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Journal of Medicinal Plants Research

Table of Contents: Volume 9 Number 21, 3 June, 2015

ARTICLES

Research Articles

academicJournals

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Review

A review on gall karkatshringi

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Karkatasringi (*Pistacia integerrima*) is a well known medicinal plant which belongs to the family of Anacardiaceae. The plant is indigenous to India and is found in the outer ranges of the North-Western Himalayas at an altitude of 500 to 2500 m. The different parts of the plant like leaf, bark, root and galls are reported to contain the secondary metabolites. Among them, the galls are more used in folk medicines. They are used in various Ayurvedic formulation like the “Dasamularista”, “Chayavanaprasa” and “Shringyadi curna” which are used in the treatment of diseases like *swasa* (asthma), *yakshma* (tuberculosis), *ajeerna* (indigestion), *hridyaroga* (heart disease), *jwara* (fever) and *yakrit roga* (liver disorder). The secondary metabolites like alkaloids, tannins, terpenoids and flavonoids are reported in the galls. Beside them, minor constituents like crystalline hydrocarbon, gum mastic, resinous substance, crystalline acids are also present. The bark contains terpenoids and flavonoids. The root and leaves contain tannins and terpenoids. Tannin being the major chemical constituent shows the strongest astringent action of the plant. The effect of the plant is due to the presence of these different secondary metabolites that are responsible for pharmacological activities. The aim of this review is to highlight the description of plant in classical literature of Ayurveda as well as therapeutic properties and chemical constituents.

Key words: Karkatasringi, galls, secondary metabolites.

INTRODUCTION

Karkatshringi is a multibranched, single stemmed, deciduous tree of *Pistacia integerrima* which belong to the family of Anacardiaceae. This plant belongs to Amra-Kula and it is a dioecious shedding tree. It is found in North-West Himalaya including the Siwalik ranges/ Rohitkhand from Indus to Kumaon between 500 to 2500 m. Altitudes (Anonymous, 2005). The plant is known as chakra, chandraspada, shikari in Sanskrit, kakra in Hindi. (Chopra and Chopra, 2006) Typical type of worms make horn shaped galls on the branches and leaves. (Vashist and Anil, 2012)These galls are pale greenish brown or pinkish, elongated, horn-shaped, hollow, twisted, curved or straight. When young they are coriaceous, but later become hard (http://www.indianmedicinalplants.info/Medicinal-Plants). This gall is caused by the insect *Dasia aedifactor* (Homoptera), (plant produce resin against insect) (http:/www.ayurvedaconsultants.com/ayurvedaherbs). They make these galls by sucking juice from the leaves (Vashist and Anil, 2012). Then they are called

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karkatshringi (Vashist and Anil, 2012). Majorly, galls contains resins, pistacienoic acid, tetracyclic triterpenes, camphene, luteolin, pistancin, pistacinin, amino acids, dihydromavalic acids, sterols and tannins (Warrier et al., 1995). These galls are useful in asthma, cough, hiccough, dysentery, diarrhoea, ulcers, bronchitis, fever, irritability of stomach, leprosy, psoriasis, skin diseases, vitiated condition of tridosha, dyspepsia, inflammation, anorexia, pharyngitis, leucorrhoea and general debility. It is also very effective in children at the time of teething. In Pakistan, the galls of *Pistacia chinensis* var. *Integerrima* are used for the treatment of hepatitis and liver. It has been reported to have depressant, analgesic anti-inflammatory activities and hyperuricemic effect disorder (Ghias et al., 2011; Ansari et al., 1993)

REVIEW OF LITERATURE

Literature brush up reveals the different classical categorization of Karkatshringi. Different Acharayas (Ancient scholars) kept it in the different categories according to their own knowledge. Some confusion arises because of all the names related to Karkatika, Karktahvaya, Karkatakhya etc. which are generally accepted as Karkatshringi.

Classical categories: Gana

Charak sahmita: In Charak sahmita Karkatshringi is kept under the gana of (Bhedaniya, Angamardaprasamana, Svedopaga, Madhuraskanda) (http://www.ayurwiki.info/wiki/karkatshringiclassical_categories_gana), (Kasahara, Hikkanigrahna and Madhura skandha) (Sharma, 1981), Charak interpreted it as the small variety of amalaki (Sharma and Bhagwan, 1996).
Sushruta sahmita: In Sushruta sahmita, Karkatshringi placed under the gana of (Vidarigandhadi, Adhibhagahara, Vatasamshama) (Sharma, 1981). It is important to note that sushruta classified it as a Kanda visa (poisnous tuber). The toxic symptoms of this plant are documented by sushruta samhita (Bhishagratha and samhita, 1991).
Bhav prakash: In this ancient text, some of the properties of the plant are mentioned (Sharma, 1981).
Asthanga Haridayam: In Asthanga Haridayam, Acharya Vagbhatta mentioned the description of Karkatashringi in Kesava Paddhati. (Kaviraj and Sangrah, 1993).

