



Research Article

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Antimicrobial Potential of Plant Extracts from the Pitangui-Mg Region, Brazil

Parreira AG^{1*}, Azevedo EJC², Pereira FR³, Ferreira LVS³ and Azevedo RS³¹University of the State of Minas Gerais, Divinópolis MG Unit and Federal University of Sao Joao Del Rei Campus CCO, Brazil²Department of Agricultural Research Company of Minas Gerais, Pitangui MG, Brazil³University of the State of Minas Gerais, Divinópolis MG Unit, Brazil***Corresponding author:** Parreira AG, University of the State of Minas Gerais, Divinópolis MG Unit and Federal University of Sao Joao Del Rei Campus CCO, Brazil.**Received Date:** May 25, 2019**Published Date:** June 04, 2019

Abstract

Brazil is recognized worldwide as one of the largest holders of biodiversity on planet, possessing more than 20% of all plant species in the world, with countless of them presenting great medicinal potential. The medicinal plants use throughout the world has reached very significant economic figures, although its usage in folk medicine has been recognized for hundreds of years in different regions of the planet. The aims of this work were to evaluate the antimicrobial potential of plant extracts of Cerrado and Atlantic Forest specimens that occurs at Pitangui City region in Minas Gerais state with emphasis on those with regional folk medicine reports. The plant material used were leaves obtained from specimens collected at EPAMIG Pitangui Experimental Fields and Technical Institute of Agriculture and Cooperatives. The extracts obtained were submitted to experiments in Petri dishes and to the diffusion disc technique to determine their antimicrobial action through the inhibition zone. For evaluation of plant extracts effects on bacterial isolates and fungal isolate growth, it was possible to see a large responses variation in a scenario of species-specific dependence amid the tendency of slight decrease in the inhibitory effect for aqueous extracts. An average increase of 20% was observed in inhibition zone formation at the presence of ethanolic plant extract. For *S. aureus*, *S. epidermidis* and *E. coli* there was a higher number of leaf extracts in ethanolic solvent that inhibited the growth of those bacterial species, compared to the same plant extracts whose solvent was the aqueous. Exception for plant species, *P. alata*, in which leaf extracts only in aqueous solvent were effective against the species *S. aureus* and *S. epidermidis*. In both extracts, there was no antimicrobial effect detected for leaf extracts of *B. forficata*. The plant extracts that demonstrated greater amplitude of bacterial growth inhibition were *A. absinthium*, *S. cordifoliae*, *T. avellanadae*, with emphasis on ethanolic extracts of these species. For fungal isolate tested, *C. glabrata*, there was no growth inhibition against any of plant extracts evaluated. Considering the species *Baccharis retusa* and *Baccharis dracunculifolia*, only ethanolic extracts were tested. Inhibition results were obtained for all organisms tested, except for *Enterobacter cloacae* in relation to ethanolic extract of *B. dracunculifolia*.

Keywords: Brazil medicinal plants; Bactericide potential; Bacteriostatic potential

Introduction

Brazil is recognized worldwide as one of the largest holders of biodiversity on planet, possessing more than 20% of all plant species in the world, with countless of them presenting great medicinal potential [1]. The medicinal plants use throughout the world has reached very significant economic figures [2], although its usage in folk medicine has been recognized for hundreds of years in different regions of the planet. Thus, investment is justified in studies related to its active principles as well as new plant species with potential medicinal application, especially in Brazil, which, despite of its wide diversity of species already described, still presents large collection to be discovered and explored.

Proof of this is the enormous effort of Brazilian scientists to study the biodiversity of higher plants in the country in recent years, reflecting in the number of publications in international journals between 2011 and 2013, totaling 10000 publications in that period [3]. As another propellant factor, there is still growing interest in the use of phytotherapies and medicinal plants worldwide, especially in Brazil, where it was observed between 2013 and 2015 growth of 161% in the search for treatments based on medicinal plants and Herbal medicines by the Unified Health System (SUS), notably for treatment of Burns, gastritis and ulcers, according to data from Ministry of Health, [3].

