NPC Natural Product Communications

2011
Vol. 6
No. 6
907 - 912

Chemical Composition and Antioxidant, Antimicrobial, and Larvicidal Activities of the Essential Oils of *Annona salzmannii* and *A. pickelii* (Annonaceae)

Emmanoel Vilaça Costa[a,*], Lívia Macedo Dutra[a], Hugo César Ramos de Jesus[a], Paulo Cesar de Lima Nogueira[a], Valéria Regina de Souza Moraes[a], Marcos José Salvador[b], Sócrates Cabral de Holanda Cavalcanti[c], Roseli La Corte dos Santos[d] and Ana Paula do Nacimento Prata[e]

[a]*Laboratório de Pesquisa em Química Orgânica de Sergipe* (LABORGANICS), *Departamento de Química*, *Universidade Federal de Sergipe*, *CEP 49100-000*, *São Cristóvão*, *Sergipe*, *Brazil*

[b]*Curso de Farmácia*, *Departamento de Biologia Vegetal*, *Universidade Estadual de Campinas*, *CEP 13083-970*, *Campinas*, *São Paulo*, *Brazil*

[c]*Laboratório de Química Farmacêutica*, *Departamento de Farmácia*, *Universidade Federal de Sergipe*, *CEP 49100-000*, *São Cristóvão*, *Sergipe*, *Brazil*

[d]*Laboratório de Parasitologia, Departamento de Morfologia, Universidade Federal de Sergipe, CEP 49100-000, São Cristóvão, Sergipe, Brazil*

[e]*Laboratório de Sistemática Vegetal*, *Departamento de Biologia*, *Universidade Federal de Sergipe*, *CEP 49100-000*, *São Cristóvão*, *Sergipe*, *Brazil*

emmanoelvilaca@yahoo.com.br

Received: January 19th, 2011; Accepted: February 22nd, 2011

The essential oils from the leaves of *Annona salzmannii* and *A. pickelii* (Annonaceae) growing in Sergipe, northeastern region of Brazil, were obtained by hydrodistillation using a Clevenger-type apparatus, and analyzed by GC/MS and GC/FID. Thirty-four compounds were identified in the essential oil of *A. salzmannii* and twenty-seven in that of *A. pickelii*; sesquiterpenes predominated in both essential oils. Bicyclogermacrene (20.3%), (*E*)-caryophyllene (19.9%), δ-cadinene (15.3%), α-copaene (10.0%), and *allo*-aromadendrene (5.7%) were the main components of *A. salzmannii*, and bicyclogermacrene (45.4%), (*E*)-caryophyllene (14.6%), and α-copaene (10.6%) of *A. pickelii*. The essential oils showed significant antioxidant capacity in the $ORAC_{FL}$ and DPPH assays. The antimicrobial activity of these essential oils was also evaluated against bacteria and fungi, as well as the larvicidal activity against *Aedes aegypti* larvae.

Keywords: *Annona salzmannii*, *Annona pickelii*, essential oil composition, antioxidant, antimicrobial and larvicidal activity.

Annonaceae is a pan-tropical family of trees, shrubs, and climbers with *ca.* 130 genera and 2500 species that are found predominantly in lowland tropical regions [1]. The species of this family are known for their edible fruits and medicinal properties [2]. Some species, such as *Cananga odorata* (Ylang-Ylang) supply raw material for cosmetics and perfumery [2]. Previous chemical and pharmacological investigations of some species of this family revealed the presence of important bioactive compounds, exhibiting pharmacological activities such as cytotoxicity against tumor cell lines [3,4], antimicrobial [4-7] and antioxidant [5] activities, insecticidal action [4,8], and antiparasitic properties against *Leishmania* sp. [6,9], *Plasmodium falciparum* [10], and *Trypanosoma cruzi* [9,11]. These activities generally are attributed to alkaloids, essential oils and acetogenins. Despite their importance in folk medicine, the number of species that have been chemically investigated is still very small.