Modern books

Botanical name: *P. integerrima* (Anonymous, 2005; Warrier et al., 1995; Sharma,1981; Sharma and Bhagwan, 1996; Anonymous, 2000; Anonymous, 2001; Family: Anacardiaceae (Anonymous, 2005; Sharma and Bhagwan, 1996; Anonymous, 2000; Anonymous, 2001; Anonymous, 2000).
Classical names: (Sharma, 1981) Karkarshringi, Shringi, Kuliravishanika, Ajashringi, Chakara, Karkatkhya, Vakra, Visanika

Classical categorization

Caraka: Kasahara, Hikkanigrahana, Madhur skandha.
Sushruta: Kakolyadi, Padmakadi.
Vagbhata: These medicinal plants were described in Kesave Paddhati. Both caraka and Sushruta consider this plant as a poison for a vegetable origin. Acharya Sushruta kept this plant in Visa khand. Similar confusion is apparent in the context of Gunja which is categorized under Mula visa (root poison). Caraka interpreted it as amalaka and the toxic symptoms are mentioned by Acharya Sushruta. Likewise, Dalhana's comments add more confusion about its identification since Mesasringi, Ajasringi and Uttamarni are equated to Karkatshringi because, the Asclepiadaceae family may have the same synonyms (Jivanti).

Botanical description

Deciduous tree, up to 18 m high, barks dark grey or blackish. Leaves alternate, pinnate, 15 to 23 cm. long, with or without terminal leaflet, leaflets 4 to 5 pairs, lanceolate, acuminate, sub-opposite, coriaceous, 7 to 12 cm long. Characteristic galls are produced on the leafy branches. Flowers small, reddish, unisexual, dioecious. malepanicles short, compact. Female flowers have long lax panicles. Drupe globose, wrinkled, rugose and grey when ripe.

Microscopic characters

Transverse section of mature root shows a wide zone of stratified cork, exfoliating at places, consisting of rectangular, thin-walled, tangentially elongated, radically arranged cells, upper few layers filled with reddish-brown contents, remaining cells colourless, cortex, a wide zone of rounded cells with fibre groups towards central and middle region, cells obliterated at places, endodermis barrel-shaped, slightly thickwalled, pericycle and phloem not distinct, xylem forms bulk of root consisting of vessels, fibres and parenchyma, medullary rays not distinct, vessels shows annular or pitted thickening, fibres thick-walled, elongated having a few simple pits. Powder-yellowish-brown, under microscope shows fragments of corks, xylem vessels and fibres.

Distribution

North-West Himalayas (Indus to Kumaon) at 350 to 2500 m. cultivated in Punjab plains.

Chemical constituents

Karkatshringi contains various important phyto-constituent for commercial value and therapeutic potential. Chiefly it contains resin two isomeric triterpenic acids-pistacienoic acids A and B, tannins, a triterpene alcohol-tirucallol, beta-sitosterol, tetracyclic triterpenes, pistacigerrimones A.B,C(galls); alpha-piene, beta-piene, camphene, dl-limonene,1:8-cinol, alpha- terpineol, beta- terpineol, aromadenddrene, lactonic stearoptene, caprylic acid, alpha-d-pinene, alpha &beta-phallandrene,amino acids, dihydromalyalic acid, protein (seeds); hydrocarbons, sterols, triterpenoids(seeds oil), tannins (leaves,bark).

Pharmacological activities

Karkatashringi is an important medicinal plant whose galls are used in traditional medicines in India for the treatment of asthma, chronic bronchitis, phthisis, diarrhea, fever and other reported activities as antispasmodic, carminative, antiamoebic and anthelmintic.

Toxicology

Essential oil of gall has a depressant action on the central nervous system of guinea-pigs and white rats when given in sub-lethal doses. The animals become deeply unconscious in about an hour. Lethal doses (m.l.d0.1cc/100 gm body wt.) cause deep narcosis leading to death within a few hours. The oil has a slight irritant action on the skin and mucous membrane.

Therapeutic evalution

In a clinical trail Brihat talisadi churna in which Karkatshringi is one of the ingredients was found useful in the patients of productive cough when administered in the dose of 500 mg QID with honey as adjuvant.