Although the pharmaceutical industries have produced an expressive number of new antibiotics in recent decades, the microbial resistance to these drugs has also increased. In general, bacteria have the genetic ability to acquire and transmit resistance to drugs used as therapeutic agents (Cohen, 1992). The use of plant and phytochemical extracts of known antimicrobial activity can guarantee significant importance in various therapeutic treatments. One of several examples and most emblematic that can be cited is the case of Boldo (*Peumus Boldus*), very employed in Brazil for gastrointestinal discomfort treatment. In recent years, studies have shown that extracts from that plant have important antimicrobial effects, due to antimicrobial peptides presence, as well as molecules with anti-inflammatory action [4].

Several studies are routinely developed in different countries to prove their efficacy [5-10]. Fungicides originated from plants have been used for centuries. The research involving the demand for fungicide obtained from plants, however, only gained momentum in recent years. It is also noteworthy the substances diversity that exist in plants in general and possibility of finding new ones with antifungal or antibiotic action, which could be used directly by producer, through the Plant cultivation, preparation and direct application of its extracts. Another possibility refers to identification of new substances in plant extracts with fungicide and/or antibiotic characteristics which would serve as a model to chemical synthesis of their active principles in a second moment [11].

Cerrado, an important Brazilian biome, contains more than 6,000 vascular plants [12], many of them with food and medicinal value [13], however there is a very limited studies number related to species identification that present medicinal properties, to obtain extracts, biological assessments, Pharmacological and toxicological studies, which provide subsidies for their safe use, especially, when considering the great existing biodiversity. On the other hand, the Atlantic Forest, another Brazilian biome, is a forest formation of most threatened in Brazil, despite its ecological importance associated with exceptional biodiversity and high endemic species rates, information about the medicinal plants potential is rare. The medicinal plants extractivism presents great potential for rational use, since it does not imply the removal of forest and compared with other uses can generate lower environmental impacts [14] (Pereira 2012). The aims of this work were to evaluate the antimicrobial potential of plant extracts of Cerrado and Atlantic Forest specimens that occurs at Pitangui City region in Minas Gerais state, with emphasis on those with regional folk medicine reports.

Material and Methods

Study area

The plant material to be used were leaves obtained from specimens collected at EPAMIG Pitangui Experimental Fields and Technical Institute of Agriculture and Cooperatives, located at Pitangui municipality, Minas Gerais state (19° 42 ' 18.4 "latitude South and 44° 53 ' 10.3 "Longitude West", in Alto São Francisco

region, bordering to the north by Pompéu city, to south with Conceição do Pará, to east with Jaguar of Pitangui and to east with Leandro Ferreira and Martinho Camosciates. After collects, the specimens were identified, and their specimens exsiccates were deposited at/AG herbarium from Minas Gerais State Agriculture Research Company (EPAMIG). It is important to emphasize that access to genetic heritage was registered in SISGEN with registration code A3FD8E7, in October 2018, after the institutional register completion at State University of Minas Gerais on that platform also in October 2018.

Preparation and partitioning of the plant extract

To obtain aqueous extract from leaves, the infusion method was used, in which the dried leaves are placed in hot water ($T > 90^{\circ}\text{C}$) (DIAS et al. 2000), in a mixture of 1g plant material for each 10 mL of water. The Maceration method was used to obtain ethanolic extract, in proportion of 1g material to 9 mL of alcohol at 70% (V/V). The next step involved the dilution of macerated material in water and DMSO (dimethyl sulfoxide) in proportion of 10:1 (Araújo et al. 2013).

Antimicrobial and antifungal activity tests

For the evaluation of bacteriostatic, bactericidal, fungicidal or fungicidal effects, the extracts obtained were submitted to experiments in Petri dishes and to the diffusion disc technique to determine their antimicrobial action through the inhibition halo. Experimental procedures will follow those described in CLSI [13].

Result

For evaluation of plant extracts effects on bacterial isolates and fungal isolate growth, it was possible to see a large responses variation in a scenario of species-specific dependence amid the tendency of slight decrease in the Inhibitory effect for aqueous extracts (table 1). An average increase of 20% was observed in inhibition halo formation at the presence of ethanolic plant extract. For *S. aureus*, *S. epidermidis* and *E. coli* there was a higher number of leaf extracts in ethanolic solvent that inhibited the growth of those bacterial species, compared to the same plant extracts whose solvent was the aqueous. Exception for plant species, *P. alata* Dryander, in which leaf extracts only in aqueous solvent were effective against the species *S. aureus* and *S. epidermidis*. In both extracts, there was no antimicrobial effect detected for leaf extracts of *B. forficata*. The plant extracts that demonstrated greater amplitude of bacterial growth inhibition were. *absinthium*, *S. cordifoliae*, *T. avellanadae*, with emphasis on ethanolic extracts of these species. For fungal isolate tested, *C. glabrata*, there was no growth inhibition against any of plant extracts evaluated (data not shown). Considering the species *Baccharis refuse* and *Baccharis dracunculifolia*, only ethanolic extracts were tested. Inhibition results were obtained for all organisms tested, except for *Enterobacter cloacae* in relation to ethanolic extract of *B. Dracunculifolia*. The positive and negative controls achieved the expected results by serving as a comparison parameter to treatments (data not shown) (Table 1 & 2).

