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Antifungal activity of plant extracts against *Microsporum canis* and *Candida* spp. strains

[Actividad antifúngica de extractos de plantas contra cepas de *Microsporum canis* y *Candida* spp.]

Bruna V. Soares[1], Selene M. Morais[1,2], Raquel O.S. Fontenelle[3,4], Erika H.S. Brito[5], Vanessa A. Queiroz[2], C.M.S. Castro[2], Nadja S. Vila-Nova[1], Carolina S.P. Cavalcante[1,7] & Marcos F.G. Rocha[1,6]

[1]*Postgraduate Program in Veterinary Sciences, State University of Ceará, Brazil*
[2]*Department of Chemistry, State University of Ceará, Fortaleza, CE, Brazil*
[3]*Centre of the Agricultural Sciences and Biological, Acaraú Valley State University, Sobral, CE and*
[4]*Postgraduate Program in Natural Recursions, State University of Ceará, Fortaleza, CE, Brazil*
[5]*International University of Integrating Lusophone African-Brazilian, Campus dos Palmares, Acarape, CE*
[6]*Departament of Pathology and Legal Medicine, School of Medicine, Specialized Medical Mycology Center, Federal University of Ceará, Fortaleza, Ceará, Brazil*
[7]*Postgraduation Program in Pharmaceutical Sciences, Federal University of Ceará, Fortaleza, CE, Brazil.*
Contactos | Contacts: **Raquel O.S. FONTENELLE - E-mail address: raquelbios@yahoo.com**

Abstract: Aiming to find a phytotherapeutic compounds to treat animal fungal infections, plants commonly found in Northeastern Brazil were evaluated *in vitro* against *Microsporum canis* and *Candida* spp. strains isolated from dogs and cats. The leaf ethanol extracts of *Momordica charantia*, *Calotropis procera*, *Peschiera affinis* and *Piper tuberculatum* and decoction of *Mangifera indica* were initially evaluated by the agar-well diffusion method. Four extracts induced growth inhibition zones against *M. canis*: *P. tuberculatum* (20 mm), *M. indica* (14 mm), *M. charantia* (13 mm) and *P. affinis* (11 mm). None of them were active against *Candida* spp. Broth microdilution tests were performed for *M. canis* strains (n=5), to find the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). The geometric means for the MIC values were 590, 370, 350, 170 µg/mL, and for the MFC values were 1190, 750, 700, 340 µg/mL for *M. charantia*, *P. affinis*, *P. tuberculatum* and *M. indica*, respectively. Therefore, extracts from *M. charantia*, *P. affinis*, *P. tuberculatum* and *M. indica* are good candidates to produce antifungal phytotherapics since these extracts demonstrated good activity against *M. canis*.

Keywords: Antifungal activity, Plant extracts, *Microsporum canis*, *Candida* spp

Resumen: Con el objetivo de encontrar compuestos fitoterapéuticos para tratar las infecciones por hongos de los animales, plantas que se encuentran comúnmente en el noreste de Brasil se evaluaron *in vitro* frente a cepas de *Microsporum canis* y *Candida* spp. aisladas de perros y gatos. Los extractos etanólicos de hojas de *Momordica charantia*, *Calotropis procera*, *Peschiera affinis* y *Piper tuberculatum* y la decocción de *Mangifera índica* fueron evaluados inicialmente por el método de difusión en pocillos de agar. Cuatro extractos indujeron zonas de inhibición del crecimiento contra *M. canis*: *P. tuberculatum* (20 mm), *M. índica* (14 mm), *M. charantia* (13 mm) y *P. affinis* (11 mm). Ninguno de ellos fue activo contra *Candida* spp. Se realizaron pruebas de microdilución en caldo para las cepas de M. canis (n = 5), para encontrar la concentración mínima inhibitoria (CIM) y la concentración fungicida mínima (CFM). Las medias geométricas de los valores de CIM fueron 590, 370, 350, 170 mg/ml, y para los valores de CFM fueron 1.190, 750, 700, 340 mg/ml de *M. charantia*, *P. affinis*, *P. tuberculatum* y *M. indica*, respectivamente. Por lo tanto, los extractos de *M. charantia*, *P. affinis*, *P. tuberculatum* y *M. indica* son buenos candidatos para la producción de fitoterápicos antifúngicos ya que estos extractos demostraron una buena actividad contra M. canis.