Formulations and preparations

Karkatshringi is one of the major ingredients of various type of Ayurvedic formulations like Shringiadi chura, Karkatadi chura, Balachaturbhadra churna, Brihat talisadi churna, Devadarvayadi kwath churna, shatavaryadi ghrit, chayanprash-awaleha, Dashmularista, Kanta karyavaleha, Siva gutika and khadiradi gutika used invarious therapeutic purposes.

Substitues and adultrants

Galls produced on the plants viz. *Rhus succedanea, Garuga pinnata, Terminalia chebula* are also used and sold under the name Karkatshringi.

Research work done

1. Oil extracted from *P. chinensis* exhibited central nervous system (CNS) depressant activity (Sharma, 1981).
2. The crude methanolic extract of *P. integerrima* bark evaluated for antipyretic activity (The crude methanolic extract of *Pistacia integerrima* bark evaluated for antipyrectic activity, Sharma and Bhagwan, 2011).
3. Phytochemical investigation of the galls of P. Integerrima (Ahmad et al., 2010).
4. Ethyl gallate isolated from *P. integerrima* Linn. inhibits cell adhesion molecules by blocking AP-1 transcription factor (Mehla et al., 2011).
5. Pharmacological basis for use of *P. integerrima* leaves in hyperuricemia and gout (Ahmad et al., 2008).
6. Analgesic and anti-inflammatory effects of *P. integerrima* extracts in mice (Ahmad et al., 2010).
7. Propagation of pistachio rootstock by rooted stem cuttings (Almehdi et al., 2002).
8. Phylogenetics and reticulate evolution in *Pistacia* (Anacardiaceae) (Phylogenetics and reticulate evolution in *Pistacia* (Anacardiaceae) (Yi et al., 2008).
9. Analgesic, anti GIT motility and toxicological activities of *P. integerrima* Stewart ex Brandis bark in mice (Ismail et al., 2012).
10. Hepatoprotective effects of berries lyceum, gallium aparine and *P. integerrima* in carbon tetrachloride treated rats (Khan et al., 2008).
11. Pharmacognostical studies on the south Indian market sample of Karkatshringi (Kadukkaipoo)- (Gaertn. Leaf gall) (Santha et al., 1991).

Observation

The profile of the medicinal plant (Karkatshringi) which is present in ancient and modern text carried little confusion related to the synonym of plant. Ancient scholar (Sushruta) kept the plant in poisonous category (Visha khand). They also mentioned the uses of plant in respiratory disorders viz. asthma, hiccough, cough etc. After reviewing the various research articles on the basis of preclinical studies it possess the properties mentioned in Ayurvedic texts and shows some other significant properties like: antipyretic, analgesic, anti-inflammatory, antioxidant, hepatoprotective, antimicrobial, GIT motility and toxicological, hyperuricemia and gout (Table 1).

CONCLUSION

The multipurpose medicinal plant (*P. integerrima*), is the unique source of various types of compounds having

Table 1. Ayurvedic properties.

Parameter	Properties
Rasa	Tikta (Pungent)
Veerya	Ushna (Hot)
Vipaka (Post digestion effect)	Katu (pungent)
Effect on tridosha	Pacifies Kapha and Pitta

Table 2. Vernacular names.

S/N	Language	Names
1	English	Crab's claw
2	Hindi	Kakdashingi,Kakarsingi,Kakra,Kakkatasimgi
3	Punjabi	Kakar,Kakarshingi,Drek,Gurgu,Kakkeran,Kakkrangehe,Kakala,Kangar Masna,Sumak,Tungu,Tanbari,Shne,Karkarshingi
4	Bengali	Kakra,Kakrashingi,Kandashringi
5	Gujrati	Kakadasingi,Kakra,Kakarshingi
6	Marathi	Karkadasringi, Kakra,Kakarsingi,Kakadshingi
7	Malayalm	Karkatasringi,Karkktakasingi
8	Tamil	Karkata,Singi,Kakkatashingi
9	Telgu	Kakarashingi,Kakatakashrungi,Kakarasimga
10	Assam	Kakiasrngi
11	Oriya	Kakadashrungi,Kakadashringi,
12	Urdu	Kakrasinghi,Kakra

Table 3. Identity, purity and strength**.**

S/N	Identity, purity and strength
1	Foreign matter not more than 2 per cent
2	Total Ash Not more than 11 per cent
3	Acid-insoluble ash Not more than 2 per cent
4	Alcohol-soluble extractive Not less than 9 per cent
5	Water-soluble extractive Not less than 16 per cent

diverse chemical structures. Very little work has been done on the biological activity and plausible medicinal applications of these compounds and hence extensive investigation is needed to exploit their therapeutic utilityto combat diseases. The present results therefore, offer a scientific basis for the traditional use of the various extracts of *P. integerrima* (Tables 2 and 3).

