Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(8):1182-1189

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antioxidant effect of plant extracts of the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray on the free radical DPPH

Mayara Tania P.[1], Deisiane Del Castilo B.[2], Christopher Douglas Serrão P.[2], Alex Bruno Lobato R.[3], Ryan da Silva R.[3], Flavia de Oliveira P.[2], Paula Stefany Ferreira S.[2], Núbia Priscila Leite Távora[2] and Sheylla Susan Moreira da Silva de A.[3]

[1]*Department of Biological and Health Sciences, Faculty of Pharmaceutical Sciences, Post Graduate Program in Biodiversity and Biotechnology of the Amazon (Bionorte), General and Analytical Chemistry Laboratory of the Federal University of Amapá, Macapá- Amapá- Brazil*
[2]*Undergraduate Course of Pharmaceutical Sciences, General and Analytical Chemistry Laboratory of the Federal University of Amapá - Brazil*
[3]*Laboratory of Pharmacognosy and Phytochemistry of the Federal University of Amapá – Brazil*

ABSTRACT

This study aims to evaluate the antioxidant and cytotoxic effect of aqueous and ethanol extracts from the leaves of Tithonia diversifolia (Asteracea) on the free radical DPPH • and larvae of Artemia salina Leach, respectively. Samples of Tithonia diversifolia (Hemsl.) A. Gray was collected in the district of Fazendinha, Macapá- Amapá. The selected leaves were dried at 40C and then ground in a mill to obtain a fine-grained powder. After obtaining the aqueous and ethanolic plant extracts, they were subjected to phytochemical analysis for identification of metabolites classes. Phytochemicals tests were conducted to verify the presence of saponins, organic acids, reducing sugars, phenols, tannins, alkaloids, cyanogenic glycosides, resins, proteins and amino acids. The evaluation of the antioxidant activity was assessed by the sequestrant ability extracts on free radical DPPH • (2,2-diphenyl-1-picryl-hidrazila). There was prepared a methanol solution of DPPH at the concentration of 40mg / ml. Where the aqueous and ethanolic crude extracts T. diversifolia were diluted in methanol at the following concentrations (5-2,5-1,0-0,75-0,5 and 0.25 mg/mL). To evaluate the cytotoxic activity the adult forms (metanauplius) of Artemia salina were incubated for 24 hours with the following concentrations of 1000 extracts, 750, 500, 250, 100 and 50 µg/mL, thereby groups were assigned according to their respective concentration and all tests were performed in triplicate. The result of phytochemical analysis of crude aqueous extract Tithonia diversifolia revealed the presence of saponins, organic acids, reducing sugars, phenols, tannins, alkaloids, cyanogenic glycosides, and resins. In crude ethanol extract was revealed the presence of those found in the aqueous crude extract plus protein and amino acids. It is observed a strong antioxidant activity of both the aqueous extract as ethanol dose dependent on the type DPPH • with IC_{50} of 2.273 and 0.630 mg / mL, respectively, for EBA and EBE. Regarding the cytotoxic activity, is observed that the aqueous crude extract of T. diversifolia was non-toxic to A. salina with CL_{50} equal to 3660 µg/mL. To the ethanolic extract to LC_{50} of 1403 µg / mL also shows no toxicity, but statistical analysis revealed that values obtained for T. diversifolia are within the probabilistic error bound suggesting cytotoxic activity by high concentrations. On the results obtained, it is concluded that the plant species is a promising source of antioxidant compounds.

Keywords: *Tithonia diversifolia*, DPPH, cytotoxic activity, antioxidant.

INTRODUCTION

The man has always sought out nature's solutions to their health problems. The use of medicinal plants in the prevention and treatment of diseases is made from the earliest days of humanity. In fact, discoveries made in the remains of early hominids, show that about 60,000 years ago they already used various plants for medicinal purposes. It is used for millennia phytotherapy as a source of medicines for the alleviation and cure of the disease [1].

Medicinal plants have had their therapeutic value researched more intensively by science in recent years and its use recommended by health professionals [2]. Among the widely studied plants are the Angiosperms, more numerous and known to be economically important. In this phylum are the Asteraceae family, which comprises about 1,600 genera and 23,000 species, well distributed in tropical, subtropical and temperate regions, representing 10% of the vascular flora world. In this phylum are several species of medicinal interest [3-6].

