

Antimicrobial and Cytotoxic Properties of Extracts from Plants Traditionally Used in North-East Brazil

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Abstract. The north-eastern region of Brazil is home to communities of diverse ethnic origins that still depend significantly on folk medicine, with emphasis on plant extracts. The present work aims at investigating medicinal plants used in the traditional medicine of north-eastern Brazil with an assessment of the antimicrobial profile of their extracts. The antimicrobial activity of 12 extracts from 11 plant species from eight higher plant families traditionally used was evaluated against three human pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*) using the Agar Disk Diffusion and Broth Microdilution assays. The cytotoxic effects were evaluated on human keratinocytes. A hydroalcoholic extract from *Anadenanthera colubrina* presented the most promising *in vitro* antimicrobial activity against *S. aureus* associated with low cytotoxicity towards human keratinocytes. The information gathered in the present study represents a starting point for further research aiming at providing scientific evidence to the empirical usage of medicinal plants in traditional practices.

Introduction

Traditional Medicine (TM), also known as non-conventional or complementary medicine, is used all over the world, and is sometimes the primary, if not the only, source of delivered healthcare [34]. Scientific studies on the rational use of traditional medicinal plants and the popular therapeutic practices of local communities can lead to a profitable identification of useful therapeutic strategies, as well as leading to better preservation and sustainable use of the local biodiversity.

Brazil is one of the richest sources in the world in terms of biological, ethnic and cultural diversity [10], wherein the north-eastern region figures as one of the most diversified for its ethnical compositions and traditional use of plants for the treatment of diverse diseases [4,5]. Thanks to its unique geographical and climatic conditions, this region has an unparalleled environment that harbors a particularly ample variety of ecosystems and habitats [1]. This has contributed to the natural selection of a large variety of plants capable of surviving under high-stress conditions, thanks to their

complex defense mechanisms and the production of an arsenal of bioactive molecules [4], which are used in TM.

Plant-derived compounds are also highly useful to contemporary conventional medicine as well. It has been estimated that up to 30% of currently available therapeutic medications are derived from natural sources, mostly from plants or microorganisms, and many classes of active principles have in fact been isolated from Brazilian medicinal plants [10]. These natural compounds can be considered as evolutionarily optimized drug-like molecules. A medicinal plant is, therefore, by definition, any plant that contains, in any of its parts, active substances that can be used in the treatment or prevention of diseases [29], as for example, digoxin from foxglove (*Digitalis purpurea*), morphine from poppy (*Papaver somniferum*), aspirin (acetylsalicylic acid) from willow bark (*Salix alba*) [25].

Ethnobotanical knowledge has paved the way for the discovery of a significant number of drugs that have transformed medicine and represent the basis of modern pharmacopeias. However, the therapeutic effect is often delivered by a complex mixture of active substances contained by the plant (the phytocomplex) rather than by a single purified molecule. These molecular mixtures can be extracted by means of a variety of traditional and domestic preparations. It is of paramount importance to define which mode of preparation, and which of the subsequent practices, are the safest and most efficient to make them available for the human organism. Sharing such knowledge could eventually lead to an empowerment of the local population thanks to these self-care tools for basic disease prevention, which also have the untapped potential to lower primary healthcare expenses [18–20,26].

The aim of this study is, therefore, to point out the importance and the need for preservation of this cultural and natural heritage. In pursuing this aim, we evaluated the antimicrobial activity of 12 north-eastern plants extracts on some important human pathogens, namely *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pneumoniae*, and also evaluated their cytotoxic effects on human keratinocytes, in an attempt to rationalize their traditional use, and possibly reveal potentially useful anti-infective properties.