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Conflict of interest

Authors have none to declare.

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Full Length Research Paper

Antimicrobial activity and synergistic effects of an ethyl acetate fraction from methanol extract of *Myracrodruon urundeuva* bark

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This study aimed to investigate the antimicrobial action of different methanolic extract fractions from *Myracrodruon urundeuva* (Anacardiaceae) bark and to evaluate the interaction between the most active fraction and some commercial antibiotics. After methanolic extraction, the extract obtained was submitted to liquid/liquid fractionation using cyclohexane, ethyl acetate, n-butanol and water. All fractions showed antimicrobial action, although the ethyl acetate fraction showed the best antimicrobial activity. This fraction inhibited all tested microorganism (*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus niger)* with minimum inhibitory concentration (MIC) values ranging from 0.097 to 0.78 mg/ml. The ethyl acetate fraction showed different effects depending on the kind of antibiotic and bacteria tested. The best interaction was observed with gentamicin which resulted in synergistic results for all tested bacteria (∑FIC values of 0.01 for both *S. aureus* and *B. subtilis* and 0.25 for *K. pneumoniae* and *E. coli*). In conclusion, *Myracrodruon urundeuva* bark is a source of antimicrobial compounds able to improve the activity of some antimicrobials, especially gentamicin, erythromycin, tetracycline and ciprofloxacin. The identification of the active compounds and the action mechanisms involved are crucial for the use of this plant in biotechnological preparations.

Key words: Antimicrobial activity, *Myracrodruon urundeuva*, Caatinga, synergism, pharmaceutical preparations.

INTRODUCTION

Microorganisms are responsible for infections which represent a major cause of morbidity and mortality in the world (Brachman, 2003; MacPherson et al., 2014), and pharmacological interventions with antibiotics comprise the most important advances to treat them (Bradford, 2001; Rice, 2009). However, the phenomenon of resistance has become fairly common throughout the world, especially in hospitals, due to the intensive and

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indiscriminate use of antibiotics. Antibiotic resistance reduces the possibility of effective treatments and increases the risk of complications or death for the patient (Woodford and Livermore, 2009).

Medicinal plants have been used as therapeutic tools to treat various pathologies, such as bacterial and fungal infections (Holetz et al., 2002). Furthermore, due to the growing ineffectiveness of many synthetic products, as well as their high costs and collateral effects, there is a great interest in medicinal plants. An option to overcome bacterial resistance is the combination of antibiotics with plant-derived compounds (Wagner and Ulrich-Merzenich, 2009), and an assessment of synergism has become a great tool in finding new active agents (Figueredo et al., 2014; Bezerra dos Santos et al., 2015).

A good example of a medicinal plant is *Myracrodruon urundeuva* (Anacardiaceae), a typical species of the Caatinga, a semi-arid biome exclusively found in Northeastern Brazil. This plant (mainly the bark) has been used by rural community to treat several injuries (Monteiro et al., 2005; Cartaxo et al., 2010) and some of these biomedical properties have been scientifically supported, especially, its anti-inflammatory, analgesic, antioxidant and antimicrobial actions (Viana et al., 2013; Sá et al., 2009a; Jandú et al., 2013). Other biotechnologic applications are also reported to extracts and compounds isolated from *M. urundeuva*. For example, lectins from different tissues of *M. urundeuva* (heartwood, bark and leaves) (Sá et al., 2009b; Napoleão et al., 2012) and m-pentadecadienyl-phenol isolated from seeds (Souza et al., 2012) showed insecticidal activity against *Aedes aegypti*. Neuroprotective action was reported for a chalcone-enriched fraction from its stem bark (Nobre-Júnior et al., 2009). Extracts from *M. urundeuva* also showed inhibitory effect against caries development in rats (Crivelaro de Menezes et al., 2010) and healing activity (Cavalcante et al., 2005). Previous studies of our group showed that methanolic extract from *M. urundeuva* (MuBME) has broad-spread antimicrobial activity and it was able to increase the action of erythromycin against clinical isolates of *S. aureus* (Jandú et al., 2013). This study aimed to investigate the antimicrobial action of different fractions obtained from a methanolic crude extract of *M. urundeuva* bark and the synergistic potential of the most active fraction with some commercial antibiotics.