In Brazil, the Annonaceae comprise nearly 26 genera (seven endemic) and 260 species, for the largest part occurring in forests, with few representatives in open areas [12]. In the State of Sergipe (Brazil) according to ASE "Herbário da Universidade Federal de Sergipe" there are four genera and thirteen species [13]. The most representative genus is *Annona* L., with six species. In our search for antioxidant, antimicrobial, and larvicidal compounds from Sergipe Annonaceous plants, we report in this work the chemical composition of the essential oils from the leaves of *Annona salzmannii* A. DC., popularly known as "araticum-da-mata" and "araticum-apé", and *A. pickelii* (Diels) H. Rainer, popularly known as "araticum-do-mato" and "jussara". These plants are typical of the Flora of Sergipe [13]. Only *A. salzmannii* has use in folk medicine. The leaves and bark of this species are used for the treatment of diabetes, tumors and inflammation [2]. Previous phytochemical studies on these species described

Table 1: Essential oil composition of *Annona salzmannii* and *A. pickelii*.

	Compound	RI[a]	RI[b]	Leaf oil %	
				Annona salzmannii	*Annona pickelii*
1	α-Pinene	931	932	0.5	0.3
2	β-Pinene	976	974		0.3
3	Sabinene	971	969	0.4	
4	Mircene	988	988	0.3	
5	Limonene	1028	1024	0.2	
6	(*E*)-β-Ocimene	1045	1044	0.3	
7	Linalool	1099	1095	0.8	
8	δ-Elemene	1331	1335	0.1	0.3
9	α-Cubebene	1347	1345		2.3
10	Cyclosativene	1368	1369	0.5	0.3
11	α-Copaene	1375	1374	10.0	10.6
12	β-Bourbonene	1383	1387	0.3	1.1
13	β-Cubebene	1387	1387		3.8
14	β-Elemene	1389	1389	1.7	0.1
15	α-Gurjunene	1406	1409	0.3	0.3
16	(*E*)-Caryophyllene	1419	1417	19.9	14.6
17	β-Copaene	1429	1430	0.2	0.4
18	Aromadendrene	1437	1439	0.4	0.8
19	α-Humulene	1455	1452	3.7	2.3
20	*allo*-Aromadendrene	1459	1458	5.7	0.9
21	*trans*-Cadina-1(6),4-diene	1472	1475		0.2
22	γ-Muurolene	1475	1478	1.5	0.8
23	Germacrene D	1481	1484	3.1	5.0
24	Viridiflorene	1490	1496	0.4	
25	Valencene	1491	1496		0.8
26	Bicyclogermacrene	1495	1500	20.3	45.4
27	(*E*,*E*)-α-Farnesene	1507	1505		0.2
28	Germacrene A	1507	1508	0.3	
29	γ-Cadinene	1512	1513	0.4	0.2
30	δ-Cadinene	1518	1522	15.3	4.8
31	*trans*-Cadina-1,4-diene	1532	1533		0.3
32	α-Calacorene	1540	1544	0.3	
33	(*E*)-Nerolidol	1561	1561		0.3
34	Spathulenol	1577	1577	2.6	1.7
35	Caryophyllene oxide	1582	1582	2.4	0.2
36	Viridiflorol	1594	1592	0.3	
37	Muurola-4,10(14)-dien-1β-ol	1626	1630	0.7	
38	α-Muurolol	1644	1644	1.0	
39	Selin-11-en-4α-ol	1659	1658	1.4	
40	Heptadec-8-ene	1677	1680	0.5	
41	Kaur-16-ene	1979	2042[c]	0.2	
42	Phytol	2008	2008[c]	0.2	
	Monoterpenes			2.5	0.6
	Sesquiterpenes			93.7	97.7
	Total Identified Compounds %			96.2	98.3

RI[a] (calc.), retention indices on ZB-5MS column calculated according to ref. [21]. RI[b] retention indices according to ref. [22]. [c]Compared with authentic standard.

the isolation of alkaloids [14] and acetogenins in *A. salzmannii* [15,16], and lignans in *A. pickelii* [17]. This is the first report on the analysis of the volatile constituents from the leaves of these plants and their biological activities.