Materials and Methods

Collection of plant samples. Plants were selected on the basis of a review carried out by Benko-Iseppon and Crovella [5], on further analysis of the ethnobotanical literature, on anecdotal reports from the local population, and on previous laboratory findings. The samples; leaves, branches and flowers of plants from the outside of protected areas, were collected by the research teams of the Genetics and Biophysics departments (Laboratory of Plant Genetics and Biotechnology and the Laboratory of Chemical Biophysics, respectively) of the Universidade Federal de Pernambuco (UFPE, Recife, Brazil), with the help of both a local field guide and an agronomist. Herbarium vouchers of the studied species were deposited in the Universidade Federal de Pernambuco (UFP) herbarium. The voucher numbers and coordinates for collected specimens are listed in Table 1. For most analyzed specimens, leave tissues were collected, while for *E. elatior* (Table 1) both leaves and flowers were collected. Part of this material was air-dried (45° C) and used for the extraction procedures, while another part was stored in a deep freezer (-80° C).

Extraction procedures. Plant leaves were submitted to three distinct extraction procedures: ethanolic, hydroalcoholic, and acidic (see Table 1), respectively applied to obtain molecules with specific physicochemical properties related to their solubility in each chosen solvent type.

Ethanolic extraction was applied specifically on *Etilingera elatior* tissues (leaves and flowers), using oven-dried material (45 °C for 7 days), which was subsequently stirred continuously for 24 h in 100% ethanol and the extract was then roto-evaporated at 45 °C.

The hydroalcoholic extraction was carried out following the method of Doosti et al. with minor adjustments [9]. Selected plant samples were dried at 45° C for 5-7 days and then crushed in a Tecnal Willye type TE-650/1 knife mill until tiny particles were obtained. The powder was placed in a percolator to which the solvent (ethyl alcohol at approximately 92%) was added and changed four times every two days, for a total of eight days of extraction. After the extraction, the solvent was

removed by roto-evaporation at $40 \pm 2^\circ\text{C}$. Finally, the resulting solid crude extract was removed from the flask and stored in Eppendorf tubes at 4°C . Aliquots of each solid ethanolic extract were then resuspended in absolute ethanol, to a final concentration of 100 mg/ml, vortexed, sonicated in a bath at 50°C , and then centrifuged. The supernatants were collected and used for the subsequent experiments. All samples resulted in light to dark green suspensions with some precipitate present.

The acid extraction was carried out as described by Díaz Dellavalle et al. with minor adjustments [8]. Samples were freeze-dried and macerated using liquid nitrogen, with the aid of a mortar and pestle. Five grams of powdered material was extracted with 50 ml of aqueous solution of 10% acetic acid for 1 hour at 50°C under stirring. The homogenate was removed by filtration and the supernatant suspension centrifuged at 7000 g for 30 minutes at 4°C until there was apparently suspended material. The supernatant was then injected in aliquots of 1 or 2 ml into disposable Waters SepPak C18 reversed-phase cartridges. The cartridges were modified in order to fit onto a GE Aktä HPLC system running at a flow rate of 0.4 ml/min to provide a pressure the cartridges could support. Fractions were collected using a gradient of 0-100% acetonitrile (0.05% TFA) in 17.5 minutes, with absorbance monitoring at 214 nm. The collected fractions were frozen at -80°C and subsequently lyophilized. The dried material was then resuspended in Milli-Q water in order to obtain stock solutions (10 mg/ml).

Antimicrobial activity assays. The plant's extracts were tested against three relevant bacterial pathogenic species: *Staphylococcus aureus* (American Type Culture Collection ATCC 25923, Gram-positive bacteria), *Escherichia coli* (ATCC 25922 Gram-negative bacteria), and *Streptococcus pneumoniae* (ATCC 6303, Gram-positive bacteria). *S. aureus* and *E. coli* were cultured using Luria-Bertani (LB) agar and broth at 37°C (Sigma Aldrich, St. Louis, Missouri, U.S.A.). *S. pneumoniae* was cultured using Brain Heart Infusion broth and Columbia blood agar (Oxoid, Thermo Fisher) with a CO_2 -enriched environment at 37°C . All strains were cultured on agar for one night before the experiments. On the day of each experiment, some colonies were collected using a sterile cotton swab and were then diluted in broth at a concentration of 0,5 McFarland for *S. aureus* and *E. coli* and 1 McFarland for *S. pneumoniae* (optical density 0,1 at 600 nm, corresponding to about 10^8 colony forming unit (CFU) of bacteria). The concentration was measured using a DensicheckTM instrument (21255, Biomériuex, Marcy-l'Étoile, France).

