



CHEMICAL COMPOSITION AND ANTI-CANDIDIASIS ACTION OF *SCHINUS TEREBINTHIFOLIUS* RADDI (AROEIRA) ESSENTIAL OIL

Mônica Batista Almeida^{1,2}, Daniela Lima Travassos¹, Patrícia Severino^{3,4*}, Carine Santana Ferreira^{3,4*}, Jessica Oliveira Carvalho¹, Jane de Jesus da Silva Moreira⁵, Rita de Cassia Trindade¹ and Edilson Divino Araújo²

¹Department of Morphology, Center for Basic Health Sciences, Federal University of Sergipe, 49100-000 São Cristóvão - SE, Brasil.

²Department of Biology at the Laboratory of Genetics and Conservation of Natural Resources Federal University of Sergipe, 49100-000 São Cristóvão - SE, Brasil.

³Laboratory of Nanotechnology and Nanomedicine (LNMed), Institute of Technology and Research (ITP), Av. Murilo Dantas, 300, 49010-390 Aracaju, Brazil.

⁴Industrial Biotechnology Program, Tiradentes University (Unit), Av. Murilo Dantas, 300, 49010-390 Aracaju, Brazil.

⁵Department of Food Engineering - Federal University of Sergipe, 49100-000 São Cristóvão - SE, Brasil.

***Corresponding Author: Dr. Patrícia Severino**

Laboratory of Nanotechnology and Nanomedicine (LNMed), Institute of Technology and Research (ITP), Av. Murilo Dantas, 300, 49010-390 Aracaju, Brazil.

Article Received on 17/02/2017

Article Revised on 11/03/2017

Article Accepted on 01/04/2017

ABSTRACT

Candida species remains the most common cause of human fungal infection. Vaginal candidiasis is difficult to treat and is often resistant to many antifungal drugs. Considering the need to obtain new therapeutic agents aimed at combatting vaginal candidiasis, the objective of this study was to investigate antifungal activity of essential oil *Schinus terebinthifolius* Raddi in species of Candida species. *Schinus terebinthifolius* Raddi essential oil was obtained by hydrodistillation and characterized by gas chromatography. The Minimum Inhibitory Concentration (MIC) and Minimal Fungicide Concentration (MFC) were performed based on Clinical and Laboratory Standards Institute. Results showed majority components of essential oil was monoterpenes. Fungistatic action were observed for essential oil extracted from dry fruits and essential oil extracted from fresh leaves or fruits presenting promising results for further research and continuity of the study of this plant. In summary, these demonstrate the potential fungistatic activity of essential oil *Schinus terebinthifolius* Raddi that may help to explain the popular use in vaginal infection.

KEYWORDS: Medicinal plant; candidiasis; essential oil; *Candida spp*, *Schinus terebinthifolius* Raddi.

INTRODUCTION

Candida sp are recognized as the yeasts most commonly involved in the etiology of mycotic infections.^[1] Vaginal candidiasis is a common mucosal infection of vagina, mainly caused by *Candida sp* species that represents one of the most frequent gynecological diseases, affecting at least once in the life about 75% of the female sexually active population.^[2]

The majority of strains isolated from the vagina correspond to *C. Albicans* species and it is estimated that the proportion of nonalbicans strains has progressively increased in recent years.^[3] Often, fungal infections are difficult to treat, a fact intrinsically related to the acquisition by their etiological agents of resistance to the action of antifungal agents.^[4]

The use of alternative medicine by the world population has been significant in recent times. Data from the World Health Organization (WHO) released 80% of developing

countries depended on medicinal plants as the only form of access to health care.^[5] Medicinal plant has been widely used for population and it has potential in efficacy in various diseases. In Brazil has a great potential in medicinal plants^[6] among theirs *Schinus terebinthifolius* Raddi highletd.

Schinus terebinthifolius Raddi is a species of the Anacardiaceae family, native to Central and South America, especially in southern Brazil, Paraguay and Argentina. It is common in the coastal vegetation of the Brazilian northeast from Rio Grande do Norte to the State of Sergipe. It can also be found in semitropical and tropical regions of the United States, Africa, beyond Asia and, in the United States, occurs in Hawaii, the States of California, Arizona and Florida where it was introduced for ornamental purposes. It presents the following botanical synonyms: *Schinus aroeira* Vell, *Sarcotheca bahiensis* Turcz., *Schinus antiarthritica* Mart., *Schinus mucromulata* Mart.