Hydrodistillation of the leaves of *A. salzmannii* and *A. pickelii* gave a light yellowish crude essential oil, with a yield of 0.1% and 0.2% (w/w), respectively, in relation to the dry weight of the plant material. As shown in Table 1, it was possible to identify 42 compounds; thirty-four in the essential oil of *A. salzmannii*, and twenty-seven in that of *A. pickelii*. The essential oils were dominated by sesquiterpenes, with 93.7% in *A. salzmannii* and 97.7% in *A. pickelii*. The major compounds identified in the essential oil of *A. salzmannii* were bicyclogermacrene (20.3%), (*E*)-caryophyllene (19.9%), δ-cadinene (15.3%), α-copaene (10.0%), and *allo*-aromadendrene (5.7%), while bicyclogermacrene (45.4%), (*E*)-caryophyllene (14.6%), and α-copaene (10.6%) were the most abundant constituents in the essential oil of *A. pickelii*.

In addition to the major constituents, β-elemene, α-gurjunene, aromadendrene, α-humulene, γ-muurolene, germacrene D, spathulenol, and caryophyllene oxide were present in both species, and have been reported in essential oils of several other species of Annonaceae, such as species of *Annona* [6,9], *Duguetia* (formerly known as *Pachypodanthium*) [10], *Guatteria* [7], *Hexalobus* [10], and *Xylopia* [10].

In recent work by *Costa et al.* [6], the authors observed the major compounds in the essential oil from the leaves of *Annona foetida* Mart. as bicyclogermacrene, (*E*)-caryophyllene, and α-copaene, which are the main compounds identified in the essential oils from the leaves of *A. salzmannii* and *A. pickelii*. The presence of these compounds [bicyclogermacrene, (*E*)-caryophyllene, and α-copaene] in the essential oils of *A. salzmannii* and *A. pickelii* suggest that these compounds could be considered chemotaxonomic markers of the genus *Annona*, since their occurrence is very common in species of this genus [6,9].

A. pickelii, formerly *Rollinia pickelii* Diels, was recently placed in *Annona* on the basis of molecular phylogenetic studies of the genus *Annona*, which included species of *Rollinia* and *Raimondia* [18]. This inclusion of *Rollinia* into *Annona* corroborates the recent work of Chatrou *et al.* [19] and Richardson *et al.* [1]. The results obtained in this work also support this change.

The essential oils of *A. salzmannii* and *A. pickelii* were shown to possess antioxidant/free-radical scavenging effectiveness. The ORAC results for the essential oils and for quercetin, isoquercitrin, chlorogenic acid and caffeic acid are summarized in Table 2.

The ORAC assay is an easy and inexpensive tool for measuring the total antioxidant activity of a biological sample (for example, body fluids, agricultural products, food products, pharmaceutical products). This assay can quantitatively measure the total antioxidant capacity as well as qualitatively measure the amount of fast versus slow acting antioxidants in the sample to be tested. The assay measures the effectiveness of various antioxidants present in the sample in preventing the loss in fluorescence intensity of the fluorescent marker fluorescein during peroxy radical induced free radical damage. Assay results

Table 2: Antioxidant capacity of the essential oils of *Annona salzmannii* and *A. pickelii*.

