

Review Article

BRIEF REVIEW ON THE MEDICINAL USES AND ANTIMICROBIAL ACTIVITY OF DIFFERENT PARTS OF *SCHINUS TEREBINTHIFOLIUS* RADDI

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ABSTRACT

Populations all over the world make use of medicinal plants to treat health problems. Plants are pertaining to the Anacardiaceae family which largely occur throughout Brazil, such as the *Schinus terebinthifolius* Raddi species, commonly known as *aroeira*, have been adopted in Brazilian folk medicine for the treatment of countless diseases and have also been indicated and studied as an antimicrobial agent. Several parts of the plant such as the leaves, the fruits and barks have their antimicrobial activity attested. The indication for the part of the plant to be used as an antimicrobial for folk medicine is yet to be made clear. Several parts are used and in some cases, for similar ends. There are no conclusive studies that describe the difference in antimicrobial action from each plant part. In this review, we investigated the antimicrobial activity of the *Schinus terebinthifolius* Raddi and then the information available in the literature was organized. The antimicrobial activity described in scientific publications according to the plant parts applied and the type of extract acquired in order to determine such activity.

Keywords: *Schinus terebinthifolius*, Anacardiaceae, Antimicrobial, Plant extracts.

INTRODUCTION

Medicinal plants have been man's most important natural resource of man to treat microbial infections and are an important source in the search for new drugs with therapeutic activities [1]. A wide range of studies has investigated plant extracts as sources of natural products that reduce the risk of toxicity and presents antimicrobial activity [2]. The immoderate and indiscriminate use of antibiotic such as methicillin, oxacillin and penicillin has led to antimicrobial resistance, making room for the promising use of plant extracts for antimicrobial activity [3-5]. The ethnopharmacological studies carried out in Brazil set forth several species with antimicrobial property have been used empirically by the population, chiefly species that belong to the Anacardiaceae family [6]. Most species with several medicinal uses are prepared in different extracts many parts of which are used and have distinguished extraction [7].

The *aroeira* (*Schinus terebinthifolius* Raddi) is a species of Anacardiaceae family native to South America and Central America [8]. Species belonging to the Anacardiaceae family are well known for providing good-quality wood and for being used in dyeing and tanning [9-10]. In Brazil the species is found from Ceará all the way down to Rio Grande do Sul [11]. It is considered a medicinal plant whose use is widespread up and down the country. Virtually all of its aerial parts are commonly used for medicinal purposes, including the fruits, the seeds, the leaves and the stem barks. The *S. terebinthifolius* Raddi's main local names in Brazil are *aroeira-vermelha*, *aroeira-mansa*, *aroeira branca*, *aroeira-da-praia*, *aroeira-do-sertão*, *aroeira-do-paraná*, *araguaraiá*, *corneiba*, *fruto-de-sabiá*, *aroeira,pimenta-rosa* and *cambuís* [11-13]. The plant grows and propagates from seeding or cutting. The tree is 5-10m high, medium-sized and carries a broad crown. The trunk is covered with a thick bark and the leaves are imparipinnate-composed ranging from 3-10 pairs of aromatic leaflets [12]. The leaflets are wider than those of the plants of the same family such as the *S. molle* and the *S. lentiscifolius*. The male and female flowers are very small, arranged in pyramidal inflorescences. The fruits are drupe-like, globose, sweet, aromatic, bright and red [11,14]. (fig. 1). For having an essential oil that produces a spicy flavor, the fruits are quite used in the French cuisine, known as "poivre rose". In Peru, they are used for syrups, vinegar, and drinks, and in Chile, for wine [15,16]. There is a great deal of studies about such species and their antimicrobial effects. However, there is a wide variety of extracts, protocols, and compounds investigated that can be exploited [17].



Fig. 1: The fruits, leaves and flower of the *S. terebinthifolius* Raddi

Medicinal uses and pharmacological properties

In folk medicine, including in some states in northeastern Brazil, it is used to treat the skin, mucous membrane injuries as a remedy for ulcers, gastroduodenal disorders, urinary infection, respiratory problems, cancer, diarrhea and arthritis. It has also been used to treat sexually transmitted diseases, uterine inflammation, as antiseptic, antioxidant and anti-inflammatory [18]. The *S. terebinthifolius* Raddi is among the most used plants for dental use, being among the most sold for such an end by indigenous people in Brazil. The decoction of the leaves is commonly used for skin treatment while the decoction of the bark is used for diarrhea, gout and rheumatism [12, 19]. Furthermore, intravaginal compresses with the aqueous extract of such a plant have been used to treat cervicitis and cervicovaginites [11, 18, 20].

