Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 6 Issue 1, January - 2019

Assessment Of Different Pharmacological Activities Of *Cayaponia Tayuya* (Vell.) Cogn.

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***Abstract* — Phytotherapy is an alternative therapeutic practice incorporated to the Brazilian Health System. Cucurbitaceae family members, including *Cayaponia tayuya*, are used in folk medicine and related to a wide range of biological activities. In this sense, the aim of this report was to evaluate pharmacological activities of *C. tayuya* aqyeous extract with *in vitro* models. In the DPPH assay, it was detected an IC_{50}=153.97 µg/mL, probably beacause of aqueous fraction. The extract was non-toxic to *Artemia salina* naupii (LC_{50}=1293.76 µg/mL). *C. tayuya* reduced the number of murine fibroblast cells (L929), however it was non-toxic on concentrations below 125 µg/mL. Moreover, the extract had low capacity to inhibit Acetylcholinesterase. These data suggest that *C. tayuya* may be a potential candidate as a safe natural product.**

***Keywords* — Cayaponia tayuya; phytotherapy; antioxidants; biological products.**

I. Introduction

The National Politics of Medicinal and Phytotherapy Plants were created in Brazil in 2006, which has allowed phytotherapy practice in the Brazilian Unique Health System (SUS) ever since. It represents beyond an alternative therapeutic to treat Brazilian population, the incorporation of a millenary practice that combines popular and scientific knowledge [1-3]. This politic stimulates the progress in regards to scientific confirmation of efficacy and safety of medicinal plants and a broader treatment options to those patients who search for alternative treatments [3-5]. There is a global market demand that supports the purchase of products elaborated with natural actives. The driving force of this market is the fact that people believe in the advantages of these products in comparison to the synthetic ones [6,7]. In this context, the development of new natural products represents a niche in the national and international market [8]. It has been observed a following average annual growth of the “green market” from 8 to 25%, while synthetic products market grows from 3 to 10% per year. Based on these numbers, industries have been developing innovative products containing natural bioactive [9].

Since 1960s, pharmacological activities of species from Cucurbitaceae family have been attracting researches attention [10]. Recent findings are emerging about this family due to mainly the presence of cucurbitacins, triterpenes, including the basic 19-(10□9β)-abeo-10α-lanost-5ene ring skeleton [11]. Cucurbitacins have shown therapeutic activities such as antitumor by inhibition of STAT3 and/or JAK3 phosporilation [10], anti-inflammatory [12,13], anti-artherosclerotic, anti-diabetic [11], antimicrobial [14], antioxidant and antiproliferative activities [15]. One of the largest genders that belongs to the Cucurbitaceae family is the Cayaponia. There are more than 60 species, including *Cayaponia tayuya,* that are found in the United States of America (USA), Argentine, Africa [16], and Amazon region in Brazil [17]. The roots from *C. tayuya* are used in traditional medicine [17,12] to treat arthritis, rheumatism, and skin infections [17,13]. Furthermore, previous studies demonstrated analgesic, diuretic and tonic properties of *C. tayuya*, and some findings also showed an anti-inflammatory role of isolated compounds of this plant [12,13]. Considering Brazilian stimulus to the use of phytotherapy, the increase of consumption of products containing natural actives, and the scarcity of literature descriptions about pharmacological potentials related to *C. tayuya*, this current study aimed to evaluate the antioxidant activity, the toxicity toward *Artemia salina* and murine fibroblasts and the potential of acetyl cholinesterase enzymatic inhibition of the aqueous extract.

II. Methods

A. Plant Material

The fibers of *C. tayuya* used in the assays were obtained from Santos Flora Comércio de Ervas Ltda (Lote TAIUR01/0111). They were macerated to prepare aqueous extract (25 g to 500 mL of water; duration: 72 h; room temperature; protected from light). After 72 h, the extract was filtered and leaded to slow

evaporation under reduced pressure in the temperature of 40 °C (Rotavapor® R-210, Buchi, Swiss), followed lyophilization (ALPHA 1 - 4 LD plus, Christ, Germany) under pression of 1.8 mbar and -14 °C. This process aimed to reach the complete elimination of the solvent and the performance was 3.83% w/w.