In Brazil, the rich flora has been studied since colonial times [7]. Listing up 2,065 species in 288 genera being considered the center of the country's diversity of Asteraceae [8]. Brazilian biodiversity is considered a major source of biologically active substances, and its preservation is essential both for the intrinsic value of this immense biological richness as by its enormous potential as a source of new drugs [9-11]. In this scenario highlights the Amazon, which has acquired today the meaning of a remarkable laboratory for several studies involving new technologies (especially biotechnology) and production systems, based on sustainable use.

The *Tithonia diversifolia* (Asteraceae) species belongs to Spermatophyta division Class: Dicotiledoneae, Subclass: Metaclamídeas Order: campanulate, Family: Asteraceae Genus: Tithonia [12]. It is described as a herbaceous plant with 1.5 to 4.0 m, it has strong branches with alternating leaves, petiole, ranging from 7.0 to 20.0 cm in length and, 4.0 to 20.0 cm wide . In its composition were found about 150 chemical compounds such as; sesquiterpenes, including lactones, terpenes, flavonoids luteolin and chlorogenic acid and derivatives [13-16]. Other smaller classes were also identified, such as phytosterols, xanthones, coumarins, ceramides, chromone and chromene [17-18].
In folk medicine, the aerial parts of *T. diversifolia* are indicated in the treatment of diabetes [19], malaria [20], and infectious diseases [20-22]. The species is of particular interest since they show various pharmacological activities, such as antiplasmodial [22. 23. 24. 25] amoebicide [26-27], antiviral [27-28], anti-inflammatory [29-30] and antidiabetic [31].

This research seeks to deepen the studies in the Asteraceae family evaluating the antioxidant and cytotoxic potential of plant species. By the above, it has been proposed as an alternative hypothesis the plant extracts of the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray, present antioxidant and cytotoxic activity. In the null hypothesis, the plant extracts of the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray did not have antioxidant and cytotoxic activity.

EXPERIMENTAL SECTION

Plant Material

Samples of *Tithonia diversifolia* (Hemsl.) A. Gray were collected in the district of Fazendinha (Lat. 00 ° 03'12.5 "S Long: 51 ° 07'59.5"W) Macapá- Amapá. The herbarium specimens obtained in the collection were identified and deposited in the Herbarium Amapaense HAMAB / Institute of Scientific and Technological Research of the State of Amapá- IEPA) under the code (IAN): 018,880. The selected sheets were dried at 40C and then ground in a mill to obtain a fine-grained powder **(Figure 1).**

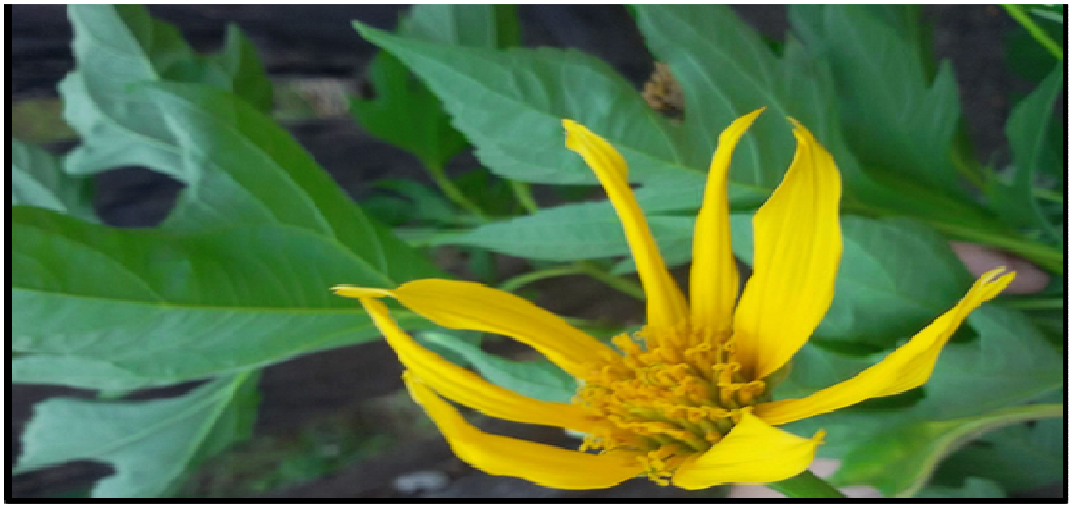

Figure 1 - Leaves and flowers of *Tithonia diversifolia* species
Source: Author's collection

Preparation of materials, solutions and reagents (Phytochemistry Prospection)
All reagents were prepared according to conventional techniques in Phytochemistry [32-35].