Table 1: Microbial growth inhibition results table in relation to aqueous and ethanolic leaf plant extracts 1. *Chaptalia l. nutans* (Arnica); 2. *Passiflora alata* Dryander (sweet passion fruit); 3. *Artemisia absinthium* L. (Losna); 4. *Sida cordifolia* L. (Malva); 5. *Tabebuia avellanedae* Lorentz ex Griseb (Ipê-roxo); 6. *Bauhinia forficata* Link (cow shank); 7. *Bidens* sp.; 8. *Baccharis retusa*; 9. *Baccharis dracunculifolia* (alecrim do campo).

Aqueous Vegetable extract									
Microorganism	Zone of Inhibition								
Staphylococcus aureus	1	2	3	4	5	6	7	8	9
		yes	Yes						
Staphylococcus epidermidis	1	2	3	4	5	6	7	8	9
		yes	yes						
Proteus mirabilis	1	2	3	4	5	6	7	8	9
	yes			yes					
Enterobacter cloacae	1	2	3	4	5	6	7	8	9
				yes			yes		
Escherichia Coli	1	2	3	4	5	6	7	8	9
	yes				yes				
Ethanolic Vegetable extract									
Staphylococcus aureus	1	2	3	4	5	6	7	8	9
	yes		Yes	Yes	Yes			Yes	Yes
Staphylococcus epidermidis	1	2	3	4	5	6	7	8	9
			yes	yes	yes				
Proteus mirabilis	1	2	3	4	5	6	7	8	9
	yes								
Enterobacter cloacae	1	2	3	4	5	6	7	8	9
							yes	yes	
Escherichia Coli	1	2	3	4	5	6	7	8	9
	yes		yes	yes	yes			yes	yes
Klebsiella oxytoca	1	2	3	4	5	6	7	8	9

Table 2: Microbial growth inhibition results table in relation to ethanolic leaf plant extracts 1. *Hyptidendrom canum* (flower); 2. *Hyptidendrom canum* (sheets); 3. *Stryphnodendron* sp; 4. *Xylopi*a sp; 5. *Gochnatia* sp; 6. *Braccharis* sp.

Ethanolic Vegetable extract						
Microorganism	Zone of Inhibition					
Enterobacter cloacae	1	2	3	4	5	6
Staphylococcus aureus	1	2	3	4	5	6
Klebsiella oxytoca	1	2	3	4	5	6
					yes	
Staphylococcus epidermidis	1	2	3	4	5	6
					yes	
Acinetobacter haemolyticus	1	2	3	4	5	6
			yes			
Candida glabrata	1	2	3	4	5	6

Tests were also carried out with other extracts as follows in table below where antimicrobial potential was found in plant ethanolic extracts of 3 and 5 species, for *Klebsiella oxytoca*, *Staphylococcus epidermidis*, *Acinetobacter haemolyticus* organisms (Figure 1).

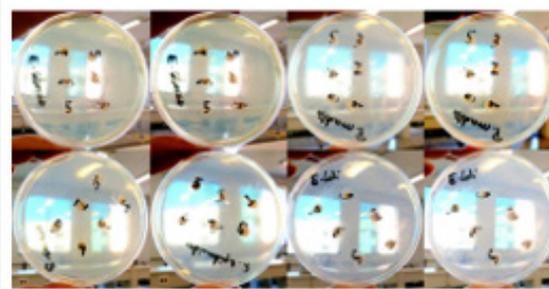


Figure 1: Ilustration of disc-diffusion test results in agar-nutrient showing inhibition halos of plants species extracts against *Staphylococcus aureus* ATCC 25923; *Staphylococcus epidermidis*; *Proteus mirabilis*; *Enterobacter cloacae* e*Escherichia colibacteria*.