Palabras clave: Actividad antifúngica, Extractos de plantas, *Microsporum canis*, *Candida* spp

INTRODUCTION

Many plants from Brazilian biomes, such as the Caatinga, Cerrado (savannah) and Atlantic and the Amazon rainforests, have been used as natural medicines by local populations to treat tropical diseases, including leishmaniasis, malaria, schistosomiasis, fungal and bacterial infections (Alves *et al*., 2000; Duarte *et al*., 2005; Fontenelle *et al*., 2008; Cavalcante *et al*., 2012; Garcia *et al*., 2012; Soares *et al*., 2012; Abrantes *et al*., 2013). Moreover, many exotic plants introduced in Brazil following colonization have been incorporated into folk medicine (Duarte *et al*., 2005).

Several effective antimicrobials have been developed over the years. Nevertheless, there has been increased development of antimicrobial drug resistance to currently available antimicrobials (Coe & Anderson, 1996; Eksi *et al*., 2013). The indiscriminate use of antibiotics worsens this problem, leading the development of drug-resistant fungal infections. Based on this fact, it is necessary to have a broader array of medicines available for the efficient control of fungal infections (Martinez-Rossi *et al*., 2008; Mehrabani *et al*., 2013).

In veterinary practice, dermatophytoses are among the most common infectious skin diseases in mammals worldwide. They are frequently observed in domestic animals, but also in captive and wild fauna. Few antifungal agents are licensed for use in veterinary practice, and the use of systemic drugs is limited in livestock due to problems of residues in products intended for the human consumption (Chermette *et al*., 2008). Yeasts of the genera *Candida* can be found as commensal microorganisms in animals and are considered one of the most important species in veterinary medicine. Strains of *Candida* spp. isolated from dogs showed high resistance to azole antifungal agents (Brito *et al*., 2007). To contribute to organic management of domestic animals, we chose some medicinal plants to evaluate their antifungal activity against common fungal infections caused by *Microsporum canis* and *Candida* spp.

Mormodica charantia was our first choice since the ethanol extract from leaves of this plant was previously tested against *Microsporum canis* infected

rabbits and showed good results (Braga *et al*., 2007). The same extract was also shown to have anthelmintic activity against *Haemonchus contortus* (Batista *et al*., 1999) and significant molluscicidal activity, causing 85% mortality at a concentration of 100 ppm (Rodrigues *et al*., 2010). The other plants chosen were *Calotropis procera*, *Mangifera indica*, *Peschiera affinis* and *Piper tuberculatum.*

The essential oil from flowers of *Calotropis procera*, and latex were tested against *Enterobacter cloacae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Aspergillus flavus*, *Curvularia luneta* [*Cochliobolus lunatus*], *Drechslera tetrâmera* [*Cochliobolus spicifer*], *Fusarium moniliforme* [*Gibberella fujikuroi*] and *Candida albicans*. Maximum inhibitory activity was observed in ethanol extracts of root bark against *Enterobacter cloacae* and of the stem against *F. moniliforme.* Leaves were not tested due to the negative findings of Jain *et al*. (1996).

Mangifera indica is widely cultivated in tropical regions of the world. Leaf decoctions are taken as remedies for fever, chest pains, diarrhea, diabetes and hypertension. Extracts of bark, leaves, stems and unripe fruits are used as antibiotics against many ailments (Aiyelaagbe & Osamudiamen, 2009).

The aim of this study was to screen plants commonly found on Northeast of Brazil against *Microsporum canis* and *Candida* spp. strains isolated from symptomatic dogs and cats to find new antifungal agents.