The first screening for antimicrobial capacity was performed using the agar disc diffusion method (ADD). After dilution, the pathogens were uniformly inoculated on agar Petri dishes using a sterile cotton swab. Five mm sterile paper discs soaked with 20 μl of each plant extract were then placed on the dishes. Extract concentrations were such that each paper disc was soaked with either 1 mg of each hydroalcoholic and ethanolic extract, or 0.2 mg of each acid extract. After incubating for 24 hours at 37°C , the diameter (in cm) of the growth inhibition halo around each paper disc was measured. For *S. aureus* and *E. coli*, each experiment was repeated four times using the hydroalcoholic and then the ethanolic extracts and twice with the acid extracts. For *S. pneumoniae*, each experiment was repeated twice for all extracts.

ADD has been reported to be unsuitable to evaluate the antimicrobial potential of some bioactive substances, mainly due to their reduced agar diffusion capacity (e.g., the polymixin antibiotics)[13]. In this case, the broth microdilution (BM) method can be more suitable to confirm the antimicrobial results. Therefore, the second antimicrobial screening of the extracts against the selected bacteria was carried out using the BM method. Bacteria were diluted in broth 1:200 from the original suspension, and 200 μl were seeded into 96 multi-well culture plates (about $1e^6$ CFU per well). Three different concentrations of 1 mg/ml, 0.1 mg/ml, and 0.01 mg/ml were evaluated for either hydroalcoholic and ethanolic extracts, whereas 0.4 mg/ml, 0.1 mg/ml, 0.01 mg/ml were used for acidic extracts. After 24 hours at 37°C , the absorbance of the suspension in each well plates was measured at 600 nm using GloMax®-Multi Detection System (Promega, Fitchburg, Wisconsin, U.S.A.). Since plant extracts were not themselves transparent, the final absorbance was calculated by subtracting the absorbance at time zero, considered as the 'blank' for each well. Each experiment was repeated four times.

Both types of antimicrobial evaluations were carried out following European guidelines [11], and literature references [3]. Minor adjustments were made to the broth microdilution procedures according to the aims of the study, namely only three concentrations were assessed for each plant.

Cytotoxicity assay. The cytotoxic evaluation of the extracts was performed on HaCaT cells (a spontaneously transformed keratinocyte cell line from human skin). The cells were cultured in DMEM medium (with 4,5 g/l glucose, Euroclone, Pero, Milan, Italy), supplemented with 10% fetal bovine serum, 100 U/ml Penicillin/Streptomycin, and 2 mM Glutamine (Euroclone, Pero, Milan, Italy). One day before the experiments, the cells were seeded into a 96 multi-well plate with 100 μ l of final volume per well with cell density of 10^4 cells/well. Each plant extract was tested at the same concentrations used for the antimicrobial activity tests. After 24 hours, the vitality of the cells was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation colorimetric assay (Trevigen, Gaithersburg, Maryland, U.S.A.) following the manufacturer's instructions. Absorbance was measured at 600 nm using a GloMax®-Multi Detection System (Promega, Fitchburg, Wisconsin, U.S.A.).

Statistical Analysis. Statistical analysis was performed using the Graphpad Prism™ 6 version 6.0 (GraphPad, La Jolla, California, U.S.A.). For the in vitro experiments, the Kruskal Wallis test was used to analyze the differences between untreated bacteria and the bacteria treated with the three different doses of plant extracts. Statistically significant results were corrected using Dunn's test. Moreover, the Mann-Whitney test was used to compare the survival of untreated bacteria and those treated with each concentration of the obtained plant extracts.

Plant extracts that presented stronger antimicrobial activity were selected using two cut-offs. We specifically choose (i) extracts presenting an inhibition halo > 1 cm in the ADD test and (ii) the extracts that showed statistically significant results in the BM method using the Kruskal Wallis test and Mann-Whitney test (for all evaluated concentrations).

After the selection based on the antimicrobial effect, the cytotoxicity was considered, i.e., only the extracts that presented nontoxic effect were selected (viability $>75\%$ in comparison to untreated cells).

