

***Baccharis* (Compositae): a review update**

Maria José Abad^{*} and Paulina Bermejo

Department of Pharmacology, Faculty of Pharmacy, University Complutense, Avda.

Complutense s/n, 28040, Madrid, Spain

E-mail: mjabad@farm.ucm.es

In honor of Professor Atta-ur-Rahman on his 65th birthday

Abstract

The *Baccharis* genus is an important source of natural medicinal products. The information collected here is an attempt to cover the most recent developments in the ethnopharmacology, pharmacology and phytochemistry of this genus. This review describes its traditional and folkloric uses, phyto-constituents and pharmacological and toxicological reports of the prominent species of the genus *Baccharis*. Flavonoids and other phenolic compounds, diterpenoids and volatile constituents have been reported as the major phyto-constituents of the *Baccharis* species. Pharmacological studies are mainly based on the anti-inflammatory, antioxidant, antimicrobial and antifungal properties. The potential for development of leads from *Baccharis* genus continues to grow. The information summarized here is intended to serve as a reference tool to practitioners in the fields of ethnopharmacology and chemistry of natural products.

Keywords: *Baccharis*, ethnopharmacology, phytochemistry, pharmacology

Contents

Introduction

1. Medicinal Uses of Selected *Baccharis* Species
 - 1.1. Medicinal uses
 - 1.2. Methods of preparing traditional herbal remedies
2. Phytochemistry
 - 2.1. Essential oils
 - 2.2. Diterpenoids
 - 2.3. Triterpenoids
 - 2.4. Phenolic compounds

- 2.4.1. Flavonoids
- 2.4.2. Coumarins
- 2.4.3. Simple phenolic derivatives
3. Pharmacological Activity
 - 3.1. Antibacterial, antifungal and antiviral activities
 - 3.2. Anti-inflammatory and antioxidant activities
 - 3.3. Other activities
4. Toxicological Considerations
5. *Baccharis* Plant Cell Cultures
6. Conclusions

Introduction

A well known objective in the study of medicinal plants is the discovery of new bioactive components in the search for promising drugs.^{1,2} It is over four years since the recompilation of our first analysis of the traditional use, scientific knowledge and major chemical constituents of several of the most commonly used medicinal plants belonging to the genus *Baccharis* L. (Compositae).³ The *Baccharis* genus has been instrumental in the discovery of natural medicinal products.⁴

Baccharis is the largest genus in the family Compositae, with over 500 species distributed throughout the North and South American continents. The species of this genus are distributed mainly in the warm temperate and tropical regions of Brazil, Argentina, Colombia, Chile and Mexico. Over the past four years since our previous review, there has been a rapid increase in the information available on the structures and pharmacological activities of novel compounds isolated and identified from *Baccharis* species since 2002. Results of various projects on South American ethnobotany and some of the subsequent pharmacological and phytochemical studies on the genus *Baccharis* are summarized, focusing both on chemistry-pharmacology as well as on the ethnopharmacological aspects of our research. The depth and breadth of research involving *Baccharis* plants has been organized into easily accessible and comparable information which is presented in three sections: traditional uses, phytochemistry and pharmacological activities of *Baccharis* plants.

1. Medicinal Uses of Selected *Baccharis* Species

1.1. Medicinal uses

The many general traditional medicinal uses of *Baccharis* include the treatment of wounds and ulcers, fever, gastrointestinal illnesses, as spasmolytics, diuretics and analgesics, and in the treatment of diabetes and bacterial/fungal infections;⁵⁻⁹ more details are given below.

Baccharis articulata (Lam.) Pers. (Carquejilla) is traditionally used as a diuretic and digestive in local folk medicine of southern of Brazil, Uruguay and Argentina. Its decoctions or infusions are also an antidiabetic remedy in Paraguay. The plant is used in the form of infusions or decoctions of the aerial parts.

Baccharis conferta (H.B.K.) (Quauhizquitzli) is used in the region of Veracruz, Mexico, to treat stomachaches. It is also recommended for use as a laxative and for stimulating urination. Tea made from the leaves is taken to assist weight loss.

Baccharis coridifolia D.C. (Mio-Mio, Romerillo) is used externally as an anti-inflammatory. Reports also indicate that the vapours of this species, when mixed with sulphur, are used to treat horse's distemper and horse's external parasites. The plant is used in the form of decoctions of the whole herb.

Baccharis crispa Spreng. (Carqueja): decoctions from the aerial parts of the plant have been reported as digestives and antiseptic for external use.

Baccharis dracunculifolia D.C.: propolis is a natural resinous exudate collected by honeybees from buds and exudates of plants. The botanical origin of Brazilian propolis was *B. dracunculifolia* (Alecrim do Campo) resinous exudates. Brazilian propolis is extensively used to improve health and prevent diseases, and in beverages in various countries.

Baccharis floribunda H.B.K. (Niquitao) is used in Venezuela as decoctions or infusions of leaves and stems for skin infections, diabetes and rheumatism.

Baccharis gaudichaudiana D.C. (Chilca melosa) is used in Paraguay as a folk medicine for the treatment of diabetes and as a tonic and for the relief of gastrointestinal ailments.

Baccharis genistelloides Lam. = *Baccharis trimera* (Lam.) Pers. (Carqueja, Charara) has been popularly used to treat liver disease, rheumatism, diabetes, as well as digestive, hepatic and renal disorders. This plant is also used in popular medicine in Argentina as an aphrodisiac. The plant is used in the form of decoctions of the whole herb.

Baccharis glutinosa (B. & P.) Pers. (Jarilla) is used in several states of Mexico for gynaecological and digestive disorders, as well as skin diseases. The leaf infusion, the traditional preparation, is preferable to the fresh and dried plant tinctures.

Baccharis grisebachii Hieron (T'ola alta, Quinchamal): the aerial parts of this shrub, in the Andean provinces in Argentina, are recommended in infusion to treat gastric ulcers, as a digestive, local antiseptic and cicatrizant.

Baccharis heterophylla H.B.K. (Curacuata, Jarakatua) is ethnomedically used in the state of Queretara, Mexico, for alleviating gastrointestinal disorders. The plant is used in the form of infusions or decoctions of the aerial parts.

Baccharis illinita D.C. (Cha-ventura, Erva milagrosa) found in Brazil's subtropical regions is widely used in folk medicine for its anti-inflammatory, skin and mucosal healing and anti-infectious properties, as well as for its protective action against stomach ulcers. The formulation most frequently used is the tea obtained from the leaves and stems. The powder obtained from macerated and dried leaves is widely used for the cicatrization of skin wounds.

Baccharis incarum Wedd. (Lejia) is used in Argentina as decoctions or poultices of leaves for healing wounds. The plant is used in the form of infusions of the leaves and/or stems.

Baccharis latifolia Pers. (Chilca) is used in the form of decoctions of leaves and/or stems in the traditional medicine of South America for the treatment of rheumatism, liver disease, wounds and ulcers.