On the other hand [21] has been observed that the cure rate for bacterial vaginosis by the vaginal gel out of an extract of *aroeira* was lower than the one obtained by metronidazole gel, regardless of being observed rare and non-severe side effects in the studied groups. Many of these uses have been proven by scientific research [18-22].

The decoction of the leaves is used in hypertension, depression and irregular heartbeat treatment. The decoction of the bark is, in turn, used in rheumatic pains and backaches [23]. Other extracts of the bark are used as a home medication for the treatment of diseases

from both the urinary and respiratory system. They are applied in postpartum baths as anti-inflammatory and healing, in treating cervicitis, genital discharge, hemoptysis and uterine hemorrhage. The leaves and the fruit are also used in the wash water of wounds and ulcers [24].

In dentistry, the aroeira is among the most commercialized plants for dental use managed by healers in Brazil [25]. Among the thirty-four plant species mentioned in the ethno-pharmacological study for the treatment of diseases of the oral cavity in Brazil, the *S. terebinthifolius* was one of the most cited [26]. Several of the scientific studies brought out of dentistry suggest that the aroeira extracts may be an effective alternative in the treatment of infections of the oral cavity such as stomatitis, tooth decay and periodontitis [13, 19].

In Brazil, the aroeira is described in the first version of the Brazilian Pharmacopoeia [27] and it is included in the National List of Essential Medicines [28,29]. The vaginal suppository or the gel containing alcoholic bark extracts are indicated as an anti-inflammatory, healing and topical antiseptic for gynecological use [28].

Although there are no reports of toxic effects when using aroeira, it is believed that prolonged use of the plant can generate toxic effects. The main adverse effect mentioned hitherto implicate skin allergy [30,31]. Toxicity tests have demonstrated that the resin produced by the plant in contact with the skin causes allergic dermatitis [32].

In addition to the antimicrobial property, aroeira has several other known pharmacological properties (fig. 2).

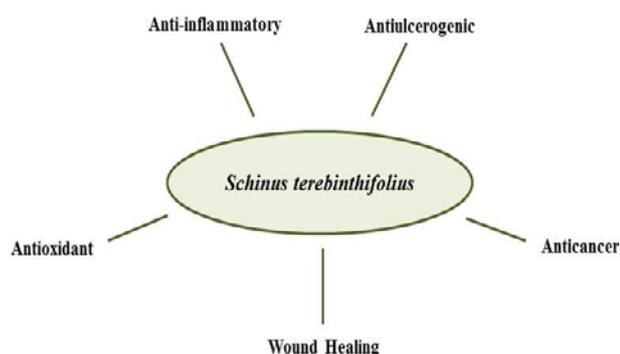


Fig. 2: Some pharmacological properties of the *S. terebinthifolius* extra antimicrobial activity

Anti-inflammatory and wound healing activity

Some researches [33] within the studies about the therapeutic effects of the hydroalcoholic extracts of the *S. terebinthifolius* leaves applied topically to the oral mucosal wound healing process of rats concluded that the extract of the *S. terebinthifolius* reduced the intensity of the inflammatory process. The studies [34] described the wound healing action, respectively, in bladder, stomach and skin surgical wounds of rats. It was concluded that the *S. terebinthifolius* extract acts by accelerating the reparation process of the epithelial tissue stimulating the keratinization and also acting for the reparation of the connective tissue, reducing the intensity of inflammation and accelerating angiogenesis and collagen maturation which are healing characteristics. In scientific studies with the *S. terebinthifolius* stem barks, it was detected the presence of tannins which gives it astringent, disinfectant and anti-inflammatory action [35, 36]. The essential oil of vegetative parts of the aroeira proved nonsteroidal anti-inflammatory activity through the inhibition of phospholipase A2 by competitive inhibition of the specific enzyme [37].

The aroeira wound healing activity was also directly related to triterpenes present in the fruits of the plant [38].

Anticancer activity

The aqueous extract of leaves and the essential oil of aroeira fruits were identified by their anti-cancer activities [22, 39]. It was

determined the cytotoxic activity *in vitro* in isolated cell cultures of human prostate cancer and human breast carcinoma cancer.

