RESEARCH ARTICLE

***In vitro* evaluation of the antifungal activity of Marco (*Ambrosia arborescens* Mill.) and Matico (*Aristeguietia glutinosa* Lam.) on the pathogenic fungi that cause dermatomycosis (ringworm) [version 1; peer review: 1 approved with reservations, 1 not approved]**

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Abstract

Background: Currently, there is a trend towards using natural and ethnopharmacological species with therapeutic potential. This investigation evaluated the antifungal activity of two species in the Ecuadorian Andes, which are used in treating dermatomycosis: *Ambrosia arborescens* Mill. (Marco) and *Aristeguietia glutinosa* Lam. (Matico).

Methods: We worked with seven concentrations (100 to 700ppm) of *Ambrosia arborescens* Mill. extract and ten concentrations (0.5 to 5%) of essential oil (EO) of *Aristeguietia glutinosa* Lam. on *Trichophyton mentagrophytes* ATCC 9533, *Trichophyton rubrum* ATCC 28188, *Microsporum canis* ATCC 36299 and *Candida albicans* ATCC 10231. The methodology used was a modified version of the Kirby-Bauer method, using diffusion in agar wells.

Results: The Tukey test, after the one-way Anova, determined effective concentrations of EO: 5% for *Trichophyton mentagrophytes*, 4.5% for *Trichophyton rubrum*, 5% for *Microsporum canis* and 2% for *Candida albicans*. In the extracts, the concentration of 700ppm was used for *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and 600ppm for *Microsporum canis* and *Candida albicans*.

Conclusions: The evaluation of the antifungal activity of the *Ambrosia arborescens* extract showed inhibition in the studied dermatophytes in each one of the planted concentrations (100 to 700ppm). The evaluation of the antifungal activity of *Aristeguietia glutinosa* EO showed inhibition in the studied dermatophytes in each of the planted concentrations (0.5 to 5%).

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Any reports and responses or comments on the article can be found at the end of the article.

Keywords

Ambrosia Arborescens, Aristeguietia glutinosa, essential oil, extract, antifungal, dermatomycosis

This article is included in the **Pathogens** gateway.

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Introduction

This research provides a technical basis that allows for plants used in the field of ethnomedicine to be used in the development of natural products in concentrations that allow for an effective therapeutic action. Plants have been identified as being useful in treating several diseases that have affected populations since ancient times, amongst which are allergies, pains, infections and skin diseases. The most studied skin lesions are related to different forms of dermatitis, skin allergies, acne and bacterial infections of the skin. Several medicinal plants, or essential oils and natural extracts obtained from them, have a curative influence on these pathologies¹.

Many plants have been granted the classification of “medicinal”, being part of the intangible natural heritage of a country, thus it is advisable to take advantage of ancestral knowledge² in the development of new therapeutic agents. Current knowledge about medicinal plants has two fundamental objectives, once their therapeutic value has been validated. The first is to discover the molecular structure of the active principles and the second is to lay down scientific bases for the direct use of the plants by large sectors of the population³. *Ambrosia arborescens* (Marco) and *Aristeguietia glutinosa* (Matico) belong to the Asteraceae family, which are characterised as being aromatic plants with medicinal properties. Marco is widely used amongst the inhabitants of our region in order to mitigate headaches, migraines, rheumatism, fever, constipation, prostate disorders, fractures and injuries as well as for vaginal baths⁴. Within agriculture, it is used as a biological means of controlling pests, related to the protection or defense against attacks of microorganisms and insects⁵. Matico is a native species of the Inter-Andean valley and is found only in Ecuador. It is attributed with having anti-inflammatory, expectorant, antitussive, healing and disinfectant properties. It is also used as an emollient and protector of the skin⁶.

In the same way that the nutritional value of certain plant products has been discovered over the years, humankind has also determined the curative or analgesic value of many plants. The extinction of plants and loss of cultural knowledge puts at risk new applications which have not yet been developed by modern science and might be valid, safe and effective⁵.