Schinus chichita Speg., *Schinus lentiscifolia* e *Schinus rhoifolus* Mart.^[7]

Schinus terebinthifolius Raddi oils and extracts are as antimicrobial^[8], anti-inflammatory^[7] antiulcerogenic, antiseptic and cicatrizant.^[9] In this perspective, the objective of this work was to evaluate the efficacy of the essential oil of fruits and leaves of *Schinus terebinthifolius* Raddi in inhibiting the growth of yeast species of *Candida*.

Material

The leaves and fruits of *Schinus terebinthifolius* Raddi were harvested at the Experimental Farm "Campus Rural of Federal University of Sergipe" located in São Cristóvão, Sergipe, Brazil, (11° 04' 08" S, 37° 08' 11" W).

METODOLOGY

Preparation of essential oil

Essential oil of *S. terebinthifolius* Raddi were prepared from fruits and leaves in dried form. The dried material was maintained a stove (Tecnal, model MA-037/18, Brazil) with forced air circulation at 40°C for five days. The methodology of oil extraction was done with adaptation.^[10] The oil extraction was obtained from dried material by steam distillation using Clevenger system, during 1.5 h. The essential oils obtained were separated from the aqueous solution (hydrolate) and transferred to amber bottle, being stored at -20°C.

Phytochemical analysis

The identification of volatile constituents was conducted by gas chromatography (Varian, model 3900, USA). It was used apolar column (HP-5MS (30 m x 25 mm x 0.25 µm) with oven temperature setting of 60 – 250°C (3°C/min), injector operating in split mode (ratio 1:10), temperature of 250°C. The detection of the analytes was performed in a mass spectrometer (Varian *Ion Trap*, model 3900, EUA) with *transferline* in temperature of 250°C, with 70 eV electron impact ionization source. The mass scanning range was 35 a 350 m/z. The delay was 4 minutes. The identification of the volatile organic compounds was performed using the Retention Index (RI) using library of mass spectra of the National Institute of Standard and Technology, 2008^[11] and "Odour and Pherobase".

Fungi

The *Candida albicans* (ATCC 18804), *Candida parapsilosis* (ATCC 22019) and *Candida glabrata* (ATCC 2001) were three standard lines obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) provided by National Institute of Quality Control in Health (INCQS). Clinical fungi (*Candida albicans*, *Candida parapsilosis* and *Candida glabrata*) belonging to Laboratory of Applied Microbiology of the Federal University of Sergipe. It was isolated in previous studies and identified using phenotype and molecular methods.

Culture media and inoculum

The strains were maintained on Sabouraud Agar Dextrose (SAD) in 4°C. In the study, approximately 10⁵ UFC/mL was standardized according to the 0.5 tube turbidity of the *McFarland* scale. The culture medium was Roswell Park Memorial Institute – 1640 (RPMI 1640) containing L-glutamine and 2% glucose. The pH was adjusted to 7.0 with morpholinepropanesulfonic acid (MOPS) in a final concentration < 1%.

Determination of Minimum Inhibitory Concentration (MIC) and Minimal Fungicide Concentration (MFC)

The evaluation of the anti-*Candida* activity was performed by the microdilution technique, according to the methodology described in the Clinical and Laboratory Standards Institute.^[12] The solubilisation of essential oil was done in dimethylsulfoxide (DMSO) concentration 1%. CIM was interpreted as the lowest concentration capable of inhibiting visible yeast growth compared to the control sample (without the antimicrobial agent). For determination of MFC the essential oil was plated on Sabouraud Agar 100 µl of concentrations that showed no visible growth after 48 h of incubation. Concentrations that did not show growth in the corresponding plates were considered as fungicides. All experiments were performed in triplicate and the mean of the calculated values.

Technique of diffusion in solid medium

To evaluate influence on fungal growth inhibition between oil essential obtained from dried material and fresh material was done technique of diffusion in solid medium. The technique of diffusion in solid medium was used SAD (20 mL) in Petri dish and the fungal strains (inoculated in saline solution) were seeded. Then, sterile absorbent discs previously immersed in 5 µL of the essential oil of *S. terebinthifolius* and inserted. Discs immersed in saline solution were used as negative control. All experiments were performed in triplicate and the mean of the calculated values.

