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Vol. 11(3), pp. 43-57, 17 January, 2017
DOI: 10.5897/JMPR2016.6313
Article Number: 4F30B6962396
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Review

The genus *Eupatorium* L. (Asteraceae): A review of their antimicrobial activity

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Received 30 November, 2016; Accepted 6 January, 2017

In recent years, the number of infectious diseases linked to the occurrence of bacterial and fungal resistance has increased, leading to extensive search for new drugs to treat these infections. Species of the Asteraceae family and the genus *Eupatorium*, have high biological potential and are used in folk medicine to treat various diseases. This review article presents the main phytochemical and biological characteristics of the Asteraceae family and the genus *Eupatorium s.l.*, whose antimicrobial activity is promising, especially antibacterial and antifungal activity. The current review was achieved using an organized search of the scientific data published on antimicrobial activity and phytochemical of the species of the genus *Eupatorium* using various databases, including PubMed, ScienceDirect, Scopus, Scielo, SciFinder and Google Scholar. The species of *Eupatorium* are rich in terpenes, phytosterols and sesquiterpene lactones, the latter being chemotaxonomic markers of the group, with broad anticancer, antiplasmodial and antimicrobial activity, making them promising for the development of new drugs. Various species of *Eupatorium* seems to hold great potential for in-depth investigation for antimicrobial activities. Many species have broad folk use, with scientific confirmation of its antimicrobial properties making these plants potential sources of safer and more effective treatments.

Key words: Compositae, *Eupatorium s.l.*, antimicrobial potential, ethnopharmacology, ethnobotany.

INTRODUCTION

The family Asteraceae, belonging to the class of eudicotyledons, is one of the largest families of angiosperm plants. Its species are known for their therapeutic, cosmetic and aromatic properties and have great importance both from the environmental and economic standpoints. They have been widely studied for their anti-inflammatory, antimicrobial, anticancer, antisyphilitic, antigonorrheal and insecticidal properties (Carrillo-Hormaza et al., 2015). Biological studies of its extracts, oils and constituents for production of phytochemicals have attracting growing interdisciplinary interest from the scientific community (Garcia-Sanchez et

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al., 2011; Yang et al., 2004; Souza et al., 2007).

Among several genera of Asteraceae studied, *Eupatorium* L. *sensu lato* stands out. Numerous studies of its species have been performed and the majority showed the presence of terpenes, phytosterols and sesquiterpene lactones (Liu et al., 2015), the latter identified as promising sources for development of new drugs due to the antimicrobial, anticancer and antiplasmodial action (Albuquerque et al., 2010; Lang et al., 2002).

In a broader context, ethnobotanical and ethnopharmacological studies are very useful for investigation of new bioactive plant sources (Silambarasan and Ayyanar, 2015). Many traditional indigenous communities have longstanding contact with plant species having medicinal properties, with information being transmitted from generation to generation. This information is useful to the scientific community in studies of natural resources, as occurred in studies of *Eupatorium s.l.* (Silva et al., 2012; Paredes-Flores et al., 2007).

Chemical compounds with biological activity isolated from plants and used by the population through extracts are promising sources to prospect for new antimicrobial drugs. These substances may also act synergistically with other drugs, potentially improving the antibiotic action (Al-Fatimi et al., 2007). However, it is necessary to establish whether the traditional use of plants for the treatment of infectious diseases is supported by pharmacological action or merely based on folklore (Pessini et al., 2003).

One obstacle to the treatment of bacterial infections is the phenomena of resistance to antibacterial drugs, caused by the selection of resistant bacteria from exposure to chemotherapeutic agents, causing transfer of resistant gene fragments between bacterial strains and clonal spread of resistant strains among hospitalized patients (Spellberg et al., 2013). The exposure of antimicrobial compound can increase bacterial resistance also by selecting for mutation in genes that help microbes detoxify antibiotics. As for antibiotics, inadequate knowledge of the pharmacokinetic and pharmacodynamic properties, in addition to factors inherent to the patient, such as immune status and non-adherence to treatment, affect the efficiency of chemotherapy against pathogenic bacteria (Muller et al., 2015; Udy et al., 2013).

The use of natural extracts in the treatment of infectious diseases is promising, but studies on antimicrobial activity of different plant extracts should be expanded, including analysis of essential oils and their constituents in order to maximize the effect studied and to discover which chemical compound plays the main role in antimicrobial action (Danielli et al., 2013). In relation to antifungal drugs, natural products are widelyused and the results are promising (Cragg and Newman, 2013). Due to the resistance of many pathogenic microorganisms, the search for new antimicrobial agents from plants is intense (Alviano and Alviano, 2009). The relevance of these studies is based on the fact that infections of bacterial etiology, and in particular fungal infections, are aggravated in immunosuppressed individuals, due to therapy against cancer and AIDS and suppression of the immune response to prevent rejection of transplanted organs (Miceli et al., 2011).

For a comprehensive literature overview, the current review was achieved by using an organized search of the scientific data published on antimicrobial activity and phytochemical, focusing on the antibacterial and antifungal activities of the species of the genus *Eupatorium*. The search was conducted using the keyword search term "Eupatorium antimicrobial activity". The searches were carried out using various databases, including PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Science Direct (http://www.sciencedirect.com/), Scopus (http://www.scopus.com/), Scielo (http://www.scielo.org/), SciFinder (https://scifinder.cas.org/) and Google Scholar (http://www.scholar.google.com/).

Based on these considerations, in the present paper, the antibacterial and antifungal potential of species of the genus *Eupatorium s.l.*, were analyzed highlighting also biological properties of the Asteraceae family.

ASTERACEAE FAMILY

Asteraceae (Compositae) includes about 1600 genera and 25000 species (Petacci et al., 2012) belonging to 17 tribes and three subfamilies. It is considered by some authors as the largest family of eudicotyledonous angiosperm plants (Nakajima and Semir, 2001; Hattori and Nakajima, 2008). The Asterales order is formed by the Calyceraceae, Menyanthaceae, Goodeniaceae, Campanulaceae and Asteraceae (Judd et al., 2009). This family has been studied intensely over the past 25 years regarding morphology, anatomy, ontogeny and ecology as well as phytochemical, cytogenetic and macromolecular aspects (Hind and Beentje, 1996).