MATERIAL AND METHODS
Plant material
Table 3 shows the names of the plants, collection site, plant part and extract type used in this study. The plant material was dried at room temperature for seven days, and powdered using a knife mill. The powdered material was extracted using 98% ethanol as solvent at room temperature for seven days. The extracts were then filtered and concentrated under vacuum in a rotary evaporator. The decoctions resulting from the extraction of the essential oil were extracted with ethyl acetate and after solvent elimination, ethyl acetate extract was obtained.

Table 3
Plant extracts used in the study

Scientific name	Common name	Part used	Type of Extract
Calotropis procera R.Br.	Jealousy, Silk flower	Leaves	Ethanol
Momordica charantia L.	São-caetano-melon	Leaves, fruits, flowers, stalk	Ethanol
Peschiera affinis (Müell.Arg.) Miers	Grain cock	Stems	Ethanol
Piper tuberculatum Jacq.	Long pepper	Leaves	Ethanol
Mangifera indica L.	Mango	Leaves	Decoct

Phytochemical study
Peschiera affinis (**Müell.Arg.**)

The ethanol extract of stems was submitted to acid-alkaline treatment for separation of alkaloids from neutral and acid compounds. Both alkaloids and other compounds were tested against *M. canis* and *C. albicans* and the first were not active. The neutral part showed activity and was fractioned in a silica gel chromatographic column. A series of mixtures of petroleum ether and ethyl acetate with increasing polarities were eluted through the column. A compound was separated in a fraction eluted with a 70:30 mixture of these two solvents, respectively. The spectroscopic data (^{1}H and ^{13}CNMR) were similar to those of the triterpene Lupeol (Mahato, 1994). The fractions obtained with a 60:40 mixture led to isolation of a second compound, an amorphous white solid characterized by spectroscopic data (^{1}H and ^{13}CNMR) as β-sitosterol based on literature data (Moreira *et al.,* 1998).

Mangifera indica L.

The aqueous solution obtained from extraction of volatile constituents of mango leaves was lyophilized. The resulting light yellow powder was submitted to phytochemical tests to detect the presence of secondary metabolites such as phenols, tannins, leucoantocianidins, flavonoids, steroids, triterpenes and alkaloids, according to Matos (2009). These tests are based on visual observation of color change or precipitate formation after addition of specific reagents. The lyophilized material was adsorbed on silica gel (60-120 mesh) in the proportion of 1:1 and submitted to chromatographic analysis with passage through a silica gel column packed with petroleum ether (60-80°). Chloroform and methanol in mixtures of increasing polarity were

eluted through the column, which gave a pale yellow amorphous powder as the main product. After crystallization from ethanol, this produced pale yellow needle crystals, mp: 268-270°, λ max: 205.6, 256.8, 238.4, 315.2, 367.2 nm. IR (KBr) cm^{-1}: 3366(O-H), 2937(C-H), 1649(C=O), 1495(C=C), 1253(-C-O), 1050 (C-O-C). NMR (δ ppm): 13.8 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.8 (Ar-H, 1H), 6.4 (Ar-H, 1H), 7.4 (ArH, 1H), 2.5 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 2H), 3.5 (-CH-, 3H). The spectroscopic data of the compound isolated from chromatographic treatment of the amorphous powder of mango leaf decoctions was revealed to be mangiferin by comparison with previously published data (Singh *et al.,* 2009).

Piper tuberculatum Jacq.

The ethanol extract had a pleasant smell due to the presence of essential oil rich in (*E*)-caryophyllene and germacrene-D and pinene. The essential oil of *P. tuberculatum*, obtained by steam distillation of leaves, was previously studied by Facundo & Morais (2005) and presented as major components (*E*)-caryophyllene (26.3%) and α-cadinol (13.7%). In another study, the most significant constituents of the oil of *P. tuberculatum* were the sesquiterpenes (*E*)-caryophyllene (37.78%) and germacrene D (11.81%), while α-pinene (4.06%) and β-pinene (4.51%) were the main constituents of the monoterpene fraction.

