Journal of Medicinal Plants Research Vol. 4(17), pp. 1810-1814, 4 September, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR09.303
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Composition and antifungal activity against *Candida albicans, Candida parapsilosis*, *Candida krusei* and *Cryptococcus neoformans* of essential oils from leaves of *Piper* and *Peperomia* species

Andreia de Araujo Morandim-Giannetti[1,2]*, Aline Ringis Pin[1], Natalia Ama Santo Pietro[1], Haroldo Cesar de Oliveira[2], Maria Jose Soares Mendes-Giannini[2], Alberto Camilo Alecio[3,5], Massuo Jorge Kato[4], Jose Eduardo de Oliveira[3] and Maysa Furlan[3]

[1]Departamento de Engenharia Química, Centro Universitário da FEI, Av. Humberto de Alencar Castelo Branco, 3972, 09850-901, São Bernardo do Campo-SP, Brazil.
[2]Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Rua Expedicionários do Brasil, 1621, 14801-902, Araraquara-SP, Brazil.
[3]Instituto de Química, Universidade Estadual Paulista, CP 355, 14800-900, Araraquara-SP, Brazil.
[4]Instituto de Química, Universidade de São Paulo, CP 26077, 05599-970, São Paulo-SP, Brazil.
[5]Universidade Paulista, Av. Alberto Benassi, 200, 14804-300, Araraquara-SP, Brazil.

Accepted 2 August, 2010

This study was addressed to investigate the composition and antifungal activity of essential oils from leaves of Piperaceae species (*Piper aduncum*, *Piper amalago*, *Piper cernuum*, *Piper diospyrifolium*, *Piper crassinervium*, *Piper gaudichaudianum*, *Piper solmsianum*, *Piper regnellii*, *Piper tuberculatum*, *Piper umbelata* and *Peperomia obtusifolia*) against *Candida albicans*, *C. parapsilosis*, *C. krusei* and *Cryptococcus neoformans*. The essential oils from *P. aduncum*, *P. gaudichaudianum* and *P. solmsianum* showed the highest antifungal activity against *Cryptococcus neoformans* with the MIC of 62.5, 62.5 and 3.9 mg.mL-1, respectively. The oil from *P. gaudichaudianum* showed activity against C. krusei with MIC of 31.25 mg.mL-1.

Key words: Piperaceae, *Piper*, *Peperomia*, essential oil composition, antifungal activity, GC-MS.

INTRODUCTION

Piperaceae species are mostly pioneer shrubs with several medicinal uses such as treatment of many diseases including gynaecological maladies, vaginitis, intestinal disorders, psychotropic, antimicrobial, antioxidant and cytotoxic effects. Their species are widely spread in tropical regions and have shown the accumulation of several classes of physiologically active natural products such as alkaloids, amides, pyrones, dihydrochalcones, flavonoids, phenylpropanoids, lignans neolignans, chromenes and terpenes (Silva et al., 2002; Batista et al., 2008; Felipe et al., 2007; Lago et al., 2004; Benevides et al., 1999; Moreira et al., 1998; Maia et al., 1998; Navickiene et al., 2006; Parmar et al., 1997).

The volatile components from aerial parts of Piperaceae species were subjected to a number of investigations showed a predominance of monoterpenes (C10) and sesquiterpenes (C15) although diterpenes (C20) and phenylpropanoids. The essential oils exhibited antimicrobial, antioxidant, antiinflammatory, antispasmodic, relaxing properties have been described in both animals and humans (Tognolini et al., 2006; Dorman et al., 2000).

The essential oils from *Ottonia anisum*, *Piper amplum, Piper arboreum, Piper aduncum, Piper tuberculatum, Piper dilalatum, Piper goesii, Piper hispidum, Piper*

*Corresponding author. E-mail: andreia.morandim@gmail.com. Tel: +55-011-43532900. Fax: +55-01141095994.

hoffmanseggianum, Piper nigrum, Piper gaudichaudianum, Piper guineense, Piper mollicomum, Peperomia blanda, Piper gaudichaudianum, Piper regnellii and *Piper cernuum* showed biological activity including strong molluscicidal activity against *Biomphalaria glabrata* besides cytotoxic, fungistatic, insecticide and antibacterial activities (Navickiene et al., 2006; Santos et al., 2001; Moreira et al., 1998; Costantin et al., 2001; Maia et al., 1987).