Results and Discussion

Despite the fact that all the selected plants were already known for their antimicrobial effect of their extracts on human pathogens, an up to date literature review clearly indicates a lack of investigations assessing antimicrobial efficacy in association with cytotoxic effects. The purpose of this study was to obtain data that will help to address this research gap and provide a solid basis for further investigation.

To begin with, the antimicrobial effect was evaluated using the ADD method, and inhibition halos ≥ 1 cm was set as the selection criterion for antimicrobial active plant extracts. These were: *A. colubrina*, *E. elatior* (leaves and flowers), *P. barbatus* and *T. catigua* against *S. aureus*; *A. zerumbet*, *A. colubrina*, *E. elatior* (leaves), *M. charantia*, *O. gratissimum*, *P. barbatus*, *P. alliacea*, *S. chilensis* and *T. catigua* against *S. pneumoniae* (Table 2).

The BM assay was then performed using three different concentrations of the extracts. The plant extracts which showed statistically significant p-values at all the concentrations tested when compared to untreated samples were: *H. impetiginosus* against *E. coli* (Table 3); *A. colubrina* and *P. alliacea* against *S. aureus* (Table 4). No statistically significant results were observed against *S. pneumoniae* (data not shown).

In consideration of such evaluations, a strong consensus between ADD and BM results was obtained only for the extracts from *A. colubrina* against *S. aureus*, and therefore it is considered to have the strongest antimicrobial efficacy in our experimental data set.

According to the information collected, *A. colubrina* represented the most promising plant extract in terms of both appreciable antimicrobial activity and low cytotoxicity. Although no previous cytotoxicity tests of *A. colubrina* were identified in the literature, one study prospected antimicrobial activity followed by hemolytic activity (human erythrocytes) from the ethanolic extract of the related species *A. macrocarpa*, with activity against *S. mitis*, *S. mutans*, *S. oralis*, *S. salivarius*, *S. sanguinis*,

and *S. sobrinus* higher than 25 µg / ml and 15.5–24.5 µg / ml hemolytic activity. This shows low toxicity in relation to the isolated compounds (tannins), suggesting that the absence of hemolytic effect may have occurred due to the lack of interaction with lipids and erythrocyte membrane proteins [2]. *A. colubrina* species belong to the family Fabaceae, is native of South America and ranges from northern Colombia, northern and northeastern Brazilian States to the southern State of Paraná [7]. It has been reported that *A. colubrina* presents a broad spectrum of antimicrobial action, being active against Gram-positive and negative bacteria [28,31,32] and fungi [17].

Studies also showed that hydroalcoholic extracts of *A. colubrina* fruits exhibited antibacterial activity against *S. aureus* [28], causing morphological changes characterized by a corrugated and thicker cell wall, with protruding blebs and perforations.

Furthermore, stem bark extracts from *A. colubrina* were active against biofilm produced by *Staphylococcus epidermidis* [31] and *Pseudomonas aeruginosa* [32]. The treatment of *P. aeruginosa* resulted in a vacuolization, wall deformation, and disruption [32]. *A. colubrina* crude extracts and ethyl-acetate fractions also inhibited the growth of *Candida albicans* and biofilm formation by this yeast, inducing a de-structuring of cellular morphology [17]. In our work, in addition to the laboratory evidence of antimicrobial capacity of the leaf extracts (that confirmed results with other tissues in previous studies), we highlighted a low cytotoxicity of the evaluated extract against human keratinocytes, with survival rates of 78.70% at the concentrations of 0.1 mg/ml and 83.39 at 0.01 mg/ml (Table 5).

The cytotoxic effects of *A. colubrina* had been previously tested using an acid heteropolysaccharide, ARAGAL, extracted from *A. colubrina* gum against mouse peritoneal macrophage cells *in vivo* and *in vitro*, and no toxicity was detected in either. On the other hand, the ARAGAL compound probably modulated host anti-tumor immune mechanisms (*in vivo* tests), as well as activated the action of natural killer cells and tumor-infiltrating lymphocytes, producing effects on cytokine production such as interferon and colony-stimulating factor [21]. Such evidence could validate the therapeutic efficacy of *A. colubrina* tissues against different types of therapeutic uses, leading us to consider this plant as a potential candidate to undergo further investigations.