M. charantia L.

Qualitative phytochemical tests of *M. charantia* were performed according to Matos (2009). In this phytochemical study, the leaf extract was mixed with ethanol and distributed in several test tubes, to which specific reagents were added, revealing the presence of alkaloids due to a precipitate formed by the

addition of Dragendorf and Mayer reagents; phenols due to formation of a dark blue color with $FeCl_3$ solution; steroids due to the green color with the Lieberman-Buchard reagent; and saponins because of formation of a constant spoon by shaking the sample with water.

Fungal strains
A total of five strains of *M. canis*, two strains of *Candida albicans* and two strains of *Candida tropicalis* were isolated from dogs and cats. The strains were stored in the fungal collection of the Specialized Medical Mycology Center – CEMM (Federal University of Ceará, Brazil), where they were maintained in saline (0.9% NaCl) at 28° C. At the time of the analysis, an aliquot of each suspension was taken and inoculated into potato dextrose agar (Difco, Detroit, USA), and then incubated at 28° C for 2-10 days.

Inoculum preparation for antifungal susceptibility tests
For the agar-well diffusion method, based on Fontenelle *et al*. (2007), stock inocula were prepared on day 2 and day 10 for *Candida* spp. and *M. canis*, respectively, grown on potato dextrose agar (Difco, Detroit, USA) at 28 °C. Potato dextrose agar was added to the agar slant and the cultures were gently swabbed to dislodge the conidia. The suspensions with blastoconidia of *Candida* spp. or suspension of hyphal fragments of *M. canis* were transferred to a sterile tube and adjusted by turbidimetry to obtain inocula of approximately 10^6 cfu/mL blastoconidia of *Candida* spp. and 10^5 cfu/mL hyphal fragments or conidia of *M. canis*. The optical densities of the suspensions were spectrophotometrically determined at 530 nm and then adjusted to 95% transmittance.

For the broth microdilution method, standardized inocula ($2.5 – 5$ x 10^3 cfu/mL for *Candida* spp. and 5 x 10^4 cfu/mL for *M. canis*) were also prepared by turbidimetry. Stock inocula were prepared on day 2 and day 10 for *Candida* spp. and *M. canis* cultures, respectively, grown on potato dextrose agar at 28° C. Sterile normal saline solution (0.9%; 3mL) was added to the agar slant and the cultures were gently swabbed to dislodge the conidia from the hyphal mat for the *M. canis* (Brilhante *et al*., 2005) and the blastoconidia from *Candida* spp. (Brito *et al*., 2007). The suspensions of conidia with hyphal fragments of *M. canis* and the blastoconidia

suspension of *Candida* spp. were transferred to sterile tubes, and the volume of both suspensions was adjusted to 4 mL with sterile saline solution. The resulting suspensions were allowed to settle for 5 min at 28° C and their density was read at 530 nm and the adjusted to 95% transmittance. The suspensions were diluted to 1:2000 for *Candida* spp. and 1:500 for *M. canis,* both with RPMI 1640 medium (Roswell Park Memorial Institute – 1640) with L-glutamine without sodium bicarbonate (Sigma Chemical Co., St. Louis, Mo.), and then buffered to pH 7.0 with 0.165M MOPS (Sigma Chemical Co., St. Louis, Mo.), to obtain the inoculum size of approximately $2.5 – 5$ x 10^3 cfu/ml for *Candida* spp. and 5 x 10^4 cfu/mL for *M. canis.*

Agar-well diffusion susceptibility test
The antifungal activity of the plant extracts was evaluated against *Candida* spp. (n=4) and *M. canis* (n=4), by the agar-well diffusion method according to Fontenelle *et al.* (2007). Petri dishes with 15 cm diameter were prepared with potato dextrose agar (Difco, Detroit, USA). The wells (6 mm in diameter) were then cut from the agar and 100 μL of essential oil was delivered into them. The extracts were weighed and dissolved in DMSO to obtain the test concentration of 10000 μg/mL. Stock solutions of griseofulvin (1000 μg/mL; Sigma Chemical Co., St. Louis, USA) and amphotericin B (5 μg/mL; Sigma Chemical Co., USA) were prepared in DMSO and tested as positive controls for *M. canis* and *Candida* spp., respectively. Each fungal suspension was inoculated on to the surface of the agar. After incubation (2 days for *Candida* spp. and 5 days for *M. canis*) at 28 °C, all dishes were examined for zones of growth inhibition and the diameters of these zones were measured in millimeters. Each experiment was repeated at least twice.