RESULTS AND DISCUSSION

The chemical composition of the essential oil obtained of dry material of *Schinus terebinthifolius* Raddi is shown in Table 1 and 2 and its chromatographic profile can be observed in Figure 1 and 2.

Gas chromatography is a system that offers excellent performance and highly reproducible separation with technology to support analysis of complex matrices such as extract samples, aromas and essential oils.

Table 1 and Figure 1 identified compounds from *Schinus terebinthifolius* Raddi essential oil obtained by dried leaves. It shows the records of 44 compounds, of which 34 were identified in the present study, corresponding to 100.0% of the essential oil. In this sample, the major compounds were: Camphene (20.121%), α-Felandreno (19.207%), R-α-Pineno (19.129%) and Beta-Cariophyllene (13.279%). Thirty-four compounds,

including mono- and sesquiterpenes were identified, with a content of about 0.45% (w/w) of dry leaves weight.

Table 1- Identified compounds from *Schinus terebinthifolius* Raddi essential oil obtained by dried leaves.

Nº	Time	Analyte	RI	Area %	[13]
1	5.489	α-Pineno	934	19.129	932
2	6.621	β-Pineno	976	1.677	974
3	7.447	α-Felandreno	1005	19.207	1002
4	7.593	β-Carene	1009	6.009	1008
5	7.74	m-Cimeno	1013	0.141	1082
6	8.365	Canfeno	1031	20.121	
7	9.151	trans-3-Caren-2-ol	1053	0.091	
8	10.184	α- Terpinoleno	1082	0.524	1086
9	11.49	NI	1117	0.141	
10	12.195	trans-Pinocarveol	1134	0.058	1135
11	12.547	Canfor	1143	0.015	1141
12	13.736	δ-Terpineol	1172	0.046	1162
13	13.889	m-Cimen-8-ol	1176	0.051	1176
14	14.019	p-Cimen-8-ol	1179	0.008	1179
15	14.126	Criptona	1182	0.035	1183
16	14.289	α-Terpineol	1186	0.295	1186
17	14.484	Mirtenol	1190	0.009	1194
18	14.740	cis-Pinocarveol	1197	0.120	1182
19	14.996	cis-Piperitol	1198	0.029	1195
20	16.910	(-)-cis-Sabinol	1245	0.467	
21	17.602	Felandral	1262	0.058	
22	18.292	Acetato de bornila	1279	0.035	1287
23	18.944	p-Cimen-2-ol=Carvacrol	1294	0.072	1298
24	20.435	-	1331	0.109	
25	22.011	-	1369	0.310	
26	22.745	-	1387	2.621	
27	23.946	beta-Cariofileno	1416	13.279	1417
28	25.184	Aloaromadendreno	1447	1.296	1458
29	26.260	Germacreno D	1475	5.446	1484
31	26.513	-	1479	0.607	1582
32	26.862	-	1488	0.525	
33	26.919	β-Selineno	1489	0.789	1489
34					
35	27.294	α-Selineno	1498	2.546	1498
36	27.985	δ-Cadineno	1517	0.476	1522
37	28.508	-	1531	0.019	
38	30.259	Óxido de cariofileno	1578	1.127	1582
39	31.232	-	1604	0.110	
40	31.54	γ-Eudesmol	1612	0.120	1622
41	32.656	Agarospírol	1642	1.666	1646
42	33.405	Eudesm-7(11)-en-4-ol	1662	0.142	1662
43	34.874	-	1701	0.400	
44	35.326	-	1714	0.043	
TOTAL				100,00%	

RI – Retention Index.