Synthetic drugs offer indisputable advantages, however it has been found that undesirable and even serious sideeffects can occur. This has encouraged a return to the study of plants in an attempt to find natural drugs that have higher therapeutic value and lower pathological risks. Although empirical knowledge is valuable, it is not reliable enough, thus scientific confirmation is necessary³. In the case of dermatoses, the most commonly used medication for lesions is Clotrimazole (a compound derived from the imidazole family) commonly used for the treatment of yeast infections, oral candidiasis, and dermatophytosis (ringworm). It is also used to treat athlete's foot and tinea corporis⁷.

Another problem is the innate and acquired resistance that has been demonstrated *in vitro* and *in vivo*, therefore the early recognition of species resistant to one or more antifungals guarantees an optimal treatment and better result. However, resistance is constantly growing and evolving, so the introduction of new antifungals is important⁸.

The fungi that produce superficial mycoses are opportunistic, thus patients with one or more of the following are more susceptible: diabetes, AIDS, cancer and any other debilitating or chronic condition. Worldwide, a prevalence of 5% to 10% of mycosis caused by *Candida* has been found by dermatology services. Other isolated species with greater frequency are *Tinea corporis* and *Tinea capitis* as well as the following fungi: *Microsporum canis* (9%), *Trichophyton mentagrophytes* (8.8%) and *Trichophyton rubrum* (8.6%)⁹.

The ethnobotanical use of species such as Marco (*Ambrosia arborescens*) and Matico (*Aristeguietia glutinosa*) in skin conditions allows them to be considered inhibitory agents of pathogenic fungi. The verification of this activity *in vitro* allowed us to determine the most effective concentrations and consequently one could standardise the use of these species as natural assets in the formulation of products for the treatment of dermatomycosis.

Methods

Vegetal material

The vegetal material was collected in Ibarra City in the province of Imbabura. *Ambrosia arborescens* was obtained from the surrounding area of the Yaguarcocha Lagoon and *Aristeguietia glutinosa* in the San Miguel de Arcángel neighbourhood on the Alto de Reyes Hill. Ibarra is 2225 metres above sea level (m.a.s.l.). Located 115km northwest of Quito and 125km south of the city of Tulcán, it has a temperate and pleasant dry climate. The historical meteorological annuals have determined an average temperature of 15, 90°C, with a minimum variation of less than 0, 3°C.. Records show a maximum mean temperature of between 20 and 25°C and a minimum mean of between 7 and 11°C (see Table 1). Maximum average wind speeds are 7m/s and the minimum are 3.5 m/s. A hydrometeorological analysis has determined that average annual precipitations are between 1000 mm and 1400 mm¹⁰.

Table 1. Geomorphologic Data.

	<i>Ambrosia arborescens</i>	<i>Aristeguietia glutinosa</i>
Location	Laguna de Yaguarcocha	Loma Alto de Reyes
Coordinates	022°0" N 78°7'0" W	0° 21'46"N 78°07'48"O
m.a.s.l	2190	2405
Temperature	15°C	17°C
Humidity	72% HR	68% HR

Data was obtained on the day of collection is presented in [Table 1](#).

The material was dried under referenced conditions that guarantee not to affect it, namely that air temperature should be moderated to avoid the temperature of the herbs exceeding critical temperature (generally between 30 and 60°C for medicinal plants). Drying interrupts the degradation processes caused by enzymes or ferments and prevents the development of microorganisms and oxidation and hydrolysis reactions¹¹.

Fungal population

Of all the fungal population, with regard to the group of fungi and complying with international regulations, depending on the incidence of skin diseases⁹, the *in vitro* analysis was carried out on the following microorganisms: *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis* and *Candida albicans*, which are fungi with high influences on treated dermatomycoses.

Obtaining extracts

We worked with two types of extracts. We used a soft extract for Marco (*Ambrosia arborescens*), obtained by placing percolation in 96% ethyl alcohol (1:4) for 72 hours at room temperature, with a subsequent evaporation in a vacuum until a consistency of thick stringy mass was achieved (15 to 25% humidity) and the concentration is 2:1 according to Osorio (2009)¹². In the case of Matico (*Aristeguietia glutinosa*), a steam distillation at 92°C was carried out in a distiller for 45 minutes until the essential oil was obtained.

