p.p.m.). Gentamicin sulfate (0.1 mg/0.1 ml) was used as a standard antibiotic and as a growth inhibition solution. As a negative control, Muller Hilton Agar and Muller Hilton Agar boxes with DMSO (0.1 ml) were prepared.

Results and discussion

Physical and chemical constants

Table 1 shows the results of density, total solids, pH and refractive index of the alcoholic extracts of the bark, leaves and latex of the two Sangre de Drago varieties analyzed.

As can be seen, there is no marked difference between the values of density, total solids and pH of the different extracts analysed. The organoleptic characteristics, on the other hand, differ clearly between the extracts of the two *Croton* species. In the same way, the capillary analysis shows a clear differentiation between the extracts and basically between the latex extracts (Figure 1). The presence of a fluorescent blue colour on the fringe of the capillary analysis image was characteristic in all the analysed extracts. When the wet paper strip was exposed to ammonia fumes, revealed with 366 nm UV

light and the environment left to dry, a weaker blue color was observed on the paper strips with 366 nm UV light. According to the consulted literature¹³, these results are characteristic of flavonoid-anthocyanidin type metabolites.

Table 2 shows the values of the physical and chemical parameters of the crude latex of the two varieties of Sangre de Drago analysed. As can be seen, the organoleptic characteristics allow the two varieties to be clearly differentiated. In terms of density parameters, total solids and pH, the results vary for each species. The refractive index cannot be determined by the physical characteristics of the samples (colour and viscous consistency).

Chromatographic analysis

Figure 2 presents the results of the thin layer chromatography, RF of the spots and colours visualized with the developers: 366 nm UV light, 254 nm UV light, ammonia fumes, Drangendorf reagent, alcoholic extracts of leaves, bark and latex.

As can be seen, the separation of the respective fractions occurs with greater clarity in the plates run in the mobile phases of BAW and 70–30 toluene-ethyl acetate. The different fractions obtained are observed as yellow at the point of application and faint orange to the naked eye during the course of the shift.

When revealed with 366 nm UV light, fluorescent orange spots are observed at different RF. When revealed with 254 nm UV light, faint grey spots are observed. The plates were passed through ammonia fumes and observed under 366nm UV light, where it was observed that the orange-coloured dots turned fluorescent blue. The phases were run in chloroform-acetone-diethylamine (7-2-1) mobile phase. Under natural light, no stain can be seen, but under the 366nm UV light, reddish spots are observed at a 0.48 RF and under 254 nm UV light, blue spots are observed. The RF of the calculated spots is closer to that reported in the literature for the alkaloid taspine (Theoretical RF of 0.50). When revealing the spots with Drangendorf's

Table 1. My caption.

SPECIES	SAMPLE	ORGANOLEPTIC CHARACTERISTICS		Other aspects	Physical-Chemical Parameters			
		Colour	Odour		σ	ST (%)	Ph	η
C. lechleri	leaves	yellowish-green	characteristic	transparent with an absence of sedimentation	0,89	1,0	7,02	1,37
C. lechleri	bark	reddish-brown	characteristic	transparent with an absence of sedimentation	0,89	2%	6,29	1,36
C. lechleri	latex	wine red	characteristic	transparent with an absence of sedimentation	0,90	WNR	WNR	1,37
C. urucurana	leaves	dark green	characteristic	transparent with an absence of sedimentation	0,89	1	7,05	1,37
C. urucurana	bark	reddish-brown	characteristic	transparent with an absence of sedimentation	0,89	1	6,62	1,37
C. urucurana	latex	reddish-brown	characteristic	transparent with an absence of sedimentation	0,93	WNR	WNR	1,37
C. urucurana	latex	reddish-brown	characteristic	transparent with an absence of sedimentation	0,98	WNR	WNR	1,37

reagent, a faint brown color was observed that disappeared after a few seconds. The presence of orange spots that turn blue in the presence of ammonia is characteristic of flavonoid-type phenolic compounds, and within these the anthocyanidins group, which are components present in Sangre de Drago¹⁴.