MATERIALS AND METHODS

Plant source, collection and identification

M. urundeuva bark was collected in Parque Nacional do Catimbau (PARNA do Catimbau), Pernambuco, Brazil, in 2010. Botanical identification was done by Dr. Alexandre Gomes da Silva, at the Herbarium of the Instituto Agronômico de Pernambuco (IPA), where the voucher specimen 84.059 is deposited. The bark was dried using an incubator at 45°C for 2 to 3 days. The dried vegetal material was ground into powder form using a grinder followed by a Waring blender.

Methanolic extraction

Samples of *M. urundeuva* bark were submitted to methanolic extraction as described in Jandú et al. (2013). The resultant extract was subjected to liquid/liquid fractionation, using cyclohexane, ethyl acetate and n-butanol. Subsequently, each solvent was evaporated and the fractions were stored at -20°C until used. The remaining aqueous fraction was dried by lyophilization and the other fractions were then concentrated in a rotary vacuum. All the extracts were kept in tightly stoppered bottles in a refrigerator until used for anti-microbial testing.

Antimicrobial assays

Microorganisms

The seven tested microorganisms were obtained from the culture collection of the Departamento de Antibióticos, Universidade Federal de Pernambuco (UFPEDA), Brazil. The following microorganisms were used as test organisms: *Staphylococcus aureus* (UFPEDA 02), *Bacillus subtlis* (UFPEDA 86), *Micrococcus luteus* (UFPEDA 100), *Enterococcus faecalis* (UFPEDA 138), *Escherichia coli* (UFPEDA 224), *Klebsiella pneumoniae* (UFPEDA 396), *Candida albicans* (UFPEDA 1007), and *Aspergillus niger* (UFPEDA 2003).

Disc diffusion method

The paper disc diffusion method was used to determine antibacterial activity (Bauer et al., 1966). Bacterial strains were grown on Mueller-Hinton Agar (MHA) at 37°C for 18 h and suspended (1.5×10^8 CFU/ml). The MHA was poured into petri dishes and inoculated with 100 µl of the suspension. Sterile paper discs (6 mm diammeter), containing 100 µg of fractions, were set on the agar. Dimethyl sulfoxide (DMSO) was used as a negative control. The plates were cultured at 37°C for 18 h. At the end of the incubation period the antimicrobial activity was evaluated by measuring the inhibition diameter plus the diameter of the disc. An inhibition zone of 14 mm or more was considered as showing high antibacterial activity.

Determination of the minimum inhibitory concentration (MIC)

MICs of all fractions and reference antibiotics (ampicillin, penicillin, gentamicin, ciprofloxacin, erythromycin, gentamicin, neomycin, kanamycin and nystatin) were determined by the microdilution method: in Mueller-Hinton broth (MHB) for bacteria and Sabouraud broth (SAB) for bacteria fungi, according to the Clinical and Laboratory Standards Institute (CLSI) Standards (2011). Inoculates were prepared in MHB, an aliquot of 10 µl of microbial suspension (1.5×10^8 CFU/ml) was added to a two-fold serial dilution of all fractions (50 to 0.09 mg/ml). After specific incubation (24 h at 37°C for bacteria; 48 and 96 h at 30°C for *C. albicans* and *A. niger*, respectively). MIC values were recorded using a resazurin solution (0.01%) as a growth indicator (CLSI, 2011).

Evaluation of combinatory effects of the ethyl acetate fraction and antibiotics

The checkerboard method was used to test the combinatory effect of the ethyl acetate fraction and commercial antibiotics (ampicillin, penicillin, gentamicin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, neomycin and tetracycline) against *S. aureus, B. subtlis* and *K. pneumonia* strains. The ethyl acetate fraction and drug were

Table 1. Antimicrobial activity of fractions obtained from methanolic extracts of *Myracrodruon urundeuva* bark.

UFPEDA Number	Microorganism	Cyclohexane fraction		Ethyl acetate fraction		n-Butanol fraction		Aqueous fraction	
		IDZ (mm)	MIC (mg/ml)	IDZ (mm)	MIC (mg/ml)	IDZ (mm)	MIC (mg/ml)	IDZ (mm)	MIC (mg/ml)
02	*Staphylococcus aureus*	18±2.08[a]	3.125	27±1[b]	0.097	18±2.31[a]	1.56	21±0.58[a]	0.78
86	*Bacillus subtilis*	11±0.00[a]	3.12	18±0.00[b]	0.097	15±0.58[c]	0.78	15±0.58[c]	3.12
100	*Micrococcus luteus*	18±6.66[a]	3.12	30±1.73[b]	0.097	23±6.66[c]	1.56	27±1[a]	0.78
138	*Enterococcus faecalis*	0[a]	3.12	18±1.53[b]	0.78	10±2.08[c]	3.12	0[a]	3.12
396	*Klebsiella pneumoniae*	0[a]	6.25	17±0.58[b]	0.39	0[a]	3.12	0[a]	1.56
1007	*Candida albicans*	8±0.00[a]	0.78	11±1.53[b]	0.097	9±2.08[b]	0.78	7±0.58[b]	0.39
2003	*Aspergillus niger*	0[a]	3.125	0[a]	0.097	0[a]	0.78	0[a]	0.39