Evaluation of antifungal activity

The method is based on the relationship between the concentration of the substance needed to inhibit a bacterial strain and the growth inhibition halo on the surface of an agar plate with a suitable culture medium, planted homogeneously with the bacteria to be tested and upon which a 6mm diameter filter paper disc has been placed, or planted in an impregnated well with the correct amount of the substance¹³. The Microbiologics-KWIK-STIK™ strains *Trichophyton mentagrophytes* ATCC 9533, *Trichophyton rubrum* ATCC 28188, *Microsporum canis* ATCC 36299 and *Candida albicans* ATCC 10231 were used for the evaluation. We carried out an activation of the lyophilized strains and a subsequent culture in the first three microorganisms. We prepared a suspension of spores, evaluating the concentration of them with the Neubauer count chamber and the planting value or inoculum for the three microorganisms was 106. In the case of *Candida albicans* ATCC 10231, the preparation of the inoculum was carried out by means of the method for the quantification of the growth of microbial populations based on the cell mass by turbidimetry using a UV Shimadzu Spectrophotometer (model: mini 1240). The bibliographically referenced conditions determine a wavelength of 625 nm, an absorbance value of 0.08 to 0.11 which is equivalent to 1-2x10⁸ UFC ml for bacteria and a wavelength of 530nm and absorbance value of 0.13 to 0.16 which is equivalent to 1-5x10⁶ cells for fungi and yeasts¹⁴. Seven successive dilutions of *Ambrosia arborescens* (Marco) extract. Concentrations of 100ppm, 200ppm, 300ppm, 400ppm, 500ppm, 600ppm and 700ppm (parts per

million) were made using dimethyl sulphoxide (DMSO) polar miscible fluid and without antifungal activity as diluent. 10 dilutions of *Aristeguietia glutinosa* (Matico) essential oil were made in percentages of 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5% and 100% using Dimethylsulfoxide (DMSO) as the diluent. Planting was executed in plates by mass homogenisation for *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum canis*. 1ml of inoculum of the fungus was deposited and 19 ml of molten Sabouraud Dextrose Agar (SDA) culture medium was added, tempered at approximately 45°C and sterile. Both were mixed by gentle rotation of the plates. In this way, the microorganisms were distributed homogeneously in the culture medium allowing growth throughout the agar¹⁵.

Once the inocula were planted, the natural extracts were placed in the indicated concentrations following the Kirby-Bauer methodology, a method which was modified for use with diffusion in agar wells. The wells were made on the surface of the agars with the help of a sterile hole puncher with a diameter of 6mm. Then, 50µl of different concentrations of extract, different concentrations of oil, as well as a positive blank (clotrimazole) and negative blank (Dimethylsulfoxide-DMSO) were poured into each of them. The plates were incubated for 2 to 5 days at 25°C to allow confluent fungal growth. All tests were done in triplicate. The inhibition halos were measured with a gauge or calliper (mm), considering them as starting from the point of complete inhibition as seen by the naked eye. The reading was taken on the back of the plate with a black background and reflected light. The interpretation of the reading was based on the Kirby-Bauer method, which relates the concentration of the substance necessary to inhibit a bacterial strain with the growth inhibition halo on the surface of an agar plate with a suitable culture medium and seeded homogeneously with the tested fungi and on which has been deposited a (6 mm diameter) filter paper disk¹⁶.

The statistical analysis was carried out with a two-way ANOVA and the Tukey Test (HSD) with the use of the [Statistix 10.0](#) General AOV/AOCV Program and the Statistix 10.0 Tukey HSD All-Pairwise Comparisons Test Program.

Results

[Table 2](#) shows the results of an average of three determinations whose deviation limit is close to 95%. The results showed antifungal activity in each of the concentrations and the positive blank (Clz) used on *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis* and *Candida albicans*.

[Table 3](#) shows the results of an average of three determinations whose limit of deviation is close to 95%. The results evidenced antifungal activity in each of the concentrations and the positive blank (Clz) used on *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis* and *Candida albicans*.

The two-factor ANOVA statistical analysis resulted in a probability of $p=0.0004$ which is less than α (0.05). The statistical test F is 8.23, higher than the Ft of 5.41. These values allow one to accept the alternative hypothesis, which is that Matico (*Aristeguietia glutinosa* Lam.) EO presents antifungal activity

Table 2. Average diameter of inhibition halos of the *Ambrosia arborescens* extract.