Asteraceae are for the most part herbs, subshrubs or shrubs, trees or vines (Roque and Bautista, 2008). Its representatives of greatest importance are *Baccharis trimera* (Less) DC. (gorse), *Matricaria chamomilla* L. (camomile), *Cynara scolimus* L. (artichoke), *Vernonia condensata* Baker (“boldo-da-bahia”) and *Arnica montana* L. (true arnica). These species are widely used and marketed as herbal products, suggesting species of the family Asteraceae have significant potential as a source of bioactive compounds (Maia et al., 2010; Mello et al., 2008). They are also sources of edible oils (*Helianthus annuus* L., sunflower), pesticides and latex, as well as being eaten as leafy vegetables (e.g.,*Lactuca sativa* L., lettuce) and used as ornamental plants (Wu et al., 2006). Phytochemical studies of *Eupatorium* have identified the presence of sesquiterpene lactones, flavonoids, triterpenes, benzofuran compounds, pyrrolizidine alkaloids, chromene and steroids (Liu et al., 2015; Zhang et al., 2008; Albuquerque et al., 2004; Lang et al., 2001).

GENUS *EUPATORIUM*

The genus *Eupatorium s.l.* (tribe Eupatorieae, subtribe Eupatoriinae) was one of the most representative of the family Asteraceae, containing around 1200 species, however a detailed survey conducted by King and Robinson (1987) found that about 1010 species would be reclassified to different new genera. When interpreted in its traditional concept, this genus form a polyphyletic group. *Eupatorium s.l.* have been a highly artificial concept, tending to include all members of the tribe with a pappus of numerous capillary bristles, five ribs on the achene, and another appendage as long as or longer that wide (King and Robinson, 1987). This genus has been restructured and was fragmented into 80 genera. Currently, the genus *Eupatorium sensu stricto* containing about 45 species, distributed primarily by arctic-tertiary region (King and Robinson,1987; Bremer, 1994).

The species of this genus are used in folk medicine in different parts of the world (Albuquerque et al., 2010), like Mexico, where over 1000 medicinal plants have been used for over 400 years in folk medicine, many are in the genus *Eupatorium* (Garcia-Sanchez et al., 2011).

Ethnopharmacological studies of medicinal plants are important for the development of new drugs (Silambarasan and Ayyanar, 2015). The main goal is to find new bioactive substances for disease control with minimal side effects (Rodríguez and Carlini, 2003). In this context, studies have been conducted of biological activities such as toxic activity of essential oils (Albuquerque et al., 2004) and plant extracts (Rozo et al., 2008) against the larvae of *Aedes aegypti* and antiplasmodial activity against *Plasmodium falciparum*, a protozoan that causes malaria (Lang et al., 2002).

In particular, the literature contains numerous studies of the genus *Eupatorium* with use of plant extracts and essential oils against strains of bacteria and fungi that infect animals and plants, for development of new drugs for veterinary and human use and new herbicides. Studies were found from 1948 to 2016 with *Eupatorium* species. *E. adenophorum*, *E. odoratum* and *E. triplinerve* are the species with the most work reporting their antimicrobial potential (Table 1).

Antimicrobial activity of *Eupatorium s.l.*

Most studies of the antimicrobial activity involved species of Asia and Europe, with fewer studies of South American plants. This is counterintuitive, given that although countries like China, Malaysia, India, Zaire, Indonesia and Madagascar can be considered megadiverse (Sandes and Di Blasi, 2000), species of *Eupatorium* are particularly diverse in tropical and subtropical areas of the Americas (Zhang et al., 2008).

For antibacterial action, tests have been conducted on activity against a wide range of microorganisms, both Gram-positive and negative bacteria. For investigations of plant extracts, the most commonly used solvents are methanol, ethanol, ethyl acetate and n-hexane, used in studies the aerial parts, branches, twigs and leaves. Nevertheless, there are reports of the use of roots to obtain the essential oil of *E. adenophorum* Spreng. (Ahluwalia et al., 2014) and the aqueous extract of *E. purpureum* L. (Carlson et al., 1948; Pates Madsen, 1955), in both cases having antibacterial action.

Referring to antifungal bioassays, the studies focused on *Eupatorium* generally also have involved tests against bacteria. The tested fungi include filamentous fungi, dermatophytes and yeasts. Tests with dermatophytes, filamentous fungi that cause dermatophytosis are promising for the genus, with reports of activity for *Eupatorium arnottianum* Griseb. (Muschietti et al., 2005), *Eupatorium aschenbornianum* S. Schauer (García et al., 2003 Rios et al., 2003), *Eupatorium buniifolium* Hook. and Arn. (Muschietti et al., 2005), *Eupatorium japonicum* Thunb. (Inouye et al., 2006), *Eupatorium laciniatum* Kitam. (Inouye et al., 2006) and *Eupatorium odoratum* L. (Taylor et al., 1996). In all these studies, extracts of plant material were used. There are few reports in the literature on the use of essential oils for susceptibility testing. As for the fungi tested, of the three genera of dermatophytes (*Trichophyton*, *Microsporum* and *Epidermophyton*), only two showed sensitivity and growth inhibition (*Trichophyton* and *Microsporum*), highlighting species *T. rubrum* and *T. mentagrophytes* in the genus *Trichophyton*.

Eupatorium adenophorum Spreng.

The species *E. adenophorum* [syn. *Ageratina adenophora* (Spreng.) R.M.King & H.Rob.], native to Mexico, is now widely distributed in the world (King and Robinson, 1970) and is used in folk medicine as an antimicrobial, antiseptic, analgesic and antipyretic (Bhattarai and Shrestha, 2009). It has also been shown to have molluscicide potential against *Oncomelania hupensis*, the intermediate host of *Schistosoma japonicum* (Zou et al., 2009). The essential oil extracted from the flowers, and roots was tested and showed inhibitory activity for the strains of *Xanthomonas oryzae*, *Erwinia chrysanthemi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*. Assays were performed using the disk diffusion and agar *dilution methods (Ahluwalia et al., 2014). The essential oil extracted from the aerial parts was also investigated against strains of Arthrobacter protophormiae, Escherichia coli, Micrococcus luteus, Rhodococcus rhodochrous* and *Staphylococcus aureus*. Assays were performed by broth dilution and the results showed growth inhibition for all strains tested (Kurade et

Table 1. Species of the genus *Eupatorium s.l.* with antimicrobial activity.