Obtaining aqueous crude extract (EAB) of *Tithonia diversifolia* (Hemsl.) A. Gray
The crude aqueous extract (EBA) was obtained from this powder ratio dilution in distilled water of approximately 1/20 (w / v) and submitted to hydrodistillation process (100 ° C) in a Clevenger type apparatus for 2 h [36]. Held extraction, the EBA was evaporated under reduced pressure and further diluted in suitable solvents and concentrations to achieve the phytochemicals and biological assays [35].

Obtaining ethanol crude extract (EAE) of *Tithonia diversifolia* (Hemsl.) A. Gray
After grinding 50 g of dried leaves of *Tithonia diversifolia* it was transferred to a suitable container which was added to ethyl alcohol (70%) until complete submersion of the material a total of volume of 500mL. The extract was macerated for 3 days. Performed the extraction, the ethanol crude extract (CEE) was rotatory evaporated and further diluted in solvents and appropriate concentrations for performing biological assays [37].

Prospection Phytochemistry
Phytochemicals tests were conducted to verify the presence of saponins, organic acids, reducing sugars, phenols, tannins, alkaloids, cyanogenic glycosides, resins, proteins and amino acids [32- 35, 37].

Determination of antioxidant activity by capturing the free radical DPPH
The evaluation of the antioxidant activity, with some modifications, was based on the methodology proposed by Sousa et al. [39] and Lopez-Lutz et al. [40] through the sequestering capacity of the DPPH (2,2-diphenyl-1-picryl-hidrazila). Preparation of methanol solution of DPPH at the concentration of 40mg / ml. The EBA and EBE from *T. diversifolia* was also diluted in methanol at the following concentrations (5-2,5-1,0-0,75-0,5 and 0.25 mg/mL). To evaluate the antioxidant activity were made triplicates with volume of 0.3 ml of extract per tube, added to 2.7 mL of the DPPH solution. Meanwhile, the white of each concentration was prepared, and this mixture 2.7 mL of methanol plus 0.3 ml of the methanolic solution of the EBA and EBE. After 30 minutes of incubation at room temperature and protected from light, the readings were performed with a spectrophotometer (Biospectro SP-22) at a wavelength of 517nm in quartz cuvette. The antioxidant activity was calculated according to Souza et al. [39] **(Figure 2).**

$$(AA\%) = 100 - \{[(Abs_{sample} - Abs_{white})100]/Abs_{control}\}$$

Figure 2; Equation to calculate the % of antioxidant activity (AA%).
AA% - Percentage of antioxidant activity
Abts sample = Sample Absorbance
Abs white-=White Absorbance
Abs control -=control Absorbance

Cytotoxicity assay with *Artemia salina* Leach

The cytotoxicity assay front *Artemia salina* Leach. Was based on the technique of Araujo et al. [41] and Lobo et al. [42] with adaptations. It was prepared a synthetic sea salt solution at 35 g / L, and in this were incubated 45 mg of *A. salina* Leach eggs. The solution was incubated in a dark and exposed the source of artificial heat container, within 24 hours to hatch larvae (nauplii). Then the nauplii were separated and placed in a bright environment at room temperature for 24 hours to achieve metanauplius stage. The mother solution of EBA and EBE from *T. diversifolia* was prepared containing 18 mg of dry extract, solubilized in 1.5 mL of Tween 80 to 5% to facilitate its solubilization, then were added 7,5mL of saline solution making a total of 9 mL of final volume, the final concentration obtained was 2mg / ml. Subsequently, to the end of the light period the metanauplius were selected and divided into 7 groups of 10 subjects in each test tube. For group were added aliquots of 2500, 1900.1250, 625, 250 and 125µL of EBA, respectively, completing the final volume to 5 mL with synthetic sea salt solution (35g / L). It was yield the final solutions with the following concentrations of 1000, 750, 500, 250, 100 and 50 µg / mL, thereby groups were designated according to their respective concentration and all tests were performed in triplicate. After 24 hours have been counted the number of survivors to determine LC_{50} through PROBIT analysis of the software SPSS®.