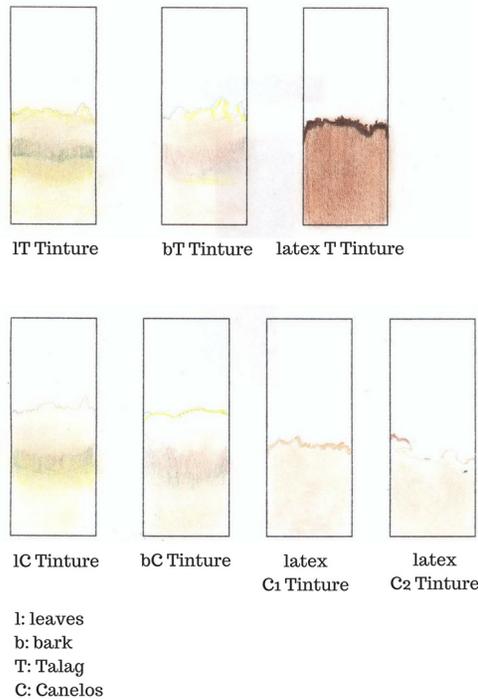


Figure 1. Capillary analysis of alcoholic extracts of leaves, bark and latex of *Croton lechleri* and *Croton urucurana*.

Microbiological control

The results obtained from the count of bacteria, fungi and yeasts of the alcoholic extracts of leaves, bark and crude latex in colony forming units/ml of sample are found in Table 3.

As can be observed, the values corresponding to the microbiological count of bacteria of the alcoholic extracts are within those established for crude drugs (see Table 4). The other two samples of latex did not present bacterial growth. The count of moulds and yeasts presents similar results to the bacterial count in the alcoholic extracts. The same cannot be said for the crude latex, because on the fifth day, the three analysed samples presented massive growth, which did not allow for the counting of the colony forming units. The presence of pathogenic bacteria was negative for the alcoholic extracts and for the latex samples from “C1” Talag and Canelos. This was not so for the “C2” Canelos latex sample, which showed growth of pink colonies, characteristic of *E. coli* in red Violet Agar. The abundant bacterial growth with a metallic luster in Mac Conkey Agar of the planting in lines of characteristic colonies in Red Violet Agar confirmed the presence of *E. coli*. It was impossible to count the colony forming units, as they surpassed what was established by the WHO for this type of sample. All samples were negative for bacterial growth in Cetrimide Agar specific for *P. aeruginosa* and in Baird Parker Agar for *S. aureus*.

Antimicrobial activity

For the determination of the antibacterial activity by means of the method described in the CYTE Research Techniques Manual⁹, a battery of six bacteria was used (*E. coli*, *S. aureus*, *P. aeruginosa*, *S. epidermidis*, *B. subtilis* and *Streptococcus sp.*). The preliminary results of each of the bioassays performed are presented in Table 5, in which the moderate antimicrobial activity

Table 2. Organoleptic characteristics and physical-chemical parameters of the latex.

Species	Sample	Organoleptic Characteristics (Colour, Odour and Appearance)	Physical-chemical parameters		
			δ	TS	pH
<i>C. lechleri</i>	Latex	Dark wine red. Viscous, dense liquid, absence of solid substances in suspension Milky-red upon contact with water. Does not allow passing of light Hardens upon contact with air and leaves a+ reddish stain when applied to the skin.	0.8972	0.03%	3.7
<i>C. urucurana</i>	Latex C1	Dark reddish brown. Lightly viscous, dense liquid, absence of solid substances in suspension. Upon contact with the water it precipitates a pinkish white colour. Under light, it is cloudy. Hardens upon contact with air and leaves a reddish-brown stain when applied to the skin.	0.9286	0.02%	4.6
<i>C. urucurana</i>	latex C2	Reddish brown. Lightly viscous, dense liquid, absence of solid substances in suspension. Upon contact with the water it precipitates a pinkish white color. Under light, it is cloudy. It presents foam on the surface. Hardens upon contact with air and leaves a reddish-brown stain when applied to the skin.	0.9826	0.03%	3.9

δ , density g/ml; ST, total solids.