Scientific name	Part used	Biological preparation	Bioactive compounds	Biological activity	Reference
Eupatorium adenophorum Spreng.	I, L, St, R	EO, OsE	Terpenoids	Antibacterial activity: Gram-positive and Gram-negative bacteria Antifungal activity: filamentous fungi	Bhattarai and Shrestha, 2009; Kurade et al., 2010; Kundu et al., 2013; Ahluwalia et al., 2014; Chauhan et al., 2015
Eupatorium altissimum L.	AP	DE	-	Antibacterial activity: Gram-positive and Gram-negative bacteria	Carlson et al., 1948; Cantrell et al., 1998
Eupatorium arnottianum Griseb.	AP	ME	-	Antifungal activity: filamentous fungi	MuschietTI et al., 2005
Eupatorium aromaticum L.	L	AqE	-	Antibacterial activity: Gram-positive bacteria	Pates and Madsen, 1955
Eupatorium aschenbornianum S. Schauer.	AP	HE, ME	Benzofurans compounds and phytosterols	Antifungal activity: yeast and filamentous fungi	García et al., 2003, Rios et al., 2003
Eupatorium ayapana Vent.	L	PE	Steroids, coumarins, tannins and saponins	Antibacterial activity: Gram-positive and Gram-negative bacteria	Gupta et al., 2002
Eupatorium ballotifolium Kunth	AP	EO	Terpenoids	Antifungal activity: filamentous fungi	Sobrinho et al., 2016
Eupatorium buniifolium Hook. ex Hook. & Arn.	AP	ME	-	Antifungal activity: filamentous fungi	MuschietTI et al., 2005
Eupatorium cannabinum L.	AP	OsE	-	Antibacterial activity: Gram-positive and Gram-negative bacteria	Freerksen and Bönicke, 1951; Senatore et al., 2001
Eupatorium capillifolium (Lam.) Small.	AP	EO	Terpenoids	Antifungal activity: filamentous fungi	Senatore et al., 2001; Tabanca et al., 2010
Eupatorium fortunei Turcz.	AP	AqE	-	Antibacterial activity: Gram-negative bacteria	Li et al., 2010
Eupatorium glutinosum Lam.	AP	HE, ME	Terpenoids	Antibacterial activity: Gram-positive and Gram-negative bacteria	Lopez et al., 2001; El-Seedi et al., 2002
Eupatorium havanense Kunth	AP	DE	-	Antibacterial activity: Gram-positive bacteria	Cantrell et al., 1998
Eupatorium intermedium	F	EO	Terpenoids	Antibacterial activity: Gram-positive and Gram-negative bactéria	Czaikoski et al.. 2015
Euparorium inulaefolium Kunth.	L	AqE, EE	-	Antibacterial activity: Gram-positive and Gram-negative bacteria	Pérez and Anesini, 1994; Sanabria-Galindo et al., 1998; Álvarez et al., 2005
Eupatorium japonicum Thunb.	L, F	EA	Terpenoids	Antifungal activity: filamentous fungi	Inouye et al., 2006
Eupatorium laciniatum Kitam.	L, F	EA	Terpenoids and coumarin	Antifungal activity: filamentous fungi	Inouye et al., 2006

Table 1. Cont'd

Eupatorium laevigatum Lam.	L	HE, EE, ME	Alkaloids, steroids, phenols, tannins and flavonoids	Antibacterial activity: Gram-positive and Gram-negative bacteria	Schmidt et al., 2009; Fabri et al., 2011
Eupatorium lindleyanum DC	AP	EE, ME, EA	-	Antibacterial activity: Gram-positive and Gram-negative bacteria	Ji et al., 2008
Eupatorium maculatum L.	AP, S	EE	-	Antibacterial activity: Gram-positive bacteria	Bishop and Macdonald, 1951; Borchardt et al., 2008
Eupatorium odoratum L.	L, AP, F	EE, ME	Polyphenols	Antibacterial activity: Gram-positive and Gram-negative bacteria Antifungal activity: filamentous fungi	Cáceres et al., 1994; Taylor et al., 1996; Suksamrarn et al., 2004; Chomnawang et al., 2005; Molina-Salinas et al., 2007; Gautam et al., 2007; Chomnawang et al., 2009; Sharma et al., 2013; Patil and Kamble, 2015
Eupatorium patens D. Don ex Hook. & Arn.	L	EO	Terpenoids	Antibacterial activity: Gram-positive bacteria	Bailac et al., 2000
Eupatorium perfoliatum L.	L, F	EE, PE	-	Antibacterial activity: Gram-positive and Gram-negative bacteria Antifungal activity: yeast	Carlson et al., 1948; Bishop and Macdonald, 1951; Madsen and Pates, 1952; Liegey, 1953; Frisby et al., 1953; Borchardt et al., 2008
Eupatorium purpureum L.	R, St, L	AqE, EE	-	Antibacterial activity: Gram-positive and Gram-negative bacteria	Carlson et al., 1948; Pates and Madsen, 1955
Eupatorium rugosum Houtt.	L, S	AqE	-	Antibacterial activity: Gram-positive and Gram-negative bacteria	Frisby et al., 1954
Eupatorium salvia Colla	AP	RE	Resinous exudate	Antibacterial activity: Gram-positive bacteria	Urzua et al., 1998
Eupatorium serratum Spreng.	L	ME, EA	Tannins, flavonoids, steroids and alkaloids	Antibacterial activity: Gram-positive bacteria	Desoti et al., 2011
Eupatorium tashiroi Hayata.	AP	GE	-	Antibacterial activity: Gram-positive bacteria	Chen et al., 1989
Eupatorium triplinerve Vahl	St, L, AP	EO, CE	Terpenoids	Antibacterial activity: Gram-positive and Gram-negative bacteria Antifungal activity: filamentous fungi	Rahman and Junaid, 2008; Begum et al., 2010; Unnikrishnan et al., 2014; Sugumar et al., 2015
Eupatorium urticaefolium Reichard	AP	AE	-	Antibacterial activity: Gram-positive and Gram-negative bacteria	Carlson et al., 1948

I- inflorescence; L- leaves; R- root; AP- aerial part; F- flower; St- stems; S- seed; ME- methanol extract; CE- chloroform extract; HE- hexane extract; EE- ethanol extract; PE- petroleum ether extract; DE- dichloromethane extract; EA- ethyl acetate extract; AqE- aqueous extract; AE- acid extract; GE- gross extract; OsE- organic solvents extract; RE- resinous exudate; EO- essential oil.

al., 2010).

Extracts from fresh leaves using organic solvents were tested against 15 bacterial strains by the agar diffusion method. The organic extracts showed antibacterial activity against *Salmonella paratyphi*, *Salmonella* spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Bacillus cereus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Enterobacter aerogenes*, *Proteus mirabilis* and *Proteus* spp. while the aqueous extract showed activity against *P. aeruginosa*, *E. coli*, *S. aureus*, *Staphylococcus* spp., *Citrobacter freundii*, *Proteus* spp., *B. subtilis*, *E. aerogenes*, *Salmonella* spp., *S. paratyphi* and *B. thuringiensis* (Bhattarai and Shrestha, 2009).