Baccharis multiflora Bent. (Quappatli, Malinalli) is used in the form of infusions of the leaves in the traditional medicine of Mexico for the treatment of catarrhs and for urinary problems.

Baccharis notoserquilla Griseb. (Carqueja) is used in the folk medicine of the northeast Argentina, Uruguay and Paraguay as a diuretic and digestive agent. The plant is used in the form of infusions of the leaves and/or stems.

Baccharis obtusifolia H.B.K. (Chilca redonda) is used in the form of decoctions of the aerial parts in the traditional medicine of Colombia for the treatment of rheumatism, liver disease, wounds and ulcers.

Baccharis pentlandii D.C. (Chilca, Mayu chilka) has been used in traditional medicine in Bolivia in the treatment of different pathologies, including inflammation and rheumatism.

Baccharis rubricaulis Rubby (Chilca) is used by the rural and indigenous populations of Bolivia for treating different types of mucous ailments. The plant is used in the form of infusions or decoctions of the leaves and/or stems.

Baccharis salicifolia (R. et P.) Pers. (T'ola alta, Chilca marga): an infusion of the leaves/leafy branches are applied externally as a remedy for inflammation. A decoction of the leaves and stems has also been used as a female hygienic agent.

Baccharis sarothroides A. Gray (Desert broom): a tea made by boiling the twigs is rubbed on muscles for relief, and is used to treat colds. Seeds are steeped and used as a tea-like drinks for refreshment.

Baccharis serraefolia D.C. (Tzajal) is a widely used plant for the treatment of diarrhoea and other gastrointestinal illnesses in Mexican traditional medicine. An infusion made from leaves of this plant is used primarily for the treatment of different types of diarrhoea, dysentery and against worms and cough.

Baccharis subalata Wedd is used in the form of decoctions of the aerial parts in the traditional medicine of South America for the treatment of rheumatism, liver disease, wounds and ulcers.

Baccharis teindalensis L. is a herbal plant which is widely used in folk medicine in Ecuador as an anti-inflammatory, analgesic and antimicrobial remedy. The plant is used in the form of infusions or decoctions of the aerial parts.

Baccharis tricuneata (L.f.) Pers. (Sanalotodo) is used in Venezuela as decoctions or infusions of leaves and stems for skin infections and diabetes.

Baccharis trinervis (Lam) Pers. (Chilca) is employed in the treatment of high fever, edema, sores and muscle cramps. The plant is used in the form of infusions of the aerial parts.

Baccharis tucumanensis (H. et A.) (Sancho blanco) is used in several regions of Argentina as an abortive.

Baccharis vaccinoides H.B.K. (Chilco) is one of the most popular remedies from the Chiapas region of Mexico for gastrointestinal disorders. The plant is used in the form of infusions or decoctions of the whole plant.

1.2. Methods of preparing traditional herbal remedies

Infusion: an infusion is generally like making a cup of tea. Boil 32 parts of water (by volume), remove from heat and steep one part (by volume) of the herb in the water for 20-30 minutes. Strain and drink warm or cold. This is approximately 1 level teaspoon of dried herb per teacupful of water.

Decoction: combine 32 parts of water with one part of dried herb (by weight), bring slowly to a boil, continue to boil for 10 to 20 minutes, cool until warm and strain. Pour additional water through the herb to return the volume to 32.

2. Phytochemistry

The phytochemistry of the *Baccharis* genus has been extensively studied since the early 1900s. Today, over 150 compounds have been isolated and identified from this genus. The most prominent compounds in *Baccharis* are diterpenoids, although it is mainly other biologically active constituents, e.g. phenolic compounds and essential oils, that have been reported in the last years. The constituents of *Baccharis* species compiled from different sources are presented below.

2.1. Essential oils

Essential oils from the *Baccharis* genus have been studied in several species from South America. More than 100 constituents were identified in studies of the composition of the essential oil of Argentinian *Baccharis* species. Malizia *et al.*^{10,11} investigated the volatile constituents of leaf oils from four species of the genus *Baccharis* collected in the Argentinean Patagonia: *Baccharis racemosa* (Ruiz et Pav.) D.C., *Baccharis linearis* (Ruiz et Pav.) Pers.,

Baker, *B. trimera* and *Baccharis usterii* Heering. Despite the morphological similarity between these species, differences could be seen in the composition of their essential oils obtained by hydrodistillation. Special attention was given to *B. trimera* and *B. crispa*, since it is impossible to differentiate them in their early stages due to the absence of synflorescences. Carquejyl acetate is suggested as a marker for *B. trimera*. There have been extensive works on the composition of the essential oil of one of these species, *B. articulata*, together with the species *B. trinervis*. The essential oil from the aerial parts of *B. trinervis* obtained by hydrodistillation was analyzed.¹⁵ α -Thujene, α - and β -pinene, sabinene, β -phellandrene, (*E*)-lachnophyllum acid methyl ester and (*Z*)-lachnophyllum acid methyl ester were found to be the main components among the identified constituents. Essential oils of *B. articulata* produced from plants harvested at full flowering were also examined.¹⁶ The main components of the female plant oil were β -pinene, spathulenol (Figure 2) and (*E*)-nerolidol. The male plant oil contained spathulenol (Figure 2), β -caryophyllene, bicyclogermacrene and (*E*)-nerolidol.

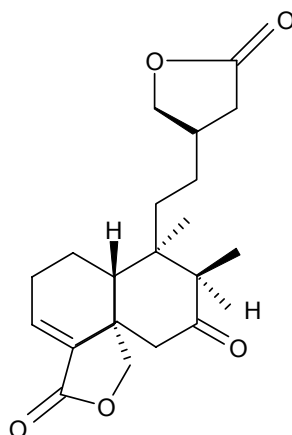


Figure 3. Structure of gaudichaunolide B

2.2. Diterpenoids

Characteristic diterpenic constituents from the *Baccharis* genus are the *neo*-clerodane type diterpenes, although kaurane and labdane derivatives have also been isolated. From *B. gaudichaudiana*, new clerodane diterpenoids, gaudichanolides A and B (Figure 3), and a new *ent*-clerodane diterpene named bacchariol (Figure 4) were isolated.^{17,18} Guo *et al.*¹⁹ isolated three new *ent*-clerodane diterpenes from this species. Their structures were elucidated as 15,16-epoxy-15 α -methoxy-*ent*-clerod-3-en-18-oic acid, 13-epi-15,16-epoxy-15 α -methoxy-*ent*-clerod-3-en-18-oic acid and 7-oxo-16-hydroxy-*ent*-clerod-3-en-15-oic acid methyl ester-18,19-olide.

