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Antimicrobial and Antioxidant Activities of Some Plant Extracts

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1. Introduction

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (N'guessan et al., 2007). The abusive and indiscriminate use of antimicrobial compounds over many years is the main factor responsible for the appearance of the phenomenon of bacterial resistance to such compounds (Andremont, 2001). With increased incidence of resistance to antibiotics, natural products from plants could be interesting alternatives (Lu et al., 2007; Mbwambo et al., 2007). Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to demonstrate such efficacy (Benoit-Vical et al., 2006; Senatore et al., 2007; Singh et al., 2007). On the other hand, free radicals are known to be the major cause of various chronic and degenerative diseases. Oxidative stress is associated with pathogenic mechanisms of many diseases including atherosclerosis, neurodegenerative diseases, cancer, diabetes and inflammatory diseases, as well as aging processes. It is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants, and it also includes their elimination by protective mechanisms, referred to as antioxidative systems. This imbalance leads to damage of important biomolecules and organs with potential impact on the whole organism. Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress (Duracková, 2010; Reuter et al., 2010). Natural antioxidants have been studied extensively for decades in order to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage. To date, many plants have been claimed to pose beneficial health effects such as antioxidant properties (Kaur & Arora, 2009; Newman & Cragg 2007). According to World Health Organization (WHO), 65 - 80% of the world populations rely on traditional medicine to treat various diseases (Kaur & Arora, 2009). The WHO recommends

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research into the use of the local flora for therapeutic purposes, with the intention of reducing the number of people excluded from effective therapy in the government health systems, which could constitute an economically viable alternative treatment of several diseases, especially in developing countries (Gonçalves et al., 2005; WHO, 2002). The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000 - 500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller (Mahesh & Satish, 2008). In this scenario, the screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases, and about 20% of the plants or their extracts in the world have been submitted to biological or pharmacological tests (Rayne & Mazza, 2007; Suffredini et al., 2004). The phytochemical research based on ethnopharmacological information is considered an effective approach in the discovery of new agents from higher plants (Chen et al., 2008; Duraipiyan, 2006). Thus, in this study, methanol extracts of different parts of 70 species, most of them commonly used in Brazil for treating conditions likely to be associated with microorganisms, were evaluated for their antimicrobial and antioxidant activity. Furthermore, a phytochemical screening of the bioactive extracts was performed.

2. Materials and methods

2.1 Plant material

Specimens of 70 species (Table 1) were collected in Juiz de Fora, Minas Gerais, Brazil. A voucher specimen was deposited at the Herbarium Leopoldo Krieger (CESJ) of Federal University of Juiz de Fora.

2.2 Preparation of plant extracts

The dried parts of the plant (50 g each) were powdered and macerated with methanol (3 x 200 mL) for five days at room temperature. After evaporation of the solvent under reduced pressure, the respective methanol extracts were obtained. All the extracts were kept in tightly stoppered bottles under refrigeration (4 °C) until used for the biological testing and phytochemical analysis.

2.3 Antioxidant activity

2.3.1 DPPH assay

The free radical scavenging activity of samples and standard α-tocopherol solutions in methanol was determined based on their ability to react with stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Govidarajan et al., 2003). The plant samples at various concentrations (7.8 to 250 µg/mL) were added to a 152 µM solution of DPPH in methanol. After incubation at 37 °C for 30 min, the absorbance of each solution was determined at 517 nm. The antioxidant activity of the samples was expressed as IC_{50} (inhibitory concentration), which was defined as the concentration (in µg/mL) of sample required to inhibit the formation of DPPH radicals by 50%. Ascorbic acid, α-tocopherol, BHT, rutin and quercetin were used as positive control.

Family	Botanical name [Voucher number]	Common name	Plant parts used[a]	Ethnomedical uses (Albuquerque, 1989; Alice, 1995; Corrêa, 1984; Camargo, 1988; Corrêa et al., 1998; Lorenzi, 2000; Lorenzi & Matos, 2002; Matos, 2000; Moreira & Guarim-Neto, 2009; Morim, 2010; Panizza, 1998)
Amaranthaceae	*Alternanthera brasiliana* (L.) Kuntze [CESJ 48585]	Acônito-do-mato, caaponga, cabeça-branca	F, L	Diuretic, digestive, depurative, liver and bladder diseases, astringent, laxative, cough
Apocynaceae	*Allamanda cathartica* L. [CESJ 47443]	Alamanda, buiussu, carolina, cipó-de-leite	B, F, L, La	Scabies and lice elimination, purgative, parasitosis, fever, treatment of jaundice, complications of malaria, enlarged spleen, laxative
	Aspidosperma olivaceum Müll. Arg. [CESJ 49229]	Guatambu, guatambu-branco, guatambu-amarelo, tambu		No use reported
Asteraceae	*Acanthospermum australe* (Loefl.) Kuntze [CESJ 47438]	Picão-da prata, carrapicho-rasteiro, mata-pasto	AP	Liver diseases, diaphoretic, gonorrhea, malaria
	Achillea millefolium L. [CESJ 46087]	Novalgina, erva-de-carpinteiro, aquiléia, milefólio	L	Fever, head and general aches, colds indigestion
	Anthemis cotula L. [CESJ 48584]	Camomila-do-campo	F, L	Fever, gastrointestinal disorders, dysenteria, gouty arthritis
	Baccharis trimera (Less.) DC. [CESJ 46074]	Carqueja	L	Gastrointestinal and liver diseases, diabetes, inflammation
	Bidens segetum Mart. ex Colla [CESJ 47437]	Picão-do-mato		No use reported
	Carduus marianus L. [CESJ 48581]	Cardo-mariano, cardo-santo, cardo-de-nossa-senhora, cardo-branco	S, SB	Appetite stimulant, diuretic, tonic, liver cell regenerator, gastrointestinal disorders, bile flow stimulant, cirrhosis, hepatitis
	Matricaria chamomilla L. [CESJ 47435]	Camomila, camomila-romana, camomila-comum	F	Digestive, sedative, colic treatment, appetite stimulant, carminative
	Piptocarpha macropoda (DC.) Baker [CESJ 49448]			No use reported
	Solidago chilensis Meyen [CESJ 678]	Arnica, erva-de-lagarto, erva-lanceta, espiga-de-ouro	L	Stomachic, astringent