Concentration (ppm)	Diameters of inhibition halos (mm)			
	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton rubrum</i>	<i>Microsporum canis</i>	<i>Candida albicans</i>
100	13.7	13.5	17.5	11.2
200	14.7	22.0	19.4	11.1
300	22.5	24.9	28.2	10.8
400	25.9	27.6	29.1	10.9
500	26.9	29.7	40.1	11.3
600	30.8	32.9	43.5	13.1
700	32.1	35.6	40.6	10.4
Clz (+)	48.8	53.2	30.6	24.9
DMSO (-)	6.0	6.0	6.0	6.0

Table 3. Average diameter of inhibition halos of *Aristeguetia glutinosa* essential oil.

Concentration (%)	Diameters of inhibition halos (mm)			
	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton rubrum</i>	<i>Microsporum canis</i>	<i>Candida albicans</i>
0.5	6.0	6.0	6.0	9.2
1	6.1	10.7	6.9	10.2
1.5	9.1	12.3	11.5	10.5
2	9.9	12.6	13.8	11.9
2.5	11.3	14.0	13.8	10.7
3	12.3	13.7	17.9	10.9
3.5	14.2	15.6	30.7	10.7
4	11.3	15.2	33.5	10.4
4.5	12.7	17.7	37.0	10.9
5	13.2	19.0	37.8	10.7
100	16.4	22.0	15.7	6.0
Clz (+)	49.9	52.0	37.5	24.5
DMSO (-)	6.0	6.0	6.0	6.0

(Figure 1). Once the alternative hypothesis was accepted, the Tukey test was carried out *a posteriori* to demonstrate whether or not there is a significant difference between the inhibition halos.

The test evidenced the existence of two groups: A and B. Group A consisted of *Microsporum canis* and group B consisted of *Candida albicans*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*. The most evident inhibition average was seen on *Microsporum canis* with an average of 20.81mm (Table 4).

The two-factor ANOVA statistical analysis resulted in a probability of $p=0.000$ which is less than $\alpha(0.05)$ and the statistic of the

F test is 24.16, higher than the F_t of 4.76. These values allow one to accept the alternative hypothesis, which is that extract of Marco (*Ambrosia arborescens* Mill.), presents antifungal activity (Figure 2). Once the alternative hypothesis was accepted, the Tukey test was carried out *a posteriori* to show whether or not there is a significant difference between the inhibition halos.

The test evidenced the existence of three groups: A, B and C. Group A consisted of *Microsporum canis* and *Trichophyton rubrum*, group B of *Trichophyton rubrum* and *Trichophyton mentagrophytes* and group C of *Candida albicans*. The most evident inhibition average was seen on *Microsporum canis* with an average of 31.20mm (Table 5).

Analysis of Variance Table for HALOS					
Source	DF	SS	MS	F	P
CEPAS	3	705.477	235.159	8.23	0.0004
ACEITE	5	727.645	145.529	5.09	0.0016
Error	31	885.878	28.577		
Total	39				
Note: SS are marginal (type III) sums of squares					
Grand Mean	13.871				
CV	38.54				

Figure 1. Analysis of two-way parametric variance (*Aristeguetia glutinosa*).

Table 4. Tukey Test *a posteriori* (*Aristeguetia glutinosa*).

Concentration (%)	Halo average (mm)	Belonging or related to group	Sample size
0.5	6.800	B	3
1	9.663	B	3
1.5	9.663	B	3
2	12.250	AB	3
2.5	12.250	AB	3
3	13.750	AB	3
3.5	13.750	AB	3
4	18.588	A	3
4.5	18.588	A	3
5	20.175	A	3
Studied strain	Mean rank (Halo average) (mm)	Belonging or related to group	Sample size
<i>Microsporum canis</i>	20.813	A	3
<i>Trichophyton rubrum</i>	13.603	B	3
<i>Trichophyton mentagrophytes</i>	10.533	B	3
<i>Candida albicans</i>	10.533	B	3

Statistix 10.0 (30-day Trial)

Analysis of Variance Table for HALOS

Source	DF	SS	MS	F	P
CEPAS	3	1529.86	509.952	24.16	0.0000
EXT	6	929.12	154.854	7.34	0.0004
Error	18	379.95	21.108		
Total	27	2838.93			

Grand Mean 23.214
CV 19.79

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	319.111	319.111	89.16	0.0000
Remainder	17	60.842	3.579		

Figure 2. Analysis of two-way parametric variance (*Ambrosia arborescens* Mill.) (*Aristeguetia glutinosa*).