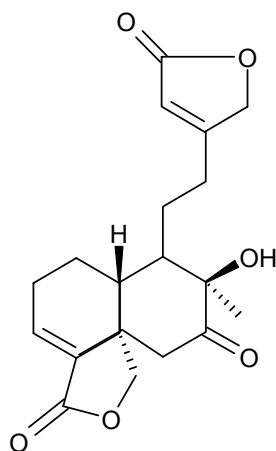


Figure 4. Structure of bacchariol.

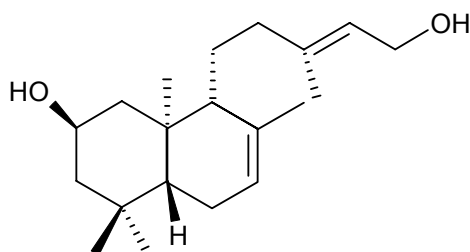


Figure 5. Structure of labdane diterpenes from *Baccharis grisebachii*.

Reports on clerodane diterpene components of other species of the *Baccharis* genus have also been found in the literature. Two clerodane-type diterpene glycoside esters, together with the known diterpene marrubiagenine, were isolated from the aerial parts of *Baccharis sagittalis* (Less),²⁰ while the acetone extract of *Baccharis flabellata* Hook. & Arn. yielded three *neo*-clerodane diterpenoid derivatives.²¹ Examples of other diterpenoids isolated from the *Baccharis* genus also included two labdane diterpenes from *B. grisebachii* (Figure 5),²² kaurane diterpenes from *B. illinita* flowers,²³ and the aerial parts of *Baccharis rufescens* Spreng., which yielded the diterpene *ent*-3,19-disuccinyloxy-kaur-16-ene (Figure 6).²⁴

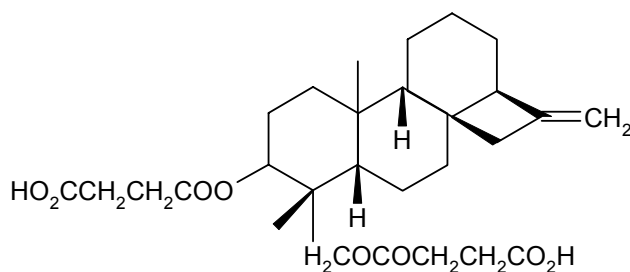


Figure 6. Structure of *ent*-3,19-disuccinyloxy-kaur-16-ene

2.3. Triterpenoids

There have only been a few reports on the triterpene composition of the *Baccharis* genus in the last years. The most widespread compounds of this group reported in the genus *Baccharis* were oleanolic triterpenoids. Phytochemical study of chloroform extract from *Baccharis pseudoteunifolia* Il Teodoro shrub led to the isolation of the triterpene oleanolic acid (Figure 7) and α -spinasterol.²⁵ Oleanolic acid (Figure 7) was also isolated from *B. rufescens*,²⁴ and *Baccharis ligustrina* D.C., which also yielded ursolic acid.²⁶

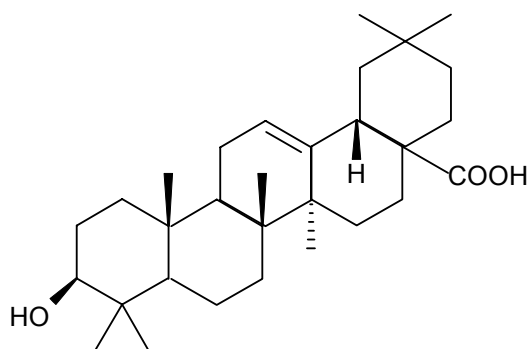


Figure 7. Structure of oleanolic acid

2.4. Phenolic compounds

Polyphenols form a complex group of molecules associated with most plant cell walls and represent an abundant antioxidant component of the human diet. They range in chemical complexity from single phenolic acids to high-molecular-weight tannins. This section deals mainly with those phenolic classes of pharmaceutical interest found in the *Baccharis* genus, namely flavones and related flavonoid glycosides, coumarins and derivatives, and simple phenolic compounds.

2.4.1. Flavonoids

Propolis is a natural resinous substance collected by honeybees from buds and exudates of plants. It has been used as a health food and in beverages in various countries. The botanical origin of Brazilian propolis was the resinous exudates of *B. dracunculifolia*.^{27,28} When the composition of its chemical ingredients was measured, Brazilian propolis contained high amounts of flavonoid aglycones that are useful dietary sources of flavonoids with a potency to prevent dioxin toxicity.²⁹

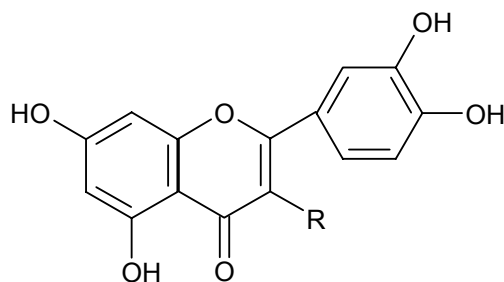


Figure 8. Structure of quercetin (R=OH) and luteolin (R=H)

The exudates and seriated extracts of the aerial parts of *B. grisebachii*, which is recommended as a digestive and to relieve gastric ulcers in Argentina, yielded the flavonoids 5,7,4'-trihydroxy-6-methoxyflavone and quercetin (Figure 8).³⁰ Following the study of *B. latifolia*, two flavonoids were isolated from the dichloromethane extract of the aerial parts.³¹ Nine flavonoids were isolated from the ethanol extract of the air-dried flowers of *B. illinita*, namely luteolin (Figure 8), aromadendrine, naringenin, 2'-methoxychrysin, apigenin, 3-O-methylquercetin, taxifolin, quercetin (Figure 8) and chrysoeriol.²³ The chloroform extract yielded the flavonoids nobiletin, tangeretin, luteolin (Figure 8), naringenin and kaempferol.

Phytochemical study of the methanol extract from the *B. pseudotenuifolia* shrub led to the isolation of the flavonoids hispidulin, naringenin, 3'-methoxyluteolin, apigenin, kaempferol, eriodyctiol, aromadendrin, quercetin (Figure 8), 3'-methoxyquercetin, quercetin-3-O-rhamnoside and quercetin-3-O-glucoside.²⁵ The flavonoids naringenin and hispidulin were also isolated from *B. ligustrina*.²⁶ Examples of other flavonoids isolated from the *Baccharis* genus also included eight known flavonoids from *B. gaudichaudiana*,¹⁷ and the species *B. rufescens*, which yielded the flavonoids cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone) and cirsilinol (5,3',4'-trihydroxy-6,7-dimethoxyflavone).²⁴

2.4.2. Coumarins

There have only been a few reports on the coumarin composition of the *Baccharis* genus in the last years. These reports concern the species *B. grisebachii*, which yielded eight *p*-coumaric acid derivatives,^{22,30} and the Colombian plant *Baccharis tricuneata* L.F., which afforded the coumarin scopolin (Figure 9).³²

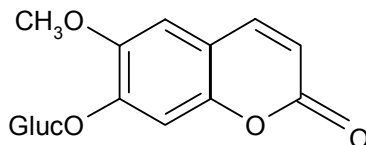


Figure 9. Structure of scopolin.