Figure 1 illustrates the microbial growth inhibition results by disc technique diffusion, in front of plant extracts tested. Figure 2 shows, in turn, details of growth inhibition halo formation of bacterium *S. epidermidis* around the disc soaked with the solution containing the foliar extracts diluted in ethanolic solvent. In relation to halos' rays observed diameter, those formed with leaf extract (*P. alata* Dryander) were that presented the largest dimension, with emphasis on ethanolic extract (Figure 1&2).



Figure 2: Illustration of Petri dish inoculated with bacterium *Staphylococcus epidermidis* illustrating the inhibition halos formation around some of paper discs (2.3 and 4) soaked with ethanolic solution of leaf extracts.

Discussion

The results obtained point to the presence of different plant components extracted in leaf extracts, especially secondary metabolites of a diverse nature, which would justify the differences in observed responses in relation to the various Microorganisms. In addition, the use of a polar solvent, water, compared to ethanol, more apolar, leads to the extraction of plant components with different degrees of polarities and different biological properties, such as anti-inflammatory, antioxidant, analgesic or Antimicrobial. Active compounds found in several plants demonstrate antiseptic action, such as Thymol and Carvacrol, Eugenol and Isoeugenol and Terpinenol-4. In some cases, the terpene of the essences, which are water soluble, have higher antibacterial power than others (Knobloch et al., 1989). Phenolic compounds, alkaloids, terpenes and sterols are considered the main family of secondary metabolites (Fumagali et al., 2008). Tannins are responsible for the astringency of many fruits and plant products, acting as antioxidant, antiseptic, cicatrizing and Vasoconstrictor (Pereira, Cardoso, 2012). Flavonoids have many biological effects such as antioxidant, anti-inflammatory and antitumoral activity, power to reduce permeability and capillary fragility and inhibition of platelet aggregation [9]. Another characteristic is its antibiotic activity, probably related to the ability to form complexes with soluble and extracellular proteins and with the walls of bacterial cells [8].

In the studies by Truiti [16], the antibacterial property of *C. nutans* raised hypotheses that justify its popular use in the treatment of wounds contaminated by bacterial infections (Gram positive bacteria), being detected that the pure compound that Antibacterial activity was identified as 7-O-B-D-Glucopiranosil-Nutanocumarina. The Oils essences also have a great value in the food and cosmetics industry, aiming its antiseptic and antimicrobial properties (Bakkali et al., 2008). In studies carried out with extracts from the root of *C. Nutans*, the bacterium *S. aureus* was considered susceptible and the bacteria *E. coli* and *P. Aeruginosa* were considered resistant to that extract [16]. Two important groups for the pharmacological area are the terpenoids and the flavonoids, the first are part of a group of secondary metabolites, characteristic of essential oils, being their use based on their antimicrobial properties, very Used by the pharmaceutical industries. The flavonoids belong to numerous classes of chemical substances in a natural way, having several pharmacological activities acting in the biological system, favoring

human health with its antioxidant and antimicrobial actions [12]. In our study it was observed that the leaf extracts of *C. Nutans* were effective in inhibiting the growth of *P. mirabilis*, *E. coli* and *S. aureus* isolates. These results are very promising in view of their action on Gram-negative and Gram-positive bacteria (*S. aureus*), having this last great importance in public health, especially due to the frequent association of this species with nosocomial infections. It is in the execution phase to perform phytochemical tests for the evaluation of secondary metabolites present in the crude extracts of that specimen to subsequently start the execution of the minimum inhibitory concentration (MIC) tests and Minimum bactericidal concentration (CBM), which will allow for more precise conclusions and associations between the secondary metabolites found and the antimicrobial effects observed.