Broth microdilution method
The minimum inhibitory concentration (MIC) for *Candida* spp. was determined by the broth microdilution method, in accordance with the Clinical and Laboratory Standards Institute – CLSI (formerly NCCLS; M27-A2, 2002). The broth microdilution assay for *M. canis* was performed as described by Brilhante *et al*. (2005), based on the M38-A document (CLSI; formerly NCCLS, 2002). The minimum fungicidal concentrations (MFC) for both *Candida* spp. and *M. canis* were determined according Fontenelle *et al.* (2007). In addition, *C.*

parapsilosis (ATCC 22019) and *C. albicans* (ATCC 1023) strains were used as quality controls for broth microdilution method.

The plant extracts (10,000 µg) were diluted in DMSO (1 mL). Amphotericin B (AMB) (Sigma, Chemical Co., USA) and griseofulvine (Sigma Chemical Co., St. Louis, USA) were prepared in DMSO. For the susceptibility analysis, the plant extracts were tested in concentrations ranging from 4 to 5,000 µg/mL.

Statistical analysis

Antifungal activity was expressed as mean ± SD of the diameter of the growth inhibition zones (mm). The antifungal activity of the plant extracts was analyzed by linear correlation for individual analysis and the two-tailed Student t-test at 95% confidence intervals was used to evaluate differences between the plant extracts and the controls.

RESULTS AND DISCUSSION

The antifungal activity of natural products obtained from common plants found in Brazil's Northeast was initially tested by the agar-well diffusion assay, at 10 mg/mL concentration, against four strains of *M. canis*, two strains of *Candida albicans* and two strains of *Candida tropicalis* isolated from dogs and cats. Of the tested substances, five induced growth inhibition zones against *M. canis*, but none of then induced significant growth inhibition zones against *Candida* spp. These results are shown in Table 1. The ethanol extract of *P. tuberculatum* (20 mm) was the most effective against *M. canis*, followed by *M. indica* (14 mm), *M. charantia* (13 mm), *P. affinis* (11 mm), while for *C. procera* the result was considered not significant. The positive control, griseofulvine, induced significant growth inhibition zones (55.25 ± 3.69 mm) against *M. canis* and amphotericin B induced a significant growth inhibition zone (10.25 ± 1.26 mm) against *Candida* spp.

Based on this initial screening, the active extracts were submitted to the broth microdilution method (Table 2) just for *M. canis* strains (n=5). For the *M. charantia* extract, the MIC ranged from 150 to 1250 µg/mL, and the MFC ranged from 310 to 2500 µg/mL; for the *P. affinis* extract, the MIC ranged from 310 to 620 µg/mL, and the MFC ranged from 620 to 1250 µg/mL; for the *P. tuberculatum* extract, the MIC ranged from 39 to 620 µg/mL, and the MFC ranged from 78 to 1250 µg/mL; and for *M. indica*,

the MIC ranged from 78 to 310 µg/mL, and the MFC varied from 150 to 620 µg/mL.

P. tuberculatum showed the lowest MIC (39 µg/mL) and MFC (78 µg/mL) values, but according to geometric means, the extract of *M. indica* was the most effective, showing the lowest values of MIC (170 µg/mL) and MFC (340 µg/mL), followed by *P. tuberculatum*, *P. affinis* and *M. charantia* (Table 2).

Both β-sitosterol and lupeol isolated from *P. affinis* have been reported as having antifungal activity: From a methanol extract of dried-ground aerial parts of *Senecio lyratus*, an antifungal and antibacterial active compound was isolated and identified as β-sitosterol (Kiprono *et al*, 2000) and the results of another study demonstrated that Lupeol failed to display appreciable activity against *Candida albicans* but demonstrated high and selective activity against *Microsporum canis* (Gallo & Sarachine, 2009).