2.4.3. Simple phenolic derivatives

Reports on the simple phenolic acid composition in the *Baccharis* genus mainly concern the species *B. dracunculifolia* (Brazilian propolis). As you can see in the present review, propolis is a resinous hiveproduct collected by honeybees from plant sources. The composition of the propolis depends on the season, vegetation and the area of collection. De Alencor *et al.*³³ investigated the phytochemical composition of Brazilian propolis produced from *B. dracunculifolia* in the states of Sao Paulo and Minas Gerais by *Africanized Apis mellifera*. A distinctive characteristic of propolis and *B. dracunculifolia* was that both had a high proportion of the phenolic compound artemillin C (3,5-diprenyl-4-hydroxycinnamic acid) and other dihydrocinnamic acid derivatives. Artemillin C was also identified in both propolis and resinous exudates, and both ethanol extracts contained the highest concentrations of this compound as compared with the rest of the chemical constituents.³⁴ Based on phytochemical evidence, *B. dracunculifolia* was found to be the main source of propolis produced in the states of Sao Paulo and Minas Gerais from Brazil. Other simple phenolic acid derivatives such as (*E*)-3-(*E*)-3-hydroxy-3-methyl-1-butenyl-4-(2,3-dihydrocinnamoyloxy)-cinnamic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid and chlorogenic acid (Figure 10) were also isolated from the aerial parts of *B. dracunculifolia*.³⁵⁻³⁷

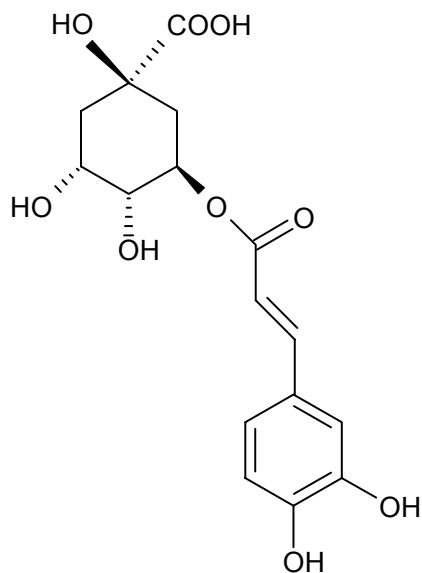


Figure 10. Structure of chlorogenic acid.

Examples of other simple phenolic acid derivatives from the *Baccharis* genus also included 4'-*O*- β -*D*-glucopyranosyl-3',5'-dimethoxy-benzylcaffeate isolated from *B. articulata*, a plant traditionally used in the local folk medicine of South of Brazil, Uruguay and Argentina,³⁸ and 3,5-dicaffeoylquinic acid from the aerial parts of *B. gaudichaudiana*.¹⁷

3. Pharmacological Activity

Pharmacological studies have generally focused on the fractions of either a plant species or a “crude drug”, considered as a preparation of either a single or a mixture of *Baccharis* plants. A number of studies, however, have focused on the bioactivity of specific isolated compound(s). Some of these compounds were bioassay-guided isolated after previously detecting activity on the part of the plant. The information in this section is organized by pharmacological activity, with reference to the crude extract or isolated constituents studied.

3.1. Antibacterial, antifungal and antiviral activities

A review of the literature concerning the evaluation of plant extracts of the *Baccharis* genus and secondary metabolites isolated from them reveals that many studies into their antimicrobial and antiviral activities have been carried out in recent years.

Propolis is natural product that may prevent dental caries triggered by *Streptococcus mutans*. *B. dracunculifolia*, a native plant from Brazil, is the main botanical origin for production of green propolis (Brazilian propolis) by honeybees. However, whether *B. dracunculifolia* has an anticariogenic effect like green propolis remains unknown. Da Silva *et al.*³⁹ investigated the *in vitro* effects of Brazilian green propolis and *B. dracunculifolia* extracts on cariogenic factors of *Streptococcus mutans*. Both extracts produced a bacteriostatic effect on *Streptococcus mutans* cultures at a concentration of 0.40 mg/ml. These results demonstrated that *B. dracunculifolia* leaf rinse and green propolis extracts have similar inhibitory effects on the *Streptococcus mutans* cariogenic factors evaluated, and suggest that *B. dracunculifolia* leaves may be a potential source for pharmaceutical products used for this purpose.

Additionally, Santos *et al.*⁴⁰ investigated the antibacterial activity on periodontopathogens of propolis samples collected in the dry and rainy seasons from an experimental apiary located in a region of Cerrado vegetation in Brazil. Antibacterial assays were performed by the method of dilution of an ethanol extract of propolis in agar, and showed that all sixteen actinomycetemcomitan strains tested were inhibited by propolis concentrations of 0.1% to 0.25%, and did not grow at all at 0.5%. The growth inhibition of six *Fusobacterium* spp. and sixteen black-pigmented anaerobes was observed at concentrations of 0.05% to 0.1%, and no growth was observed at 0.25%.

Orsi *et al.*⁴¹ investigated the effects of Brazilian and Bulgarian propolis on the bactericidal activity of macrophages against *Salmonella typhimurium*. Macrophages play an important role in the early phase of *Salmonella* infection. Propolis from Brazil and Bulgaria enhanced the bactericidal activity of macrophages depending on its concentration. Brazilian propolis seemed to be more efficient than Bulgarian, due to their different chemical composition. In Bulgaria, bees collect the material mainly from the bud exudates of the poplar tree, while in Brazil *B. dracunculifolia* was shown to be the main source of propolis.

Gekker *et al.*⁴² reported that propolis inhibited viral expression of human immunodeficiency virus type-1 (HIV-1) in a concentration-dependent manner. Maximal suppression of 85 and 98% was observed at 66.6 µg/ml propolis in CD4(+) lymphocyte and microglial cell cultures, respectively. The mechanism of the antiviral property of propolis in CD4(+) lymphocytes appeared to involve, in part, inhibition of viral entry. While propolis had an additive antiviral effect on the reverse transcriptase inhibitor zidovudine, it had no noticeable effect on the protease inhibitor indinavir. The results of this *in vitro* study support the need for clinical trials of propolis or one or more of its components in the treatment of HIV-1 infection.