	Vernonanthura divaricata (Spreng.) H. Rob. [CESJ 49450]	Cambará-açu		No use reported
	Vernonia condensata Baker [CESJ 46086]	Boldo, alumã, alcachofra, figatil, cidreira-da-mata	L	Carminative, liver insufficiency, inflammation of the gallbladder, analgesic, syphilitic, appetite stimulant, liver and stomach disorders
Bignoniaceae	*Stenolobium stans* (L.) Seem [CESJ 46071]	Ipê-de-jardim, ipê-amarelo-de-jardim, ipêzinho-de-jardim	B, L	Diabetes, diuretic, tonic, antisyphilitic, vermifuge pains in the stomach
Bixaceae	*Bixa orellana* L. [CESJ 46077]	Urucum	S	Expectorant, laxative, stomachic, anti-bleeding, healing, dyspepsia liver and heart disorders, tuberculosis, skin problems, fever, inflammation
Commelinaceae	*Commelina robusta* Kunth [CESJ 50021]	Batata-ovo, manobi-açu, trapoeraba-açu	AP, L, R	Back pain, urinary tract infections with fever, trauma, wounds illnesses prevention
Euphorbiaceae	*Alchornea triplinervia* (Spreng.) Müll. Arg. [CESJ 49442]	Tapiá-vermelho, tapiá-guaçu-branco, pau-óleo	L	Gastric disturbances
	Acalypha brasiliensis Müll. Arg. [CESJ 50011]	Tapa-buraco		No use reported
Fabaceae	*Chamaecrista desvauxii* (Collad.) Killip [CESJ 23372]	Sene, acácia, carqueja-do-tabuleiro, flor-de-lilás, capim reis	L, R	Wounds in the uterus, worms, bowel, arthritis
	Samanea tubulosa (Benth.) Barneby & J.W. Grimes [CESJ 49743]	Amendoim-de-veado, árvore-da-chuva e pau-de-cangalha	L, SB	Colds and high blood pression
	Senna macranthera (DC. ex Collad.) H.S. Irwin & Barneby [CESJ 46159]	Manduirana, pau-fava, aleluia, mamangá, fedegoso		No use reported
	Senna multijuga (Rich.) H.S. Irwin & Barneby [CESJ 49783]	Pau-cigarra, canafístula, aleluia	S	Ophthalmic and skin infections
	Stylosanthes scabra Vogel [CESJ 47436]	Alfafa do nordeste, alfafa do campo		No use reported

Flacourtiaceae	*Casearia sylvestris* Sw. [CESJ 49218]	Guaçatonga, bugre-branco, café-bravo, café-de-frade	L, SB	Burns, cutaneous injuries, herpes, tonic, depurative, rheumatism, inflammation, analgesic, hemostatic, gastritis
Hypericaceae	*Vismia magnoliifolia* Schltdl. & Cham. [CESJ 49759]			No use reported
Lacistemataceae	*Lacistema pubescens* Mart. [CESJ 49751]	Espeto-vermelho, canela- vermelha, sabonete, cafezinho		No use reported
Lamiaceae	*Hyptis suaveolens* (L.) Poit [CESJ 46089]	Bamburral, erva-canudo, arbusto-selvagem	AP	Cramps, skin infections, respiratory tract infections, nasal congestion, fever, flu
	Ocimum basilicum L. [CESJ 46161]	Manjericão, alfavaca	L	Gastrointestinal disorders, fever, digestive, bacterial infections, parasitosis
	Peltodon radicans Pohl [CESJ 46158]	Paracari, hortelã-do-mato, rabugem-de-cachorro	AP	Expectorant, pertussis, cough, asthma, sneezing, carminative, dermatites, scorpion and snake bites, antispasmodic, syphilitic, parasitosis, diuretic
	Plectranthus neochilus Schltr. [CESJ 46580]	Boldo	L	Treatment of respiratory infections or related symptoms
	Salvia officinalis L. [CESJ 46579]	Sálvia, salva	AP	Infections diseases, astringent
Lauraceae	*Nectandra rigida* (Kunth) Nees [CESJ 49221]	Canela-amarela	B	Rheumatism
Lythraceae	*Cuphea ingrata* Cham. & Schltdl. [CESJ 47432]	Sete-sangrias-do-campo	WP	Fever, venereal diseases, rheumatism
Malpighiaceae	*Byrsonima variabilis* A. Juss. [CESJ 49240]	Murici		No use reported
Malvaceae	*Sida glaziovii* K. Schum. [CESJ 47439]	Guanxuma-branca		No use reported
Melastomataceae	*Miconia latecrenata* (DC.) Naudin [CESJ 49990]	Pixirica-preta		No use reported
	Tibouchina grandifolia Cogn. [CESJ 40445]	Orelha-de-onça		No use reported