The other plant extracts did not show a consensus between ADD and BM tests; nevertheless, it is important to note the significant activity of *H. impetiginosus* against *E. coli* and *P. alliacea* against *S. aureus* in the BM assay. *H. impetiginosus* is one of the most exploited species and, therefore one of the most important members of the Bignoniaceae family [12]. It has a broad spectrum of biological properties due to the great diversity of described metabolites, as reported by dos Santos et al. [27]. However, there is no literature report to date of antimicrobial activity of ethanolic extract, whereas anti-inflammatory and chondroprotective activities have been described [24]. Park et al. [23] showed the activity of methanolic extract, hydrophobic fractions and isolated bioactive compounds (2-(Hydroxymethyl) anthraquinone and lapachol) from the inner bark of *H. impetiginosus* against *Helicobacter pylori*, a Gram-Negative bacterium responsible for gastroduodenal disease. Accordingly, our results detected an antibacterial effect only against the Gram-Negative *E. coli*, and not against the Gram-Positive *S. aureus* and *S. pneumoniae*. No toxic effect on HaCaT cells was observed.

In our screening, another species with potential antimicrobial activity was *Petiveria alliacea*. Previous studies evaluating its antimicrobial potential identified several antifungal and antibacterial labile organosulfur compounds from roots [16], such as thiosulfinates and sulfines with minimum inhibitory concentration (MIC) ranging between 16-128 µg/ml. The authors found these hydrophobic molecules are present in minor concentrations in this species, which may explain the relatively lower antimicrobial activity of *P. alliacea* in our work and also explain why some previous literature reports required higher extract concentrations to obtain relevant results [22].

The previously reported cytotoxic activity of *P. alliacea* was somewhat different from our results using ethanolic extract and showed a high cell mortality at a concentration of 1mg/ml [33]. However, those studies used purified methanolic fractions from leaves and stems of the plant to test cytotoxic activity in peripheral blood mononuclear cells, fibroblasts and also tumor cells. The authors found that the F4 fraction was the most promising due to the significant difference in cytotoxicity in

strains of neoplastic compared to healthy cells against which F4 was less deleterious, indicating the potential of *P. alliacea* to be explored.

The other nine tested extracts showed no relevant antimicrobial activity, possibly due to the plant component used in the extraction, the type of solvent, extraction procedure and/or a significant presence of compounds that may have reduced or inhibited the expected activity [6]. Antagonistic interactions may occur due to the combination of chemical constituents and/or their dosage. These factors can suppress or inhibit bioactive molecules [6]. For example, Suberu et al. have tested different combinations of compounds from *Artemisia annua* (Asteraceae) extract against *Plasmodium falciparum* (protozoan responsible for malaria) [30]. They observed that the action of artemisinin (a potent anti-plasmodium sesquiterpene) when combined with 9-*epi*-artemisinin and artemisitene was suppressed when tested against *P. falciparum* strains. Although the reason for such an antagonistic interaction is still unclear, these molecules have minor structural differences and are likely to have identical molecular targets and compete for them [6,30].

Nevertheless, additional information about chemical features and concentration of compounds is also necessary to understand antimicrobial activity or its lack thereof. Hydrophobic bioactive molecules from extracts probably would not spread easily through the solid medium in the ADD method, and their lower concentration may not be enough to promote the activity against the tested bacteria.

The extraction method should also be taken into account as it impacts significantly on the recovery yield and on the characteristic of the molecules extracted. For example, plant phytochemicals present different diffusivity and solubility dependent on the solvents used. Moreover, high temperatures and long extraction times can increase the diffusion but are deleterious for thermolabile components [14,35].

Intriguingly, the part of the plants employed for extraction seems to be fundamental. For example, both flower and leaf extracts from *Etilingera elatior* were utilized in the current work. The flower possessed an antimicrobial action in the ADD test and no cytotoxicity, while the leaf had no effect on bacteria but a high cytotoxicity at 1 mg/ml. These variations are likely due to different phytoconstituents of the two components of the plant displaying distinct bioactive properties [15].