In this paper, we wish to describe the essential oil composition from leaves of *Piper amalago, P. aduncum, P. cernuum, P. diospyrifolium, P. crassinervium, P. gaudichaudianum, P. regnellii, P. solmsianum, P. tuberculatum, P. umbellata* and *Peperomia obtusifolia*. Additionally, their antifungal activities were evaluated against the fungi *Candida albicans, C. parapsilosis, C. krusei* and *Cryptococcus neoformans* by microdilution method. Furthermore, this is the first report for the composition of essential oil from *Piper umbelata* and *Peperomia obtusifolia*.

MATERIALS and METHODS

Plant material

P. aduncum L. (Cordeiro-PA0) and *P. umbelata* (L.) MIQ. were collected at the Institute of Chemistry (UNESP) in Araraquara, in São Paulo State, Brazil. The voucher specimens are deposited in the Herbarium of the Botanical Institute, in São Paulo city, Brazil. *P. cernuum* Vell., *P. regnellii* (Miq) C.D.C., *P. crassinervium* Kunth and *P. tuberculatum* Jacq were collected at the Campus of the University of Sao Paulo, in Sao Paulo city, Brazil and they were identified by Dr. Guillermo E. Delgado (Universidad Nacional Pedro Ruiz Gallo, Peru). Voucher specimens Kato-0137, E. Guimarães-1961, Kato-84 and Kato-163, respectively, are deposited in the Herbarium of the Botanical Institute, in Sao Paulo city, Brazil.

P. solmsianum (Miq.) Yunck, *P. amalago* L., *P. diospyrifolium* Kunth, *P. gaudichaudianum* Kunth and *P. obtusifolia* (L.) A. Dietr. were collected at the Campus of the University of Sao Paulo (Brazil) and were identified by Dr Elsie Franklin Guimaraes (Rio de Janeiro Botanical Garden, in Rio de Janeiro city, Brazil) as voucher specimens Kato-27, Kato-244, Kato-431, Kato-489, Kato-70, respectively. They are deposited in the Herbarium of the Rio de Janeiro Botanical Garden, in Rio de Janeiro city, Brazil.

Extraction of essential oil

Plant material (100 g of each *Piper* or *Peperomia* species) was subjected to hydro-distillation in a Clevenger-type apparatus for 2 h. The obtained oil layers were dried out over anhydrous Na_2SO_4. The average yields were achieved after three experiments and they were calculated on a dried weight material basis.

Essential oil analysis

Gas chromatography-mass spectrometryanalyses

Analyses were carried out using a Shimadzu Gas chromatography-mass spectrometry (GC-MS) automated chromatograph model GC-17A/QP-5050A, connected to an auto-sampler model AOC20i. Their operation was managed by using the software GCM Solutions v.1.02 workstation (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on a fused-silica capillary nonpolar column DB–5-MS (30 m x 0,25 mm i.d. x 0,25 µm film thickness; J and W Scientific, Folsom, CA, USA) with phenyl arylene polymer virtually equivalent to (5% - Phenyl)-methylpolysiloxane at the stationary phase. All of the analyses were carried out using EI/MS (in positive mode) with the scanning acquisition mode (40 to 500 m/z). The oven temperature was initially at 60°C, and then increased at the rate of 3°C min^{-1} to 240°C. This was kept for 10 min, and the analysis was completed within 70 min (Adams, 1995). The carrier gas was helium at a constant flow rate of 1 mL min^{-1} and, sample aliquots (1 µL) were injected in the split mode (1:20) without pre-treatment solvent delay. The injector and detector temperatures were maintained at 220 and 240°C, respectively.

Antifungal experiment

The test organisms included *C. albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019 and *C. neoformans* ATCC 90012. The microorganisms were originally obtained from the Mycology Laboratory of the Department of Clinical Analysis of the Sao Paulo State University (UNESP). The yeasts were grown and kept on Sabouraud-dextrose agar for 24 h to 48 h, at room temperature. The antifungal activity tests were performed using the broth micro-dilution method described in the M27-A2 document of the Clinical and Laboratory Standards Institute (CLSI) with minimum modifications (Rodriguez-Tudela et al., 1996). The medium used was RPMI 1640 with L-glutamine buffered to pH 7.0 with 0.165 $mol.L^{-1}$ morpholinepropanesulfonic acid (MOPS), supplemented with 2% glucose.