Reports on antimicrobial activity in other *Baccharis* species have also been found in the literature. Thirty-two organic and aqueous extracts belonging to twelve Argentinian medicinal plants were tested for their *in vitro* trypanocidal activity on epimastigote forms of *Trypanosoma cruzi*.⁴³ Among the selected species, the organic extracts of *Baccharis spicata* (Lam.) Baill. showed trypanocidal activity with a percentage of growth inhibition higher than 70% at a concentration of 100 µg/ml. Examples of other antimicrobial extracts of the *Baccharis* genus also included *B. heterophylla*, a plant used in Mexican traditional medicine,⁴⁴ and *B. glutinosa* which inhibited the growth of *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium expansum*, *Fusarium moniliforme* and *Fusarium poae* moulds.⁴⁵

The compounds responsible for the reported antimicrobial activity in the *Baccharis* species are terpenoids, the main constituents of their essential oils. Derno *et al.*⁴⁶ evaluated the antibacterial and antifungal activity of essential oils obtained from medicinal plants from Argentina. The microorganisms used were *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus cereus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa* and the yeast *Candida albicans*. Among the plants tested, the essential oil of *B. flabellata* was effective against *Staphylococcus aureus*. Examples of other antimicrobial essential oils of the *Baccharis* genus also included the essential oil of *B. latifolia* which showed interesting activity against *Staphylococcus aureus*,³¹ and the essential oil from aerial parts of *B. trinervis* which were active against four bacteria and one fungus.¹⁵

Some of the antimicrobial compounds from the *Baccharis* genus were bioassay-guided isolated after previously detecting activity on the part of the plant. Da Silva *et al.*⁴⁷ performed a trypanocidal bioactivity-guided study of *B. dracunculifolia*, the main botanical origin of Brazilian green propolis. The leaf rinse extract of *B. dracunculifolia*, at a concentration of 3 mg/ml, displayed 100% lysis of trypomastigote forms of the Y strain of *Trypanosoma cruzi*. The chromatographic fractionation of the leaf rinse extract afforded the isolation of isosakuranetin, aromadendrin-4'-methylether, baccharis oxide (Figure 11), ferulic acid, dihydrocinnamic acid, 3-prenyl-4-(dihydrocinamoyloxy)-cinnamic acid and friedelanol. Isosakuranetin and baccharis oxide (Figure 11) were the most active in the trypanocidal assay, showing IC₅₀ values (inhibitory concentration required for 50% inhibition) of 247.6 and 249.8 µM, respectively. Examples of other antimicrobial terpenoids of the *Baccharis* genus also included clerodane diterpenes from *B. sagittalis*,²⁰ and the diterpene labda-7,13E-dien-2β,15-diol from *B. grisebachii* which was active

towards *Epidermophyton floccosum*, *Trichophyton rubrum*, *Microsporus canis* and *Trichophyton mentagrophytes* with IC_{50} values between 12.5-25 $\mu\text{g/ml}$.²²

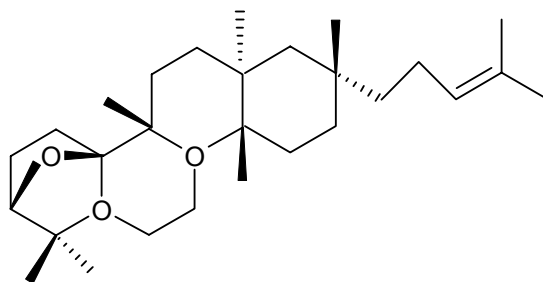


Figure 11. Structure of baccharis oxide.

Besides diterpenoids and essential oils, compounds belonging to other structural types have also been isolated from *Baccharis* species as antimicrobial constituents, such as 3-prenyl-*p*-coumaric acid and 3,5-diprenyl-*p*-coumaric acid from *B. grisebachii* which were active towards *Epidermophyton floccosum* and *Trichophyton rubrum* with IC_{50} values of 50 and 100-125 $\mu\text{g/ml}$, respectively.²²

3.2. Anti-inflammatory and antioxidant activities

Various research groups initiated anti-inflammatory screening programmes on plants used in traditional medicine all over the world as anti-inflammatory agents in the treatment of several pathologies, although other uses have also been suggested. Some of these studies concern species belonging to the genus *Baccharis*.

Fifty four different extracts of nine Bolivian plants were evaluated for their radical scavenging activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitro-blue tetrazolium/hypoxanthine superoxide and hydroxyl/luminal chemiluminescence methods, and for their antioxidant activity by the β -carotene bleaching test.⁴⁸ Some ethyl acetate extracts such as those from *B. pentlandii* and *Baccharis platypoda* D.C. exhibited activities comparable to those of commercial extracts/compounds, thus making it possible to consider some of the studied plants as a potential source of antioxidants of natural origin.

Organic and aqueous extracts from four Bolivian *Baccharis* species, *B. obtusifolia*, *B. latifolia*, *B. pentlandii* and *B. subalata* were studied for their *in vitro* anti-inflammatory activity in cellular systems.⁴⁹ These extracts were tested for their ability to inhibit the generation of several inflammatory mediators in two experimental systems: calcium ionophore A23187-stimulated mouse peritoneal macrophages validated as a source of cyclooxygenase-1 (prostaglandin E_2) and 5-lipoxygenase (leukotriene C_4) enzymes, and elicited mouse peritoneal macrophages stimulated with *Escherichia coli* lipopolysaccharide for testing cyclooxygenase-2

(prostaglandin E₂), nitric oxide synthase (nitric oxide) and tumour necrosis factor- α activity. Most of the extracts were active in all assays.

Coelho *et al.*⁵⁰ investigated the anti-arthritis effect of the aqueous extract of *B. genistelloides*. The treatment of the collagen-induced arthritis group with 4.2 mg/Kg of extract induced an important decrease (75%) in severity in all animals, while the 42 mg/Kg dose was effective in only 50% of the animals. Examples of other anti-inflammatory and antioxidant extracts of the *Baccharis* genus also included *B. articulata* extracts,³⁸ and propolis, a beehive product with a very complex chemical composition, widely used in folk medicine because of its several therapeutic activities.⁵¹

The compounds responsible for the reported anti-inflammatory and antioxidant activities in the *Baccharis* species are polyphenolic compounds, such as phenolic acids, flavonoids and coumarins. Some of these compounds were bioassay-guided isolated after previously detecting activity on the part of the plant. The aqueous extracts of aerial parts of *B. trimera*, *B. crispa* and *B. usterii* displayed significant radical scavenging activity in a DPPH assay.⁵² In order to identify the active principles rapidly, the crude extracts were analyzed by high performance liquid chromatography (HPLC). An HPLC-micro-fractionation of the extract of *B. usterii* was performed and a series of radical-scavenging quinic acid derivatives were identified as active constituents.

The antioxidant activity of crude ethanol and aqueous extracts, together with dichloromethane, ethyl acetate and *n*-butanol fractions obtained from aqueous extract of *B. articulata* was detected using a DPPH assay.³⁸ The *n*-butanol fraction was found to be the most active and its main compound was isolated, identified and assayed. This compound, 4'-*O*- β -*D*-glucopyranosyl-3',5'-dimethoxybenzylcaffeate, presented a similar antioxidant capacity when compared to the reference compound, trolox.