	Tibouchina granulosa (Desr.) Cogn. [CESJ 49761]	Quaresmeira		No use reported
	Tibouchina mutabilis (Vell.) Cogn. [CESJ 46175]	Manacá		No use reported
	Trembleya parviflora (D. Don) Cogn. [CESJ 49219]	Manacá		No use reported
Monimiaceae	*Mollinedia schottiana* (Spreng.) Perkins [CESJ 48921]			No use reported
	Siparuna guianensis Aubl. [CESJ 49778]	Capitiú, caá-pitiú, erva-santa, fedorenta, negramina, negra-mena	SB	Carminative, stimulant, fever, antidispeptic, diuretic, muscle spasms prevention, headache, inflammation
Myrtaceae	*Eugenia cumini* (L.) Druce [CESJ 46601]	Jambolão, cereja, jamelão, jalão	B, Fr	Diabetes
	Myrcia splendens (Sw.) DC. [CESJ 49230]	Guamirim, folha-miúda		No use reported
Piperaceae	*Piper corcovadensis* (Miq.) C. DC. [CESJ 49993]	João-brandinho	L	Mucous membranes anesthesia (mouth), rheumathism, cough
Poaceae	*Cymbopogon citratus* (DC) Stapf. [CESJ 46582]	Capim-cheiroso, erva-cidreira, capim-cidreira, capim-limão	L	Calmant, gastrointestinal disorders, infections diseases, colic treatment, anxiety
Rosaceae	*Eriobotrya japonica* (Thunb.) Lindl. [CESJ 47434]	Nespereira, ameixeira	FR, L	Cough, asthma, chronic bronchitis, phlegm, high fever and gastroenteric disorders
	Rubus rosifolius Sm. [CESJ 48580]	Morango-silvestre, amora-do-mato	AP	Infectious and dolorous diseases
	Rubus urticifolius Poir. [CESJ 46583]	Nhambuí, árvore-preta, amora-do-silva	Fr	Throat diseases, diuretic
Rubiaceae	*Amaioua intermedia* Mart. [CESJ 49994]	Canela-de-veado, vachila, carvoeiro, pimentão-bravo, marmelada-brava		No use reported
Rutaceae	*Zanthoxylum rhoifolium* Lam. [CESJ 49782]	Mamica-de-cadela	L, SB	Toothache, earache, malaria

Sapindaceae	*Allophylus semidentatus* (Miq.) Radlk [CESJ 49774]	Fruta-de-faraó		No use reported
	Cupania oblongifolia Mart. [CESJ 49447]	Pau-magro, caboatã	B, L	Weight loss
	Sapindus saponaria L. [CESJ 46172]	Sabão-de-soldado	Fr, R, SB	Antitussive, adstringent, , calmant, diuretic, expectorant
Solanaceae	*Solanum sellowianum* Dunal [CESJ 49225]			No use reported
	Solanum swartzianum Roem. & Schult. [CESJ 49226]	Barbaso, fruta-de-pombo		No use reported
Tropaeolaceae	*Tropaeolum majus* L. [CESJ 46586]	Capuchinha, chaguinha, alcaparra-de-pobre, chagas, mastruço-do-peru	L	Scurvy, sepse, expectorant, urinary, gastrointestinal and dermatological disinfectant
Turneraceae	*Turnera subulata* Sm. [CESJ 47442]	Chanana, flor-do-Guarujá	R	Amenorrhea
Typhaceae	*Typha dominguensis* Pers. [CESJ 49773]	Taboa	F, R	Treatment of burns, wounds and inflammation, kidney stones and diarrhea
Urticaceae	*Cecropia pachystachya* Trécul [CESJ 46591]	Embaúba, umbaúba, torém	B, L	Cough, expectorant, asthma and diabetes
Verbenaceae	*Lippia pseudo-thea* Schauer [CESJ 46171]	Capitão-do-matto, câmara, chá-de-frade, chá-de-pedestre, cidrilha	L	Gastrointestinal disorders, expectorant, stimulant, rheumatism
	Lippia hermannioides Cham. [CESJ 46088]			No use reported
	Lippia alba (Mill.) N.E. Br. ex Britton & P. Wilson [CESJ 46177]	Erva-cidreira, erva-cidreira-do-campo, alecrim-do campo, salsa	L, R	Hypertension, stomach cramps, nausea, coughs, colds
	Lippia rubella (Moldenke) T.R.S. Silva & Salimena [CESJ 46178]			No use reported

	Lippia sidoides Cham. [CESJ 46180]	Alecrim-pimenta, alecrim-do-nordeste, estrepa-cavalo, alecrim-bravo	F, L	Allergic rhinitis, throat and mouth infections, antiseptic, skin and scalp disorders
	Lantana camara L. [CESJ 47441]	Camará, cambará, chumbinho, camará - de-chumbo	L	Treatment of respiratory diseases such as cough, bronchitis, pertussis, colds, flu, asthma, hoarseness, expectorant, antispasmodic, rheumatism, digestive, diuretic
	Aloysia floribunda M. Martens & Galeotti [CESJ 46584]			No use reported
Vitaceae	*Cissus verticillata* (L.) Nicolson & C.E. Jarvis [CESJ 46587]	Anil-trepador, cipó-pucá, cipó-puci, puçá, insulina, insulina-vegetal	AP, L	Tachycardia, hypertension, dropsy, anemia, leakage, tremors, activator of blood circulation, diabetes, anticonvulsant
Zingiberaceae	*Hedychium coronarium* J. König [CESJ 50022]	Gengibre-branco, lírio-do-brejo, lágrima-de-moça, lírio-branco, borboleta, lágrima-de-vênus	Fr, Rh	Arthritis, diabetes, headache and hypertension

[a]AP, Aerial Parts; B, Bark; F, Flowers; Fr, Fruits; L, Leaves; La, Latex; R, Root; Rh, Rhizome; S, Seeds; SB, Stem Bark; WP, Whole Plant

Table 1. Ethnomedical data on medicinal plants.