Statistical analysis

The results were expressed by mean ± standard error (SEM) organized according to the relevance, in tables, graphs, tables and figures. Significant differences between treatments were carried out using ANOVA A Critério followed by Student-Newman-Keuls test. The analysis of correlation and regression wereapplied to evaluate the DE_{50} and CL_{50}. The CL_{50} was determined in PROBIT regression, using SPSS (Statical Package for Social Sciences) with probabilistic limit of $p \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical analysis of aqueous and ethanol extracts from *Tithonia diversifolia*

The result of phytochemical analysis of crude aqueous extract of *Tithonia diversifolia* revealed the presence of saponins, organic acids, reducing sugars, phenols, tannins, alkaloids, cyanogenic glycosides and resins. In crude ethanol extract was revealed the presence of those found in the aqueous crude extract plus protein and amino acids **(Table 1).**

Table 1 Classes of secondary metabolites found after phytochemical analysis of aqueous and ethanol crude extract of the leaves of *Tithonia diversifolia.* (+) Present / (-) Absent

Secondary Metabolite	*Tithonia diversifolia* extracts	
	Aqueous crude extract (EBA)	Crude extract ethanol (EBE)
Saponins	+	+
Organic acids	+	+
Reducing sugar	+	+
Phenols	+	+
Tannins	+	+
Alkaloids	+	+
Cyanogenic glycosides	+	+
Resins	+	+
Proteins and amino acids	-	+

Antioxidant activity of aqueous and crude ethanol extract of *Tithonia diversifolia* in eliminating free radical DPPH •.

Table 2 shows the result of the antioxidant activity of the EBA and EBE *Tithonia diversifolia* on the free radical DPPH •. It is observed strong antioxidant activity of both the aqueous extract as ethanol dose dependent on the type DPPH • with IC_{50} of 2.273 and 0.630 mg / mL, respectively, for the EBA and EBE.

Table 2 Average and standard deviation of the percentage of antioxidant activity of ethanol aqueous and crude extract of *Tithonia diversifolia* leaves on the free radical DPPH •

Concentration	% Antioxidant Activity (% AA)	
	EBA *T. diversifolia*	EBE *T. diversifolia*
5mg/mL	93,0±0,72a	96,1±3,75[a]
2,5mg/mL	65,9±1,61[a], b	95,0±2,65 [b]
1,0mg/mL	25,9±1,47[a], b	86,8±10,73 [a,b]
0,75mg/mL	12,0±1,21[a], b, c	60,4±5,13[a, b, c, d]
0,5mg/mL	12,5±1,70[a], b, c	46,9±3,32[a, b, c]
0,25mg/mL	12,8±1,76[a], b, c	32,3±1,27[a, b, c, d]
IC_{50}	2,273*	0,630*
***p*-valor**	0,000	0,000

Vertically, values (% AA) followed by the same letter do not differ significantly for ANOVA (P <0.05).*

Cytotoxicity in vitro and aqueous crude ethanol extract of *Tithonia diversifolia* against *Artemia salina* Leach.
Cytotoxic activity of the the EBA and EBE *T. diversifolia* was evaluated against larvae of the parasite *Artemia Salina* Leach. at different concentrations of the extracts. As Table 3 shows, the aqueous crude extract of *T. diversifolia* was non-toxic to *A. salina* with CL_{50} equal to 3660 mg / mL. The LC_{50} ethanolic extract of 1403 µg/mL also shows no toxicity, but statistical analysis revealed that values obtained for *T. diversifolia* are within the probabilistic error bound suggesting cytotoxic activity by high concentrations.

Table 3 – Toxicity test of aqueous and crude ethanol extract of *Tithonia diversifolia* species front of the larvae of *Artemia salina*

Concentration (µg/mL)	**Mortality percentage of** *Artemia salina* **(% M)**	
	EBA of *T. diversifolia*	EBE of *T. diversifolia*
1000	23,40	43,28
750	6,72	24,55
500	3,41	13,42
250	1,72	6,38
100	0,68	1,50
50	0,57	0
CL_{50}	3660	1403*
***p* - value**	0,124	0,018

** P <0.05 establishes CL50 in error Probabilistic limit.*

The phytochemical analysis in ethanol and aqueous extracts of leaves of *T. diversifolia* confirm the presence of phenolic compounds like tannins, alkaloids and reducing sugars, saponins and organic acids able to scavenge free radicals by acting as an antioxidant [43- 44].