(±) for *B. subtilis* can be observed in the seven samples analysed (extracts and crude latex). For the remaining five bacteria, the antibacterial activity was negative (-) for the alcoholic extracts. The Sangre de Drago latex coded “T” and “C1” were active (+) for *S. epidermidis* at doses of 2500 p.p.m. and 5000 p.p.m. and the “C2” latex is active (+) at a dose of 1250 p.p.m. For the four remaining bacteria, they did not exhibit antimicrobial activity. The negative activity (-) for *E. coli* does not agree with previous reports¹⁵⁻¹⁷. Regarding *B. subtilis*, it could be said that it is similar to results from other authors’ studies. The literature does not present information relating to activity on *S. epidermidis*. As for fungal activity, this test was not performed due to growth problems that arose with the *C. albicans* strain.

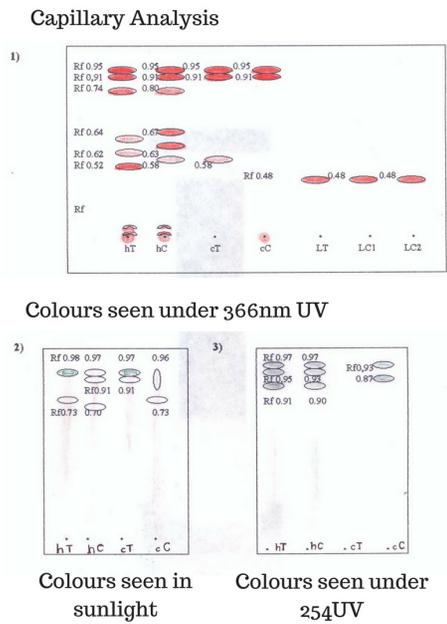


Figure 2. Chromatograms obtained for the alcoholic extract of the plant material and latex of the two Sangre de Drago species analysed in the following systems: 1) chloroform-acetone-diethylamine 70:20:10; 2) BAW 4:1:5 and 3) toluene ethylacetate 70:30.

Table 3. Microbiological count (total aerobics, moulds and yeasts) of tinctures of the bark, leaves and crude latex of Sangre de Drago.

Sample	Total bacterial count (cfu/ml) on 2nd incubation day at 37°C	Total yeast and mould count (cfu/ml) on 5th day of incubation at room temperature
Tincture of leaves (T)	51	53
Bark tincture (T)	40	Absence of growth
Tincture of leaves (C)	153	11
Bark tincture (C)	50	Absence of growth
Latex (T)	Absence of growth	Innumerable
Latex (C1)	Absence of growth	Innumerable
Latex (C2)	113	Innumerable

T, Talag; C, Canelos; C1, latex from unidentified tree; C2, latex from identified tree.

Table 4. Microbiological limits acceptable for (a) pre-treated plants and (b) for raw plants.

a)	MICROORGANISMS	LIMIT
	Total aerobic count	Maximum 10 E7 cfu/g
	Moulds and yeasts	Maximum 10 E4 cfu/g
	<i>Escherichia coli</i>	Maximum 10 E2 cfu/g
	Other enterobacteria	Maximum 10 E4 cfu/g
	<i>Salmonella</i> spp	Absent
b)	MICROORGANISMS	LIMIT
	Total aerobic count	Maximum 10 E4 cfu/g
	Moulds and yeasts	Maximum 10 E5 cfu/g

Source: Quality Control Methods for Medicinal Plant Materials⁹

Table 5. Results of the antimicrobial activity in alcoholic extracts and latex of *Croton lechleri* and *Croton urucurana*.

Species	Sample	Concentration (ppm)	Bacteria Strain					
			Ec	Sa	Se	Ssp	Pa	Bs
<i>C. lechleri</i>	Tincture of leaves	5000	R	R	R	R	R	I
		2500	R	R	R	R	R	I
		1250	R	R	R	R	R	I
		1000	R	R	R	R	R	I
		500	R	R	R	R	R	I
		250	R	R	R	R	R	I
		125	R	R	R	R	R	I