Cadinene derivatives extracted from leaves of *E. adenophorum* showed antifungal activity against four plant pathogenic fungi strains: *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium oxysporum* and *Macrophomina phaseolina*. Cadinene sesquiterpenes were isolated by column chromatography and preparative thin layer chromatography. Less polar cadinene sesquiterpenes were active against *S. rolfsii* and polar cadinene derivatives with mono or dihydroxy groups were more inhibitory towards *R. solani* (Kundu et al., 2013).

Ahluwalia et al. (2014) analyzed the antifungal action of the essential oil of *E. adenophorum* against five pathogenic fungi: *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Pythium debaryanum* and *Fusarium oxysporum*. They found the strongest inhibitory activity against *S. rolfsii*. Regarding toxicity, they evaluated the phytotoxic action of the essential oil, which inhibited the growth and seed germination of *Phalaris minor* Retz. and *Triticum aestivum* L., used for phytotoxicity assays in a dose-dependent concentration.

In another study, the essential oil from aerial parts exhibited moderated antifungal activity against *Fusarium oxysporum* by disk diffusion method (Chauhan et al., 2015). Twenty-two components were identified in the composition with an abundance of monoterpenes p-cymene (14.56%) followed by phellandrene (12.25%), camphene (10.42%), bornyl acetate (9.76%) and δ-2-carene (5.39%).

Eupatorium altissimum **L.**

In a study on the species collected in the state of Louisiana in the United States, a dichloromethane extract was tested against *Mycobacterium tuberculosis* bacterium, causative agent of most cases of tuberculosis (Cantrell et al., 1998). In a bioprospecting study of 550 plants collected in the states of Ohio and Oregon in the United States, action was reported on the extract of aerial parts of *E. altissimum* against strains of *S. aureus* and *E. coli* (Carlson et al., 1948).

Eupatorium arnottianum **Griseb.**

E. arnottianum [syn. *Chromolaena arnottiana* (Griseb.) R.M. King & H. Rob.] is an herb native to Latin America, common in the North-East and Centre of Argentina and South of Bolivia, traditionally used to treat stomach ailments (Clavin et al., 2007), against asthma, bronchitis, colds and topically in plasters for bone fractures and dislocations by healers from the bolivian altiplano (Giraoult, 1987), in addition to having anti-inflammatory and analgesic properties (Muschietti et al., 2005).

A methanol extract of the aerial parts showed antifungal activity against the dermatophytes *Microsporum gypseum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* according to broth microdilution tests (Muschietti et al., 2005).

Eupatorium aromaticum **L.**

E. aromaticum [syn. *Ageratina aromatica* (L.) Spach.] is a perennial herb, medium-sized, native to America, common in the southeastern United States, known as “Small-leaved white snakeroot” or “Lesser snakeroot” (Blackwell and Mcmillan, 2013). The ethyl extract of the leaves of *E. aromaticum* showed antibacterial activity against Gram-positive bacteria (Pates and Madsen, 1955).

Eupatorium aschenbornianum **S. Schauer.**

E. aschenbornianum [syn. *Ageratina pichinchensis* (Kunth) R.M. King & H. Rob.] is a plant traditionally used in Mexico to treat skin lesions, mouth ulcers and tumors (Rios et al., 2003). Studies of hexane and methanol extracts obtained from the aerial parts point to the antifungal potential of *E. aschenbornianum* against the dermatophytes, *T. mentagrophytes*, *T. rubrum*, the yeast *C. albicans* and the filamentous fungus *Aspergillus niger* (Rios et al., 2003; García et al., 2003).

Yeasts belonging to the genus Candida are involved in the etiology of candidiasis (Netea et al., 2015). Studies on the species E. aschenbornianum showed activity against C. albicans, using hexane and methanol extracts of the aerial parts (Figure 1). Benzofurans compounds (5-acetyl-3β-angeloyloxy-2β-(1-hydroxyisopropyl)-2,3-dihydrobenzofurane and 5-acetyl-3β-angeloyloxy-2β-(1-hydroxyisopropyl)-6-methoxy-2,3-dihy-drobenzofurane) and phytosterols were isolated (Rios et al., 2003; García et al., 2003).

Eupatorium ayapana **Vent.**

The leaves of *E. ayapana* [syn. *Ayapana triplinervis* (Vahl) R.M. King & H. Rob.] are used in folk medicine as a heart stimulant, laxative and anticoagulant (Gupta et al., 2002). In rainforests of South America, its leaves are used in decoctions, infusions, tea and baths as tonic, stimulant, sedative, febrifuge and anti-inflammatory, especially in Brazilian folk medicine (Melo et al., 2013). The first preliminary studies of this species were performed by Oswaldo Gonçalves de Lima, then director

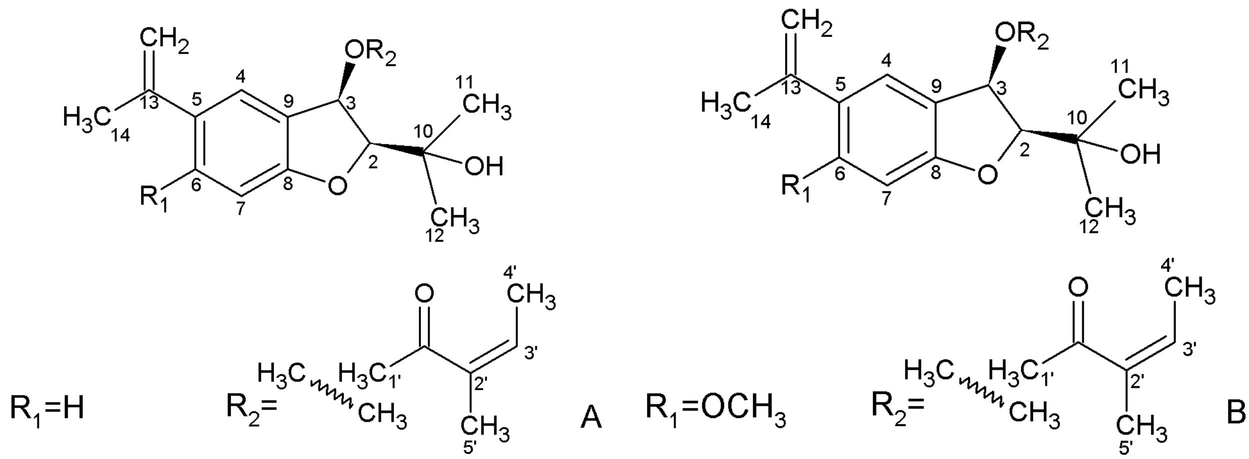

Figure 1. Structures of two benzofurans compounds from aerial parts of *Eupatorium aschenbornianum*. **A.** 5-acetyl-3β-angeloyloxy-2β-(1-hydroxyisopropyl)-2,3-dihydrobenzofurane. **B.** 5-acetyl-3β-angeloyloxy-2β-(1-hydroxyisopropyl)-6-methoxy-2,3-dihy-drobenzofurane.

of the Antibiotics Institute of the University at Recife, Brazil (Lima, 1963).