M. charantia (*Cucurbitaceae*), commonly known as balsam pear, bitter gourd or karela, is used for several purposes in traditional medicine. The fruits, leaves and roots of this plant have been used as laxatives and anthelmintics, and some studies have shown hypoglycemic potential in normal and diabetic rats (Begun *et al*., 1996; El-Batran *et al*., 2006). Schormoulo *et al*. (2005) reported use in folk medicine to treat scabies, and the aqueous extracts were effective against *C. albicans* and *C. neoformans in vitro*, showing 150 and 180 mg/mL MICs values. *In vivo* studies the ethanol extract showed good action against *Mycrosporum canis* but in this study the ethanol extract of *M. charantia* did not show significant activity against the four stains of *Candida* spp tested.

Aqueous extracts of the leaves of *M. charantia* did not show activity against *Epidermophyton flocosum*, *M. canis* and *T. mentafrophytes* (Bhakuni *et al*., 1998). *M. charantia* showed antimicrobial activity at 0.18 mg/10mL plate of medium, with activity being most prominent with the ethanol extracts and negligible with the hexane extracts. The results of the above study suggest that the ethanol extracts of *M. charantia* might be effective as herbal medicines to control *E. coli* and *S. aureus* induced diseases, although clinical trials are necessary to confirm the initial findings. Many compounds like saponins, steroids, alkaloids and phenols display antifungal activity and could be

responsible for the antifungal activity of the ethanol extract of *M. charantia* found in the present trial.

Several *Piper* species have been used in traditional medicine to treat many diseases, including gynecological maladies like vaginitis, intestinal disorders and others (Maia *et al*., 1998; Moreira *et al*., 1998). The chemical studies carried out of Brazilian Piperaceae species have revealed the occurrence of pyrones, lignoids and chromenes besides various amides, which have shown potent insecticidal and antifungal properties. The essential oils from leaves, stems and fruits of *P. tuberculatum*, *P. aduncum* and *P. arboreum* were found to be active against *Cladosporium sphaerospermum* and *C. cladosporioides* (Debonsi *et al*., 2006). The ethanol extract of *P. tuberculatum* leaves tested in this study showed good activity against *M. canis* strains, corroborating that this plant has antifungal properties.

With respect to the compounds present in the essential oil *P. tuberculatum*, some studies have shown that alpha-cadinol possesses antifungal activity against a broad spectrum of plant pathogenic fungi and could be used as a potential antifungal agent for the control of fungal diseases in plants, and also that α-pinene and β-pinene display several activities including fungicidal (Silva *et al*., 2012).

M. indica (*Anacardiaceae*) grows in tropical and subtropical regions and its parts are commonly used in folk medicine for a wide variety of ailments (Coe & Anderson, 1996). Vimang® is an anti-inflammatory produced in Cuba from *M. indica* compounds. This property is mainly associated with a metabolite also found in mango leaves, mangiferin, which is the predominant component of the aqueous extract (Garrido *et al*., 2004). Mangiferin, traditionally used by native inhabitants of Bolivia, Southern Guiana, the Antilles, Colombia, the Philippines and India in the treatment of a number of diseases, has subsequently been proved to have varied pharmacological activities, such as antiviral and antitumor, spasmolytic, antioxidant, antidiabetic, immunostimulating, antifungal and antibacterial activities (Stoilova *et al*., 2005). The phytochemical study of *Mangifera indica* leaf extract revealed the presence of steroids, flavonoids, reducing sugar and cardiac glycosides in the hexane extract; anthraquinone, tannin and reducing sugar in the ethyl acetate extracts; and saponin, steroids, tannin, flavonoid, reducing sugars and cardiac glycosides in the methanol extracts (Aiyelaagbe & Osamudiamen, 2009). Tannins are reported to exhibit antiviral,

antibacterial, antitumor activities and cardiac glycosides are known to work by inhibiting the Na^+/K^+ pump. This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for contraction of the heart muscle, which improves cardiac output and reduces distention of the heart. Thus, these compounds are used in the treatment of congestive heart failure and cardiac arrhythmia. They are also used to strengthen weakened hearts and allow them to function more efficiently, though the dosage must be controlled carefully, since the therapeutic dose is close to the toxic dose (Denwick, 2002).