2.3.2 Reducing power assay

The reducing power was determined by the method of Oyazu (1986), based on the chemical reaction of Fe(III) to Fe(II). Ten mg of each sample were mixed with potassium phosphate buffer (0.2 M, pH 6.6) (2.5 mL) and potassium ferricyanide (10 g/L) (2.5 mL). The mixture was incubated at 50 °C for 20 min. A 2.5 mL aliquot of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3.000 g for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% $FeCl_3$ (0.5 mL), and the absorbance was measured at 700 nm. Ascorbic acid was used as reference material. All tests were performed in triplicate. Increase in absorbance of the reaction indicated the reducing power of the samples. A higher absorbance indicated a higher reducing power. EC_{50} (effective concentration) values (μg/mL) were calculated and indicate the effective concentration at which the absorbance was 0.5 for reducing power.

2.3.3 β-carotene - linoliec acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene/linoleic acid mixture was prepared as follows: 50 μL of β-carotene (10 mg/mL) in chloroform (HPLC grade), 20 μL linoleic acid, 200 μL Tween 40 and 1 mL of chloroform was added. Chloroform was completely evaporated using a vacuum evaporator. Then, 30 mL of distilled water saturated with oxygen (30 min 100 mL/min) were added with vigorous shaking, and 250 μL of the reactive mixture and 10 μL of the extracts (40 μg/mL) were added in a microplate and

incubated at 45 °C to accelerate oxidation reactions and start the bleaching of β-carotene. The absorbance readings were taken immediately at intervals of 15 min for 120 min in spectrophotometer at 470 nm (Duarte-Almeida et al., 2006). The same procedure was repeated with the antioxidant flavonoid quercetin as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were expressed as percentage inhibition (1).

$$\text{Inhibition } (\%) = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100 \quad (1)$$

2.4 Antimicrobial assay

2.4.1 Microbial strains

The samples were evaluated against a panel of microorganisms, including the bacterial strains *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* serovar Typhimurium (ATCC 13311), *Shigella sonnei* (ATCC 11060), *Klebsiella pneumoniae* (ATCC 13866), *Escherichia coli* (ATCC 10536), *Bacillus cereus* (ATCC 11778), and the yeasts *Candida albicans* (ATCC 18804) and *Cryptococcus neoformans* (ATCC 32608).

2.4.2 Serial dilution assay for determination of the minimal inhibitory concentration (MIC)

The MIC of each extract was determined by using the broth microdilution techniques for bacteria and yeasts, respectively (Bouzada et al., 2009; NCCLS, 2002). MIC values were determined in RPMI 1640 buffered to pH 7.0 with MOPS for yeasts and Mueller Hinton broth (MHB) for bacteria. Bacterial strains were cultured overnight at 37 °C in Mueller Hinton agar (MHA). Yeasts were cultured for 48 h at 30 °C in Sabouraud dextrose agar (SDA). Sample stock solutions were two-fold diluted from 500 to 2.0 μg/mL (final volume = 80 μL) and a final DMSO concentration ≤ 1%. Then, RPMI or MHB (100 μL) was added onto microplates. Finally, 20 μL of 10^6 CFU/mL (values of 0.08 - 0.10 at 625 nm, according to McFarland turbidity standards) of standardized yeasts and bacterial suspensions were inoculated onto microplates and the test was performed in a volume of 200 μL. Plates were incubated at 30 °C for 48 h for yeasts and at 37 °C for 24 h for bacteria. The same tests were performed simultaneously for growth control (RPMI + yeast and MHB + bacteria) and sterility control (RPMI or MHB + extract). The MIC values were calculated as the highest dilution showing complete inhibition of the tested strain. Chloramphenicol and Amphotericin B were used as reference drugs for bacteria and yeasts, respectively.

2.5 Phytochemical studies

A portion of each extract that was subjected for the biological screening was used for the identification of the major secondary metabolites employing the protocols described by Matos (1997). Briefly, the extract (1 mg/mL) was submitted to the following identification reactions: The characterization for tannins was performed by gelatin, iron salt and lead acetate reactions. Triterpenoids and sterols were investigated by Liebermann-Burchard reagent and the alkaloids analysis was done by precipitation reactions with the reagents of Dragendorff, Bouchardat, Mayer and Bertrand. For the research of flavonoids, the reactions

of Shinoda and aluminum chloride were employed and the presence of saponins was determined by the formation of foam.

2.6 Statistical analysis

DPPH, reducing power and β-carotene/linoleic acid assays were carried out in triplicates. The results were expressed as mean ± standard deviation (SD). All statistical analysis were conducted using Graph Pad Prism software.

3. Results and discussion

The paper describes the antimicrobial and antioxidant activities and the phytochemical profile of some methanol extracts belonging to Brazilian traditional medicinal plants, most of them commonly used for treating conditions likely to be associated with microorganisms.

The major classes of phytocompounds of the bioactive extracts are presented in Table 2.

Plant species	Part tested[a]	Phytocompounds[b]					
		Al	Tr	St	Ta	Sa	Fl
Alternanthera brasiliana	AP	-	-	+	-	-	+
Allamanda cathartica	L	+	-	+	+	-	+
Acanthospermum australe	AP	+	-	+	+	+	+
Achillea millefolium	L	+	-	+	-	-	+
Anthemis cotula	L	-	+	-	-	-	+
Anthemis cotula	F	-	-	+	-	-	-
Baccharis trimera	AP	-	-	+	+	-	-
Bidens segetum	L	-	-	+	+	-	+
Carduus marianus	L	-	-	+	+	-	+
Matricaria chamomilla	L	+	+	-	+	-	+
Piptocarpha macropoda	L	+	+	-	+	-	+
Solidago chilensis	L	+	+	-	+	+	+
Vernonanthura divaricata	L	+	-	+	+	-	+
Stenolobium stans	L	+	-	+	+	-	+
Bixa orellana	L	+	-	+	+	+	+
Alchornea triplinervia	L	+	+	-	-	-	-
Acalypha brasiliensis	L	+	+	-	+	-	+
Chamaecrista desvauxii	L	+	+	-	+	-	+
Samanea tubulosa	L	+	+	-	+	-	+
Senna macranthera	L	+	-	+	+	-	+
Senna multijuga	F	+	+	-	+	-	+
Stylosanthes scabra	A	-	-	+	+	-	+
Casearia sylvestris	L	+	-	+	+	+	+
Vismia magnoliifolia	L	-	+	-	+	-	+