Essential Oil / Positive Control	ORAC assay[a] (μmol of TE g^{-1})[b]	DPPH assay[c]
A. salzmannii	1451.7 (1.70)	strong
A. pickelii	1844.3 (1.74)	strong
Quercetin[d]	5.6 (0.90)	strong
Isoquercitrin[d]	5.2 (1.80)	strong
Caffeic acid[d]	2.9 (2.05)	strong
Chlorogenic acid[d]	2.7 (1.50)	strong

[a]Mean (%RSD, relative standard deviation) of triplicate assays. [b]ORAC data expressed as μmol of Trolox equivalents per g of essential oil (μmol of TE g^{-1}). [c]TLC-based 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenger antioxidant assay.[d]Positive controls: ORAC data expressed as relative Trolox equivalent, mean (%RSD, relative standard deviation) of triplicate assays.

are quantitated by allowing the reaction to reach completion and then integrating the area under the kinetic curve relative to a blank reaction containing no added antioxidants. The area under the curve is proportional to the concentration of all the antioxidants present in the sample. Each reaction is calibrated using known standards of Trolox, a water soluble vitamin E analog. The essential oils were also subjected to screening for their possible antioxidant activity by DPPH free radical scavenging. It is well known that antioxidants can seize free radicals to form stable free radicals, which do not initiate or propagate further oxidation, and DPPH has been used extensively as a free radical to evaluate reducing substances. The DPPH free radical scavenging ability of the essential oils applied on silica gel TLC plates was evaluated. All samples produced yellow spots after spraying with the DPPH reagent, suggesting antioxidant activity for these oils. Some isolated terpenes, such as β-pinene and α-pinene, have been previously tested individually in order to determine their antioxidant nature, but none exhibited activity (data not published). Thus the results presented suggest possible synergism that might occur involving the main components with each other or with other minor components, since the essential oils are a complex mixture of components.

The antimicrobial activity results obtained for the essential oils of *A. salzmannii* and *A. pickelii* are shown in Table 3. *A. salzmannii* was the most effective oil, exhibiting a significant antimicrobial activity against most of the microorganisms tested, probably as a result of a synergistic action of compounds present in the oil. The antimicrobial activity of this oil might be attributed to its monoterpene and sesquiterpene content. Various biological properties are credited for monoterpenoids, sesquiterpenoids, and their derivatives, including antimicrobial, anti-inflammatory, antioxidant, and antineoplastic activities [6].

The larvicidal activity of the essential oils was determined against 3rd instar *Aedes aegypti* larvae. However, no larval mortality was detected at concentrations up to 1000 μg.mL^{-1}. This could probably be attributed to the high concentration of (*E*)-caryophyllene, bicyclogermacrene, and germacrene D [20]. Recent studies have demonstrated

Table 3: Antimicrobial activity of the essential oils of *Annona salzmannii* and *A. pickelii*.

Microorganism	MIC[a] (mg.mL^{-1})		
	Annona salzmannii	*Annona pickelii*	Controls[b]
Staphylococcus aureus (ATCC14458)[c]	0.5	0.5	0.025
S. aureus (ATCC6538)[c]	-	-	0.025
Staphylococcus epidermidis (ATCC1228)[c]	0.5	0.5	0.05
S. epidermidis (6epi)[d]	1	-	0.05
Escherichia coli (ATCC 10538)[c]	1	-	0.05
Pseudomonas aeruginosa (ATCC 27853)[d]	-	-	1
Candida albicans (ATCC 10231)[c]	1	-	0.012
Candida tropicalis (ATCC 157)[c]	0.5	-	0.012
Candida glabrata (ATCC 30070)[c]	-	-	0.012
Candida parapsilosis (ATCC 22019)[c]	-	-	0.012
Candida dubliniensis (ATCC 777)[c]	1	-	0.012
Candida dubliniensis (ATCC 778157)[c]	-	-	0.012

[a]MIC minimum inhibitory concentration in mg.mL^{-1}; [b]Positive controls: chloramphenicol for bacterial strains and ketoconazole for yeast strains; [c]standard strain; [d]field strain; (-) no inhibition of development.

that high concentrations of these compounds are required to produce a larvicidal effect. For example, (*E*)-caryophyllene gave an LC_{50} value of 1038 μg.mL^{-1} [20].