According to Schormoulo *et al*. (2005), the decoction of *M. indica* did not show antifungal activity against *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton rubrum*, but in this study the ethyl acetate fraction of this decoction showed good activity against five strains of *M. canis*. This antifungal action probably is related to the presence of compounds such as tannins and cardiac glycosides, which have correlated actions.

C. procera is used in traditional medicine as a purgative, anthelmintic, anticoagulant, antiinflammatory, antipyretic, analgesic, antimicrobial and anticancer agent, and to treat leprosy, leucoderma, ulcers, tumors, piles, and diseases of the spleen, liver and abdomen. Ethanol extracts of leaves, stems, roots, flowers, fruits and root bark contain steroids, flavonoids, cardenolides, anthocyanins (Jain *et al*., 1996). Leaf oil is dominated by tyranton (54.4%), 1- pentadecene (9.5%) and 1-heptadecene (8.2%). The most abundant compounds in stem oil are Z-13-docosenamide (31.8%), isobutyl nonane (13.7%) and 2,7,10-trimethyldodecane (12.3%). Both leaf and stem volatile oils contain octadecenamide and its saturated form in appreciable amounts. Also characteristic of these oils is the presence of long chain fatty acids and amides, sulfates, halogenated compounds and ketones (Moronkola *et al.*, 2011).

Plant steroids are known to be important for their cardiotonic activities. They also possess insecticidal and anti-microbial properties and are used in nutrition, herbal medicine and cosmetics (Aiyelaagbe & Osamudiamen, 2009). They are routinely used in medicine because of their profound biological activities. Flavonoids have been referred to as nature's biological response modifiers due to

strong experimental evidences of their inherent ability to modify the body's reaction to allergies, viruses and carcinogens. These compounds have shown antiallergic, antiinflammatory, antimicrobial and anticancer activities (Denwick, 2002). Both types of compounds, besides others, could responsible for the antifungal action of *C. procera*.

Table 1
Antifungal activity of the plant extracts and fractions against *Microsporum canis* and *Candida* spp. in the agar-well diffusion assay.

Extracts and fractions	Test Concentration (µg/mL)	Growth inhibition zones (mm) (mean ± SD):	
		M. canis	*Candida* spp.
C. procera	10.000	NI	NI
M. charantia	10.000	13	NI
P. affinis	10.000	11	NI
P. tuberculatum	10.000	20	11
M. indica	10.000	14	NI
*Griseofulvine	1000	(55.25 ± 3.69)	-
*Amphotericin B	5	-	(10.25 ± 1.26)

Each experiment was performed in duplicate
(NI): No inhibitory activity
(-): Not tested
(*): Control group

Table 2
Minimum inhibitory and fungicidal concentrations of plant extracts against *M. canis* strains.

Strains	Plant extracts							
	M. charantia		*P. affinis*		*P. tuberculatum*		*M. indica*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
M. canis								
CEMM 01-5-190	150	310	310	620	39	78	78	150
CEMM 01-4-104	310	620	310	620	150	310	78	150
CEMM 01-3-188	620	1250	310	620	310	620	78	150
CEMM 01-3-186	620	1250	310	620	620	1250	310	620
CEMM 01-3-165	125	2500	620	1250	620	1250	310	620
(Geometric mean)	590	1190	370	750	350	700	170	340

MIC: Minimum inhibitory concentration expressed in µg/mL ;
MFC: Minimum fungicidal concentration expressed in µg/mL;
CEMM: Specialized Medical Mycology Center.
Each experiment was repeated at least twice.
Broth microdilution method

CONCLUSIONS
In veterinary practice, dermatophytoses are among the most common infectious skin diseases in mammals worldwide. They are frequently observed in domestic animals, but also in captive and wild fauna (Chermette *et al*., 2008). Therefore extracts from *M. charantia*, *P. affinis*, *P. tuberculatum* and *M. indica,* are good candidates to produce a phytoterapic since these extracts demonstrated good antifungal activity against *M. canis*.

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