Extracts and fractions were prepared in DMSO and to each well of 96-well U-bottom culture plate was added 100 µL of culture together with 100 µL of 2-fold serial diluted test compound. The cell suspension was prepared in 0.85% saline with an optical density equivalent to McFarland 0.5 and diluted 1:100 in RPMI for the final concentration of 1 x 10^5 to 5 x 10^5 $CFUmL^{-1}$. This suspension was inoculated on a micro-dilution plate previously prepared with the oils diluted in the concentration between 1000 and 0.48 μgmL^{-1}. The plates were incubated with agitation at 37°C for 24 h for *C.* species and 48 h for *C. neoformans*.

Amphotericin B (Sigma) was used as a reference compound with concentrations ranging from 16 to 0.03 $\mu g.ml^{-1}$. The Minimum individual Inhibitory Concentration (MIC) was calculated as the highest dilution showing complete inhibition of the tested strain. The reference value of amphotericin's MIC was obtained in accordance with the guidelines of CLSI document M27-A2. The MIC for each oil was defined as the lowest concentration that is able to inhibit any visible fungal growth. Results were visually and spectrophotometric analyzed. The antimicrobial activity was considered high, moderate, weak or inactive when MIC values were lower than 100; from 100 to 500; from 500 to 1000 or higher than 1000 $\mu g.mL^{-1}$, respectively (Holetz et al., 2002).

RESULTS AND DISCUSSION

The yields of essential oils for 14 species obtained by hydro-distillation of leaves and calculated on a dry weight basis were variable: *P. aduncum* (0.81%), *P. amalago* (0.22%), *P. cernuum* (0.23%), *P. diospyrifolium* (1.46%), *P. crassinervium* (0.94%), *P. gaudichaudianum* (1.80%),

Table 1. Composition of the essential oils from different *Piper* and *Peperomia* species.

Compound	RI	A	B	C	D	E	F	G	H	I	J	K
Yield (%)		**92.80**	**99.09**	**99.40**	**99.68**	**85.87**	**95.76**	**91.51**	**78.16**	**98.80**	**54.10**	**95.04**
tricyclene	914	-	-	-	1.40	-	-	-	-	-	-	-
α-pinene	939	1.50	-	2.10	1.06	-	0.23	0.54	-	3.10	-	-
camphene	953	-	-	-	-	-	0.64	-	-	-	-	-
β-pinene	980	2.40	-	-	2.10	-	-	13.34	-	3.20	-	-
myrcene	991	-	-	-	-	-	-	15.45	-	-	-	-
p-cymene	1026	-	-	-	-	-	-	0.99	-	-	-	-
limonene	1031	1.70	-	-	3.20	-	-	1.60	-	-	-	-
β-phellandrene	1031	-	0.54	-	-	-	-	0.77	-	-	-	-
Z-β-ocimene	1040	3.50	-	0.59	-	-	-	-	-	-	-	-
E-□-ocimene	1050	5.00	-	-	-	-	-	-	-	2.50	-	-
γ-terpinene	1061	-	-	0.38	-	-	-	-	-	-	-	-
linalool	1098	31.80	-	-	-	-	-	1.65	-	-	-	-
α-cubebene	1351	-	-	-	-	-	0.37	-	-	-	-	-
α-copaene	1376	0.50	2.55	2.73	1.31	1.06	1.59	1.27	0.29	-	-	-
β-bourbonene	1384	-	1.03	0.56	-	-	-	-	-	-	-	-
β-cubebene	1390	-	0.61	0.73	-	-	-	-	-	-	-	-
β-elemene	1391	-	-	7.15	0.78	-	1.64	-	0.62	-	-	-
cyperene	1398	-	-	-	-	-	0.50	-	-	-	-	-
α-gurjunene	1409	-	-	-	0.45	-	2.21	-	-	1.06	-	-
β-caryophylene	1418	9.30	2.39	22.23	8.11	-	15.64	7.21	2.88	2.73	2.13	3.00
β-gurjunene	1432	-	-	0.38	0.71	-	0.71	-	-	-	-	-
aromadendrene	1439	0.90	-	0.45	1.65	4.85	4.44	8.27	2.16	-	-	2.26
geranyl acetone	1453	-	-	-	-	4.40	-	-	-	-	-	-
α-humulene	1454	5.50		2.51	3.03	1.24	23.42	0.30	0.35	4.68	-	1.08
β-farnesene	1458	-	-	-	-	-	-	-	-	-	2.42	-
seychellene	1460	1.20	-	-	-	-	4.89	-	-	-	-	-
allo-aromadendrene	1461	-	-	-	-	-	-	-	1.41	-	-	-
γ-muurolene	1477	-	7.27	-	3.25	0.43	-	-	-	-	0.54	8.94
germacrene D	1480	4.30	9.94	9.30	14.04	-	2.15	-	1.68	4.01	1.35	34.19
β-selinene	1485	-	-	1.49	-	-	6.56	1.33	0.93	-	-	-
valencene	1491	-	-	-	0.92	-	-	-	-	1.41	-	3.50
biclyclogermacrene	1493	11.30	27.91	25.10	9.17	-	-	9.66	1.27	-	-	8.95
viridiflorene	1493	-	-	-	-	-	8.08	-	-	-	-	-
epi-cubebol	1493	-	-	-	-	0.57	-	-	-	-	-	-
α-selinene	1494	-	-	-	-	-	-	-	-	1.39	-	-
α-muurolene	1499	0.50	1.34	-	-	-	-	-	-	3.42	-	1.33
Z-α-bisabolene	1504	-	-	5.69	0.76	-	-	-	-	-	-	-
germacrene A	1505	-	-	-	-	-	-	-	0.35	-	-	-
γ-cadinene	1513	1.80	0.65	-	1.78	0.53	2.56	3.07	-	2.49	0.75	5.89
cubebol	1514	-	2.05	-	0.60	0.73	-	-	0.75	-	-	1.45
epi-α-selinene	1516	-	-	-	5.04	-	-	-	0.50	-	-	-
cis-calamenene	1521	-	-	-	1.02	-	-	-	-	1.55	-	-
δ-cadinene	1524	-	4.77	3.00	-	-	4.43	3.55	-	-	3.33	15.02
α-cadinene	1538	-	-	-	0.43	-	0.52	-	-	-	0.72	1.51
α-calacorene	1542	-	-	-	1.27	-	0.36	-	-	-	-	-
elemol	1549	-	-	-	3.17	-	-	-	-	-	-	-
germacrene B	1556	-	-	-	0.46	-	0.45	0.37	-	1.63	-	-