Regarding the genus *Passiflora*, phytochemical assays previously performed with the hydroalcoholic extracts of the aerial parts (leaves, stems, epicarp, pulp and seeds) established that *Passiflora Cincinnata* has the following classes of metabolites Secondary: Condensate tannins, flobabenic, Flavones, Flavononois, Flavonois, Xantonas, Chalcones, Aurones, Flavanones, Leucoanthocyanidine, catechins and alkaloids. These, because they are phytoconstituents derived from the secondary metabolism of vegetables, which almost always act in defense of it against pathogens, can present interesting biological activities. Biological assays using isolated combinations reveal that the flavonoids exhibit a great action on biological systems demonstrating antimicrobial, antiviral, antiulcerogenic, cytotoxic, anticancer, antioxidant, Antihepatotoxic, antihypertensive, hypolipidemic, anti-inflammatory and antiplatelet (Djahanguiri et al. 1969). The activity demonstrated in the in vitro assays performed with the hydroalcoholic extracts of *P. Cincinnata* evidences a probable action of phenolic compounds present in all parts, confirming the antimicrobial activity attributed to this class of Secondary metabolites. The Association of natural products to antibiotics can exert direct activity against many bacterial species, modulating or even increasing the activity of a specific antibiotic, reversing the natural resistance of bacteria. The potentialization of the activity or the reversal of antimicrobial resistance allows the classification of these compounds as modifiers of the antibiotic activity. The use of extracts becomes interesting because they present low possibility of microorganisms acquire resistance to their action, since they are complex mixtures, which hinders the microbial adaptability [17]. In our study, the growth inhibition of *S. aureus* and *S. Epidermidis* was observed in relation to aqueous extracts of *Passiflora alata* Dyander, demonstrating interesting results from the scientific point of view with few reports associated with the effects of those extracts on *S. Epidermidis*. As previously described, studies on the phytochemical composition of these extracts are executing in order to elucidate the secondary compounds present.

A. Absinthium L. (Asteraceae), commonly known as "Absinthe", is a plant traditionally used as an anthelmintic, antiseptic, antispasmodic, for the treatment of cancer, bacillary dysentery and neurodegenerative diseases. Its essential oil has antimicrobial,

antiparasitic, insecticide and herbicidal properties [18]. Studies developed by Sandra [16] showed that leaves of *A. Absinthium* showed poor antimicrobial activity against *Staphylococcus aureus* (7 mm halo), *Staphylococcus Epidermidis* (7 mm halo) and *Enterococcus faecalis* (7 mm Halo), Previous studies [19] revealed a potential activity of *A. Absinthium* versus *S. Aureus* corroborating the results obtained in this study in which the growth inhibition of *S. aureus* was observed both for the ethanolic extract and for the aqueous extract. It is important to emphasize that extracts of *A. Absinthium* obtained in this work demonstrated antimicrobial activity for most of the microbial isolates evaluated, except *P. Mirabilis* and *E. Cloacae*, demonstrating a broad spectrum of performance and, thus, large Pharmacologic potential.

With regard to *T. Avellanadae*, popularly known as Purple ipê, the bioactive compound Lapachol, I described for the first time in 1882 by Paternò [20] was already extracted. In popular use, it has produced evident results in the treatment of diabetes and gastric ulcers, besides being used as analgesic, anti-inflammatory and even as anti-mutagênico. It has also been used in hospitals, in the form of fluid extract and powder or ointment [21]. More recent studies have demonstrated the antimicrobial potential of *T. Alba*, whose ethanolic extract obtained from its flowers demonstrated bactericidal action against *S. Epidermidis*, while the ethanolic extract of the leaf was moderately active with effect Bacteriostatic on *S. Epidermidis* and *S. aureus* [14].

Sandra (2013) observed that the bark of *T. Avellanadae* showed a very significant activity within the established criteria against the microorganism *S. Epidermidis*, with an average of 10 mm inhibitory halo, and, against *S. aureus*, *E. Faecalis* and *B. Cereus* presented a little activity Significant. *T. Avellanadae* bark showed significant antimicrobial activity against *Staphylococcus aureus* (11 mm halo), *B. Cereus* (10 mm halo), *S. Epidermidis* (10 mm halo) and *E. Faecalis* (11 mm halo), although there are reports of studies about its activity against *Helicobacter pylori* (Saad et al, 2009), the analysis of the antimicrobial activity performed shows that the results obtained are quite promising for the continuation of the studies Antimicrobial activity against the microorganisms analyzed. In our study, we observed expressive antimicrobial activity of leaf extracts of *T. Avellanadae* in front of *S. aureus*, *S. Epidermidis* and *E. coli*, demonstrating also a broad spectrum of action whose biological components with antimicrobial action are Studies of new antimicrobial drugs.