*Same superscript letter ([a,b,c]) indicates no significant difference ($p > 0.05$) between IDZ values from different solvents against each pathogen (same row).

added together to the medium to give a final concentration of 10 and 0.1 mg/ml, respectively, and a two-fold serial dilution was prepared (10 to 0.02 mg/ml for EAF and 0.1 to 0.0002 mg/ml for each drug). The interaction was assessed algebraically by determining the Fractional Inhibitory Concentration indices (ΣFIC) according to the following equation:

$$\Sigma FIC = (MIC_{F+D}/MIC_F) + (MIC_{D+E}/MIC_D)$$

MIC_F or MIC_D: MIC fraction or MIC drug; MIC_{F+D}: MIC_F when in combination with each drug; MICD/F: is the MIC of each drug when in combination with fraction.

Data interpretation: ΣFIC ≤ 0.5: synergism (syn); 0.5 < ΣFIC ≤ 1: addition (add); 1 < ΣFIC < 4: non-interaction (non); ΣFIC ≥ 4: antagonism (ant) (Vuuren and Viljoen, 2011).

Statistical analysis

Each experiment was performed in triplicate. The results obtained from disc diffusion test were expressed as the mean ± standard deviation (SD) and analyzed using two-way analysis of variance (ANOVA). All analyses were carried out using software GraphPrism, version 4. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

M. urundeuva is a valuable medicinal plant widely distributed in Brazil and well-known due to its traditional use (especially its bark) in the treatment of infections of the urogenital, respiratory, and digestive systems, skin, subcutaneous tissues and neoplasia (Lucena et al., 2012). In this work, the antimicrobial action of fractions obtained from crude methanolic extracts of the bark was reported and the combinatory effects of the most active antimicrobial fraction with some commercially available drugs were evaluated.

The first step of this study was to evaluate the antimicrobial activity of fractions obtained from the crude methanolic extract of *M*. *urundeuva* bark (Table 1). Our previous results have demonstrated that this extract has broad-spectrum antimicrobial activity and high concentrations of gallic, fumaric, catechuic, proto-catechuic and chlorogenic acids (Jandú et al., 2013). These phytochemicals have been detected in various bioactive extracts and some of them had the antimicrobial action confirmed when tested separated (Jayaraman et al., 2010; Daglia, 2012; He et al., 2013; Yu and Shengrong, 2015).

All fractions obtained showed antimicrobial activity and the best antimicrobial action was observed for the ethyl acetate fraction, which showed inhibition diameter zones (IDZ) of 18±0.0, 18±0.0, 27±1 and 30±1.73 mm against *B. subtilis, E. faecalis, S. aureus,* and *M. luteus*, respectively. Its MIC values were 0.097 mg/ml for *B. subtilis, S. aureus*, and *M. luteus*; and 0.78 mg/ml for *E. faecalis*. This fraction was also active against *K. pneumoniae* (IDZ of 17±0.58 mm and MIC of 0.39 mg/ml). Regarding antifungal action, the ethyl acetate fraction inhibited *C. albicans* with an IDZ of 11±1.53 mm and MIC of 0.097 mg/ml, the same MIC value as with *A. niger* (Table 1). The cyclohexane fraction showed IDZ values ranging from 0 mm (*E. faecalis*, *K. pneumonia* and *A. niger)* to 18 mm (*S. aureus* and *M. luteus*) and MIC of 3.125 mg/ml for most of the tested pathogens, except for *K. pneumoniae* (6.25 mg/ml) and *C. albicans* which showed the best potential with MIC value of 0.78 mg/ml. On the order hand, the n-butanol fraction exhibited IDZ values of 0 mm (*K. pneumoniae* and *A. niger*), 9±2.08 mm (*C. albicans*), 10±2.08 mm (*E. faecalis*), and 15±0.58 mm (*B. subitilis*). Its MIC values were 0.78 mg/ml (*B. subtilis*, *C. albicans* and *A. niger*), and 3.125 mg/ml (*E. faecalis* and *K. pneumoniae*). For *S. aureus* and *M. luteus*, this fraction showed MICs values of 1.56 mg/ml and IDZ values of 18±2.31 and 23±6.66 mm, respectively. Finally, the aqueous fraction showed IDZ values ranging from 0 mm (*E. faecalis, K. pneumoniae, A. niger*) to 27±1 mm (*S. aureus*); and MIC values of 0.39 mg/ml (*C. albicans* and *A. niger*), 0.78 mg/ml (*S. aureus* and *M. luteus*) and 3.125 mg/ml (*B. subtilis* and *E.*