This is the first report on the analysis of the volatile constituents from leaves of *A. salzmannii* and *A. pickelii*, and their biological activities. The presence of α-copaene, (*E*)-caryophyllene, and bicyclogermacrene in these oils suggest that they could be considered chemotaxonomy markers of the genus *Annona*. The results obtained for the essential oil of *A. pickelii* support the molecular phylogenetic data [1, 18, 19] that was used to remove this species from *Rollinia* into *Annona*. The significant antioxidant and antimicrobial activities of the essential oils of *A. salzmannii* and *A. pickelii* confirm that species of Annonaceae are a natural source of biologically active compounds.

Experimental

Plant material: Leaves of *A. salzmannii* and *A. pickelii* were collected in March 2010 in the "Mata do Crasto", Municipality of Santa Luzia do Itanhy, Sergipe State, Brazil, coordinates: [S 11°23'12" W 037°25'05"] and [S 11°23'01" W 037°25'13"], respectively. The species were identified by Dra Ana Paula do Nascimento Prata, a plant taxonomist of the Departamento de Biologia of the Universidade Federal de Sergipe (UFS). Voucher specimens, numbers 15438 and 15439, respectively were deposited at the Herbarium of the UFS (ASE/UFS). Leaves were obtained from flowering species.

Hydrodistillation of the essential oils: The essential oils from dried leaves (for 72 h) of *A. salzmannii* and *A. pickelii* (each 200 g) were obtained by hydrodistillation for 3 h using a Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulfate and the percentage content was calculated on the basis of the dry weight of plant material. The essential oils were stored in a freezer until further analysis. The extraction of the oils was performed in duplicate.

GC analysis: GC analyses were carried out using a Shimadzu GC-17A fitted with a flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a ZB-5MS fused capillary column (30 m X 0.25 mm X 0.25 μm film thickness) coated with 5%-phenyl-arylene-95%-methylpolysiloxane. Helium was the carrier gas at 1.2 mL/min flow rate. The column temperature program was: 50°C/2 min, a rate of 4°C/min to 200°C, then a rate of 15°C/min to 300°C, and then 300°C/15min. The injector and detector temperatures were 250°C and 280°C, respectively. Samples (0.5 μL in CH_2Cl_2) were injected with a 1:100 split ratio. Retention indices were generated with a standard solution of *n*-alkanes (C_9-C_{18}). Peak areas and retention times were measured by an electronic integrator. The relative amounts of individual compounds were computed from GC peak areas without FID response factor correction.

GC/MS analysis: GC/MS analyses were performed on a Shimadzu QP5050A GC/MS system equipped with an AOC-20i auto-injector. A J&W Scientific DB-5MS (coated with 5%-phenyl-95%-methylpolysiloxane) fused capillary column (30 m X 0.25 mm X 0.25 μm film thickness) was used as the stationary phase. MS were taken at 70 eV with a scan interval of 0.5 s and fragments from 40-500 Da. The other conditions were similar to the GC analysis.

Identification of constituents: Essential oil components were identified by comparing the retention times of the GC peaks with standard compounds run under identical conditions, and by comparison of retention indices [21] and MS [22] with those in the literature, and by comparison of MS with those stored in the NIST and Wiley libraries.

Antioxidant activity:

$ORAC_{FL}$ kinetic assay – The antioxidant capacities of the essential oils of *A. salzmannii* and *A. pickelii* were assessed through the Oxygen Radical Absorbance Capacity (ORAC) assay. This measures scavenging activity against the peroxyl radical [Azobis (2-amidinopropane) dihydrochloride (AAPH), Aldrich, Milwaukee, WI], using fluorescein (Aldrich, Milwaukee, WI) as the fluorescent probe. The ORAC assays were carried out on a Synergy 2 (Biotek, Winooski, VT) multidetection microplate reader system. The temperature of the incubator was set at 37°C. The procedure was carried out according to the method established by Ou *et al*. [23] with modifications [24]. The data are expressed as μmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich, Milwaukee, WI) equivalents (TE) per g of oil on a dry basis (μmol of TE/g). In these tests, quercetin, isoquercitrin, and caffeic acid were used as positive controls. The analyses were performed in triplicate.