The phytochemical study of petroleum ether extract showed presence of steroids, coumarins, tannins and saponins. For the species *E. ayapana*, the petroleum ether extract inhibited growth of bacteria *B. subtilis*, *Staphylococcus epidermidis*, *S. aureus*, *M. luteus*, *E. coli*, *P. aeruginosa*, *Salmonella typhi*, *Shigella* sp., *Vibrio cholerae* and *Vibrio parahaemolyticus*. The extract also inhibited the growth of *A. niger*, *A. flavus*, *Alternaria solani* and *Fusarium solani*, pathogenic fungi causing black mold in fruits and vegetables, deterioration by mycotoxins in grains, alternaria spot in solanaceous plants and root and stem rot, respectively (Gupta et al., 2002).

Eupatorium ballotifolium Kunth

Eupatorium ballotifolium Kunth (syn. *Lourteigia ballotaefolia* (Kunth) R.M. King & H. Rob.) popularly known in Brazil by many names, such as “maria-preta”, “maria-preta-verdadeira” and “picão-roxo” is a perennial herbaceous species whose vertical growth ranges from 40 to 80 cm (Cardoso et al., 2013).

The essential oil extracted from the aerial parts, was tested by broth microdilution method and showed antifungal activity against strains fungal dermatophytes, *Trichophyton rubrum*. The chromatographic analysis showed 25 components, accounting for 93.84%, among them mono- and sesquiterpenes, with prevalence of sesquiterpenes (Sobrinho et al., 2016).

The modulatory activity assay was performed to determine the combined effect of the essential oil with the ketoconazole, standard antifungal drug, against strains of *T. rubrum* by the checkerboard technique. The combination of ketoconazole with essential oil reduced the MICs for both strains of *T. rubrum* indicating a synergistic effect (Sobrinho et al., 2016).

Eupatorium buniifolium Hook. ex Hook. & Arn.

E. buniifolium [syn. *Acanthostyles buniifolius* (Hook. ex Hook. & Arn.) R.M. King & H. Rob.] is a shrubby species widely used in folk medicine, such as to treat rheumatic pains and liver problems with tea obtained by decoction. Preparations from this plant are also used as disinfectants (Ríos et al., 1993).

The methanol extract obtained from the aerial parts was used to investigate antifungal activity against strains of the dermatophytes, *M. gypseum* (MIC = 250 µg/mL), *T. rubrum* (MIC = 100 µg/mL) and *T. mentagrophytes* (MIC = 250 µg/mL). The assays were performed by the broth microdilution method (Muschietti et al., 2005).

Eupatorium cannabinum L.

E. cannabinum is a perennial herbaceous species distributed in the northern hemisphere, especially in Europe and North America (Senatore et al., 2001). A study of the essential oil of *E. cannabinum* subsp. *cannabinum* L. obtained from the aerial parts identified high percentage of terpenoids, such as germacrene, monocyclic sesquiterpene, found in large amounts. The oil showed antibacterial activity against strains of Gram-positive bacteria (Senatore et al., 2001).

In another investigation, the extract of the aerial parts showed antimicrobial activity against strains of Gram-positive and negative bacteria, the main ones tested being *S. typhi*, *Micrococcus aureus*, *B. subtilis* and

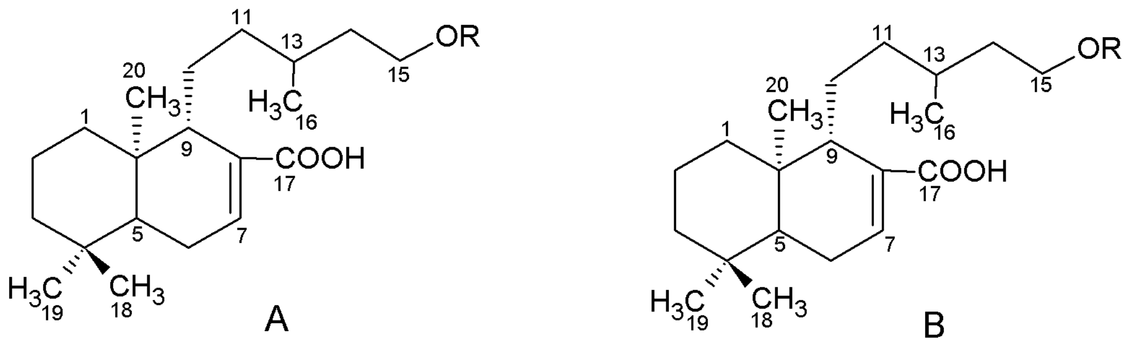

Figure 2. Structures of two isolated compounds from leaves of *E. glutinosum*. A. 15-hydroxy-7-labden-17-oic acid: R = H; B. 15-acetoxy-7-labden-17-oic acid: R = Ac.

Proteus spp. (Freerksen and Bönicke, 1951).

Eupatorium capillifolium (Lam.) Small. ex Porter & Britton

E. capillifolium is a perennial herbaceous species found in North America. It is used as food for livestock in the southeastern United States when other fodder is lacking, but it is generally considered a weed (Sellers et al., 2009).

Studies of the effect of plant extracts against pathogenic fungi are important to find new agents against fungi causing plant diseases in agricultural crops. Tabanca et al. (2010) investigated, by contact bioautography, the antifungal activity of the essential oil of *E. capillifolium*, which inhibited growth of the fungus *Colletotrichum acutatum*, the cause of strawberry anthracnose. The main constituents were thymol methyl ether (36.3%), 2,5-dimethoxy-p-cymene (20.8%) and myrcene (15.7%).

Eupatorium fortunei Turcz.

E. fortunei is a traditional Chinese medicinal herb, common in the northeastern of China, used in folk medicine to treat many diseases (Liu et al., 1992). In a study of bacteria that produce volatile sulfur compounds (VSCs) that cause halitosis, Li et al. (2010) investigated extracts of 40 plants found in China, of which 14 inhibited bacterial growth and production of VSCs. The main compounds found were hydrogen sulfide, dimethyl sulfide and methylmercaptan. Among the 14 plants that were active in the screening was *E. fortunei*, which was used to obtain the aqueous extract.