Conclusions

This research extends our knowledge on some medicinal plants used by the north-eastern Brazilian population with respect to their antimicrobial potential. At the same time, it provides a useful selection of the most promising plants that can be subjected to further laboratory and epidemiological investigations. Among the investigated plant extracts, *A. colubrina* proved to be the best candidate thanks to its low cytotoxic effect and strong antimicrobial activity against *S. aureus*. This *in vitro* evidence provides a possible explanation for its widespread employment in local folk medicine as a remedy against different ailments [1,4,5].

Although some species did not show relevant activity under our experimental conditions, our work doesn't necessarily exclude their therapeutic potential. For example, *H. impetiginosus* and *P. alliacea* probably do possess useful antibacterial properties, although a consensus in our ADD and BM tests was not achieved. There are several reasons that could explain this lack of activity, for example the complexity of their natural chemistry should be taken into account, as well as a possible inhibitory effect of the solvents.

Future studies are warranted to identify and characterize each substance responsible for the antibacterial action highlighted in the current study; nevertheless, our experimental approach, employing the crude extracts, is worthy of consideration. Indeed, identifying single active compounds may not be sufficient to model the complexity of their natural chemistry [6], also considering the uses (i.e. topical application, tea assumption) of the medicinal plant extracts in traditional medicine, where all the plant component is commonly employed.

Conflict of Interest

The authors declare they have no conflict of interest.

Acknowledgments

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Table 1. Analysed plants with taxon, popular names in Portuguese (Brazil) and in the scientific literature (English), collection site and voucher number.

Ord.	Taxon/Author	Family/Class	Popular Name		Collection Site	Voucher #
			Portuguese	English		
1	<i>Alpinia zerumbet</i> (Pers.) B.L.Burt & R.M.Sm.	Zingiberaceae / Monocot	Colônia	Shellflower	8°09'32.2"S 34°57'30.1"W	90444
2	<i>Anadenanthera colubrina</i> (Vell.) Brenan	Fabaceae / Dicot	Angico	Curupay	8°08'45.3"S 36°22'14.7"W	48663
3	<i>Etlingera elatior</i> (Jack) R.M. Sm.	Zingiberaceae / Monocot	Bastão do Imperador	Ginger Flower	7°58'3"S 34°59'54"W	AMBI- 1796*
4	<i>Gymnanthemum amygdalinum</i> (Delile) Sch.Bip ex Walp.	Asteraceae / Dicot	Alcachofra	Bitterleaf	8°09'32.2"S 34°57'30.1"W	90433
5	<i>Handroanthus impetiginosus</i> (Mart. ex DC.) Mattos	Bignoniaceae / Dicot	Ipê-Roxo	Pink Trumpet Tree	8°3'4"S 34°56'48"W	RO- 101**
6	<i>Momordica charantia</i> L.	Curcubitaceae / Dicot	Melão-de- São-Caetano	Bitter Cucumber	8°09'32.2"S 34°57'30.1"W	90161
7	<i>Ocimum gratissimum</i> L.	Lamiaceae / Dicot	Alfavaca de Caboclo	Clove Basil	8°09'32.2"S 34°57'30.1"W	90163
8	<i>Petiveria alliacea</i> L.	Petiveriaceae / Dicot	Atipim	Gully Root	8°09'32.2"S 34°57'30.1"W	90164
9	<i>Plectranthus barbatus</i> Andrews	Lamiaceae / Dicot	Falso-Boldo	Coleus	8°08'45.3"S 36°22'14.7"W	90642
10	<i>Solidago chilensis</i> Meyen	Asteraceae / Dicot	Erva-Lanceta	Goldenrod	8°09'32.2"S 34°57'30.1"W	90441
11	<i>Trichilia catigua</i> A.Juss.	Meliaceae / Dicot	Catuaba	Catuaba	Purchased	***

Voucher: Leg/Det. *AMBI= Ana Maria Benko Iseppon; **Rodrigo Oliveira. ***Purchased from “Cantinho da Semente MEI”, endereço Av. Nossa Senhora da Penha, 250 (sala 407), CEP 29055-050, Vitória, ES., web-site <https://cantinhodasemente.com.br/>

Table 2. Antimicrobial activity by Agar Disc Diffusion method (ADD) of plant extracts against *S. aureus*, *E. coli*, and *S. pneumoniae*.