The presence of organic acids in the species *T. diversifolia* emphasizes its antifungal, antimicrobial and antioxidant application [45-46]. Several plants have the ability to accumulate organic acids found in citrus fruit juices, due to the presence of citric acid, but these acids are not only present in fruit but also in the leaves of some plant species [47-48].

The citric acid has great potential antioxidant, which shows that the species *T. diversifolia* is a promising source of this substance. The same functions are assigned to phenolic compounds abundant in many plant species. They are strongly associated with reduced risk of cardiovascular disease, cancer, and other chronic diseases that regardless of the class all have antioxidant potential [49- 51]

The ability of these substances to scavenge free radicals and pro-oxidant metals (antioxidant) explains in part this association. Recent evidence suggests that these compounds may act through other mechanisms besides the antioxidant capacity as modulating the activity of different enzymes such as telomerase, lipoxygenase and cyclooxygenase interactions with receptors and signal transduction pathway, cell cycle regulation, among others, essential for the maintenance of homeostasis of living organisms [52].

The reducing sugars also exhibit antioxidant properties, the supposed mechanism is the ability it has to bind to free radicals reducing them and promoting excretion from the body, without the aid of carriers, reducing cell activity without causing oxidative stress and aging premature cell [53-54].

Steroids are also recognized for their antioxidant properties, among its benefits to human health highlights the reduction of dietary cholesterol absorption, with a consequent reduction in blood levels; reducing the risk of cardiovascular disease, and inhibiting the growth of certain types of malignant tumors [55].

They are also attributed to tannins antioxidant activity [56]. The activity is strongly related to flavonoids, tannins and volatile oils [57]. The antioxidant mechanism attributed to tanning helps in the healing process since free radicals are a major factor in the formation of various degenerative diseases such as cancer, multiple sclerosis, and atherosclerosis [58-60]. Tannins act as radical scavengers, intersect the active oxygen to form stable radicals [61-62].

In classes of saponins, there are also important metabolites able to scavenge free radicals [63]. The mechanism of action could be explained by increased excretion of cholesterol by complex formation with saponins administered orally or by increasing fecal elimination of bile acids with greater use of cholesterol for synthesis of these substances which suggests an indirect antioxidant action [64].

The cytotoxic potential Castro et al. [65] demonstrated antifungal activity against Candida albicans hydroalcoholic extract of *Tithonia diversifolia*, the minimum inhibitory concentration (MIC) was 128 µg /mL. In toxicity test on

Artemia salina, obtained 100% mortality with the LC50 of 10 µg / ml demonstrating high toxicity, and advising against the internal use of this species. Recently Passoni et al. [66] corroborate the previous statement by observing that prolonged use of *T. diversifolia* leaf extracts promoted kidney and liver changes in rodents. Previous studies have shown an antiplasmodial activity of this plant can be attributed mainly sesquiterpene lactone, Tagitinina C [23, 66- 67]. The results obtained do not rule out the possible cytotoxic effect of species, but corroborate the exposed by the authors above the isolated substances express better biological activity.

Compounds identified in plant extracts here as saponin, organic acids, reducing sugars, phenols, tannins, and alkaloids, depending on the concentration of these, may block the effects of the known-alkylamides of sesquiterpene lactones LSTs (triterpenoid class, *T. diversifolia*). Since most of the activities reported for this species are assigned to these compounds [68. 69]. The cytotoxic activity can also be ascribed to phenolic compounds which exhibit bacteriostatic and fungicidal [70] Sundarraj et al. [71] demonstrated in vitro cytotoxic activity of phenolic compounds against lung and breast cancer cells. Boutennoun et al. [72] also confirmed cytotoxic and antioxidant activity of phenolic compounds of the methanol extract of *Achillea odorata* (Asteraceae).

According to Nascimento et al. [73] and Araújo et al. [41] This activity can also be assigned to saponins which have anthelmintic activity, antiviral, spermicidal and hemolytic molusquicida [45-46]. Due to the amphipathic behavior of saponins and the ability to form complexes with steroids, proteins, and fofoslipídeos membrane. It is suggested that some saponins have the ability to disrupt the cell membrane of microorganisms, resulting in leakage of cellular contents and eventually death [74].