The plant species *S. Cordifolia* is native to tropical America but is widespread in several regions of tropical and subtropical climate of the world. It is used in folk medicine for the treatment of Stomatitis, asthmatic bronchitis and nasal congestion. The anti-inflammatory and analgesic effects were tested and confirmed in the aqueous extract of the plant, proving its popular use for that purpose [8]. In the microscopy of the white mauve powder, fragments of several fibers were detected. In the phytochemical prospecting of the white mauve observed the presence of alkaloids, phenols and saponins. The polyphenols content was 0.84% and

the foam index was 100%. In phytochemical analysis of Leaves of *S. Cordifolia* the presence of sympathomimetic amines, ephedrine and pseudoephedrine, a potent vasoconstrictor, called Vasocinone [22] was detected. There are few reports in the literature focused on the evaluation of antimicrobial effects of leaf extracts of *S. Cordifolia*, and the antimicrobial effect of extracts of that species in the present study was observed in comparison with all microbial isolates tested, Involving in this case the two extractors used, demonstrating a great antimicrobial potential of the components of their foliar extract being in execution phytochemical studies in the sense of elucidating them demonstrate their relationship with the results raised.

Regarding the gender AIDS, in Waldilleny Studies (2010) It was observed that the ethanolic extract of *S. Santaremnensis* (SSan-EtOH) and *Copaifera luetzelburgii* (Cl-EtOH), it can be concluded that these species have anti-edematogênica activity evidenced in the models of ear and paw edema. Within this perspective, there is the need for studies in order to determine the possible mechanisms of action of the extracts studied. Regarding the antimicrobial activity of extracts of that genus, there are few studies evaluating its inhibitory effects. In the present study we detected inhibitory action of leaf extracts of *S. Cordifolia* against *S. aureus*, *S. Epidermidis* and *E. coli*, in different degrees, with emphasis on the ethanolic extracts produced. This antimicrobial property represents poorly exploited property and can be aggregated the biological activities already elucidated for extracts of that genus.

The genus *Bidens* is composed of about 240 species distributed in the tropical and subtropical zone of the globe. In Brazil, this species is native to Rio Grande do Sul and occurs in the physiographic regions of Campos de Cima da Serra, Middle Plateau, Central Depression, Serra do Sudeste and Campanha; Inhabiting fields, forest margins, roadside ruderal, wasteland and agricultural areas [23]. *Bidens* spp. It is used in traditional medicine for the treatment of hepatitis, jaundice, fever, throat affections and coughs [12]. In this sense, several studies have confirmed the medicinal potential of *Bidens* spp. with anti-inflammatory actions [7] Analgesics [18], Anti-hiperglicêmica and antioxidant [3]. However, the biological activities of *B. Subalternans* have not been evaluated and the traditional uses of this species still need to be confirmed. Species of the genus *Bidens*, in addition to the traditional effects, have an important antimicrobial action. Deba et al. (2008) showed that the essential oils of leaves and flowers of *Bidens pilosa* have a significant activity against the Gram-positive bacteria *Micrococcus flavus*, *Bacillus subtilis*, *Bacillus cereus* and *Bacillus pumilus*, as well as against the rods Gram-negative *E. coli* and *Pseudomonas ovalis*. In addition, the extracts of leaves [24], Deba et al, 2008). In our study we obtained a significant result of inhibition of the growth of the bacterium *E. Cloacae*, both for the ethanolic extract and for the aqueous extract. Considering the importance of this bacterial species as an opportunistic and also nosocomial pathogen, associated with the absence of reports in the literature on the effects of leaf extracts of *Bidens* against that microbial

species [25-30], this result aroused Interest in deepening studies in relation to the compounds eventually involved in that response. The phytochemical analysis of aqueous and ethanolic extracts obtained from specimens of *Bidens* SP is underway [31-35].

Conclusion

Based on the results obtained, it is possible to conclude the great antimicrobial potential of Cerrado and Mata Atlantica extracts from the region of Pitangui MG, Brazil. Extracts of *B. forficata*, *A. absinthium*, *S. cordifoliae*, *T. avellanadae*, *B. retusa* and *B. dracunculifolia* showed interesting antibacterial and antifungal effects against several microbial species of medical importance. Complementary experiments are necessary to elucidate the constituents of those extracts responsible for the important results observed.

Acknowledgement

None.

Conflicts of Interest

No conflicts of interest.

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