TLC autographic assay for DPPH radical-scavenging: Ten μL of a 1:250 dilution of the essential oils of *A. salzmannii* and *A. pickelii* in methanol were applied to TLC plates (silica gel 60 GF254, Fluka, AG, Switzerland). These were sprayed with a 0.2% 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St Louis, MO) solution in MeOH and left at room temperature. Active compounds, observed as yellow spots against a purple background, were observed 30 min after spraying. Relative radical-scavenging activity was assigned as "strong" (samples that produced an intense bright yellow zone), "medium" (samples that produced a clear yellow spot), "weak" (samples that produced only a weakly visible yellow spot), and "not active" (samples that produced no sign of any yellow spot) [25,26].

Antimicrobial activity: Essential oils of *A. salzmannii* and *A. pickelii* were evaluated for antimicrobial activity using the broth microdilution method (96-well microtiter plates), as previously described by Salvador *et al*. [27], to give a concentration between 0.012 and 5 mg mL^{-1}. The minimal inhibitory concentration (MIC) was calculated as the lowest concentration showing complete inhibition of a tested strain. In these tests, chloramphenicol and ketoconazole were used as experimental positive controls, while the solution of DMSO-sterile distilled water (5:95, v/v) served as the negative control. Each sensitivity test was performed in duplicate for each microorganism evaluated and repeated 3 times. The strains of microorganisms utilized are shown in Table 3.

Larvicidal assay: Eggs of *Ae. aegypti*, known to be resistant to temephos, were field collected in Aracaju city, Sergipe state, Brazil and laboratory-reared at the Universidade Federal de Sergipe insectary at 27°C and 80-85% relative humidity under a 12:12 h light:dark cycle. Adults were provided with a 10% sucrose solution *ad libitum*. Assay eggs were obtained attached to paper strips. The paper strips (1000 eggs/L) were placed in a rectangular polyethylene container with natural mineral water. The container was kept in the insectary for hatching and monitoring of larvae development for 3 to 4 days. Larvae were fed with cat food (PurinaTM) to allow regular development. All bioassays were conducted in a walk-in environmental chamber with these environmental conditions.

The larvicidal assay was performed according to Santos *et al.* [28] Third-instar larvae were used in the experiment. The concentration ranges were determined from a previous concentration-response curve with 20 larvae. A 20,000 ppm stock solution was prepared using each essential oil (20 mg mL^{-1}), Tween-80 (10% v/v), and natural mineral water (90% v/v). The stock solution was used to make 20 mL water solutions ranging from 10 to 1000 ppm. Twenty larvae were collected with a Pasteur pipette, and placed in a 25 mL graduated cylinder. The volume was made up to 20 mL with natural mineral water and transferred to

disposable cups containing variable volumes of the stock solution. A mortality count was conducted 24 h after treatment. Controls were prepared with Tween-80 (0.1 mL), and water (19.9 mL). Three replicates were used for each concentration and the control. For a positive control, the commonly used organophosphorate insecticide, temephos was used at final concentrations ranging from 0.015 to 0.135 ppm.

Probit analysis [29] was conducted on mortality data collected after 24 h exposure to different concentrations of testing solutions to establish the lethal concentration for 50% mortality (LC_{50}) and 95% confidence intervals (CI) values for the respective compounds and temephos (Table 1). In all cases where deaths had occurred in the control experiment, the data were corrected using Abbott's formula (% Deaths = [1-(test/control)] x 100). Compound activity is considered significantly different when the 95% CI fail to overlap.