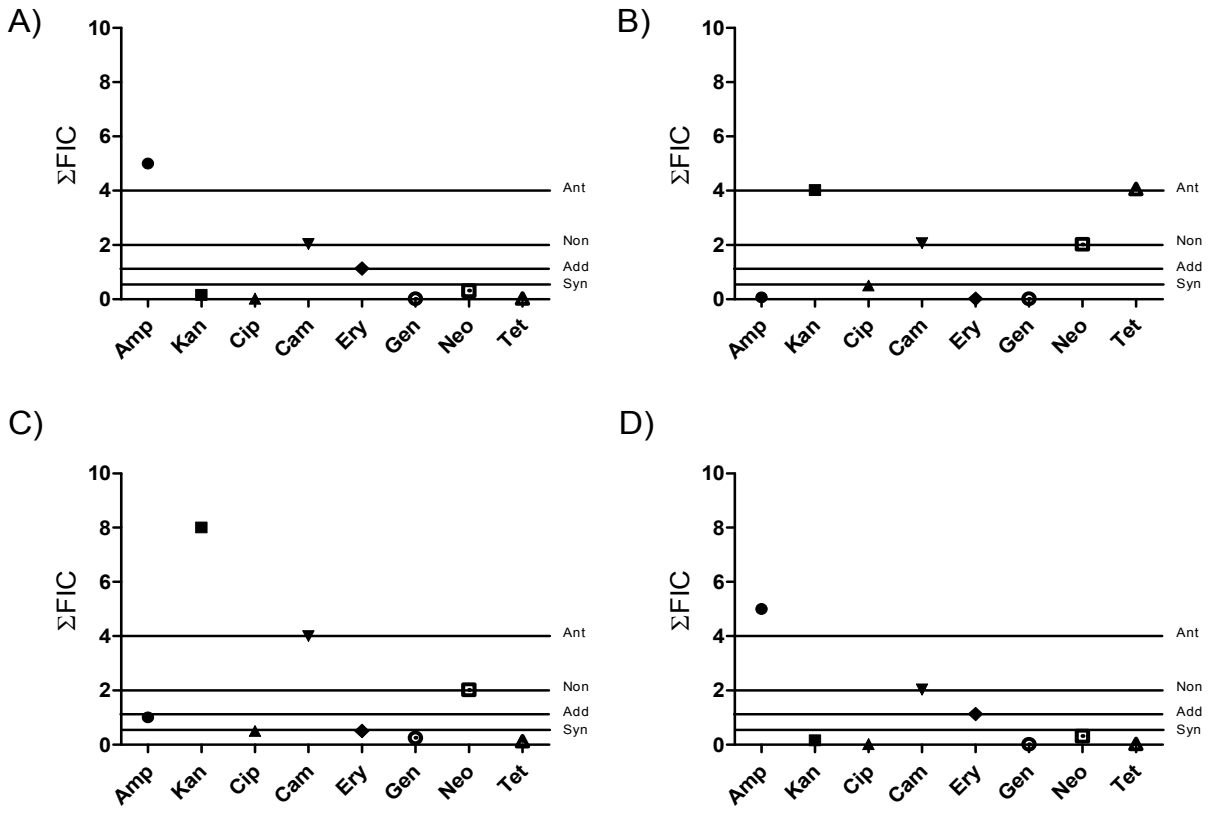

Figure 1. Combinatory effects of ethyl acetate fraction and antibiotics against *B. subtilis* (A), *S. aureus* (B), *E. coli* (C), *K. pneumoniae* (D). Amp: Ampicillin; Kan: kanamycin; Cip: ciprofloxacin; Cam: chloramphenicol; Ery: erythromycin; Gen: gentamicin; Neo: neomycin; Tet: tetracycline; non: non-interactive effect; add: additive effect; syn: synergistic effect; ant: antagonistic effect.

faecalis) (Table 1).