Species	Sample	Concentration (ppm)	Bacteria Strain					
			Ec	Sa	Se	Ssp	Pa	Bs
<i>C. lecheri</i>	Tinture of bark	5000	R	R	R	R	R	I
		2500	R	R	R	R	R	I
		1250	R	R	R	R	R	I
		1000	R	R	R	R	R	I
		500	R	R	R	R	R	I
		250	R	R	R	R	R	I
		125	R	R	R	R	R	I
<i>C. urucurana</i>	Tinture of leaves	5000	R	R	R	R	R	I
		2500	R	R	R	R	R	I
		1250	R	R	R	R	R	I
		1000	R	R	R	R	R	I
		500	R	R	R	R	R	I
		250	R	R	R	R	R	I
		125	R	R	R	R	R	I
<i>C. urucurana</i>	Tinture of bark	5000	R	R	R	R	R	I
		2500	R	R	R	R	R	I
		1250	R	R	R	R	R	I
		1000	R	R	R	R	R	I
		500	R	R	R	R	R	I
		250	R	R	R	R	R	I
		125	R	R	R	R	R	I
<i>C. lecheri</i>	Latex	5000	R	R	R	R	R	I
		2500	R	R	I	R	R	I
		1250	R	R	I	R	R	I
		1000	R	R	I	R	R	I
		500	R	R	R	R	R	I
		250	R	R	R	R	R	I
		125	R	R	R	R	R	I
<i>C. urucurana</i>	C1 latex	5000	R	R	I	R	R	I
		2500	R	R	I	R	R	I
		1250	R	R	R	R	R	I
		1000	R	R	R	R	R	I
		500	R	R	R	R	R	I
		250	R	R	R	R	R	I
		125	R	R	R	R	R	I
<i>C. urucurana</i>	C2 latex	5000	R	R	R	R	R	I
		2500	R	R	I	R	R	I
		1250	R	R	I	R	R	I
		1000	R	R	I	R	R	I
		500	R	R	R	R	R	I
		250	R	R	R	R	R	I
		125	R	R	R	R	R	I

Leyenda: Ec=Escherichia coli, Sa= Staphylococcus aureus, Pa= Pseudomona aeruginosa, Se= Staphylococcus epidermidis, Bs= Bacillus suptilis, Ssp= Streptococcus sp.

R=resistant

I= intermediate sensitivity

Dataset 1. Images and raw data from all chromatography experiments under all development methods with RF values, in addition to complete microbiological counts and physical/chemical characteristics of extracts

<http://dx.doi.org/10.5256/f1000research.14575.d211358>¹⁸

Also included are images of the plates used in this study, although it should be noted that these images were captured after storage for 2 or more years. Chromatography data 1 contains data generated using a mobile phase of Butanol:acetic acid:water (4:1:5) visualized using ferric chloride. Chromatography data 2 contains data generated using a mobile phase of chloroform:acetone:water (7:3:1) visualized using ammonia vapor; Chromatography data 3 contains data using a mobile phase of chloroform:acetone:diethylamine (7:3:1) visualized using ammonia vapor.

Conclusions

1. The results of the physical-chemical constants of the alcoholic extracts do not allow one to differentiate the two *Croton* species studied. However, the organoleptic characteristics and the capillary analysis allow for differentiation between the alcoholic extracts and latex of *C. lechleri* and *C. urucurana*.
2. The organoleptic characteristics of the latex of the two varieties allow one to differentiate them from each other. Regarding physical-chemical parameters, the values are very similar, which allows them to be used for their differentiation.
3. The average pH value, of 3.7 for *C. lechleri* latex, 4.6 for *C. urucurana* C1 latex and 3.9 for *C. urucurana* C2 latex, are similar to that found in the literature (pH 4.4).
4. The chromatographic analysis of the extracts of bark, leaves and latex of the two Sangre de Drago species allowed for the separation of this species' characteristic components, alkaloids and phenols. These compounds make up the metabolites responsible for the biological properties of the Sangre de Drago.
5. The antimicrobial activity of the alcoholic extracts of leaves, bark and latex was negative for *S. epidermidis* and

E. coli, but not for *B. subtilis*, which was moderately positive.

6. The three latex samples show antimicrobial activity for *S. epidermidis*. However, the sample of Canelos latex, obtained from the same tree that leaves and bark were collected from, presented antimicrobial activity at lower doses, 1250 p.p.m. Antimicrobial activity was not found in the reviewed literature. In relation to *B. subtilis*, moderate activity was observed in the three latex samples, an activity that was also evident in the alcoholic extracts of leaves and bark.