Antitumorogenic activity

According [40], the *S. terebinthifolius* species is used in skin and mucosal injury and ulcer treatment. The decoction of the *S. terebinthifolius* barks was tested in rats, orally and intraperitoneally, with the anti-ulcerogenic effect started at a dose of 50 mg/kg i. p. where the animals treated with the extract at high doses did not develop gastric injuries [41]. The pH increase of the gastric fluid and the reduction of the gastric bleeding have also been observed [41, 42].

Antioxidant activity

The plant extract of the *S. terebinthifolius* contains tannins, terpenes, flavonoids, and saponins, among these components, potential antioxidant properties were attributed to flavonoids [18]. The essential oils of the *Schinus* species also presented important antioxidant activity [22, 23, 43].

Antimicrobial activity

Medicinal plants have been man's most important natural resource to treat microbial infections [1]. Some plants of the Anacardiaceae family present antimicrobial and antioxidant properties. The ethanol and aqueous extracts of the *Spondias mangifera* (Anacardiaceae) showed antimicrobial activity against gram-positive bacteria viz. *Bacillus subtilis* and *Staphylococcus aureus* and important antioxidant activity. Saponins, flavonoids and tannins were positive for both extracts. Alkaloids were detected only in methanol extract [44]. As for the antimicrobial activity of the *S. terebinthifolius*, many studies confirm its therapeutic property. Accordingly to [45], in a study of some species of Cuban plants, the species that showed the most effective antimicrobial activity was the *S. terebinthifolius* Raddi acting against the *Staphylococcus aureus*. In another study by [46], alcoholic and aqueous extracts of the *S. terebinthifolius* incorporated into gels presented antimicrobial activity against standard strains of the *S. aureus* ATCC 6538, ATCC 9144. As investigated by [35], the ethanol extract of the *S. terebinthifolius* barks is effective as an antimicrobial agent against gram-positive and gram-negative microorganism.

The antifungal properties of eight plant extracts used in Brazilian traditional medicine were tested by [47] against the fungi *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Sporothrixschenckii* and *Cryptococcus neoformans*. The dichloromethane and ethyl acetate partitions derived from ethanol extract (80%) of the leaves and stems of *S. terebinthifolius*, proved to be the most active of the species examined with MICs in the range 15-125 mg/ml. The same authors also conducted a study with the pathogenic fungus *Paracoccidioides brasiliensis* responsible for paracoccidioidomycosis a lung disease prevalent in countries in South America and often fatal. The extracts have shown activity against the fungus, and two active components were identified as schinol and 4'-ethyl-4-methyl-2,2',6,6'-tetrahydroxi-[1,1'-biphenyl]-4,4'-dicarboxylate, the first being the most active (MIC between 7.5 and 125 g/mL as fungus strain) [48, 49].

Oral biofilms are precursors of most oral infectious diseases and the ability to impair the adhesion of bacteria represents an important strategy in combating bacterial pathogenesis. The examination of the *S. terebinthifolius* extracts indicated excellent effect in this direction, with a high non-adherent potential [13]. The tannins are one of the main constituents of aroeira, being considered chemical markers in the quality control of the species. [18]. The mechanism action of the tannins may be related to the ability of these compounds to inactivate microbial adhesions, the proteins, and enzymes transport as well as complexing with polysaccharides [50]. Monoterpenes as linalyl acetate, menthol, and thymol can affect microorganisms by a disturbance in membrane proteins, inhibition of respiration and changes in the process of ion transport as well as the expansion of the membrane with increased fluidity and permeability [51]. The essential oil presented in the *S. terebinthifolius* fruits has high-concentrated monoterpenes as α -pinene, sabinene, α -salven, β -pinene, α -funebrene, (R)-(+)-limonene, myrcene, and alpha-phellandrene [52]. According to [2] 91.15% of the total oil of the *S.*

terebinthifolius fruit were monoterpenes and showed antibacterial properties against wild strains tested. The essential oil is comprised of the variety of chemical compounds, so antimicrobial activity can be a result of the synergy among many chemical compounds present in oils. A synergistic effect was observed by [48-49], they observed antifungal activity for schinol as well as a synergistic effect between schinol and itraconazole against *P. brasiliensis*.