Using the classification proposed by Holetz et al. (2002), ethyl acetate fraction showed the best antimicrobial activity as it had MIC values less than 100 µg/ml for the most tested pathogens. The MIC average of this fraction was about 13, 7 and 6 times less than the values found to cyclohexane, n-butanol and aqueous fractions, respectively. The results found for the ethyl acetate fraction are also better than those reported for the methanolic extract as a whole (Jandú et al., 2013). Taking these factors together, we decided to evaluate the combinatory effects of ethyl acetate fraction with some commercial drugs against two Gram-positive (*S. aureus* and *B. subtilis)* and two Gram-negative (*E. coli* and *K. pneumoniae*) bacteria. The combinatory action of antibiotics and plant-derived products has been indicated as a new approach in the fight against bacteria, mainly to counteract the phenomenon of resistance (Hemaiswarya et al., 2008; Zuo et al., 2011). Synergistic effects may result from (i) multi-target effects (each compound has a different target); (ii) improvement of pharmacokinetic or physico-chemical features (such as solubility, bioavailability, target recognition and binding); (iii) inhibition of resistance mechanisms (e.g., drug efflux inhibition) (Wagner and Ulrich-Merzenich, 2009). Furthermore, several plants have been shown to be sources of compounds which are potentially able to increase the action of different kinds of antibiotics (Adwan et al., 2009; Bezerra dos Santos et al., 2015).

The best fraction/drug interaction was found with the protein inhibitor gentamicin, having ∑FIC values of 0.01 (for both *S. aureus* and *B. subtilis*) and 0.25 (*E. coli* and *K. pneumoniae*), these results represent synergistic effects for all tested bacteria. When combined with ciprofloxacin, the ethyl acetate fraction had synergistic (∑FIC values of 0.02 for *B. subtilis* and 0.50 for *E. coli and S. aureus*) and additive (*K. pneumoniae*, ∑FIC= 1.0) effects. Combination with erythromycin resulted in synergistic effects against *S. aureus, E. coli* and *K. pneumonia* (∑FIC value of 0.02, 0.5 and 0.16, respectively), while for *B. subtilis* non-interactive effects were observed (∑FIC= 1.13). The fraction was also able to enhance the action of tetracycline against *B. subtilis* (∑FIC= 0.25), *E. coli* (∑FIC= 0.13) and *K. pneumoniae* (∑FIC= 0.13), but showed an antagonistic effect against *S. aureus* (Figure 1). Similarly, combination with neomycin resulted in synergistic (∑FIC of 0.03 against *B. subtilis*), non-interactive (∑FIC of 2.02 for *S. aureus* and

E. coli) and antagonistic (∑FIC of 4.02 for *K. pneumoniae*) effects, while both synergistic and antagonistic effects were found for ampicillin and kanamycin, with ∑FIC values (respectively) of 0.07 and 4.02 for *S. aureus*; 5.03 and 0.02 for *B. subtilis*; 1.0 and 8.0 for *E. coli*; 8.25 and 4.01 for *K. pneumoniae*. Non-interactive and antagonistic effects were also found for the combination of the ethyl acetate fraction with chloramphenicol against all tested pathogens (Figure 1).

Generally, ethyl acetate was able to enhance the activity (by synergetic or additive efects) of 6 antibiotics against *B. subtilis*, 5 antibiotics against *E. coli* and 4 antibiotics against both *S. aureus* and *Klebsiella pneumoniae*. The synergetic effect of some constituents of *M. urundeuva* leaves have been reported, for example protocatechuic acid and gallic acid increased the action of sulfamethoxazole against the Gram-negative bacteria *Pseudomonas aeruginosa*. Gallic acid also increased the activity of tetracycline against this pathogen (Jayaraman et al., 2010). Our results showed that ethyl acetate fraction had synergetic effect when combined with tetracycline against all tested Gram negative bacteria. Additionally, the combinatory effects of ethanolic extract and essential oils from *M. urundeuva* leaves were evaluated against *S. aureus* and *E. coli*. Both samples were only able to enhance the activity of gentamicin, amikacin and clindamycin against *S. aureus* (Figueredo et al., 2013). In this sense, compounds present in the bark seem to be more effective as drug enhancers.

Conclusion

This study demonstrates that all fractions obtained from a methanolic extract of *M. urundeuva* bark are sources of antimicrobial compounds able to improve the activity of some commercial antimicrobials, especially gentamicin, erythromycin, tetracycline and ciprofloxacin. The development of pharmaceutical preparations containing products derived of *M. urundeuva* bark constitute a possible sustainable alternative to overcome resistance and others undesirable effects related to conventional antibiotics therapy. Further studies aiming to identify the active compounds and action mechanisms involved should be performed.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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