The exudates and seriated extracts from the aerial parts of *B. grisebachii*, which is recommended as a digestive and to relieve gastric ulcers in Argentina, showed activity as free radical scavengers and inhibited lipoperoxidation in erythrocytes.³⁰ Assay-guided isolation led to seven *p*-coumaric acid derivatives and six flavonoids as the main active constituents of the crude drug. The activity towards the superoxide anion was mainly due to the flavonoid constituents 5,7,4'-trihydroxy-6-methoxyflavone and quercetin (Figure 8). Other antioxidant flavonoids from the *Baccharis* genus are quercetin-3-*O*-*L*- α -rhamnoside, eupafolin, rutin and 3,5-dicaffeoylquinic acid from *B. gaudichaudiana*, which moderate scavenging activities towards DPPH radical.¹⁷

3.3. Other activities

Several other pharmacological actions have been documented from the *Baccharis* genus and compounds isolated from its species. Many plants are used in traditional medicine as active against various effects induced by snakebite. Few attempts have been made, however, to identify the nature of natural plant products with anti-ophidian properties. *B. trimera*, known in Brazil as "carqueja", has been popularly used to treat liver disease, rheumatism and diabetes, as well as in

digestive, hepatic and renal disorders. The active component was identified as the clerodane diterpene 7 α -hydroxy-3,13-clerodadiene-16,15:18,19-diolide. Januario *et al.*⁵³ reported the anti-proteolytic and anti-hemorrhagic properties of this compound against snake venoms. The compound was able to neutralize the hemorrhagic, fibrinogenolytic and caseinolytic activities of class P-1 and III metalloproteases isolated from snake venoms. However, the potential use of this inhibitor to complement anti-venom as an alternative treatment for snakebite poisoning needs to be evaluated in more detail.

With this species, *B. trimera*, Oliveira *et al.*⁵⁴ investigated the effects of its extracts and fractions on the glycaemia of diabetic and non-diabetic mice. In diabetic mice, the aqueous fraction of *B. trimera* (2000 mg/Kg twice daily) reduced the glycaemia after seven days of treatment. This effect was not associated with a reduction in body weight. These results suggest that *B. trimera* has a potential antidiabetic activity and indicate that food intake and body weight must be determined when evaluating metabolic parameters after prolonged administration of plant extracts. Dichloromethane and methanol extracts of *B. trimera* also had a relaxant effect on the smooth muscle of the *corpus carvenosum* of guinea pigs.⁵⁵

B. teindalensis is a herbal plant which is widely used in folk medicine in Ecuador as an anti-inflammatory, analgesic and antimicrobial remedy. Vidari *et al.*⁵⁶ investigated the antidiarrhoeal and antiulcer activities of their extracts in different mouse models. These results confirm the gastrointestinal protection afforded by *B. teindalensis* and suggest that the antiulcer effect could be partially due to its anti-inflammatory properties. Another *Baccharis* species with gastroprotective action is *B. illinita*, a plant used in folk medicine to treat gastric disorders.⁵⁷ This plant has a protective action against gastric lesions of the mucosa involving the maintenance of protective factors such as mucus, bicarbonate and blood flow, besides the reduction of gastric acid secretion.

Finally, medicinal plants, including propolis and *B. grisebachii*, contain 3-prenyl-cinnamic acid derivatives which are claimed to induce cancer cell apoptosis and used in antitumour drugs, health foods and cosmetics.^{58,59}

4. Toxicological Considerations

Though herbal products are generally regarded as safe, their toxicity in humans, plants and animals can not be ruled out. A few reports are available on the toxicological evaluation of *Baccharis* species.

The poisonous plant *B. coridifolia* causes necrosis in lymphoid tissues and the gastrointestinal tract of cattle, horses, sheep and rabbits. Rissi *et al.*⁶⁰ reported two outbreaks of *B. coridifolia* poisoning in cattle in southern Brazil. Cases occurred when stressed, hungry and thirsty cattle brought from pastures free of *B. coridifolia* were placed into pastures heavily infested by this poisonous plant. In the two outbreaks, morbidity was 21.73 and 22.51%, and lethality was virtually 100%. Clinical signs included mild bloating, instability of hind limbs,

muscle tremors, dry muzzle, dry faeces or diarrhoea, polydipsia and restlessness. Consistent necrosis findings included dehydration, large amounts of ruminal fluid, reddening and erosions of the mucosae of the forestomach. Degeneration and necrosis of the lining epithelium of the forestomach and of lymphoid tissue were the main histopathological changes encountered. Varaschin and Alessi⁶¹ reported an experimental poisoning of mice in order to establish an experimental model in a laboratory animal species. Mice reproduce most of the lesions observed in naturally poisoned cattle, and the use of this species as an experimental model is valid.

Other *Baccharis* species and compounds isolated from them have been reported to have phytotoxic properties. The inhibitory effects on plant growth by acetophenones and tremetones isolated from the aerial parts of *B. linearis*, *Baccharis magellanica* (Radin) and *Baccharis umbelliformis* D.C. collected in Chile were assayed.⁶² The effects on seedling growth, germination and respiration of the weedy target species of ryegrass, lettuce, green tomato and red clover were measured. These results indicate that the compounds interfere with the dicot preemergence properties, mainly energy metabolism of the seeds at the level of respiration. These compounds appear to have selective effects on the radicle rather than on the shoot growth of dicot seeds.

Baccharis boliviensis (Wedd.) Cabr., a plant with a wide distribution in the arid regions of north-western Argentina, inhibited the germination of the cacti *Trichocereus pasacana*.⁶³ Examples of another phytotoxic *Baccharis* species included the essential oil of *B. salicifolia*, which had toxic and repellent effects on *Trilobium castaneum*.⁶⁴

5. *Baccharis* plant cell cultures

Cultured plant cells may serve as sources of the various compounds characteristic of intact plants. Although the technology for plant cell culture exists, industrial applications have been limited to date, due to low economic viability and technological feasibility on a large scale. However, several examples of plant cell cultures have been reported from the genus *Baccharis*. These reports concern mainly the species *B. trimera*.

The influence of various growth regulators and medium concentrations, in different quantities, on the *in vitro* callus induction of *B. trimera* was evaluated.⁶⁵ It was found that the callus initiation was dependent on both the growth regulator and medium concentration. The highest callus induction and development were obtained by using 15 μM of 1-naphthaleneacetic acid as growth regulator and half strength of salts, vitamins and myo-inositol of Murashige and Skoog medium. *In vitro* shoot proliferation was obtained by using thidiazuran. An experiment was carried out under greenhouse conditions to evaluate the effects of arbuscular mycorrhizal fungi on growth and production of total phenols in *B. trimera*.⁶⁶ Arbuscular mycorrhizal fungi significantly influenced the production of total phenols in the *B. trimera* plants. The largest production of total phenols was obtained, independently of the manuring method, in plants inoculated with the species *Glomus claruma*.