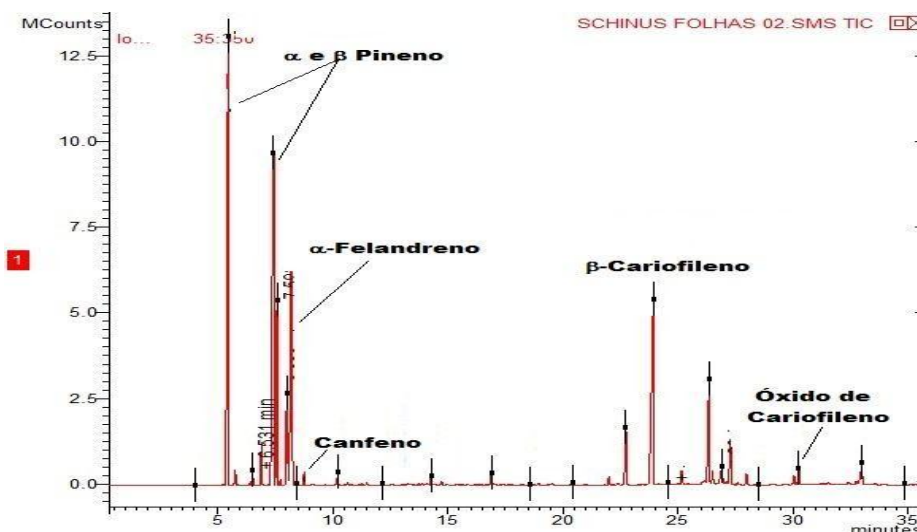


Figure 1. Chromatographic profile of the essential oil of the dried leaves of *Schinus terebinthifolius* Raddi.

The essential oil present in the fruit sample shown in Table 2 and the chromatographic profile observed in Figure 2. It is 38 compounds, of which 33 were identified in the present study, corresponding to approximately 100% of the oil. In this sample, the compounds: R- α -pinene (22.149%), β -Pinene (18.276%),

α -Felandreno (17.412%), Camphene (11.519%), 3-Carene (5.614%) represent approximately 75% of the oil composition. Thirty-three components, including mono- and sesquiterpenes were identified, with a content of about 2.8% (w/w) of dry mature fruit weight.

Table 2. Identified compounds from *Schinus terebinthifolius* Raddi essential oil obtained by dried fruits.

Nº	Time	Analyte	RI	Area %	[13]
1	5.404	α -Pineno	931	22.149	932
2	6.710	β -Pineno	986	18.276	974
3	7.347	α -Felandreno	1002	17.412	1002
4	7.520	3-Careno	1007	5.614	1008
5	7.977	m-Cimeno	1020	4.422	1082
6	8.131	Canfeno	1024	11.519	946
7	10.223	α -Terpinoleno	1083	1.126	1086
8	12.048	cis-p-Menta-2,8-dien-1-ol	1130	0.175	1133
9	12.180	trans-Pinocarveol	1134	0.508	1135
10	12.433	Canfor	1140	0.082	1141
11	12.578	-	1145	0.048	
12	13.037	trans-3-Caren-2-ol	1155	0.130	
13	13.310	-	1162	0.723	
14	13.387	-	1164	0.173	
15	13.530	-	1167	0.896	
16	13.733	δ -Terpineol	1172	0.880	
17	13.930	m-Cimen-8-ol	1177	0.184	1176
18	14.058	p-Cimen-8-ol	1180	0.201	1179
19	14.144	Criptona	1182	0.145	1183
20	14.294	α -Terpineol	1186	0.772	
21	14.535	Mirtenol	1191	0.238	1194
22	14.740	cis-Pinocarveol	1197	1.316	1182
23	14.994	cis-Piperitol	1198	0.082	1195

24	15.641	Diidro acetato de mircenal	1214	1.636	
25	16.210	-	1228	3.272	
26	17.794	Felandral	1266	0.239	
27	18.290	Acetato de bornila	1278	0.473	1287
28	18.945	p-Cimen-2-ol=Carvacrol	1295	0.182	1298
29	23.786	beta-Cariofileno	1413	1.487	
30	25.159	Aloaromadendreno	1446	0.110	1458
31	26.277	Germacreno D	1473	0.461	1484
32	28.978	Elemol	1543	2.032	1548
33	30.243	Óxido de cariofileno	1577	0.375	1582
34	31.616	γ-Eudesmol	1614	0.075	1622
35	32.077	epi-Cubenol	1627	0.901	
36	32.744	Cubenol	1644	0.728	1645
37	32.845	Agarospírol	1647	0.830	1646
38	33.398	Eudesm-7(11)-en-4-ol	1662	0.070	
TOTAL				100%	

RI – Retention Index.