Gram-positive species are more sensitive to the essential oil of aroeira due to a lower structural complexity of the cell walls [2]. In addition, the aroeira extracts reduced the number of pathogenic microorganisms in the vaginal microbiota showing some 84% cure rate. However, the presence of alkyl phenols in preparations based

on the aroeira materials can cause allergic reactions on the skin and mucous membranes which seem to suggest caution when using [12-20]. Ethnobotanical uses of the *S. terebinthifolius* Raddi are scientifically proven but like most parts of the plant as bark, leaves and fruits are used, the correct way to use appears to be confusing.

As described above, the antimicrobial activity of the aroeira bears scientific evidence nonetheless there is no systematization that allows for correlating the part of the plant used with the type of extract tested and the reported antimicrobial activity. The following table presents accumulated data based on the review of the literature (table 1).

Table 1: Antimicrobial activity of the different parts of *Schinus terebinthifolius* Raddi

Plant part assayed	Microorganism (strain)	Extract or fraction assayed	References
Fruits Leaves	<i>Bacillus</i> sp., <i>Corynebacterium</i> sp., <i>Enterobacter</i> sp., <i>Enterobacter agglomerans</i> (not cited), <i>Escherichia coli</i> (not cited) <i>Klebsiella oxytoca</i> (not cited), <i>Nocardia</i> sp., <i>Pseudomonas</i> sp., <i>Staphylococcus aureus</i> (not cited), <i>Streptococcus</i> group D (not cited).	Essential oil	[2]
	<i>Bacillus cereus</i> (ATCC 11778), <i>Staphylococcus aureus</i> (ATCC 6538)	Alcoholic extract	[14]
	<i>Mycobacterium bovis</i> BCG strain Moreau	Crude extract (methanol)	[53]
	<i>Escherichia coli</i> (WDCM 00013), <i>Klebsiella pneumoniae</i> (ATCC 29665)	Lectin isolated	[3]
	<i>Proteus mirabilis</i> (WDCM 00023), <i>Pseudomonas aeruginosa</i> (WDCM 00025), <i>Salmonella enteritidis</i> (MM 6247), <i>Staphylococcus aureus</i> (WDCM 00032)		
	<i>Candida albicans</i> (ATCC 10231), <i>Streptococcus mutans</i> (UA159)	Crude extract (methanol and petrol ether)	[13]
	<i>Aspergillus niger</i> (not cited), <i>Aspergillus parasiticus</i> (not cited), <i>Candida albicans</i> (not cited), <i>Escherichia coli</i> (not cited), <i>Pseudomonas aeruginosa</i> (not cited), <i>Staphylococcus aureus</i> (not cited)	Essential oil	[23]
	<i>Corynebacterium pseudotuberculosis</i> (T1), <i>Corynebacterium pseudotuberculosis</i> (VD57)	Methanolic extract	[42]
	<i>Candida albicans</i> (collected from the oral cavity of patients with denture stomatitis), <i>Candida albicans</i> (ATCC 18804), <i>Candida krusei</i> (ATCC 20298), <i>Candida tropicalis</i> (ATCC 750), <i>Candida glabrata</i> (ATCC 2001)	Ethyl acetate fraction	[47][47][47]
	<i>Sporothrix schenckii</i> (ATCC 20679)	Ethanol extract and dichloromethane fraction	[47]
	<i>Paracoccidioides brasiliensis</i> (ATCC MYA-826)	Ethanol extract and dichloromethane fraction	[48-49]
	<i>Paracoccidioides brasiliensis</i> (ATCC 32069)	Hexane fraction and Aqueous fraction	[48-49]
	<i>Paracoccidioides brasiliensis</i> (from the fungal collection of the Faculty of Medicine of the University of São Paulo, São Paulo, SP, Brazil), <i>Candida albicans</i> (ATCC 18804)	Hexane fraction, ethyl acetate fraction, and dichloromethane fraction	[48-49]
	<i>Cryptococcus neoformans</i> (ATCC 32608)	Hexane fraction	[48-49]
	<i>Acinetobacter calcoaceticus</i> (NCIB 8250), <i>Aspergillus niger</i> (ACTCC 10535), <i>Aspergillus flavus</i> (ACTCC 10124), <i>Bacillus subtilis</i> (NCIB 3610), <i>Candida albicans</i> (ATCC 10231), <i>Citrobacter freundii</i> (NCIB 11490), <i>Clostridium perfringens</i> (ACTCC 3620), <i>Clostridium sporogenes</i> (NCIB 10696), <i>Escherichia coli</i> (NCIB 8879), <i>Klebsiella pneumoniae</i> (NCIB 4184), <i>Penicillium notatum</i> (ACTCC 9178), <i>Proteus vulgaris</i> (NCIB 4175), <i>Pseudomonas aeruginosa</i> (NCIB 950), <i>Salmonella Typhii</i> (ACTCC 6539), <i>Staphylococcus aureus</i> (NCIB 6751), <i>Yersinia enterocolitica</i> (NCIB 10460)	Crude extract (methanol)	[50]
	<i>Candida albicans</i> (ATCC 10231)	Crude extract (methanol)	[50]
	<i>Escherichia coli</i> (ATCC 25922), <i>Pseudomonas aeruginosa</i> (ATCC 14207)	Essential oil	[54][54]
	<i>Staphylococcus aureus</i> (ATCC 15008)		
	<i>Bacillus subtilis</i> (not cited), <i>Candida albicans</i> (not cited), <i>Cryptococcus neoformans</i> (not cited), <i>Escherichia coli</i> (not cited), <i>Klebsiella pneumoniae</i> (not cited), <i>Pseudomonas aeruginosa</i> (not cited), <i>Salmonella setubal</i> (not cited), <i>Saccharomyces cerevisiae</i> (not cited), <i>Staphylococcus aureus</i> (not cited), <i>Staphylococcus epidermidis</i> (not cited)	Fluid extracts (ethanol 80 %)	[55]
	<i>Staphylococcus aureus</i> (ATCC 25923)	Fluid extracts (ethanol 80 %)	[55]
	<i>Staphylococcus intermedius</i> (isolated from dogs, LMI-S9)	Essential oil	[56]
	<i>Candida albicans</i> (ATCC 289065), <i>Candida tropicalis</i> (ATCC 40147), <i>Candida krusei</i> (ATCC 40042)	Ethanol extract	[57]
	<i>Escherichia coli</i> (ATCC), <i>Staphylococcus aureus</i> (ATCC 6538), <i>Pseudomonas aeruginosa</i> (ATCC 8027)	Essential oil	[58]
<i>Bacillus corineforme</i> (not cited) <i>Enterococcus group D Staphylococcus</i>	Alcoholic tincture (10%)	[1]	
	Hydroalcoholic extract	[5]	
	dry ethanolic extract and	[35]	