Another metabolite that may be involved in cytotoxic activity are the alkaloids, which due to its toxicity bitterness and act as amoebicide, antitumor and antiviral [45]. The functions of these compounds in plants are not well understood. Initially, they were attributed to alkaloids coated paper, resulting from the high toxicity that gives the plant. However, it is believed that the alkaloids act also as a reserve protein synthesis stimulants or growth regulators, internal metabolism, or reproduction and also final agents detoxification and simple transformation of other substances, the accumulation of which can be harmful to the plant. [69] Based on these results, it is concluded that the plant species is a promising source of natural antioxidant compounds and cytotoxic.

REFERENCES

[1] TK Karam; LM Dalposso; DM Casa; GBL Freitas. *Revista Brasileira de Plantas Medicinais*. **2013**, 5 (2), 280-286.
[2] AH Arnous; AS Santos; RPC Beinner. *Espaço Saúde*. **2005.** 2 (6), 01-06.
[3] K Bremer. *Portland: Timber Press*. **1994**, 752.
[4] AA Andenberg; JW Kadereit; C Jefrey. *The Families and Genera of Vascular Plants*. **2007**.
[5] SC Frerreira; RM Carvalho-Okano; JN Nakajima. *Rodriguésia*. **2009**. 60 (4),903-942.
[6] AA da Silva; LHC Andrade; *Biotemas*, **2013**, vol. 26 (2), 93-104.
[7] RBL Silva. A etnobotânica de plantas medicinais da comunidade quilombola de Curiaú, Macapá-AP, Brasil. Masters Dissertation–Universidade Federal Rural da Amazônia, Belém, **2002**.
[8] J Nakajima; B Heiden; G Dematteis; M Hattori; M Magenta; M.R Ritter; C.A Mondin; N Roque; SC Ferreira; AM Teles; RAX Borges; M Monge; Jr Bringel; JBA; CT Oliveira; PN Soares; G Almeida; A Scheider; G Sancho; MM Saavedra; RM Liro. *Asteraceae . In: Lista de espécies da flora do Brasil.Jardim Botânico do Rio de Janeiro*, **2010**, 1, 678-750.
[9] EJ Barreiro; CAM Fraga. *Química Nova*. **1999**. 22 (5), 744-759.
[10] CRD Correia; PRR Costa; VF Ferreira. *Química Nova*. **2002**. 25 (1), 82-89.
[11] EJ Barreiro; VS Bolzani. *Quimica. Nova*. **2009**. 32 (3), 679-688.
[12] CM Sousa; HR Silva; GM Vieira-Jr; MCC Ayres; CLS da Costa; DS Araújo; LCD Cavalcante; EDS Barros; PBM Araújo, MS Brandão; MH Chaves. *Química Nova*. **2007**, 30 (2), 351- 355.
[13] SR Ambrosio; Y Oki; VC Heleno; JS Chaves; PG Nascimento; JE Lichston; MG Constantino; EM Varanda; FB da Costa. *Phytochemistry*. **2008**. 69 (10), 2052-2060.
[14] DA Chagas-Paula; RB de Oliveira; VC da Silva; L Goggo-Neto; TH Gasparoto; AP Campanelli; LH Faccioli; FB da Costa. *Journal of Ethnopharmacology*. **2011**, 136 (2), 355-362.
[15] M Kourda; A Yokosuka; R Kobayashi; M Jitsuno; H Kando; K Nosaka; I Ishii; T Yamori; Y Mimaki. *Chemical and Pharmaceutical Bulletin*. **2007**, 55 (8), 1240–1244.
[16] DA Chagas-Paula; RB Oliveira; BA Rocha; FB da Costa. *Chemistry & Biodiversity*. **2012**, 9 (2), 210-235.
[17] MY Bouberte; K Krohn; H Hussain; E Dongo; B Shulz; HU Qunxiu. *Zeitschrift für Naturforschung B*. **2006**, 6 (1), 78-82.
[18] CY Ragasa; MM Tepora; JA Rideoutb, J. A. *Journal of Research in Science Computing, and* Engineering. **2007**. 4 (1), 17.