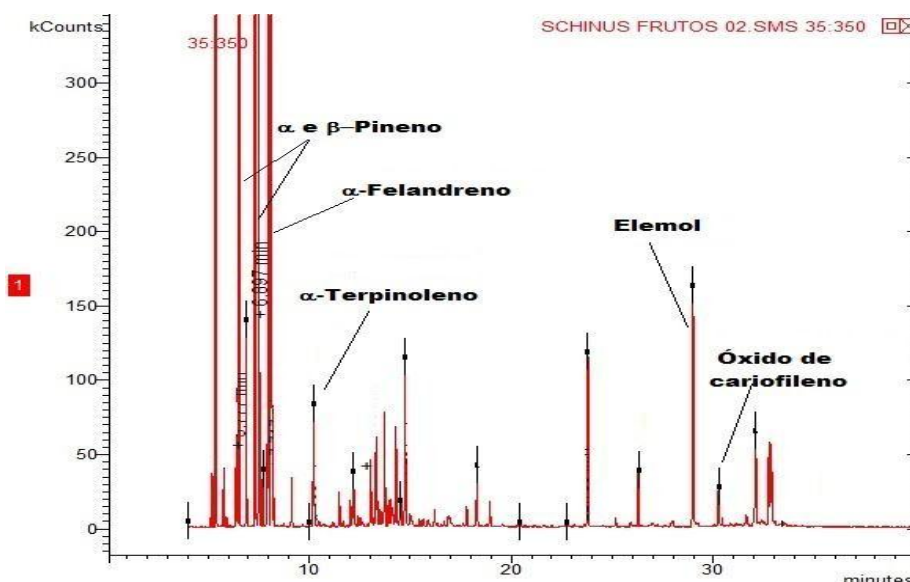


Figure 2. Chromatographic profile of the dried ripe fruits of *Schinus terebinthifolius* Raddi.

The phytochemical profile of the essential oil obtained in this work identified the predominance of monoterpenes camphene, α -felandreno and R- α -pinene. However, majority of the essential oil from leaves, flowers and/or fruits of *S. terebinthifolius* Raddi collected at different sites revealed presence of α -pinene as the component.^[14,15]

Studies described in the literature identified compounds as p-menth-1-en-9-ol, o camphene, o germacreno-D, o hediciariol, o α -gurjuneno, o α -eudesmol e o β -eudesmol as majority components.^[16] Another's studies^[7,17] identify a variety of components highlighting the genetic characteristics of the plant as seasonal and environmental influences (temperature, humidity, soil and time of collection) significantly interfere in the chemical composition of the essential oil.

Antifungal essays

In the antifungal activities, it was observed that the fungal strains evaluated were more susceptible to the action of the essential oil from dry fruits of the aroeira. Table 3 shows values of MIC and MFC obtained in microdilution tests. The essential oil from dry leaves no activity has been found in all strains.

The essential oil of the dry fruit showed activity against *C. parapsilosis* (ATCC 22019) (MIC de 25 mg/ml), *C. parapsilosis clinical isolate* (MIC de 25 mg/ml), *C. albicans* (ATCC 18804) (CIM 200 mg/ml) and *C. glabrata* (ATCC 2001) (MIC 200 mg/ml). But, no activity has been found for *C. albicans* (clinical isolate) and *Candida glabrata* (clinical isolate).

In the case of the standard strains, the observed

fungistatic action may indicate an intrinsic resistance, because they are not in constant and direct interaction with the environment, they maintain their genetic characteristics preserved with the extreme possibility of

acquiring resistance external factors. The behavior of the strains suggests an inherent resistance to yeasts, also called natural resistance.

Table 3. MIC and MFC of essential oils of fruits and leaves of *Schinus terebinthifolius* Raddi

Candida strains	Essential oil leaves		Essential oil fruits	
	MIC*	MFC**	MIC	MFC
<i>C.albicans</i> (ATCC 18804)	I	I	200	I
<i>Candida glabrata</i> (ATCC 2001)	I	I	200	I
<i>Candida parapsilosis</i> (ATCC 22019)	I	I	25	I
<i>C.albicans</i> (clinical isolate)	I	I	I	I
<i>Candida glabrata</i> (clinical isolate)	I	I	I	I
<i>Candida parapsilosis</i> (clinical isolate)	I	I	25	I