	<i>aureus</i> (not cited) <i>Streptococcus viridans</i> (not cited) <i>Streptococcus</i> β . <i>Hemoliticus</i>	hydroalcoholic extract	
	<i>Candida tropicalis</i> (ATCC 750)	Ethyl acetate fraction	[47]
	<i>Candida krusei</i> (ATCC 20298)	Ethanol extract and ethyl acetate fraction	[47]
	<i>Cryptococcus neoformans</i> (ATCC 32608)	Hexane and ethyl acetate fractions	[47]
	<i>Sporothrix schenckii</i> (ATCC 20679)	Ethanol extract ethyl acetate fraction and hexane fraction	[47]
	<i>Paracoccidioides brasiliensis</i> (ATCC MYA-826) <i>Paracoccidioides brasiliensis</i> (ATCC 32069)	Hydroalcoholic extract, ethyl acetate fraction, aqueous fraction and dichloromethane fraction	[48-49][48-49]
	<i>S. aureus</i> (RN4220) resistant strain MsrAS. <i>aureus</i> (SA-1199B) resistant strain NorA	Chloroform fraction, ethyl acetate fraction, hexane fraction and hydro methanolic fraction ethanolic extract	[57]
	<i>Candida tropicalis</i> (ATCC 40042)	Alcoholic tincture	[60]
	<i>Staphylococcus aureus</i> (ATCC 25923)	Hexane fraction	[62]
	<i>Streptococcus mutans</i> (ATCC 25175)	Tincture	[63]
	<i>Enterococcus faecalis</i> (ATCC 29212)	hydroalcoholic extract	[63]
Stem	<i>C. krusei</i>	Aqueous	[64]
Mixture of plant parts (Stem barks and leaves)	<i>C. albicans</i> (ATCC 18804) <i>Aggregatibacter actinomycetemcomitans</i> (ATCC 33384) <i>S. aureus</i> (ATCC 12692) <i>S. mutans</i> (ATCC 70069)	ethanol extract ethanol extract and hexane extract and butanol extract	[65] [65][65]

Among all the species of microorganisms tabulated above fifteen are gram negative and nineteen gram positive indicating a non-selectivity of the aroeira for these types of microorganism indeed. The leaves seem to be the most studied plant parts and/or with higher antimicrobial activity than the stem bark and fruits.