Eupatorium glutinosum Lam.

This species *E. glutinosum* [syn. *Aristeguietia glutinosa* (Lam.) R.M. King & H. Rob.] is widely used in folk medicine, with preparations obtained by decoction used as antimicrobial, antirheumatic and antidiarrheal agents and to heal stomach ulcers (El-Seedi et al., 2002).

The methanol extract of leaves of *E. glutinosum* presented antibacterial activity against strains of *Mycobacterium phlei*, *B. subtilis* and *S. aureus*, by the disk diffusion method (Lopez et al., 2001). The antibacterial activity of the hexane extract of leaves and stems and of two isolated compounds (15-hydroxy-7-labden-17-oic acid and 15-acetoxy-7-labden-17-oic acid) were investigated by diffusion in agar (Figure 2). The tests showed activity against *S. aureus*, *B. cereus*, *E. coli* and *P. aeruginosa* (El-Seedi et al., 2002).

Eupatorium havanense Kunth

E. havanense [syn. *Ageratina havanensis* (Kunth) R.M. King & H. Rob.] is a native shrub found in North America (Zamudio and Villanueva, 2011) and Central America (Córdoba et al., 1995) known as “Havana snakeroot”. Previous study identified the flavone sakuranetin (Wollenweber and Dietz, 1981) and flavonols, flavones and flavonoid glucosides (Yu et al., 1987).

In an antimicrobial prospecting study of plants of the Americas, the dichloromethane extract of leaves of *E. havanense*, collected in the state of Tamaulipas, Mexico, inhibited the growth of the pathogenic microorganisms *M. tuberculosis* and *M. avium*, the latter an opportunistic pathogen related to infections in immunocompromised individuals (Cantrell et al., 1998).

Eupatorium intermedium DC.

E. intermedium [syn. *Grazielia intermedia* (DC.) R.M. King and H. Rob.] is a branchy shrub, native to southern Brazil with upright growth between 1.0 to 1.5 m high and it is densely leafy until close to the inflorescences, which are composed of white flowers (Czaikoski et al., 2015).

The extracts obtained by supercritical extraction from the flowers with $scCO_2$, propane and petroleum ether as solvents were tested and showed antibacterial activity against two Gram-positive bacterial strains, *S. aureus* and *L. monocytogenes*, and two Gram-negative bacterial strains, *E. coli* and *S. typhimurium*. The assays were performed using the disk diffusion method (Czaikoski et al., 2015). The extracts were effective against all bacteria strains, particularly the extract obtained with $scCO_2$ and compressed propane.

The essential oil was tested against Gram-negative bacterial strains using the agar well method. The main constituents of essential oil were α-pinene, sabinene, β-pinene, limonene, (*E*)-caryophyllene, germacrene D, spathulenol and caryophyllene oxide (Czaikoski et al., 2016).

Eupatorium inulaefolium Kunth.

E. inulaefolium [syn. *Austroeupatorium inulaefolium* (Kunth) R.M. King & H. Rob.] is a species found in Latin America, whose common name in Argentina is “sanalotodo” or “yerba de Santa María”. This species is used in folk medicine for treatment of skin infections due to its antimicrobial properties (Lancelle et al., 2009) and throat diseases (Álvarez et al., 2005).

In a study of 122 plant species from 54 families, Pérez and Anesini (1994) investigated the antibacterial activity against the microorganism *S. typhi*, causative agent of typhoid fever. Of the plants tested in the initial screening, *E. inulaefolium* showed inhibitory activity against the growth of *S. typhi*. In an investigation of the *in vitro* antimicrobial activity of Colombian angiosperms, the inhibitory activity against the microorganisms *S. aureus* and *B. subtilis*, was investigated at a concentration of 3 mg/mL, by the agar diffusion method (Sanabria-Galindo et al., 1998). In a third study, the ethyl extract of the leaves of *E. inulaefolium* were tested against the strains of *S. aureus*, *E. coli* and *P. aeruginosa*. The authors only observed growth inhibition against *S. aureus*, at a concentration of 50 mg/mL (Álvarez et al., 2005).

Eupatorium japonicum Thunb.

E. japonicum [syn. *Eupatorium chinense* L.] is a perennial herb, native to Japan, Korea and northeastern area of China. It is a medicinal herb used in folk medicine as a decoction for the treatment of measles, rheumatic bone pains, colds and cough (Roeder, 2000).

The ethyl acetate extract of the leaves and flowers of *E. japonicum* was used to investigate the antifungal activity against the dermatophyte *T. mentagrophytes*, the most common cause of *Tinea pedis*. At the concentration of 0.25 mg/mL, the authors observed an inhibition zone of 11 mm (Inouye et al., 2006).

Eupatorium laciniatum Kitam.

E. laciniatum [syn. *Eupatorium chinense* L.] an annual herbal plant, native to Asia is distributed in the south of China, used in Chinese folk medicine for the treatment of cold, snakebite and inflammation (Yang et al., 2005).

The antidermatophytic activity of this plant species was tested against the fungus, *T. mentagrophytes*, using the ethyl acetate extract of the leaves and flowers at a concentration of 0.25 mg/mL. The extract caused an inhibition zone of 12 mm (Inouye et al., 2006).

Eupatorium laevigatum Lam.

E. laevigatum [syn. *Chromolaena laevigata* (Lam.) R.M. King & H. Rob.], native to South America is widely distributed in the South part of Brazil and Argentina, whose common name in Brazil is “erva-de-santa-maria” or “camara”, is used in folk medicine for wound healing and as an antifungal (Maia et al., 2002). In Argentina, it is known as “caá-hú” or “doctorcito” and used in folk medicine as an anticefalalgic, analgesic, cathartic, emenagogue and purgative agent (Clavin et al., 2000).

The ethanol and hexane extracts of the leaves were tested against *B. subtilis*, by the agar diffusion method, with moderate results for inhibition of bacterial growth by both solvents (Schmidt et al., 2009). In a chemical and antimicrobial prospecting study, the methanol extract of the leaves of *E. laevigatum* showed the presence of alkaloids, steroids, phenols, tannins and flavonoids. The antimicrobial activity was performed by the broth microdilution method against strains of Gram-positive and negative bacteria, showing inhibitory activity against *P. aeruginosa*, *B. cereus*, *Shigella sonnei* and *Salmonella enterica* sorovar *typhimurium* (Fabri et al., 2011).

Eupatorium lindleyanum DC.