De Bona *et al.*⁶⁷ studied the effect of indolbutiric acid and naphthaleneacetic acid from auxins on the rooting of cuttings from three *Baccharis* species: *B. articulata*, *B. trimera* and *B. stenocephala*. However, these results suggested that auxins did not affect rooting cuttings of these species.

6. Conclusions

The growing significance of natural products in drug discovery and development is obvious. *Baccharis* has been proved to be a very valuable genus to the discovery and utilization of medicinal natural products. As the investigation continues, this genus may prove to be a rich source of lead compounds needed for the development of new therapeutical agents. The collected information provides a means to understand the latest developments in the pharmacology and phytochemistry of the genus. The information summarized here is intended to serve as a reference tool to people in all fields of ethnopharmacology and natural products chemistry.

Acknowledgements

This work was supported by Programa de Cooperación Científica con Iberoamérica (PR 77/00-8992) from Ministerio de Educación, Cultura y Deporte. The technical assistance of Ms. P. Brooke-Turner is gratefully acknowledged.

References

1. Heinrich, M. *Curr. Top. Med. Chem.* **2003**, *3*, 141.
2. Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022.
3. Abad, M. J.; Latourrette, A.; Bermejo, P. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman Ed.; Elsevier: Oxford, 2005; Vol. 30, p 703.
4. Gonzaga, L.; Costa, I. M.; Pizzolatti, M. G. *Quim. Nova* **2005**, *28*, 85.
5. De Luca, M.; Zalles, J. *Flora Medicinal Boliviana. Diccionario Enciclopédico*; Los Amigos del Libro: Bolivia, 1992.
6. Gupta, M. P. *270 Plantas Medicinales Iberoamericanas*; Convenio Andrés Bello: Colombia, 1995.
7. Gomes, U. E.; De Carvalho, D. A. *Plantas Mediciniais no Dominio Dos Cerrados*; Editora UFPA: Brazil, 2001.
8. Di Stasi, L. C.; Akiko, C. *Plantas Mediciniais na Amazonia e na Mata Atlantica*; Editora UNESP: Brazil, 2002.

9. Lorenzi, H.; Abreu, F.J. *Plantas Mediciniais no Brazil, Natives e Exoticas*; Grafico Osmar Gomes: Brazil, 2002.
10. Malizia, R. A.; Cardell, D. A.; Molli, J. S.; Gonzalez, S.; Guerra, P. E.; Grau, R. J. *J. Essent. Oil. Res.* **2005**, *17*, 103.
11. Malizia, R. A.; Cardell, D. A.; Molli, J. S.; Gonzalez, S.; Guerra, P. E.; Grau, R. J. *J. Essent. Oil. Res.* **2005**, *17*, 194.
12. Biurrun, F.; Julián, R. H.; Lopez, M. L.; Zygadlo, J. A. *J. Essent. Oil Res.* **2005**, *17*, 122.
13. Arze, J. B. L.; Garneau, F. X.; Collin, G.; Jean, F. I.; Gaynon, H. *J. Essent. Oil Res.* **2004**, *16*, 429.
14. Simoes, C. A.; Debenedetti, S.; Spegazzini, E.; Mentz, L. A.; Matzenbacher, N. I.; Limberger, R. P.; Henriques, A. T. *Plants Syst. Evol.* **2005**, *253*, 23.
15. Alburquerque, M. R. J. R.; Souza, E. B.; Lins, M. V. D. S.; Nogueira, N. A. P.; Lemos, T. L. G.; Silveira, E. R.; Pessoa, O. D. L. *Arkivoc* **2004**, *6*, 59.
16. Zunino, M. P.; Lopez, M. L.; Zygadlo, J. A.; Lopez, A. G. *J. Essent. Oil Res.* **2004**, *16*, 29.
17. Akaike, S.; Sumino, M.; Sekine, T.; Seo, S.; Kimura, N.; Ikegami, F. *Chem. Pharm. Bull.* **2003**, *51*, 197.
18. Hayashi, K. I.; Kanamori, T.; Yamazoe, A.; Yamada, M.; Nozaki, H. *J. Nat. Prod.* **2005**, *68*, 1121.
19. Guo, Y.; Li, Y.; Xu, J.; Watanabe, R.; Oshima, Y.; Yamakuni, T.; Ohizumi, Y. *J. Nat. Prod.* **2006**, *69*, 274.
20. Cifuentes, D. A.; Borkowski, E. J.; Sosa, M. E.; Gianello, J. C.; Giordano, D. S.; Tonn, C. E. *Phytochemistry* **2002**, *61*, 899.
21. Juan, V. F.; Rossomando, P. C.; Giordano, O. S.; Saad, J. R. *Phytochemistry* **2002**, *61*, 389.
22. Feresin, G. E.; Tapia, A.; Giménez, A.; Ravelo, A. G.; Zacchino, S.; Sortino, M.; Schmeda-Hirschmann, G. *J. Ethnopharmacol.* **2003**, *89*, 73.
23. Verdi, L. G.; Brighente, I. M. C.; Schripsema, J.; Braz Filho, R.; Pizzolatti, M. G. *Biochem. Syst. Ecol.* **2004**, *32*, 837.
24. Simirgiotis, M. J.; Garcia, M.; Sosa, M. E.; Giordano, O. S.; Tonn, C. E. *J. Argent. Chem. Soc.* **2003**, *91*, 109.
25. Moreira, F. P. M.; Voutinho, V.; Pimentel, A. B.; Caro, M. S. P.; Costa, I. M.; Pizzolatti, M. G.; Delle, F. *Quim. Nova* **2003**, *26*, 309.
26. Moreira, F. P. M.; Branco, A.; Pizzolatti, M. G.; Morais, A. A.; Monache, F. D. *Biochem. Syst. Ecol.* **2003**, *31*, 319.
27. Kumazawa, S.; Yoneda, M.; Shibata, I.; Kanaeda, J.; Hamasaka, T.; Nakayama, T. *Chem. Pharm. Bull.* **2003**, *51*, 740.
28. Kumazawa, S.; Moneda, M.; Nakayama, T. *FFI Journal* **2004**, *209*, 132.
29. Park, Y. K.; Fukuda, I.; Ashida, H.; Nishiumi, S.; Yoshida, K.; Dausch, A.; Sato, H. H.; Pastore, G.M. *J. Agric. Food Chem.* **2005**, *53*, 10306.
30. Tapia, A.; Rodríguez, J.; Theoduloz, C.; Lopez, S.; Feresin, G. E.; Schmeda-Hirschmann, G. *J. Ethnopharmacol.* **2004**, *95*, 155.