Phytochemicals

Medicinal plants with antimicrobials effect have important therapeutic potential. They are effective to cure various ailments and exhibit fewer side effects than synthetic antimicrobials [67]. Phytochemicals are bioactive substances synthesized by plants that usually occur in complex mixtures that differ among plant organs and stages of development [68]. These bioactive substances, in addition to exercising the functions, own vegetables such as defense and reproduction adaptive functions; they may also have activity in other organisms.

The concentration of active ingredients in the plant depends on the genetic control (capacity inherent to the plant) and the stimulus provided by the environment [69]. Typically these stimuli are characterized as stress situations as excess or deficiency of some production factor to the plant. Once the plant has the power to produce the active ingredients, its concentration of active substances can be altered by climate, soil factors, and exposure to micro-organisms, insects, herbivores, and pollutants [70, 71]. Some authors [72] demonstrated that antibacterial potential of different medicinal plants varied with the species and solvents used for the extraction of phyto constituents. Triterpenes, phenolic lipids, and bioflavonoids are the most frequent substances present in the Anacardiaceae family. The essential oil of the *S. terebinthifolius* leaves collected in different regions has shown distinct chemical compounds [58]. Other researchers [54] illustrated some differences in the chemical content of *S. terebinthifolius* growing in Africa and those growing in another part of the world particularly Brazil.

Table 2: Some phytochemical compounds of the different extracts of the *Schinus terebinthifolius*

Plant part assayed	Phytochemicals	References
Leaves and bark	Alkaloids, triterpenoids; tannins; flavonoids, anthraquinones	[50]
	Schinol (1), and new biphenyl identified as 4'-ethyl-4-methyl-2,2',6,6' tetrahydroxy[1,1'-biphenyl]-4,4'-dicarboxylate (2)	[48-49]
	Saponins, flavonoids, triterpenes, steroids, and tannins.	[47]
	1, 2, 3, 4, 6-pentagalloylglucose and methyl gallate, which are aromatic compound phenol, flavones, flavonoids, xanthenes and leucoanthocyanidins, flavanones and free steroids.	[74]
Fruits	Terpens, phenols, an flavonoids anthraquinones, xanthenes and free steroids (ethanolic extract of the stem bark)Flavones, flavonoids and xanthenes, free steroids, anthraquinones and pentacyclic triterpenes (hexane extract of the stem bark)Phenols, flavones, flavonoids, xanthenes and leucoanthocyanidins, flavanones and free steroids (ethanolic extract of the leaves)	[57]
	Phenolics compounds, flavone apigenin, ellagic acid and syringic acid	[14, 53]
Fruits oil	Monoterpenes (85.81%), presenting as major constituents-3-carene (30.37%), limonene (17.44%),-phellandrene (12.60%),-pinene (12.59%), myrcene (5.82%) ando-cymene (3.46%); sesquiterpenes appeared as minor proportion (5.34%).	[2]
Leaves essential oil	Monoterpenes, sesquiterpenes, oxygenated monoterpenes, and oxygenated sesquiterpenes coumaric acid and syringic acid	[23]
	Pinene, 3-carene, limonene, and-phellandrene, p-cymene and terpinolene monoterpene alcohols and ketones, sesquiterpene hydrocarbons, alcohols and ketones, and triterpene alcohols and ketones	[75,76]

The authors observed the presence of chemical constituents such as 3-Cyclohexen-1-ol and trans- β -ocimene which were not observed in the oils of such a plant collected in Brazil. In another review research about *S. terebinthifolius* several phytochemical compounds were described [18]. In the bark of the *S. terebinthifolius* was detected the presence of tannins and the resin turpentine consists of terpene hydrocarbons, gallic acid and essential oils (camphene, limoleno, phellandrene, etc.) [73]. The aroeira carries tannin, bioflavonoids and triterpene acids in the barks and it is rich in oil with sesquiterpenes and monoterpenes in their fruit and leaves [12]. Just below in the table 2 some of the main phytochemical compounds in the aroeira are found available.

CONCLUSION

In conclusion, the aim of this review was to exploit the potential use of the *Schinus terebinthifolius* Raddi (aroeira) as medicinal and antimicrobial plant, and as source of phytochemical compounds as well as making available information about their traditional and scientific use.

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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare

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