[19] H Hui; G Tang.; VLW GO. *Chinese Medicine*. **2009**, 4 (1), 11-21.
[20] GN Njoroge; RW Bussmann. *Journal of Ethnobiology and Ethnomedicine*. **2006**, 2 (1), 8-14.
[21] M Heinrich; A Anklj; B Frei; C Weimann; O Sticher. *Social Science & Medicine*. **1998**, 47 (11), 1859-1871.
[22] SM Maregesi; OD Ngassaoa; L Pieters; AJ Vlietink. *Journal of Ethnopharmacology*. **2007**, 113 (3), 457-470.
[23] E Goffin; E Ziemons; P De Mol; C de Madureira; AP Martins; AP da Cunha ; G Philippe; M Tits; L Angenot L**.** *Planta Medica*. **2002**, 68 (6), 5.43-545.
[24] MC Madureira; PA Martins; M Gomes; J Paiva; AP Cunha; V Rosário. *Journal of Ethnopharmacology*. **2002**, 81 (1), 23-29.
[25] TO Elufioye; JM Agbedahunsi. *Journal Ethnopharmacol*. **2004**, 93, 67-171.
[26] R Muganga; L Angenot; M Tits; M Frédérich. *Journal of Ethnopharmacology*. **2010**, 128 (1), 52-57.
[27] L Tona; K Kambu; N Ngimbi; K Mesia; O Penge; M Lusakibanza; K Cimanga; T Bruyne; S Apers; J Totte; L Piters; AJ Vlietink. *Phytomedicine*. **2000**, 7 (1), 31-38.
[28] L Tona; K Kambu; N Ngimbi; K Cimanga; AJ Vlietink. *Journal of Ethnopharmacology*. **1998**, 61 (1), 57-65.
[29] P Cos; N Hermans. S Apers; JB Sindambiwe; M Witvrow; CE De; BD Vanden; L Piters; Vlietinck. *Phytomedicine*. **2002**, 9 (1), 62-68.
[30] LC Chiang. *The American Journal of Chinese Medicine*. **2004**, 32 (5), 695-704.
[31] P Rüngeler; G Lyss; V Castro; G Mora; HL Pahl; I Merfort. *Planta Medica*. **1998,** 64 (7), 588-593.
[32] VB Owoyele; CO Wuraola; AO Soladoye. *Journal of Ethnopharmacology*. **2004**, 90 (2/3), 317-321.
[33] T Miura; K Nosaka; H Ishii; T Isshida. *Biological & Pharmaceutical Bulletin*. **2005**, 28 (11), 2152-2154.
[34] T Miura; K Nosaka; H Ishii; T Isshida. *The American Journal of Chinese Medicine*, **2002**, 30 (1), 81-86.
[35] FJA Matos. Introdução à fitoquímica experimental. *Fortaleza: Edições UFC*. **1988**, 150.
[36] H Wagner; S Bladt; EM Zgainski. *Plant Drug Analysis*. **1996**, 320.
[37] AF Costa. *Farmacognosia*. **2001**, 3, 1032.
[38] WLR Barbosa;E Quignard; ICC Tavares; LN Pinto; FQ Oliveira; RM Oliveira. *Manual for Phytochemical Analysis and Chromatographic of Plant Extracts* . *Scientific Journal of UFPA*. **2001**,4, 1-19.
[39] Farmacopéia Brasileira,, fifth ed. Brasil. Agência Nacional de Vigilância Sanitária. Brasília: Anvisa, **2010**, 1, 546.
[40] HCA Souza; L Wagner; R. Barbosa; JM Vieira. *Revista Científica da UFPA*. **2004**, 4, 1-19.
[41] CM Simões. Pharmacognosy plant to medicine.**2004**, 5.ed. Florianópolis, 1104.
[42] CMM Sousa; et al. *Química Nova*. **2007**, 30 (2), 51- 355.
[43] D Lopes-Lutz; et al. *Phytochemistry*. **2008**, 69, 1732-1738.
[44] MGF Araújo; WR Cunha; RCS Veneziani. *Rev. Ciênc. Farmac. Bás. Aplic.*, **2010**, 31(2), 205-209.
[45] KMS Lôbo; et al. *Revista Brasileira de Plantas Medicinais*. **2010**, 12(2), 227-233.
[46] MLP Bianchi; LMG Antunes. *Revista Nutrição*. **1999**, 2 (1), 123-130.
[47] NF Romão; FC da Silva; RN Viana; ABF Ferraz.*South American Journal of Basic Education, Technical and Technological*. **2015,** 2 (2), 23-32.
[48] COM Simões; EP Schenkel. *Farmacognosia: da planta ao medicamento*. **2010**,6 (1).
[49] DSB Oliveira; RS Ramos; SSMS Almeida. *Biota Amazônia*. **2013**, 3(3), 76-82.
[50] GO Ihejirika. *Archives Phytopathology and Plant Protecion*. **2011**, 44 (19), 1894-1900.
[51] A Kumar; et al. *International Journal of Food Science and Technology*, **2011,** 46 (9), 1840-1846.
[52] D Bandoniene; M Murkovic. *Journal Agricultural and Food Chemistry*. **2002**, 2001(50), 2482–2487.
[53] JP Spencer, J. P; MMA el Mohsen; AM Minihane.;JC Mathers. *British Journal of Nutrition*. **2008**, 12-22
[54] M D'Archivio; C Filesi ; R Di Benedetto; R Gargiulio; C Giovannini ; R Masella, R. *Ann. Ist. Super Sanitá.* **2007**, 43 (1), 348-361.
[55] RN Silva; et al. *Magazine Science and Food Technology*. **2003**, 23 (3), 337-341.
[56] DA Quadros. et al. *Acta Scientiarum Technology*. **2010**, 32 (4), 439-443.
[57] JM Salgado, Guia dos Funcionais: Dieta Alimentar para Manter a Saúde e Evitar Doenças. *Rio de Janeiro: Ediouro- Thesis Doctoral*. **2009**, 192.
[58] AP Loguercio; A Battistin; AC Vargas; A Henzel; NM Witt. *Ciência Rural*. **2005**, 35 (2), 371-376.
[59] RS Donatini. *Revista Brasileira de Farmacognosia*. **2009**, 19 (1), 89-94.
[60] F Borrelli; AA Izzo. *Phytotherapy Research*. **2000**, 14 (8), 581-591.
[61] CA Carconezi; L Hamerski; AAL Gunatilaka; A Cavalheiro; I Castro-Gamboa; DHS Silva; M Furlan; MCM Young; MN Lopes; VS Bolzani. *Revista Brasileira Farmacognosia*. **2007**, 17 (3), 319-324.
[62] JCP Mello. *Farmacognosia: da planta ao medicamento*. **2001**, 3 (6), 517-543.
[63] U Siedentopp. *Revista Internacional de Acupuntura*. **2008**, 2 (2), 249-252.
[64] FV Castejon. Tannins and saponins. the seminar brought together Applied Seminars discipline of the Graduate Program in Animal Science School of Veterinary Medicine and Animal Science of the Federal University of Goiás, **2011**.
[65] NM Castro; SEM Barros; G Rosana; RD Dolôres; R Stefani. *Revista Eletrônica de Farmácia*. **2010**, 7, 72-81.