Plant species	Part tested[a]	Phytocompounds[b]					
		Al	Tr	St	Ta	Sa	Fl
Lacistema pubescens	L	-	+	-	+	-	+
Hyptis suaveolens	L	+	-	+	-	-	+
Ocimum basilicum	L	+	-	+	+	-	+
Peltodon radicans	L	-	+	-	-	-	+
Salvia officinalis	L	+	-	+	-	+	+
Nectandra rigida	L	-	+	-	+	-	+
Cuphea ingrata	AP	+	-	+	+	+	+
Byrsonima variabilis	L	+	+	-	+	+	+
Sida glaziovii	AP	-	-	+	-	+	+
Miconia latecrenata	L	+	-	+	-	+	+
Tibouchina grandifolia	L	+	+	-	+	-	+
Tibouchina granulosa	L	-	-	+	+	-	-
Tibouchina mutabilis	L	+	-	+	+	+	-
Eugenia cumini	L	+	+	-	-	-	+
Myrcia splendens	L	+	-	+	+	-	-
Piper corcovadensis	L	-	-	+	-	+	-
Eriobotrya japonica	L	+	-	+	-	-	+
Rubus rosifolius	L	+	-	+	+	+	+
Amaioua intermedia	L	-	+	-	-	-	+
Cupania oblongifolia	L	-	+	-	+	-	+
Sapindus saponaria	Fr	+	+	-	+	+	-
Solanum swartzianum	L	+	-	+	+	-	+
Tropaeolum majus	F	+	+	-	-	-	+
Turnera subulata	L	+	-	+	+	-	+
Cecropia pachystachya	L	+	+	-	+	-	+
Lippia pseudo-thea	L	+	+	-	+	+	+
Lippia hermannioides	L	+	+	-	+	-	+
Lippia alba	AP	+	-	+	+	+	+
Lippia rubella	AP	+	+	-	+	+	+
Lippia sidoides	AP	+	+	-	+	-	+
Lantana camara	L	-	-	+	+	-	+
Lantana camara	F	-	+	-	+	-	+
Aloysia floribunda	L	-	+	-	-	-	+
Cissus verticillata	L	+	-	+	-	-	+

[a]AP, Aerial Parts; F, Flowers; Fr, Fruits; L, Leaves. [b]Al, Alkaloids; Tr, Triterpenes; St, Sterols; Ta, Tannins; Sa, Saponins; Fl, Flavonoids

Table 2. Phytocompounds of methanol extracts of the active medicinal plants.

Plant species	Part tested[a]	MIC (μg/mL)[b,c]								
		Sa	Pa	Bc	Ss	St	Ec	Kp	Ca	Cn
Alternanthera brasiliana	AP	-	-	-	-	-	-	-	39	-
Allamanda cathartica	L	-	-	-	-	-	-	-	39	-
Achantospermum australe	AP	-	-	-	-	-	-	-	39	-
Anthemis cotula	L	-	-	-	-	-	-	-	39	-
Anthemis cotula	F	-	-	-	-	156	-	-	39	-
Baccharis trimera	AP	-	-	-	-	-	-	-	-	39
Bidens segetum	L	-	156	156	5	156	-	-	-	-
Carduus marianus	L	-	-	-	-	-	-	-	39	-
Matricaria chamomilla	L	300	78	-	-	-	-	-	-	-
Piptocarpha macropoda	L	-	-	78	-	-	-	-	78	300
Solidago chilensis	L	-	-	-	-	-	-	-	39	-
Vernonanthura divaricata	L	-	-	-	-	-	-	-	-	156
Bixa orellana	L	-	-	-	-	-	-	-	156	-
Alchornea triplinervia	L	-	-	-	-	-	-	-	-	78
Acalypha brasiliensis	L	-	-	-	-	-	-	-	-	78
Chamaecrista desvauxii	L	5	5	-	5	78	-	300	-	-
Samanea tubulosa	L	39	39	39	-	-	-	-	300	156
Senna macranthera	L	-	-	156	300	156	-	-	-	-
Senna multijuga	F	300	78	39	156	78	300	39	-	20
Stylosanthes scabra	AP	-	2	39	5	-	-	-	-	-
Casearia sylvestris	L	-	-	-	-	-	-	-	-	-
Vismia magnoliifolia	L	-	-	-	39	-	-	-	300	156
Lacistema pubescens	L	-	-	-	39	-	-	-	-	-
Nectandra rigida	L	-	-	300	-	-	-	-	-	-
Cuphea ingrata	AP	-	-	39	-	-	-	-	39	-
Sida glaziovii	AP	-	-	-	-	-	-	-	39	-
Miconia latecrenata	L	-	-	-	-	-	-	-	-	300
Tibouchina grandifolia	L	5	-	-	-	300	-	-	-	-
Tibouchina granulosa	L	-	39	39	39	-	-	-	-	-
Eugenia cumini	L	-	-	-	-	-	-	-	-	39
Myrcia splendens	L	-	-	300	-	-	-	-	-	-
Piper corcovadensis	L	-	-	-	-	-	-	-	-	78
Rubus rasaefolius	L	-	-	-	-	-	-	-	39	-
Amaioua intermedia	L	-	-	-	-	-	-	-	-	78
Cupania oblongifolia	L	-	39	39	39	-	39	-	-	-
Sapindus saponaria	Fr	-	-	-	-	-	-	-	156	300
Solanum swartzianum	L	-	-	-	-	-	-	-	-	78
Tropaeolum majus	F	-	-	-	-	-	-	-	-	39
Turnera subulata	L	-	-	-	-	-	-	-	78	-
Cecropia pachystachya	L	-	-	-	-	-	-	-	-	39
Lippia pseudothea	L	-	-	156	-	-	-	-	-	-
Lippia hermannioides	L	-	-	78	-	-	-	-	-	-
Lippia sidoides	AP	-	-	78	-	-	-	-	-	-
Lantana camara	L	-	-	-	-	-	-	-	39	-
Lantana camara	F	-	-	-	-	-	-	-	39	-