B. Antioxidant Activity

Scavenging activity of *C. tayuya* was measured according to 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) [18]. The sample (50 µl) at different extract concentrations (0.97 – 250 µg/mL) was added to each well of a 96-well microplate and mixed with 150 µl of 50 µM DPPH in ethanol solution. The reaction mixture was then kept for 30 min in the dark at room temperature. Its spectrophotometer (□=510 nm) absorbance was measured in comparison to the negative control (ethanol). Ascorbic acid was used as positive control (Sigma-Aldrich, USA) at the same concentrations. Inhibition of DPPH radical was calculated using Equation 1:

$$IC_{50}\ (\%) = 100 \times (A0 - As) / Ao \quad (1)$$

being A0 negative control absorbance and As test-sample absorbance. The IC_{50} demonstrate the concentration of the extract that inhibit 50% of the DPPH radical and was calculated in accordance to the linear equation of the linear dispersion graph.

C. Cell Viability Assay

Immortalized fibroblasts (L929) were cultivated in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% of fetal bovine serum (FBS), 100 U/mL of penicillin, 100 µg/mL of streptomycin, HEPES 10 mM, and kept in humidified atmosphere at 37 °C with CO2 5% and pH 7.4. The cellular viability study was performed by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT). The cell plating was made in 96-well plate format with density of 5 x 104 cells/mL in 100 µL of medium per well. After 24 h, the culture medium was changed by fresh medium with the treatments (*C. tayuya* extract, concentrations varying from 15.62 to 1000 µg/mL, in sextuplicate). A control experiment was performed in the same conditions, but without the incorporation of the extract. The incubation of the plates was done in laboratory ovens at 37 °C with CO2 5%. After 24 h, the medium was removed, was added 200 µl of DMEM with 50 µl of MTT coloring solution (5 mg/mL), and it was incubated during 3 h (37 ° C). The precipitated formazan was then dissolved in DMSO and the absorbance was measured at 570 nm using a microplate reader. The cellular viability was determined by the Equation 2.

$$Cell\ viability\ (\%) = (A\ sample / A\ control) \times 100 \quad (2)$$

D. Brine Shrimp Lethality Assay

The brine shrimp lethality assay was carried out following procedure described by Meyer et al. [19] with some modifications. Encysted eggs of the brine shrimp Artemia salina Leach were incubated in artificial seawater (48 h; at room temperature). Ten nauplii were placed in each well, adding the *C. tayuya* extract (10 – 1,000 µg/mL) dissolved in 0.1% of DMSO. The final volume was completed by saline until 5 mL. Thymol was used as positive control and DMSO 0.1% as negative control. After 24 h, it was counted the number of survivors with the aid of binocular microscopy and the percentage of lethality was calculated. The lethal concentration 50% (LC_{50} value) and the standard error were calculated by Probit analysis [20].

E. AchE Inhibitory Activity

The *in vitro* AChE inhibition activity of *C. tayuya* extract was measured by adaptation of the method first described by Ellmam et al. [21]. Acetylcholine iodide (AChI, Sigma Aldrich) was used as substrate and 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma Aldrich) as color reagent. Briefly, to a flat bottom 96-well plate were added 80 µl of Tris-HCl buffer (50 mM, pH 8.0), 120 µL of extract solution in different concentrations ranges, and 25 µl of DTNB (3 mM in Tris-HCl buffer pH 8.0) + AChI (15 mM in Tris-HCl buffer) 1:1 solution. Then, 25 µL of AChE solution (0.22 U/mL in Tris-HCl buffer) were merged in order to activate the reaction. Control wells contained Physostigmin® (Eserin) in place of the extract. The concentration of yellow 5-thio-2-nitrobenzoate anion formed by the reaction between DTNB and thiocholine, a resulting product from the hydrolysis of ACh, was measured in a spectrophotometer at a wavelength of 415 nm every 15 s for 41 cycles at 37 °C. IC_{50} for *C. tayuya* extract was determined using nonlinear regression in GraphPad Prism 6.0 and it represents in which extract concentration the hydrolysis of acetylcholine is inhibited in 50%. The experiments were performed in triplicate for each concentration.