Extract Type	Zones of growth inhibition (cm)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. pneumoniae</i>
Hydroalcoholic			
<i>Alpinia zerumbet</i>	0.90±0.14	0.00±0.00	1.05±0.07
<i>Anadenanthera colubrina</i>	1.27±0.64	0.00±0.00	1.20±0.00
<i>Gymnanthemum amygdalinum</i>	0.00±0.55	0.00±0.00	0.90±0.14
<i>Momordica charantia</i>	0.87±0.44	0.00±0.00	1.35±0.07
<i>Ocimum gratissimum</i>	0.75±0.07	0.43±0.75	1.50±0.00
<i>Petiveria alliacea</i>	0.00±0.00	0.00±0.00	0.95±0.07
<i>Plectranthus barbatus</i>	1.30±0.14	0.00±0.00	1.40±0.14
<i>Solidago chilensis</i>	0.80±0.00	0.00±0.00	1.00±0.00
Ethanollic			
<i>Etligeria elatior (flowers)</i>	1.00	0.20±0.40	0.90±0.14
<i>Etligeria elatior (leaves)</i>	0.00±0.00	0.00±0.00	1.00±0.00
Acid			
<i>Handroanthus impetiginosus</i>	0.00±0.00	0.00±0.00	0.00±0.00
<i>Trichilia catigua</i>	1.00±0.00	0.90±0.14	1.00±0.00

The data are reported as mean ± standard deviation.

Table 3. Antibacterial activity of the plant extracts against *E. coli* using the broth microdilution method. The p-values from the Kruskal-Wallis test, Dunn's multiple comparison test, and Mann-Whitney test are reported.

Extract Type	Kruskal Wallis test	Dunn's post-test			Mann-Whitney test		
		1 mg/ml	0.1 mg/ml	0.01 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml
Hydroalcoholic							
<i>Alpinia zerumbet</i>	0.026	0.022	NS	NS	0.029	NS	NS
<i>Anadenanthera colubrina</i>	0.011	0.004	NS	NS	0.029	NS	NS
<i>Gymnanthemum amygdalinum</i>	0.034	NS	NS	NS	NS	NS	NS
<i>Momodica charantia</i>	NS	NS	NS	NS	NS	NS	NS
<i>Ocimum gratissimum</i>	0.023	NS	NS	NS	0.029	NS	NS
<i>Petiveria alliacea</i>	0.024	NS	NS	NS	0.029	NS	NS
<i>Plectranthus barbatus</i>	NS	NS	NS	NS	NS	NS	NS
<i>Solidago chilensis</i>	NS	NS	NS	NS	NS	NS	NS
Ethanollic							
<i>Etligeria elatior (flowers)</i>	NS	NS	NS	NS	NS	NS	NS
<i>Etligeria elatior (leaves)</i>	NS	NS	NS	NS	NS	NS	NS
Acid							
		0.4 mg/ml	0.1 mg/ml	0.01 mg/ml	0.4 mg/ml	0.1 mg/ml	0.01 mg/ml
<i>Handroanthus impetiginosus</i>	0.004	NS	0.005	NS	0.029	0.029	0.029
<i>Trichilia catigua</i>	0.0005	NS	NS	NS	0.029	NS	NS

NS, not significant

Table 4. Antibacterial activity of the plant extracts against *S. aureus* using the broth microdilution method. The p-value from the Kruskal-Wallis test, Dunn's multiple comparison test, and Mann-Whitney test are reported.