Plant species	Part tested[a]	MIC (µg/mL)[b,c]								
		Sa	Pa	Bc	Ss	St	Ec	Kp	Ca	Cn
Aloysia floribunda	L	5	-	-	-	-	-	-	39	-
Cissus verticillata	L	-	-	-	-	-	-	-	-	156
Positive Controls										
Chloramphenicol		63	16	1.0	1.0	1.0	16	4.0		
Amphotericin B									0.08	0.04

[a]AP, Aerial Parts; F, Flowers; Fr, Fruits; L, Leaves
[b]Sa, *Staphylococcus aureus*; Pa, *Pseudomonas aeruginosa*; St, *Salmonella enterica* serovar Typhimurium; Ss, *Shigella sonnei*; Kp, *Klebsiella pneumoniae*; Ec, *Escherichia coli*; Bc, *Bacillis cereus*. [c]means MIC ≥ 300 µg/mL

Table 3. Antimicrobial activity of methanol extracts of the medicinal plants.

The results of the antimicrobial screening of the most active extracts are summarized in Table 3. The MIC values presented in this study for the extracts tested ranged from 300 to 5 µg/mL. All the extracts exhibited activity against at least one organism tested. According to Cos et al. (2006), plant extracts with MIC values below 100 µg/mL are very promising. So, *Bidens segetum, Chamaecrista desvauxii* and *Stylosanthes scabra* presented a very strong activity against *Shigella sonnei* with MIC of 5 µg/mL. *Chamaecrista desvauxii* and *Stylosanthes scabra* were also very active against *Pseudomonas aeruginosa* with MIC of 5 and 20 µg/mL, respectively. Against *Staphylococcus aureus*, the extracts of *Tibouchina grandifolia, Chamaecrista desvauxii* and *Aloysia floribunda* presented an outstanding activity with MIC of 5 µg/mL**.** On the other hand, *Senna multijuga* displayed a broader spectrum of antibacterial activity, showing activity against all bacteria tested with MIC values varying from 300 to 39 µg/mL (Table 3). Infections still cause about one-third of all deaths worldwide and are the leading cause of death, mainly because of disease in developing countries.

S. sonnei, a gram-negative bacterium, is a significant cause of gastroenteritis in both developing and industrialized countries (Boumghar-Bourtchai et al., 2008). People infected with *Shigella* develop diarrhoea, fever and stomach cramps starting a day or two after they are exposed to the bacterium. It is typically associated with mild self-limiting infection (DeLappe et al., 2003). Recently, there has been a rise in strains resistant to multiple antibiotics. *P. aeruginosa*, an increasingly prevalent opportunistic human pathogen, is the most common gram-negative bacterium found in nosocomial infections. Three of the more informative human diseases caused by *P. aeruginosa* are bacteremia in severe burn victims, chronic lung infection in cystic fibrosis patients, and acute ulcerative keratitis in users of extended-wear soft contact lenses (Lyczak et al., 2000). *S. aureus* is a gram-positive bacterium that commonly colonises human skin and mucosa (e.g. inside the nose) without causing any problems. However, if either of these is breached due to trauma or surgery, *S. aureus* can enter the underlying tissue, creating its characteristic local abscess lesion, and if it reaches the lymphatic channels or blood can cause septicaemia (Harris et al., 2002). Antifungal properties were presented by 35 extracts. Among them, *Acanthospermum australe, Sida glaziovii, Cuphea ingrata, Lantana camara, Allamanda cathartica, Anthemis cotula, Carduus marianus, Alternanthera brasiliana, Rubus rosifolius, Solidago chilensis,* and *Aloysia floribunda* demonstrated a strong anti-candida activity with MIC of 39 µg/mL. By the other side, extracts from *Cecropia pachystachya, Eugenia cumini, Baccharis trimera,* and *Tropaeolum majus* were active against *C. neoformans* with MIC values of 39 µg/mL, being *Senna multijuga* the most active with MIC of 20 µg/mL. Candidiasis is a common infection of the skin, oral