Table 1. Cond't.

E-nerolidol	1564	10.30	4.18	1.46	8.23	18.18	2.61	8.37	-	12.72	0.94	4.41
spathulenol	1576	0.80	19.22	7.24	9.82	25.37	2.01	7.80	5.17	15.77	-	2.30
caryophylene oxide	1581	-	1.87	-	-	7.67	2.43	-	1.78	-	-	-
globulol	1583	0.50	-	1.16	-	6.64	-	-	-	1.72	-	-
viridiflorol	1590	-	-	-	-	1.82	2.41	-	-	13.54	-	-
guaiol	1595	-	-	-	5.80	-	-	-	-	0.96	8.65	-
humulene epoxide II	1606	-	-	-	-	6.88	2.95	-	-	-	-	-
dillapiole	1622	-	-	-	-	-	-	-	-	0.84	-	-
1-epi-cubenol	1627	-	-	0.81	-	-	-	-	-	-	-	-
epi-α-cadinol	1640	-	1.74	-	-	-	-	-	-	6.33	-	-
cubenol	1642	-	2.41	-	-	-	-	-	-	-	-	-
epi-α-muurolol	1644	-	-	2.14	-	-	-	1.56	4.59	-	-	-
α-muurolol	1645	-	1.02	-	-	-	0.91	0.40	-	1.31	-	-
β-eudesmol	1649	-	-	-	10.10	-	-	-	-	-	-	-
α-cadinol	1653	-	7.60	2.18	-	-	1.12	-	-	-	-	-
valerianol	1655	-	-	-	-	-	-	-	-	-	29.40	-
7-epi-α-eudesmol	1658	-	-	-	-	5.50	-	-	-	-	-	1.22
E-isoelemicin	1660	-	-	-	-	-	-	-	53.45	-	-	-
bulnesol	1666	-	-	-	-	-	-	-	-	-	3.86	-
cadalene	1674	-	-	-	-	-	-	-	-	1.39	-	-

**Piper aduncum* (A), *Piper amalago* (B), *Piper cernuum* (C), *Piper crassinervium* (D), *Piper diospyrifolium* (E), *Piper gaudichaudianum* (F), *Piper regnellii* (G), *Piper solmsianum* (H), *Piper tuberculatum* (I), *Peperomia obtusifolia* (J) and *Piper umbelata* (K)

P. solmsianum (2.57%), *P*. regnellii (0.30%), *P. tuberculatum* (0.70%), *P. umbellata* (0.11%) and *P. obtusifolia* (1.00%). A total of 14 monoterpenes and 55 sesquiterpenes were identified by automated interpretation of mass spectra of each constituent and by the retention index (Table 1).