*MIC - Minimum Inhibitory Concentration

**MFC - Minimal Fungicide Concentration I=Inative

However, are no reports in the literature on the use essential oil of *Schinus terebinthifolius* Raddi for the treatment of Candidiase. It is an important woman disease that affect public health due to its high incidence. Azoles drugs that has clinical activity against most *Candida* sp. However, there are strains resistant to this drug. With this, the use of essential oil can be used as co-adjuvant in the treatment because the monoterpene pinene has antimicrobial according this paper and literature.^[18,20]

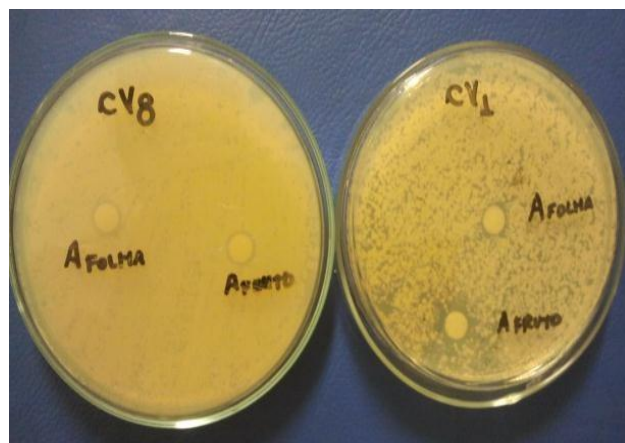
Technique of disk diffusion method

Performance evaluation of disk diffusion method for determining the antifungal activity of essential oil produced by dry and fresh material (leaves or fruits), against yeasts agents of *Candida* sp.

This technique the growth inhibition halo is measured starting from the circumference of the disk to the edge.^[21] Results from *disk diffusion method* on solid medium of essential oil. Figure 3 (A) essential oil provided leaves and fruits without dried is observed presence of fungal without halo definition. Figure 3 (B) essential oil provided fresh material was observed the presence of a halo. The diameters of inhibition halos ranged from 0.5 to 14 mm for fresh leaf oil and 7 to 12 mm for fresh fruit oil demonstrate in Table 4. Comparing the antifungal activity obtained for the essential oil from dry and fresh material concluded that the fresh material is more promising for use.



(A)



(B)

Figure 3. Diffusion test on solid medium of essential oil. (A) leaves and fruits without dried (B) leaves and fruits dried

Table 4. Inhibition halos diameter (mm) of the essential oils of leaves and fresh fruits of *S.terebinthifolius* against the studied *Candida* spp.

	CV1 (<i>C.glabatra</i>)	CV4 (<i>C.albicans</i>)	CV8.3 (<i>C.parapsilosis</i>)	(<i>C.glabatra</i>) ATCC	(<i>C.albicans</i>) ATCC	(<i>C.parapsilosis</i>) ATCC
LEAVES	07 mm	05 mm	07 mm	07 mm	14 mm	08 mm
FRUITS	12 mm	07 mm	07 mm	Resistente	10 mm	07 mm

It is important to emphasize that the production process influences the essential oil components. This can be

attributed to the process of mechanical fragmentation, drying and storage conditions. Also, not least the harvest

time, the time a medicinal plant is harvested, represents an important factor in the production of bioactive substances. With this, it opens possibility for new studies for antifungal action of *S. terebinthifolius* Raddi essential oil.

CONCLUSION

It was observed that the chemical composition of the essential oils of *Schinus terebinthifolius* Raddi showed variations which may be related to environmental and genetic factors, thus varying the chemical characteristics of the active constituents. Fungistatic action was observed for essential oil extracted from dry fruits and essential oil extracted from fresh leaves or fruits presenting promising results for further research and continuity of the study of this plant.