7. The values of antimicrobial activity against *E. coli* and *B. subtilis* are found in the literature¹⁵⁻¹⁷ for *C. Lechleri*, but not for *C. Urucurana*, a species for which no information is available.

Data availability

Dataset 1. Images and raw data from all chromatography experiments under all development methods with RF values, in addition to complete microbiological counts and physical/chemical characteristics of extracts. Also included are images of the plates used in this study, although it should be noted that these images were captured after storage for 2 or more years. Chromatography data 1 contains data generated using a mobile phase of Butanol:acetic acid:water (4:1:5) visualized using ferric chloride. Chromatography data 2 contains data generated using a mobile phase of chloroform:acetone:water (7:3:1) visualized using ammonia vapor; Chromatography data 3 contains data using a mobile phase of chloroform:acetone:diethylamine (7:3:1) visualized using ammonia vapor. DOI: [10.5256/f1000research.14575.d211358](http://dx.doi.org/10.5256/f1000research.14575.d211358)¹⁸.

Competing interests

No competing interests were disclosed.

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<http://www.doi.org/10.5256/f1000research.14575.d211358>

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Gabriel Trueba 

Institute of Microbiology, College of Biological and Environmental Sciences, University of San Francisco, Quito, Quito, Ecuador

The paper entitled "The antimicrobial activity of alcoholic extracts of leaves and bark from two varieties of "Sangre de Drago" compared with the antimicrobial activity present in the latex of the same species" describes some chemical analysis and antimicrobial properties of the alcoholic extract and latex from a medicinal plant from Ecuador. The topic of the paper is interesting but there is little additional information compared with previously published reports. Additionally, the purification methods and physiochemical analysis are simple and insufficient to provide useful information. The paper should not have the results of standard microbiological parameters accepted by Ecuadorian institutions.

Minor points

- Before considering for indexing, the paper requires a lot of improvement in style.
- The authors should pay attention to the references. References 8 and 9 don't correspond with the text.
- Page 3: This should be eliminated "Under the same conditions, the physical chemical constants of the crude latex of Sangre de Drago samples were determined. For the determination of the refractive index, a Clean-Prison series 003021 ABBE refractometer was used. pH determination was carried out in a Metrohm potentiometer, type 1.744.0010."
- Page 4, thin layer chromatography results are insufficient to identify taspine as indicated in the text
- Page 5. The methods utilized to detect E. coli are not adequate.
- Table 1 . legend requires a lot more information
- Figure 1. This figure does not provide any relevant information.
- Table 5. There is no explanation about what intermediate sensitivity means

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbial genetics

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 16 August 2018

<https://doi.org/10.5256/f1000research.15862.r36857>

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Dinithi C. Peiris

Department of Zoology (Centre for Biotechnology), University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Geuvara et al., describes a comparison of antimicrobial activities of leaves and bark of 2 varieties “Sandre de Dargo” to that of latex of the same species. However, several points should be corrected/clarified before acceptance.

1. The name of the botanist with complete affiliation after the scientific name
2. “in Gram-positive bacteria has been demonstrated, such as *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228” – Better to revise the sentence.
3. Better to include the time and the period of collection.
4. Did authors deposit a voucher specimen and allocated a herbarium number?
5. Why did authors conducted alcoholic extraction? Was it based on previous studies?
6. The introduction should include a survey of previous work of the plant the authors are investigating
7. What are the gram-negative and gram-positive bacteria used? Please separate the strains accordingly.
8. Better to expand antimicrobial testing experiment
9. Did authors only count the colonies formed.
10. You can go through the following paper for methodological details. Bandara K.V., Padumadasa C., Peiris L.D.C. (2018)¹.

11. Authors should report how they measure diameter of inhibition zone and MIC.
12. Figure 1 – Please italic the scientific name and also better to indicate what the colors denote.
13. What is the title of the table 1

References

1. Bandara KRV, Padumadasa C, Peiris DC: Potent antibacterial, antioxidant and toxic activities of extracts from *Passiflora suberosa* L. leaves. *PeerJ*. 2018; **6**: e4804 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cancer research, diabetes, antimicrobial, signalling pathways etc..

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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