E. lindleyanum is a perennial herbaceous plant, native to China, known as “Yemazhui” and used in folk medicine to relieve fever, remove toxic substances, treat coughs, promote urination, and lower blood pressure (Xia et al., 2004). In traditional Chinese medicine, its aerial part is used as an antipyretic and detoxicant (Yang et al., 2010).

The extracts of the aerial parts obtained by use of ethanol, methanol and ethyl acetate were tested by the broth dilution method against eight bacterial strains: four Gram-positive (*B. subtilis*, *S. aureus*, *B. cereus* and *E. faecium*) and four Gram-negative (*E. coli*, *S. typhimurium*, *P. vulgaris* and *Pseudomonas fluorescens*). The extracts inhibited growth of all strains tested, with the best results for *B. subtilis*, *S. aureus* and *B. cereus* (Ji et al., 2008).

Eupatorium maculatum L.

Native to United States, *E. maculatum* known as “Joe-pye weed" is widely found in moist places, especially in calcareous soils (Goebel et al., 2012). The first report of antimicrobial activity of *E. maculatum* dates back to the work of Bishop and MacDonald (1951), who reported that the ethanol extract of the aerial parts showed activity against strains of Gram-positive bacteria.

In prospective study with 336 native species of the states of Minnesota and Wisconsin in the United States, ethanol extracts of leaves and stems of *E. maculatum* were tested against strains of *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*, presenting significant action only against *S. aureus*. The assays were performed using the disk diffusion method (Borchardt et al., 2008).

Eupatorium odoratum L.

E. odoratum [syn. *Chromolaena odorata* (L.) R.M. King & H. Rob.] is a free standing shrub which is mostly distributes in America, tropical Asia and West Africa. It is widely used in traditional medicine as immunemodulator, antispasmodic, hepatoprotective, antiprotozoal, antidiabetic, antihypertensive, anti-inflammatory (Umukoro and Ashorobi, 2006). *E. odoratum* also can be used in treatment of skin diseases, dysentery, malaria fever, tooth ache and diabetes (Omoregie et al., 2014). In Mexican folk medicine, it is used for chest complaints and pulmonary affections, known as "crucita" (Molina-Salinas et al., 2007).

Various studies of *E. odoratum* have investigated the plant's antimicrobial activity against bacteria that cause various infectious diseases. Cáceres et al. (1995) observed activity against the bacterium *Neisseria gonorrhoeae*, which causes gonorrhea, with an excellent growth inhibition zone.

Four flavanones extracted from flowers of *E. odoratum* exhibited antibacterial activity against *Mycobacterium tuberculosis*. Flavanones were isolated by silica gel chromatography and analyzed with infrared spectroscopy and nuclear magnetic resonance (Suksamrarn et al., 2004). Chomnawang et al. (2005) tested the antiacne activity (crude extracts with chloroform and ethylacetate) against strains of *Propionibacterium acnes*, *S. epidermidis*, while Molina-Salinas et al. (2007) investigated the antibacterial activity (methanol extract; leaves, flowers and roots) against *Streptococcus pneumonia* and Chomnawang et al. (2009) tested it against methicillin-resistant *S. aureus*.

Methanol extract of the aerial parts of *E. odoratum* inhibited the growth of *M. phlei* by disk diffusion method, with inhibitory activity at concentrations of 300 mg/mL (Gautam et al., 2007). In another study using the aerial parts, the methanol extract inhibited microbial growth of *B. subtilis*, methicillin-resistant *S. aureus*, methicillin-sensitive *S. aureus*, *T. mentagrophytes* and *M. phlei*, in the last case only when exposed to UV radiation (Taylor et al., 1996). The essential oil from the dried leaves of *E. odoratum* showed *in vitro* antibacterial activity against both Gram-positive and negative bacterial strains (Sharma et al., 2013). The major components analyzed by gas chromatography-mass spectrometry (GC-MS) were geijerene (25.10%), germacrene D (20.27%), trans-β-caryophyllene (10.04%), α-pinene (9.64%) and β-pinene, (4.85%).

In a screening study, the aqueous leaf extract of *E. odoratum* was tested for their fungitoxicity against spores of *Uromyces vignae* and showed the better result for the inhibition of spore germination (Patil and Kamble, 2015).

Eupatorium patens D. Don ex Hook. & Arn.

E. patens [syn. *Austrobrickellia patens* (D.Don ex Hook. & Arn.) R.M. King & H. Rob.] is a densely branched shrub that is distributed from Bolivia and Paraguay to Patagonia in Argentina, known by the common names of "acancio" or "bejuco" (Cabrera and Correa, 1971).

The essential oil of the leaves was tested against the microorganisms *S. aureus, E. coli*, *B. subtillis*, *C. albicans* and *A. niger* by the agar diffusion method. Only the strains of *B. subtilis* showed significant growth inhibition zones after incubation for 24 h (Bailac et al., 2000).

Eupatorium perfoliatum L.

Native to North America, *E. perfoliatum* is a medicinal herb, known as "boneset" or "thoroughwort", used in folk medicine by the native inhabitants for the treatment of fever and flu (Maas et al., 2008). The first record in the literature on the antimicrobial potential of *E. perfoliatum* was performed by Carlson et al. (1948). The authors found that the ether extract obtained from the aerial parts inhibited the growth of *S. aureus*. Later studies investigated extracts from leaves and stems using organic solvents (ethanol, ether and acetone) and water, demonstrating antimicrobial activity against *C. albicans*, Gram-positive and Gram-negative bacteria and *Mycobacterium* spp. (Bishop and MacDonald, 1951; Madsen and Pates, 1952; Liegey, 1953; Frisby et al., 1953).

In prospective study, the ethanol extract of the leaves and flowers of *E. perfoliatum* was tested against strains of *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans.* The extract only had significant action against *S. aureus*, with inhibition zones of 11 and 7 mm for extracts of the leaves and inflorescences, respectively. The assays were performed using the disk diffusion method (Borchardt et al., 2008).

Eupatorium purpureum L.

E. purpureum is a wildflower perennial plant, native to the eastern United States, known as "joe-pye weed", "sweet joe-pye weed", and "sweetscented joe-pye weed"

(Sabanadzovic et al., 2010). It is used in traditional medicine for rheumatoid arthritis and several other disease conditions (Habtemariam, 1998).

The saline extract of the stems of *E. purpureum* L. showed antibacterial activity against *S. aureus* and *E. coli*, by the agar diffusion method (Carlson et al., 1948). The saline extract of the leaves was also investigated against *S. aureus* and *E. coli*, with positive results by the agar diffusion method (Pates and Madsen, 1955).

Eupatorium rugosum Houtt.