Acknowledgments – The authors are grateful to FAPITEC/SE (Editais N° 07/2009 and 10/2009), CNPq, CAPES, and FAPESP for financial support.

References

[1] Richardson JE, Chatrou LW, Mols JB, Erkens RHJ, Pirie MD. (**2004**) Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamaceae. *Philosophical Transactions of the Royal Society B*, ***359***, 1495–1508.

[2] Corrêa MP. (**1984**) *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*. IBDF, Rio de Janeiro, RJ.

[3] Stévigny C, Bailly C, Quetin-Leclercq J. (**2005**) Cytotoxic and antitumor potentialities of aporphinoid alkaloids. *Current Medicinal Chemistry - Anti-Cancer Agents*, ***5***, 173–182.

[4] Rupprecht JK, Hui YH, McLaughlin, JL. (**1990**) Annonaceous acetogenins: a review. *Journal of Natural Products*, ***53***, 237–278.

[5] Costa EV, Pinheiro MLB, Barison A, Campos FR, Salvador MJ, Maia BHLNS, Cabral EC, Eberlin MN. (**2010**) Alkaloids from the bark of *Guatteria hispida* and their evaluation as antioxidant and antimicrobial agents. *Journal of Natural Products*, ***73***, 1180–1183.

[6] Costa EV, Pinheiro MLB, Silva JRA, Maia BHLNS, Duarte MCT, Amaral ACF, Machado GMC, Leon LL. (**2009**) Antimicrobial and antileishmanial activity of essential oil from the leaves of *Annona foetida* (Annonaceae). *Quimica Nova*, ***32***, 78–81.

[7] Costa EV, Teixeira SD, Marques FA, Duarte MCT, Delarmelina C, Pinheiro MLB, Trigo JR, Maia BHLNS. (**2008**) Chemical composition and antimicrobial activity of the essential oils of the Amazon *Guatteriopsis* species. *Phytochemistry*, ***69***, 1895–1899.

[8] Feitosa EMA, Arriaga AMC, Santiago GMP, Lemos TLG, Oliveira MCF, Vasconcelos JN, Lima JQ, Malcher GT, Nascimento RF, Braz-Filho R. (**2009**) Chemical composition and larvicidal activity of *Rollinia leptopetala* (Annonaceae). *Journal of the Brazilian Chemical Society*, ***20***, 375–378.

[9] Siqueira CAT, Oliani J, Sartoratto A, Queiroga CL, Moreno PRH, Reimão JQ, Tempone AG, Fischer DCH. (**2011**) Chemical constituents of the volatile oil from leaves of *Annona coriacea* Mart., Annonaceae, and *in vitro* antiprotozoal activity. *Brazilian Journal of Pharmacognosy*, ***21***, 33−40.

[10] Boyom FF, Ngouana V, Zollo PHA, Menut C, Bessiere JM, Gut J, Rosenthal PJ. (**2003**) Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. *Phytochemistry*, ***64***, 1269–1275.

[11] Pinheiro MLB, Xavier CM, Souza ADL, Rabelo DM, Batista CL, Batista RL, Costa EV, Campos FR, Barison A, Valdez RH, Ueda-Nakamura T, Nakamura CV. (**2009**) Acanthoic acid and other constituents from the stem of *Annona amazonica* (Annonaceae). *Journal of the Brazilian Chemical Society*, ***20***, 1095–1102.

[12] Maas PJM, Kamer HM, Junikka L, Mello-Silva R, Rainer H. (**2001**) Annonaceae from central-eastern Brazil. *Rodriguésia*, ***52***, 61–94.

[13] Herbário da Universidade Federal de Sergipe (ASE/UFS). *http://www.splink.org.br/index/* (19 October 2010).