cavity, esophagus, gastrointestinal tract, vagina and vascular system of humans. Although most infections occur in patients who are immunocompromised or debilitated in some other way, the organism most often responsible for disease, *Candida albicans*, expresses several virulence factors that contribute to pathogenesis (Calderone & Fonzi, 2001).*Cryptococcus neoformans* is an encapsulated basidiomycete yeast responsible for disseminated infections in immunosuppressed patients. Meningoencephalitis and pneumonia are the most frequent visceral presentations of the disease, but other rare presentations have been reported (Braga et al., 2007; Charlier-Woerther et al., 2011). Some of the most active species had already been studied for their antimicrobial effects elsewhere. The essential oil of different parts of *B. segetum* presented antifungal activity (Nascimento et al., 2008). Flavonoids isolated from the leaves of *T. grandifolia* demonstrated antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum* (Kuster et al., 2009). Dichlorometane extract of *A. australe* showed positive results against *Bacillus subtilis, Micrococcus luteus, Listeria monocytogenes* and *S. aureus* (Vivot et al., 2007). Antimicrobial efficacy of flavonoids and crude alkaloids of *L. camara* was found against *C. Albicans, Proteus mirabilis, S. aureus, E. coli,* and *Trichophyton mentagrophytes* (Sharma & Kumar, 2009). The iridoid isolated from *A. cathartica* presented fungitoxicity against some dermatophytes that causes dermatomycosis (Tiwari et al., 2002). The wound healing activity of this specie has also been tested, and it presented significant results in tests *in vivo* (Nayak et al., 2006). Flavonoids from *A. cotula* flowers showed interesting antimicrobial activity against both gram-negative and gram-positive microorganisms (Quarenghi et al., 2000). Quercetin isolated from the ethyl acetate extract of *A. brasiliana* presented antibacterial action against *S. aureus* (Silva et al., 2011). Antimicrobial activity of aqueous and hydroalchoolic fractions from *R. rosifolius* leaves showed activity against *E. coli, S. aureus, P. aeruginosa* and *C. albicans* (Mauro et al., 2002) and *B. trimera* was active against *S. aureus* and *E. coli (*Avancini et al., 2000). The antifungal activity of the leaf oil of *S. chilensis* was assayed by paper disk agar diffusion test and showed that human pathogenic dermatophytes were very sensitive (Vila et al., 2002). The crude hydroalcoholic extract of *S. cumini* was active against *Candida krusei* and against multi–resistant strains of *P. aeruginosa*, *K. pneumoniae* and *S. aureus* (de Oliveira et al., 2007). However, antimicrobial activity for *C. desvauxii, S. scabra, A. floribunda, S. multijuga, S. glaziovii, C. ingrata, C. marianus, C. pachystachya* and *T. majus* were reported here for the first time. Preliminary phytochemical analysis revealed that almost all the antimicrobial extracts showed flavonoids and tannins in their chemical composition (Table 2). Flavonoids are a broad class of plant phenolics that are known to possess antimicrobial activity, essentially by enzyme inhibition of DNA gyrase (Cushnie & Lamb, 2005). The mode of tannins antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport protein, etc. They also complex with polysaccharides (Ya et al., 1988). Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity (Jones et al., 1994). However, the extracts tested also contain triterpenoids, sterols, saponins and alkaloids. Saponins are known to interact with cell membranes, increasing permeability and producing cell damage (Francis et al., 2002). In this sense, saponins may be involved in antimicrobial properties. The mechanism of action of some alkaloids is attributed to their ability to intercalate with DNA (Phillipson & O`Neill, 1989). The antimicrobial activity of triterpenes and sterols may be related to lipophilic components of plant extracts. This components increase permeability and loss of cellular components, and a change variety of enzyme systems, including those involved in the production of cellular energy and synthesis of structural components, inactivating or destroying genetic material (Bagamboula et al., 2004; Kim et al., 1995). The antioxidant hability of the extracts was also measured.

Natural antioxidants have been studied extensively for decades in order to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration (Gutteridge & Halliwell, 2000). There is a number of assays designed to measure overall antioxidant activity/reducing potential, as an indication of host total capacity to withstand free radical stress. DPPH assay is very convenient for the screening of large numbers of samples of different polarity because of its high throughput. It evaluates the ability of antioxidants to scavenge free radicals. These antioxidants donate hydrogen to free radicals, leading to non-toxic species and therefore to inhibition of the propagation of lipid oxidation. Hydrogen-donating ability is an index of primary antioxidants (Lugasi et al., 1998). Among all extracts, 24 showed an outstanding antioxidant activity with $IC_{50} \leq 10$ µg/mL. *Cecropia pachystachya, Tibouchina mutabilis, Cupania oblongifolia,* and *Myrcia splendens* were the most active ($IC_{50} \leq 3$ µg/mL) (Table 4).

Plant species	Part tested[a]	DPPH (IC_{50} µg/mL ± SD	Reducing power (EC_{50} µg/mL ± SD)	β-carotene/linoleic acid (% I ± SD)
Achillea millefolium	L	12.30 ± 1.16	14.86 ± 0.33	37.82 ± 8.70
Bidens segetum	L	6.52 ± 2.61	24.43 ± 0.06	67.67 ± 4.60
Stenolobium stans	L	7.45 ± 0.67	16.35 ± 0.30	41.98 ± 3.27
Bixa orellana	L	8.07 ± 0.71	23.42 ± 0.03	78.75 ± 3.30
Alchornea triplinervia	L	11.20 ± 1.09	> 53.64	60.69 ± 1.16
Hyptis suaveolens	L	11.70 ± 1.43	30.48 ± 0.34	51.42 ± 9.82
Ocimum basilicum	L	8.17 ± 1.46	14.66 ± 0.01	32.76 ± 11.20
Peltodon radicans	L	4.46 ± 1.32	23.23 ± 0.07	50.33 ± 14.30
Salvia officinalis	L	9.59 ± 0.50	19.44 ± 0.06	61.66 ± 2.80
Nectandra rigida	L	6.63 ± 0.63	13.19 ± 0.08	52.10 ± 12.10
Byrsonima variabilis	L	10.7 ± 2.47	33.71 ± 0.08	31.67 ± 1.80
Tibouchina granulosa	L	7.50 ± 0.42	10.05 ± 0.61	62.37 ± 3.17
Tibouchina mutabilis	L	1.56 ± 0.24	5.54 ± 0.10	69.05 ± 8.60
Myrcia splendens	L	2.90 ± 0.20	12.31 ± 0.38	49.34.±.2.31
Eriobotrya japonica	L	11.90 ± 0.87	13.98 ± 0.34	65.50 ± 2.00
Cupania oblongifolia	L	2.22 ± 0.10	6.29 ± 0.08	47.48 ± 4.8
Cecropia pachystachya	L	2.11 ± 0.40	7.70 ± 0.22	79.28 ± 2.80
Lippia hermannioides	L	3.99 ± 0.30	13.68 ± 0.42	54.90 ± 5.22
Lippia alba	AP	5.43 ± 0.34	14.40 ± 0.02	47.62 ± 27.50
Lippia rubella	AP	3.79 ± 0.27	10.27 ± 0.10	10.60 ± 5.60
Lantana camara	L	4.54 ± 0.26	14.04 ± 0.,02	55.03 ± 8.80
Lantana camara	F	9.82 ± 1.79	27.99 ± 0.07	61.84 ± 9.20
Amaioua intermedia	L	8.41 ± 1.22	12.22 ± 0.08	58.13 ± 0.70
Positive controls				
Ascorbic acid		1.80 ± 0.12	4.27 ± 0.06	
α-tocopherol		2.26 ± 0.14		
BHT		10.5 ± 1.06		
Quercetin		0.98 ± 0.20		91.52 ± 1.50
Rutin		2.52 ± 0.60		