P. aduncum and *P. regnelli* showed higher percentages of monoterpenes (45.90 and 34.34%, respectively) but sesquiterpenes were very abundant and structurally diversified in the oils extracted from leaves of every species. It is very important to emphasize that the essential oil of *P. solmsianum* presented accumulation of *E*-isoelemicin that was isolated earlier in the inflorescence of this specie.

Analyzing the composition of essential oil of *P. aduncum*, the most representative compound was linalool (31.80%), an important substance that is used in the fragrance industry. Regarding *P. amalago* and *P. cernuum* essential oils, the main compound in both cases was the bicyclogermacrene (27.91 and 25. 10%, respectively). Germacrene D was the predominant compound in *P. crassinervium* and *P. umbelata* (14.04 and 34.19%, respectively).

P. diospyrifolium and *P. tuberculatum* presented spathulenol as the most representative compound (25.37 and 15.77%, respectively). Lastly, *P. solmsianum, P. regnellii, P. gaudichaudianum* and *Peperomia obtusifolia* showed the larger existence of *E*-isoelemicin (53.45%), myrcene (15.45%),α-humulene (23.42%) and valerianol (29.40%), respectively.

The *in vitro* antifungal activity of the essential oils was evaluated by the micro-dilution method, described in the M27-A2 document of the CLSI with minimum modifycations (Rodriguez-Tudela et al., 1996). The detection limits of the samples (Table 2) were obtained according to the described methodology. The essential oil from *P. aduncum*, *P. gaudichaudianum* and *P. C. neoformans* (62.50, 3.90 and 62.50 $\mu m.mL^{-1}$, respectively) and the essential oil from *P. gaudichaudianum* also showed high antifungal activities against *Candida krusei* (31.25 $\mu m.mL^{-1}$). The other essential oils showed moderate activities against *C. albicans*, *C. parapsilosis, C. krusei* and *C. neoformans.* The essential oils from *P. amalago*, *P. diospyrifolium, P. regnelli, P. umbelata* and *Peperomia obtusifolia* did not show any activity.

The results demonstrated that *P. aduncum, P. gaudichaudianum* and *P. solmsianum* are important plants for studying the antifungal activities, mainly *P. gaudichaudianum*, that presented the best activity not only against *C. neoformans,* but also against *C. krusei*. It is also important to note that the non-phytotoxic effect of the essential oils has been published in literature and in this context these oils become important sources for the future development of antifungal products.

Table 2. Antifungal activity of essential oil of leaves from *Piper* and *Peperomia* species against *C.albicans, C. krusei, C. parapsilosis* and *C. neoformans.*

Specie	*C. albicans*	*C. krusei*	*C. parapsilosis*	*C. neoformans*
P. aduncum	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}	62.50 µg.mL^{-1}
P. amalago	n.i.	n.i.	n.i	n.i
P. cernuum	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}
P. diospyrifolium	n.i	n.i	n.i	n.i
P. crassinervium	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}
P. gaudichaudianum	> 250 µg.mL^{-1}	31.25 µg.mL^{-1}	> 250 µg.mL^{-1}	> 3.90 µg.mL^{-1}
P. solmsianum	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}	> 250 µg.mL^{-1}	62.50 µg.mL^{-1}
P. regnellii	n.i	n.i	n.i	n.i
P. tuberculatum	> 1000 µg.mL^{-1}	> 1000 µg.mL^{-1}	> 1000 µg.mL^{-1}	> 1000 µg.mL^{-1}
P. umbelata	n.i	n.i	n.i	n.i
P. obtusifolia	n.i	n.i	n.i	n.i
Amphotericin B (Positive control)	2.00	2.00	1.00	0.06

Conclusions

In this study a total of 14 monoterpenes and 55 sesquiterpenes were identified. *P. aduncum* and *P. regnelli* showed high percentages of monoterpenes, but sesquiterpenes were the most abundant and structurally diversified compounds in the oils extracted from leaves of all the species. The essential oils from *P. aduncum*, *P. gaudichaudianum* and *P. solmsianum* presented high antifungal activities against *C. neoformans* (62.50, 3.90 and 62.50 µm.mL^{-1}, respectively) and the essential oil from *P. gaudichaudianum* also showed high antifungal activities against *C. krusei* (31.25 µm.mL^{-1}).