[66] G Bidla, V PK Titanji, B Joko, G El Ghazali, A Bolad, K Berzins. *Indian Journal of Pharmacology*. **2004**, 36 (4), 245-246.
[67] TO Elufioye; JM Agbedahunsi. *Journal Ethnopharmacol*. **2004**, 93, 167-171.
[68] HHG Silva; IG Da Silva; RMG dos Santos; ER Filho; CN Elias. *Revista da Sociedade Brasileira de Medicina Tropical*. **2004**, 37(5), 396-399.
[69] AA da Silva; LHC Andrade; *Biotemas*, **2013**, vol. 26(2), 93-104.
[70] M Zhao; B Yang; J Wang; Y Liu; L Yu; Y Jiang. *International Immunopharmacology*.**2007**, 7, 162–166.
[71] S Sundarraj; R Thangam; V Sreevani; K Kaveri; P Gunasekaran; SJ Kannan. *Ethnopharmacol.* **2012,** 141 (3), 80–809.
[72] H Boutennoun; L Boussouf ; A Rawashdeh; K Al-Qaoud; S Abdelhafez; M Kebieche; K Madani. *Arabian Journal of Chemistry* , **2014**, 1-7.
[73] JE Nascimento; AFM Melo; TCL Silva; JV Filho; EM Santos; UP Albuquerque; ELC Amorim. *Rev. Ciênc. Farm. Básica Apl.* **2008**, 29(1), 145- 150.
[74] S Kaiser; C Pavei; GG Ortega. *Rev. Bras Farmacogn*. **2010**, 20 (3), 300-309.