E. rugosum [syn. *Ageratina altissima* (L.) R.M. King & H. Rob.] is a perennial herb commonly found in the midwestern and eastern United States, known as "white snakeroot" (Lee et al., 2012). The saline extract of the stems of *E. rugosum* showed antibacterial activity against strains of Gram-positive bacteria by the agar diffusion method (Frisby et al., 1954).

Eupatorium salvia Colla

E. salvia [syn. *Aristeguietia salvia* (Colla) R.M. King & H. Rob.] is a species used in folk Chilean medicine, known as "sálvia macho", "pegajosa" or "pega-pega" (Hoffmann, 1995). The resinous exudate, 7-hydroxy-8(17)-labden-15-oic acid (salvic acid) and 7-acetoxy-8(17)-labden-15-oic acid (salvic acid acetate), isolated from *E. salvia*, were tested against five bacterial strains, with activity against all tested microorganisms: *S. aureus*, *B. cereus*, *B. subtilis*, *M. luteus* and *Clavibacter michiganensis* subsp. *Michiganensis*. These diterpenoids do not affect Gram-negative bacteria and the activity of these compounds on filamentous fungi has not been reported (Urzua et al., 1998).

Eupatorium serratum Spreng.

E. serratum [syn. *Grazielia serrata* (Spreng.) R.M. King & H. Rob.], popularly known as "erva-milagrosa" or ("miraculous herb") in Brazil, is used in folk medicine to treat snake bites, as an analgesic, healing agent, antimicrobial agent and for the treatment of stomach problems, liver disorders, diabetes, cancer and bronchial asthma (Desoti et al., 2011).

The hexane, methanol and ethyl acetate extracts obtained from fresh leaves of *E. serratum* were tested against *S. aureus*, *M. luteus*, *E. coli* and *S. typhi*. The best inhibitory halo results were produced by the methanol extract against *M. luteus* (Desoti et al., 2011).

Eupatorium tashiroi Hayata

E. tashiroi, native to Asia is a wild herb which has been used in a folk medicine for treating edema and hemoptysis in Taiwan (Wu et al., 1985). The aqueous extract of the entire plant was tested by the agar diffusion method against two serotypes of *S. mutans*, bacteria associated with the development of dental caries. The extracts inhibited bacterial growth of both serotypes (Chen et al., 1989).

Eupatorium triplinerve Vahl

E. triplinerve [syn. *Ayapana triplinervis* (Vahl) R.M. King & H. Rob.] commonly found in Asia is an erect perennial herb with narrow lanceolate leaves and large number of pedicelled flower-heads at the top of the branch, known as "ayapana" (Selvamangai and Bhaskar, 2012). The species was introduced in Indian as ornamental species. In Indian, folk medicine is used as a stimulant, tonic, laxative and for the treatment of yellow fever (Unnikrishnan et al., 2014).

The antimicrobial activities of the essential oil obtained from the leaves and the stem and thymohydroquinone dimethylether were evaluated using disk diffusion assay against strains of Gram-positive and negative bacteria, showing inhibitory activity against *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *E. coli*. The essential oil showed antifungal activity against fungal strains of *Penicillium chrysogenum* and *C. albicans*; however thymohydroquinone dimethylether only inhibited the growth of *P. chrysogenum* (Unnikrishnan et al., 2014).

The chloroform extract of leaves exhibited antibacterial activity by the broth microdilution and disk diffusion methods against strains of Gram-positive and negative bacteria *Bacillus megaterium*, *B. subtilis*, *B. cereus*, *S. aureus*, *E. coli*, *S. dysenteriae*, *S. sonnei*, *S. typhi*, *S. paratyphi* and *P. aeruginosa*, that exhibited the largest zone of inhibition against *Vibrio* (22 mm in diameter with 1000 µg/disc extract). The antifungal activity was performed with filamentous fungi *Alternaria alternata*, *Curvularia lunata*, *Colletotrichum corchori*, *Fusarium equiseti*, *Macrophomina phaseolina* and *Botryodiplodia theobromae*, with highest inhibition of fungal radial mycelial growth against *C. corchori*, 73.5% with 100 µg extract/ml medium (Rahman and Junaid, 2008).

In others studies, the essential oil from aerial parts and fresh leaves exhibited moderate antibacterial activity against *B. megaterium*, *B. subtilis*, *B. cereus*, *S. aureus*, *E. coli*, *V. cholerae*, *S. dysenteriae*, *S. sonnei*, *S. typhi, Pseudomonas* sp. and *S. paratyphi*. The essential oil demonstrated strong antifungal activities against *A. alternata*, *C. lunata*, *B. theobromae*, *C. corchori*, *F. equiseti* and *M. phaseolina* (Begum et al., 2010; Sugumar et al., 2015).

Eupatorium urticaefolium Reichard

E. urticaefolium [syn. *Ageratina altissima* (L.) R.M. King &

H. Rob.] is a species native to North America, it is commonly found throughout the eastern half of continent. Ingestion of the plant causes a disease called trembles in livestock (Lee et al., 2010). The ethyl acetate extract of the aerial parts of *E. urticaefolium* inhibited the growth of *S. aureus* and *E. coli*, by the agar diffusion method (Carlson et al., 1948).

Conclusion

Thirty *Eupatorium* species were identified, some already reclassified to other genera. The aerial parts are the part of plant with more studies and so far the best source for anti-microbial product, being the essential oils and fixed compounds the main natural products. Most of the studies were carried by the broth microdilution and disk diffusion methods, according to Clinical and Laboratory Standards Institute (CLSI).

Microbial infections caused by bacteria and fungi are a growing public health problem, and increasingly common in routine medicine. Outbreaks of infections by methicillin-resistant *S. aureus* in hospital and social environments have been reported in several regions of the world. Regarding fungal infections, the search for new more efficient therapies is particularly urgent considering that the available antifungal chemicals are much less than antibacterial drugs and also typically have more side effects, besides the possible development of fungal resistance.

This review shows that the Asteraceae family, and especially the genus *Eupatorium s.l.*, contains many species with antimicrobial activity, making these plants potential sources of safer and more effective treatments. In short, *Eupatorium s.l*. contains a diverse array of species with antimicrobial potential. New studies involving chemical and biological bioprospecting are necessary to develop effective and less toxic herbal products.

Conflict of interest

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to the Postgraduate Program in Biotechnology-Renorbio of State University of Ceará for the financial support of CAPES, the Microbiology Laboratory of Vale do Acaraú State University and the Herbarium Prof. Francisco José de Abreu Matos (HUVA) of Vale do Acaraú State University.

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