ACKNOWLEDGEMENTS

This work was funded by grants provided by FEI and the Quality Monitoring and Research Center of Fuels, Crude Oil and Derivatives - CEMPEQC, Organic Chemistry Department, Institute of Chemistry, Sao Paulo State University – UNESP, JEO, MF and MJK are grateful to CNPq for the research fellowships.

REFERENCES

Adams RP (1995). Identification of essential oil components by gas chromatography / mass spectroscopy. Academic Press, San Diego.

Batista Jr JM, Lopes AA, Ambrósio DL, Regasini LO, Kato MJ, Bolzani VS, Cicarelli RMB, Furlan M (2008). Natural Chromenes and Chromene Derivatives as Potential Anti-trypanosomal Agents. Biol. Pharmaceutical. Bull., 31: 538-540.

Benevides PJC, Sartorelli P, Kato MJ (1999). Phenylpropanoids and neolignans from *Piper regnellii*. Phytochemistry, 52: 339-343.

Costantin MB, Sartorelli P, Limberger R, Henriques AT, Steppe M, Ferreira MJP, Ohara MT, Emerenciano VP, Kato MJ (2001). Essential oils from *Piper cernuum* and *Piper regnellii*: Antimicrobial activities and analysis by GC/MS and 13C-NMR. Planta. Med., 67: 771-773.

Dorman HJ, Deans SG (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol., 88: 308-316.

Felipe FCB, Sousa Filho JT, Souza LEO, Silveira JA, Uchoa DEA, Silveira ER, Pessoa ODL, Viana GSB (2007). Piplartine, an amide alkaloid from *Piper tuberculatum*, presents anxiolyticand antidepressant effects in mice. Phytomedicine, 14: 605-612.

Felipe LG, Baldoqui DC, Kato MJ, Bolzani VS, Guimarães EF, Cicarelli RMB, Furlan M (2008). Trypanocidal tetrahydrofuran lignans from Peperomia blanda. Phytochemistry., 69: 445-450.

Lago JHG, Ramos CS, Casanova DCC, Morandim AA, Bergamo DCB, Cavalheiro AJ, Bolzani VS, Furlan M, Guilharães EF, Young MCM, Kato MJ (2004). Benzoic acid derivatives from *Piper* species and their fungitoxic activity against Cladosporium cladosporioides and C. sphaerospermum. J. Nat. Prod., 67: 1783-1788.

Maia JGS, Silva ML, Luz AIR, Zoghbi MGB, Ramos LS (1987). Espécies de *Piper* da Amazônia ricas em safrol. Química Nova., 10: 200-204.

Moreira DL, Guimarães EF, Kaplan MAC (1998). A chromene from *Piper aduncum*. Phytochemistry, 48: 1075-1977.

Navickiene HMD, Morandim AA, Alécio AC, Regasini LO, Bergamo DCB, Telascrea M, Cavalheiro AJ, Lopes MN, Bolzani VS, Furlan M, Marques MOM, Young MCM, Kato MJ (2006). Composition and antifungal activity of essential oils from *Piper aduncum*, *Piper arboreum* and *Piper tuberculatum*. Química Nova, 29: 467-470.

Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen CE, Bol lPM (1997). Phytochemistry of the genus Piper. Phytochemistry, 46: 597-673.

Rodriguez-Tudela JL, Berenguer J, Martinez-Suarez JV, Sanchez R (1996). Comparison of a spectrophotometric microdilution method with RPMI-2% glucose with the National Committee for Clinical Laboratory Standards reference macrodilution method M27-P for *in vitro* susceptibility testing of amphotericin B, flucytosine, and fluconazole against Candida albicans. Antimicrobial. Agents. Chemotherapy, 40: 1998-2003.

Santos PRD, Moreira DL, Guimarães EF, Kaplan MAC (2001). Essential oil analysis of 10 Piperaceae species from the Brazilian Atlantic forest. Phytochemistry, 58: 547-551.

Silva RV, Navickiene HMD, Kato MJ, Bolzani VS, Méda CI, Young MCM, Furlan M (2002). Antifungal amides from *Piper arboreum* and *Piper tuberculatum*. Phytochemistry, 59: 521-527.

Tognolini M, Barocelli E, Ballabeni V, Bruni R, Bianchi A, Chiavarini M, Impicciatore M (2006). Comparative screening of plant essential oils: Phenylpropanoid moiety as basic core for antiplatelet activity. Life. Sci., 78: 1419-1432.