Extract Type	Kruskal Wallis test	Dunn's post-test			Mann-Whitney test		
		1 mg/ml	0.1 mg/ml	0.01 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml
Hydroalcoholic							
<i>Alpinia zerumbet</i>	0,009	0,018	NS	NS	0,030	0,030	NS
<i>Anadenanthera colubrina</i>	0,0000004	0,001	NS	NS	0,030	0,030	0,030
<i>Gymnanthemum amygdalinum</i>	0,0003	0,007	NS	0,022	0,029	NS	0,029
<i>Momodica charantia</i>	NS	NS	NS	NS	0,030	NS	NS
<i>Ocimum gratissimum</i>	0,029	NS	NS	0,022	NS	0,030	0,030
<i>Petiveria alliacea</i>	0,016	NS	0,022	NS	0,030	0,030	0,030
<i>Plectranthus barbatus</i>	0,003	0,011	NS	NS	0,030	NS	NS
<i>Solidago chilensis</i>	NS	NS	NS	NS	NS	NS	NS
Ethanollic							
<i>Etligeria elatior (flowers)</i>	0,032	NS	NS	NS	NS	NS	NS
<i>Etligeria elatior (leaves)</i>	NS	NS	NS	NS	NS	NS	NS
Acid							
		0.4 mg/ml	0.1 mg/ml	0.01 mg/ml	0.4 mg/ml	0.1 mg/ml	0.01 mg/ml
<i>Handroanthus impetiginosus</i>	NS	NS	NS	NS	NS	NS	NS
<i>Trichilia catigua</i>	0,048	0,034	NS	NS	NS	NS	NS

NS, not significant

Table 5. Cytotoxicity of plant extracts against HaCaT cell line. The data are reported as mean \pm standard deviation of the absorbance (percentage of surviving cells as compared to the not treated ones).

Extract Type	1 mg/ml	0.1 mg/ml	0.01 mg/ml
<i>Not treated cells</i> (Negative Control)	1.26 \pm 0.03 (100.0%)	1.10 \pm 0.03 (101.4%)	1.23 \pm 0.03 (103.9%)
Hydroalcoholic			
<i>Alpinia zerumbet</i>	0.20 \pm 0.01 (18.2%)	0.80 \pm 0.01 (74.3%)	0.83 \pm 0.02 (76.9%)
<i>Anadenanthera colubrina</i>	0.27 \pm 0.01 (24.6%)	0.85 \pm 0.2 (78.7%)	0.90 \pm 0.03 (83.4%)
<i>Gymnanthemum amygdalinum</i>	0.33 \pm 0.12 (31.7%)	0.19 \pm 0.01 (87.7%)	0.82 \pm 0.01 (94.7%)
<i>Momodica charantia</i>	0.13 \pm 0.01 (12.5%)	0.86 \pm 0.07 (79.7%)	0.36 \pm 0.06 (33.6%)
<i>Ocimum gratissimum</i>	0.26 \pm 0.05 (23.9%)	0.55 \pm 0.27 (51.4%)	0.84 \pm 0.003 (77.9%)
<i>Petiveria alliacea</i>	0.13 \pm 0.01 (12.1%)	0.85 \pm 0.04 (79.2%)	0.8 \pm 0.03 (78.0%)
<i>Plectranthus barbatus</i>	0.18 \pm 0.03 (16.7%)	0.94 \pm 0.07 (87.5%)	0.95 \pm 0.09 (88.3%)
<i>Solidago chilensis</i>	0.19 \pm 0.02 (15.6%)	0.85 \pm 0.07 (79.2%)	0.80 \pm 0.04 (74.2%)
Ethanolic			
<i>Etligeria elatior (flor)</i>	1.09 \pm 0.10 (101.2%)	1.09 \pm 0.01 (100.9%)	1.03 \pm 0.02 (95.8%)
<i>Etligeria elatior (folha)</i>	0.41 \pm 0.09 (38.4%)	1.07 \pm 0.01 (98.9%)	1.02 \pm 0.02 (94.9%)
Acid			
<i>Handroanthus impetiginosus</i>	1.02 \pm 0.03 (94.6%)	0.97 \pm 0.15 (90.0%)	1.10 \pm 0.02 (102.2%)
<i>Trichilia catigua</i>	0.91 \pm 0.07 (84.1%)	1.04 \pm 0.04 (96.6%)	0.97 \pm 0.13 (90.0%)

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