[14] Paulo MQ, Barbosa-Filho JM, Lima EO, Maia RF, Barbosa RC, Kaplan MA. (**1992**) Antimicrobial activity of benzylisoquinoline alkaloids from *Annona salzmanii* D.C. *Journal of Ethnopharmacology*, ***36***, 39–41.

[15] Queiroz EF, Roblot F, Laprévote O, Paulo MQ, Hocquemiller R. (**2003**) Two unusual acetogenin from the roots of *Annona salzmanii*. *Journal of Natural Products*, ***66***, 755–758.

[16] Queiroz EF, Roblot F, Cavé A, Hocquemiller R, Serani L, Laprévote O, Paulo MQ. (**1999**) A new bistetrahydrofuran acetogenin from the roots of *Annona salzmanii*. *Journal of Natural Products*, ***62***, 710–713.

[17] Mesquita LM, Roque NF, Quintana LMB, Paulo MQ, Barbosa Filho JM. (**1988**) Lignans from *Rollinia* species (Annonaceae). *Biochemical Systematics and Ecology*, ***16***, 379–380.

[18] Rainer H. (**2007**) Monographic studies in the genus *Annona* L. (Annonaceae): Inclusion of the genus *Rollinia* A.St.-Hil. *Annalen des Naturhistorischen Museums in Wien*, ***108B***, 191–205.

[19] Chatrou LW, Escribano MP, Viruel MA, Maas JW, Richardson JE, Hormaza JI. (**2009**) Flanking regions of monomorphic microsatellite loci provide a new source of data for plant species-level phylogenetics. *Molecular Phylogenetics and Evolution*, ***53***, 726–733.

[20] Doria, GAA, Silva, WJ, Carvalho, GA, Alves, PB, Cavalcanti, SCH. (**2010**) A study of the larvicidal activity of two *Croton* species from northeastern Brazil against *Aedes aegypti*. *Pharmaceutical Biology*, ***48***, 615–620.

[21] van Den Dool H, Kratz PDJA. (**1963**) Generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography A*, ***11***, 463–471.

[22] Adams RP. (**2007**) *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed. Allured Publ. Corp., Carol Stream, IL.

[23] Ou B, Hampsch-Woodill M, Prior RL. (**2001**) Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, ***49***, 4619–4626.

[24] Salvador MJ, Ferreira EO, Mertens-Talcott SU, Castro WV, Butterweck V, Derendorf H, Dias DA. (**2006**) Isolation and HPLC quantitative analysis of antioxidant flavonoids from *Alternanthera tenella* Colla. *Zeitschrift für Naturforschung*, ***61c***, 19–25.

[25] Cavin, A, Potterat O, Wolfender J, Hostettmann K, Dyatmyko W. (**1998**) Flavonoids and phenylpropanoid derivatives from *Campanula barbata. Journal of Natural Products*, ***61***, 1497–1501.

[26] Takamatsu, S, Hodges, TW, Rajbhandari, I, Gerwick, WH, Hamann, MT, Nagle, DG. (**2003**) Marine natural products as novel antioxidant prototypes *Journal of Natural Products*, ***66***, 605–608.

[27] Salvador, MJ, Ferreira, EO, Pral, EMF, Alfieri, SC, Albuquerque, S, Ito, IY, Dias, DA. (**2002**) Bioactivity of crude extracts and some constituents of *Blutaparon portulacoides* (Amaranthaceae). *Phytomedicine*, ***9***, 566–571.

[28] Santos, SRL, Silva, VB, Melo, MA, Barbosa, JDF, Santos,RLC, Sousa, DP, Cavalcanti, SCH. (**2010**) Toxic effects on and structure-toxicity relationships of phenylpropanoids, terpenes, and related compounds in *Aedes aegypti* larvae. *Vector-Borne and Zoonotic Diseases*, ***10***, 1049−1054.

[29] Finney, DJ, Stevens WL. (**1948**) A table for the calculation of working probits and weights in probit analysis. *Biometrika*, ***35***, 191–201.