[a]AP, Aerial Parts; F, Flowers; L, Leaves

Table 4. Antioxidant activity of methanol extracts of the selected medicinal plants.

The total antioxidant activity of the extracts is constituted by individual activities of each of the antioxidant compounds. Moreover, these compounds render their effects via different mechanisms such as radical scavenging, metal chelating activity, inhibition of lipid peroxidation, quenching of singlet oxygen, and so on to act as antioxidants. Even if a sample exhibits high activity with one method, it does not always show similar good results with all other methods. Therefore, it is essential to evaluate samples accurately by several methods. Hence, the antioxidant activity for those extracts was also evaluated by reducing power and β-carotene/linoleic acid assays. The reducing ability of a compound generally depends on the presence of reductants, which exhibited antioxidative potential by breaking the free radical chain, by donating a hydrogen atom. Antioxidant action of the reductones is based on the breaking of free radicals chain by the donation of a hydrogen atom. Reductones are believed not only to react directly with peroxides, but also prevent peroxide formation by reacting with certain precursors (Jamuna et al. 2010). The results found using this assay showed an outstanding antioxidant property of *C. pachystachya, T. mutabilis, C. oblongifolia,* and *M. splendens* and suggested that compounds present in those extracts were good electron and hydrogen donors, and could terminate the radical chain reaction by converting free radicals into more stable products. When employing β-carotene/linoleic acid assay, the more active inhibitors of β-carotene bleaching were *C. pachystachya, T. mutabilis* and *B. orellana* which showed values greater than 75% of inhibition. Interestingly, *C. oblongifolia* and *M. splendens* were not so effective in quenching β-carotene. It is well known that the value of this method appears to be limited to less polar compounds. They exhibit stronger antioxidative properties in emulsions because they concentrate at the lipid:air surface, thus ensuring high protection of the emulsion itself. On the other hand, polar antioxidants remaining in the aqueous phase are more diluted and are thus less effective in protecting the lipid (Koleva et al., 2002). It is well known that plants which possess antioxidative and pharmacological properties are related to the presence of phenolic compounds, specially phenolic acids and flavonoids (Fabri et al., 2009). Antioxidant activity had also been detected for *C. pachystachya* (Aragão et al., 2010) and *B. orellana* (Chisté et al., 2011). For *T. mutabilis, C. oblongifolia and M. splendens,* the antioxidant capacities were reported here for the first time. Polyphenolic compounds such as flavonoids and tannins found in the extracts (Table 2) are considered to be the major contributors to the antioxidant activity of medicinal plants. The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers, as well as their metal chelating abilities (Vladimir-Knezevic et al., 2011). It would seem that a great part of the extracts tested in this study for antimicrobial activity does not possess antioxidant effects (Table 3 and 4).

3. Conclusion

The results obtained represent a worthwhile expressive contribution to the characterization of antimicrobial and antioxidant activity of plant extracts of traditional medicinal plants from Brazilian flora and justify, in part, the popular uses of some of these species.

4. Acknowledgment

The authors are grateful to Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) and the Universidade Federal de Juiz de Fora (UFJF)/Brazil for financial

support, to Dr. Fatima Regina Salimena for the botanical identification of the species, and to Delfino Antônio Campos for his technical assistance.

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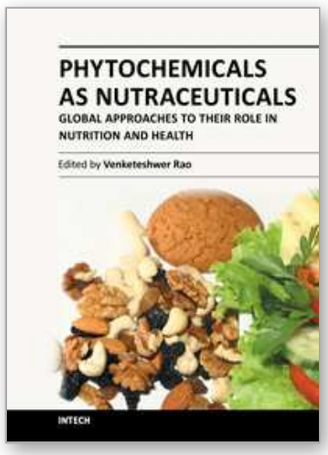

Phytochemicals as Nutraceuticals - Global Approaches to Their Role in Nutrition and Health

Edited by Dr Venketeshwer Rao

ISBN 978-953-51-0203-8
Hard cover, 278 pages
Publisher InTech
Published online 23, March, 2012
Published in print edition March, 2012

Phytochemicals are biologically active compounds present in plants used for food and medicine. A great deal of interest has been generated recently in the isolation, characterization and biological activity of these phytochemicals. This book is in response to the need for more current and global scope of phytochemicals. It contains chapters written by internationally recognized authors. The topics covered in the book range from their occurrence, chemical and physical characteristics, analytical procedures, biological activity, safety and industrial applications. The book has been planned to meet the needs of the researchers, health professionals, government regulatory agencies and industries. This book will serve as a standard reference book in this important and fast growing area of phytochemicals, human nutrition and health.

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