31. Salcedo, L.; Pillco, A.; Rodrigo, G.; Sterner, O.; Almanza, G. R. *Rev. Boliv. Quim.* **2003**, *20*, 43.
32. Cordero, C. P.; Gomez-Gonzales, S.; Leon-Acosta, C. J.; Morantes-Medina, S. J.; Aristizabal, F. A. *Fitoterapia* **2004**, *75*, 225.
33. De Alencor, S. M.; De Aguiar, C. L.; Paredes-Guzman, J.; Park, Y. K. *Ciencia Rural* **2005**, *35*, 909.
34. Park, Y. K.; Paredes, J. F.; Aguiar, C. L.; Alencao, S. M.; Fujiwara, F. Y. *J. Agric. Food Chem.* **2004**, *52*, 1100.
35. Nagatani, Y.; Warasina, T.; Noro, T. *Nat. Med.* **2002**, *56*, 195.
36. Nagatani, Y.; Warasina, T.; Noro, T. *Chem. Pharm. Bull.* **2002**, *50*, 583.
37. Midorikawa, K.; Banskota, A. H.; Tezuka, Y.; Matsushige, K.; Message, D.; Huertas, A. A. G.; Kadota, S. *Wakan Iyakugaku Zasshi* **2003**, *20*, 187.
38. De Oliveira, S. Q.; Dal-Pizzol, F.; Gosmann, G.; Guillaume, D.; Moreira, J. C. F.; Schenkel, E. P. *Free Rad. Res.* **2003**, *37*, 555.
39. Da Silva Leitao, D. P.; Da Silva Filho, A. A.; Polizello Morselli, A. C. M.; Basto, J.K.; Spadaro, A.C.C. *Biol. Pharm. Bull.* **2004**, *27*, 1834.
40. Santos, F. A.; Bastos, E. M. A. F.; Maia, A. B. R. A.; Uzeda, M.; Carvalho, M. A. R.; Farias, L. M.; Moreira, E. S. A. *Phytother. Res.* **2003**, *17*, 285.
41. Orsi, R. O.; Sforcin, J. M.; Funari, S. R. C.; Bankova, N. *Int. Immunopharmacol.* **2005**, *5*, 359.
42. Gekker, G.; Hu, S.; Spivak, M.; Lokensgard, J. R.; Peterson, P. K. *J. Ethn- pharmacol.* **2005**, *102*, 158.
43. Sulsen, V.; Guida, C.; Coussio, J.; Paveto, C.; Muschietti, L.; Martino, V. *Parasitol. Res.* **2006**, *98*, 370.
44. Rojas, A.; Mendoza, S.; Moreno, J.; Arellano, R. O. *Int. J. Phytother. Phytopharmacol.* **2003**, *10*, 416.
45. Tequida, M.; Cortez, M.; Rosas, E. C.; Lopez, S.; Corrales, C. *Rev. Iberoam. Micol.* **2002**, *19*, 84.
46. Derno, M.; Oliva, M. M.; Lopez, M. L.; Zunino, M. P.; Zygadlo, J. *Pharm. Biol.* **2005**, *43*, 129.
47. Da Silva, A. A.; Bueno, P. C. P.; Gregorio, L. E.; Andrade, M. L.; Albuquerque, S.; Bastos, J. K. *J. Pharm. Pharmacol.* **2004**, *56*, 1195.
48. Parejo, I.; Viladomat, F.; Bastida, J.; Rosas Romero, A.; Saavedra, G.; Murcia, M. A.; Jiménez, A. M.; Codina, C. *Life Sci.* **2003**, *73*, 1667.
49. Abad, M. J.; Bessa, A. L.; Ballarin, B.; Aragón, O.; Gonzales, E.; Bermejo, P. *J. Ethnopharmacol* **2006**, *103*, 338.
50. Coelho, M. G. P.; Reis, P. A.; Gava, V. B.; Marques, P. R.; Gayer, C. R.; Laranja, G. A. T.; Felzenswalb, I.; Sabino, K. C. C. *Toxicol. Lett.* **2004**, *154*, 69.
51. Sforcin, J. M.; Orsi, R. O.; Bankova, V. *J. Ethnopharmacol.* **2005**, *98*, 301.

52. Simoes, C. A.; Queiroz, E. F.; Henriques, A. T.; Hostettmann, K. *Phytochem. Anal.* **2005**, *16*, 307.
53. Januario, A. H.; Santos, S. L.; Marcusi, S.; Mazzi, M. V.; Pietro, R. C. L. R.; Sato, D. N.; Ellena, J.; Sampaio, S. V.; Franca, S. C.; Soares, A. M. *Chem. Biol. Interact.* **2004**, *150*, 243.
54. Oliveira, A. C. P.; Endringer, D. C.; Amorin, L. A. S.; Brandao, M. G. L.; Coelho, M. M. J. *Ethnopharmacol.* **2005**, *102*, 465.
55. Hnatsyzyn, O.; Moscatelli, V.; Garcia, J.; Rondina, R.; Costa, M.; Arranz, C.; Balaszczuk, A.; Ferraro, G.; Coussio, J. P. *Int. J. Phytother. Phytopharmacol.* **2003**, *10*, 669.
56. Vidari, G.; Vita, P.; Zarzuelo, A.; Galvez, J.; Zafra, C.; Chiriboga, X.; Berenguer, B.; La Casa, C.; Alarcón de la Lastra, C.; Motilva, V.; Martin, M. J. *Pharm. Biol.* **2003**, *41*, 403.
57. Hatsuko, C.; Setim, C.; Rieck, L.; Andrade, M. C. *Pharmacol. Res.* **2003**, *47*, 93.
58. Maruyama, H.; Araki, Y.; Mishima, S.; Asao, S. U.S. Patent 4,026,760, 2004.
59. Shibata, K.; Kaneeda, J.; Kawai, M.; Yoshimura, K.; Tazawa, S. Patent 1,617,06, 2004.
60. Rissi, D. R.; Pech, R. R.; Figuera, R. A.; Cagnini, D. Q.; Kommers, G. D.; Barros, C. S. L. *Pesp. Vet. Bras.* **2005**, *25*, 111.
61. Varaschin, M. S.; Alessi, A. C. *Vet. Hum. Toxicol.* **2003**, *45*, 42.
62. Cespedes, C. L.; Uchoa, A.; Salazar, J. R.; Perich, F.; Pardo, F. *J. Agric. Food Chem.* **2002**, *50*, 2283.
63. Cazon, A.; De Viana, M. L.; Gianello, J. C. *Ecol. Austral* **2002**, *12*, 73.
64. Garcia, M.; Donadel, O. J.; Ardanaz, C. E.; Tonn, C. E.; Sosa, M. E. *Pest Manag. Sci.* **2005**, *61*, 612.
65. Silva, F.G.; Pinto, J. E. B. P.; Sales, J. F.; Divino, S. P.; Bertolucci, S. K. V. *Cienc. Agrotec.* **2003**, *27*, 541.
66. Freitas, M. S. M.; Martins, M. A.; Carvalho, A. J. C.; Carneiro, R. F. V. *Rev. Bras. Plantas Med.* **2004**, *6*, 30.
67. De Bona, C. M.; Biasi, L. A.; Zanette, F.; Nakashima, T. *Rev. Bras. Plantas Med.* **2005**, *7*, 26.