Table 5. Tukey Test a posteriori (*Ambrosia arborescens*).

Concentration (ppm)	Halo average (mm)	Belonging or related to group	Sample size
700	29.675	A	3
600	30.075	A	3
500	27.000	AB	3
400	23.375	ABC	3
300	21.600	ABC	3
200	16.800	BC	3
100	13.975	C	3
Studied strain	Mean rank (Halo average) (mm)	Belonging or related to group	Sample size
<i>Microsporum canis</i>	31.200	A	3
<i>Trichophyton rubrum</i>	26.600	AB	3
<i>Trichophyton mentagrophytes</i>	23.800	B	3
<i>Candida albicans</i>	11.257	C	3

Dataset 1. Diameter of inhibition halos of *Ambrosia arborescens* extract.. Diameter (mm) of inhibition halos of *Ambrosia arborescens* EO. positive control (Clz +) and negative control (DMSO -) used on *Trichophyton mentagrophytes*. *Trichophyton rubrum*. *Microsporum canis*. *Candida albicans*

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Dataset 2. Diameter of inhibition halos of *Aristeguetia glutinosa* essential oil against fungus.. Diameter (mm) of inhibition halos of *Aristeguetia glutinosa* essential oil. positive control (Clz +) and negative control (DMSO -) used on *Trichophyton mentagrophytes*. *Trichophyton rubrum*. *Microsporum canis*. *Candida albicans*

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Conclusions

The evaluation of the antifungal activity of the *Ambrosia arborescens* extract showed inhibition in the studied dermatophytes in each one of the planted concentrations (100 to 700ppm). The data discrimination of the Tukey test, after the one-way Anova, showed that the most effective concentrations were at 700ppm for *Trichophyton mentagrophytes* and *Trichophyton rubrum* and 600ppm for *Microsporum canis* and *Candida albicans*. The two-way Anova statistical analysis proved that the extract presented a greater homogeneity at concentrations of 300 and 400ppm over the studied dermatophytes in comparison to the other concentrations, obtaining an average halo of 23.37mm. The evaluation of the antifungal activity of *Aristeguetia glutinosa* EO showed inhibition in the studied dermato-

phytes in each of the planted concentrations (0.5 to 5%). The data discrimination from the Tukey test, after the one-way Anova, showed that the most effective concentrations were 5% for *Trichophyton mentagrophytes*, 4.5% for *Trichophyton rubrum*, 5% for *Microsporum canis* and 2% for *Candida albicans*. The two-way ANOVA (analysis of variance) proved that the EO has a greater uniformity of inhibition at 2, 2.5 and 3% in relation to the other concentrations, for which an average halo of 15.75mm was determined on the studied dermatophytes.

Data availability

Dataset 1. Diameter of inhibition halos of *Ambrosia arborescens* extract. Diameter (mm) of inhibition halos of *Ambrosia arborescens* EO. positive control (Clz +) and negative control (DMSO -) used on *Trichophyton mentagrophytes*. *Trichophyton rubrum*. *Microsporum canis*. *Candida albicans*. [10.5256/f1000research.14354.d201492](https://doi.org/10.5256/f1000research.14354.d201492)¹⁷

Dataset 2. Diameter of inhibition halos of *Aristeguetia glutinosa* essential oil against fungus. Diameter (mm) of inhibition halos of

Aristeguetia glutinosa essential oil. positive control (Clz +) and negative control (DMSO -) used on *Trichophyton mentagrophytes*. *Trichophyton rubrum*. *Microsporum canis*. *Candida albicans*. [10.5256/f1000research.14354.d201493](https://doi.org/10.5256/f1000research.14354.d201493)¹⁸

Author endorsement

Matteo Radice confirms that the author has an appropriate level of expertise to conduct this research, and confirms that the submission is of an acceptable scientific standard. Matteo Radice declares they have no competing interests. Affiliation: Faculty of Agroindustry and Faculty of Agricultural and Zootechnics, Universidad Estatal Amazonica, Puyo, Ecuador.