REFERENCES

- McCarty TP and Pappas PG. Invasive candidiasis. *Infect Dis Clin North Am*, 2016; 30(1): 103-124.
- Johal HS, Garg T, Rath G, Goyal AK. Advanced topical drug delivery system for the management of vaginal candidiasis. *Drug delivery*, 2016; 23(2): 550-563.
- Fornari G, Vicente VA, Gomes RR, Muro MD, Pinheiro RL, Ferrari C, Herkert PF, Takimura M, de Carvalho NS, Telles FQ. Susceptibility and molecular characterization of *Candida* species from patients with vulvovaginitis. *Braz J Microbiol*, 2016; 47(2): 373-380.
- Sobel JD, *Recurrent vulvovaginal candidiasis*. *Am Journal of Obstetr and Gyn*, 2016; 214(1): 15-21.
- WHO, World Health Organization (WHO) guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. 2004.
- Dutra RC, Campos MM, Santos AR, Calixto JB. Medicinal plants in Brazil: pharmacological studies, drug discovery, challenges and perspectives. *Pharmacol Res*, 2016; 112: 4-29.
- Feuereisen MM, Gamero BM, Zimmermann BF, Schieber A, Schulze-Kaysers N. Pressurized liquid extraction of anthocyanins and biflavonoids from *Schinus terebinthifolius* Raddi: A multivariate optimization. *Food Chem*, 2017; 214: 564-571.
- da Silva GBPG, Silvino KF, Bezerra JDP, Farias TGS, de Araújo JM, Stamford TLM. Antimicrobial activity of *Phoma* sp. URM 7221: An endophyte from *Schinus terebinthifolius* Raddi (Anacardiaceae). *African Journal of Microbiology Research*, 2017; 11(1): 1-7.
- Scheibe CL, Ribas-Filho JM, Czezko NG, Malafaia O, Barboza LED, Ribas FM, Wendler E, Torres O, Lovato FC, Scapini JGS. *Schinus terebinthifolius raddi* (Aroeira) and *Orbignya phalerata mart.* (Babassu) effect in cecorrhaphy healing in rats. *Acta Cir Bras*, 2016; 31(6): 402-410.
- Feizbakhsh A, Pazoki H, Ebrahimzadeh M. Effect of gibberellic acid on composition of *S. ebulus* leaf essential oil (Caprifoliaceae). 2016; 2: 137-142.
- Smith R, Martell A, Motekaitis R. NIST standard reference database 46. NIST Critically Selected Stability Constants of Metal Complexes Database Ver, 2004; 2.
- Watts JL, Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard. 2008: National Committee for Clinical Laboratory Standards.
- Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 2007.
- Piccinelli AC, Santos JA, Konkiewitz EC, Oesterreich SA, Formagio AS, Croda J, Ziff EB, Kassuya CA. Antihyperalgesic and antidepressive actions of (R)-(+)-limonene, α -phellandrene, and essential oil from *Schinus terebinthifolius* fruits in a neuropathic pain model. *Nutr Neurosci.*, 2015; 18(5): 217-224.
- Gois FD, Cairo P, Cantarelli V, Costa LB. Effect of Brazilian red pepper (*Schinus terebinthifolius* Raddi) essential oil on performance, diarrhea and gut health of weanling pigs. *Livestock Science*, 2016; 183: 24-27.
- Oliveira Junior LFG, Santos RB, Reis FO, Matsumoto ST, Bispo WMS, Machado LP, Oliveira LFM. Fungitoxic effect of essential oil from aroeira (*Schinus terebinthifolius* Raddi) on *Colletotrichum gloeosporioides*. *Rev Bras Plantas Med*, 2013; 15(1): 150-157.
- Carneiro MJ, López BGC, Lancellotti M, Franchi GC, Nowill AE, Sawaya ACHF. Evaluation of the chemical composition and biological activity of extracts of *Tetragonisca angustula propolis* and *Schinus terebinthifolius Raddi* (Anacardiaceae). *J Apicultural Res*, 2016; 55(4): 315-323.
- Jiang Y, Wu N, Fu YJ, Wang W, Luo M, Zhao CJ, Zu YG, Liu XL. Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environ Toxicol Pharmacol*. 2011; 32(1): 63-68.
- Duarte MC, Figueira GM, Sartoratto A, Rehder VL, Delarmelina C. Anti- *Candida* activity of Brazilian medicinal plants. *J Ethnopharmacol*, 2005; 97(2): 305-311.
- Ngassapa OD, Runyoro DKB, Vagionas K, Graikou K, Chinou LB. Chemical composition and antimicrobial activity of *Geniosporum rotundifolium* Briq and *Haumaniastrum villosum* (Bene) AJ Paton (Lamiaceae) essential oils from Tanzania. *Trop J Pharm Res*, 2016; 15(1): 107-113.
- de Menezes, KAT, Lima, SMRR, Ueda SMY. Activity of the aqueous extract of *Schinus terebinthifolius* Raddi on strains of the *Candida* genus. *Rev Bras Gynecol Obstet*, 2016; 38(12): 593-599.