Competing interests

No competing interests were disclosed.

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The paper refers to the in vitro antifungal efficacy of both an ethanol extract from *Ambrosia arborescens* and *Aristeguietia glutinosa* essential oil. The topic is of interest, unfortunately the paper is not well written and deep editing by a native English speaker is needed. Furthermore references and data about these ethnobotanical species are too scanty. The composition of these products is not reported, making it difficult to reproduce the study. The authors should provide some data about the composition, related to healing ability, as well as the composition of specimens used for the present study.

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?

No

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

Yes

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

No

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mycology, parasitology

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

Reviewer Report 21 May 2018

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To be appropriate, this scientific work and the manuscript produced needs important improvements. The authors do not explain the reason why an organic extract from *A. arborescens* and an essential oil from *A. glutinosa* are studied. The results are not compared with the current knowledge of the plant species selected. In scientific literature there are hundreds of references regarding the bioactivity and secondary metabolites production from *A. arborescens* and *A. glutinosa*. The authors apply a well-known method to assay the antifungal activity. I strongly recommend to review the guidelines provided by the CLSI (Clinical and Laboratory Standards Institute) to set the conditions to perform an antifungal assay. In this document, the standard conditions to evaluate antifungal agents are clearly stated, including inoculums concentration and dilutions range for antimicrobial agents.

The introduction of the manuscript needs revisions. This sections needs to highlight the valuable information resulted from this work. The relevance of medicinal plants for traditional medicine, the side effects of current antifungal agents are well known. The first paragraph must be at the end of this section, presented according to a frame constructed from the current knowledge of plant-based antifungal agents.

In the Methods sections there are several bulk mistakes

- Vegetal material: "plant material" is correct, rather than "vegetable material". The geographic (not geomorphologic) data from the collection sites of the plant material are not suitable to be presented in a table. Besides, the data are repeated in the text and the table. The consideration for drying conditions are confusing.
- Fungal population. The use of this title is completely wrong. I guess the authors tried to state the fungal strains used for the bioassay. In this section the ATCC codes must be provided.
- Obtaining extracts: Please change this title as well. In this work one plant extract and one

essential oil were obtained from the plants studied. Start with the plant extract preparation. I assume the authors preparing the extract contacting the plant material with 96% ethanol. "placing percolation" is not correct. Do not use the first-person plural for writing this section (this advice applies for all the manuscript!)

- Evaluation of antifungal activity: The writing style need serious improvements. This is not a laboratory report, the audience of this papers understand that the concentration of the substance and the inhibition halo are related. In the first paragraph, the authors refers the work with bacteria (?) , since the target microorganisms are fungi. The activation of lyophilized spores is routine lab work, please drop it from this section. Why was the concentration of the inoculum was not tested in the same way? The use of a Neubauer chamber allows to express the inoculum concentration in UFC as well. Expressing the "planting value" of the inoculum as 106 makes no sense. Then comes another situation: parts per million as concentration unit for plant extracts dilutions in an antimicrobial assay is never used. If the authors review the literature, they will note that the unit used is micrograms per milliliter (mg/mL). Please, use an space between the unit and the figure. DMSO is a solvent, not a diluent. The extracts and essential oils were placed, not planted, in the plate prepared for the bioassay. Please read more scientific papers regarding the agar well diffusion method to present a coherent methodology.

In the results, the authors are presenting tables copied directly from the software used to process the data. This is not admissible, even for undergraduate work. Besides, the antifungal activity of the extract and the essential oil are rather questionable. The highest antifungal activity of *A. arborescens* extract towards *T. metagrophytes*, *T. rubrum* and *M. canis*, according to the results of this paper is 700 mg/mL. An antimicrobial activity for a plant extract is considered promising in concentrations below 100 mg/mL (Rios & Recio, 2005). Since the authors do not present the yield the essential oil extraction, is not possible to assess the feasibility of obtaining matico essential oil to scale the bioactivity in a pharmaceutical product. The results sections just limit to present the statistical analysis, without an evaluation of the real potential of the bioactivity of the plants studied.

The conclusions are a mere repetitions of the results of the laboratory work and statistical analysis.

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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?

No

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

Partly

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

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and 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.

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