III. Results and Discussion

Antioxidant compounds have different benefits in relation to quality of life. They protect the entire organism against damages induced by free radicals and normally prevent or retard the onset of diseases, such as atherosclerosis, neurodegenerative conditions, and arthritis rheumatoid [22,23]. The antioxidant activity of *C. tayuya* extract was expressed by its minimum inhibitory concentration in 50% (IC_{50}) or, in other words, the quantity of extract necessary to reduce in 50% the oxidant activity of DPPH. It was observed IC_{50}=153.97 µg/mL (Table 1), which was statistically different from positive control used – ascorbic acid (IC_{50}=1.45 µg/mL). Variations in antioxidant capacity of Cucurbitaceae family is found when comparing studies, and it may be attributed to some factors as part of the plant, extract fraction and methodology. In consensus, however, the scavenging action is associated to the presence of phenolic compounds [24]. Flavonoids have been detected in *C. tayuya* extracts [25,13]. These compounds are known as free radical scavengers, and metal chelators, as

well as it reduces hydrogen donors and singlet oxygen quenchers [26], and this property is related to an anti-inflammatory action. It was demonstrated that flavonoid rich extracts of *C. tayuya* was capable of exerting anti-inflammatory activity *in vivo*, probably by inhibiting the COX-2 and NOS induction [13].

TABLE 1: ANTIOXIDANT ACTIVTY OF C. TAYUYA EXTRACT AND REFERENCE DRUG.

Sample	Antioxidant activity (IC_{50} = µg/mL)
C. tayuya extract	153.97 ± 0.55 *
Ascorbic acid	1.45 ± 0.32

The superscript (*) indicates a statistically significant difference between ascorbic acid and C. tayuya extract at p<0.05, as analyzed by Student's t-test (mean ± SD, n=3).

The evaluation of the plant extracts toxicity is essential to determine a treatment safety. Our screening of *C. tayuya* lethality on *Artemia salina* has found a LC_{50}=1293.76 µg/mL (Table 2), being considered, then, to be non-toxic [19]. It suggests a low or inexistent presence of cytotoxic compounds in the *C. tayuya* aqueous fraction, and it could make a safe natural therapy. Controversially, a previous study performed by Dantas et al. [27] showed that two cucurbitacins isolated from *Cayaponia racemosa*, a specie from Cucurbitaceae family, was highly toxic (cucurbitacin P (LC_{50}=29.6 µg/mL); 2,3,16,20(R),25-pentahydroxy-22-oxocucurbita-5-en (LC_{50}=38.8 µg/mL). These opposed effects could be explained by considering that Dantas et al. [27] used isolated compounds from *C. racemosa* and not its extract.

TABLE 2: LC_{50} VALUE OF C. TAYUYA EXTRACT AND POSITIVE CONTROL AGAINST BRINE SHRIMP AFTER 24 H.

Sample	LC_{50} (µg/mL)
C. tayuya extract	1293.76*
Thymol	33.0

The superscript (*) indicates a statistically significant difference between Thymol and C. tayuya extract at p<0.05, as analyzed by Student's t-test (mean ± SD, n=5).

Cucurbitacins are well documented in the scientific literature as a potential antitumor drug [28,29]. In their report, Dantas and collaborators [27] showed that those isolated cucurbitacins isolated from *C. racemose* and toxic against *A. salina* were also capable of inhibiting tumor cells proliferation. Different studies suggests a cytotoxic activity of Cayaponia against several tumor cells [30-34]. Jimenez et al. [35] demonstrated that different extract fractions of *C. tayaya* were toxic against malanoma cell line B16F10, and once more the results were attributed to the metabolic composition of the extracts that includes cucurbitacins and flavonoids. However, the mechanism by which Cayaponia exerts its effect is still unclear. It is suggested that STAT3 oncogene suppression could be related to proliferative inhibition, although it does not exclude alternative mechanisms [34]. For Militão and colleagues [34], compounds that are present in Cayaponia have important pharmacological characteristics, including inhibition of cellular adhesion, proliferation of T lymphocytes, anti-inflammatory action, and immunomodulatory propriety, besides anti-angiogenic activity. In spite of the evidences demonstrating toxic effects of species from Cucurbitaceae family on tumor cell lines, Batista et al. [36] showed that oral administration of *C. tuyaya* infusion was hepatotoxic to mice. Although more studies are needed in this sense, this result suggests the search for different strategies of the extract utilization.

Direct reprogramming of somatic cells into induced pluripotent stem (iPS) cells has emerged as a priceless method for generating patient-specific stem cells of any lineage without using embryonic materials. To perform it, murine fibroblasts have been used and its cellular viability measured. Here, cellular viability assay of murine fibroblasts (L929) showed a significant cell viability reduction from 78.5% to 28.9% in relation to control group (Fig. 1). It denotes an important cytotoxic effect of *C. tayuya* extract in the concentrations between 125 and 1000 µg/mL.

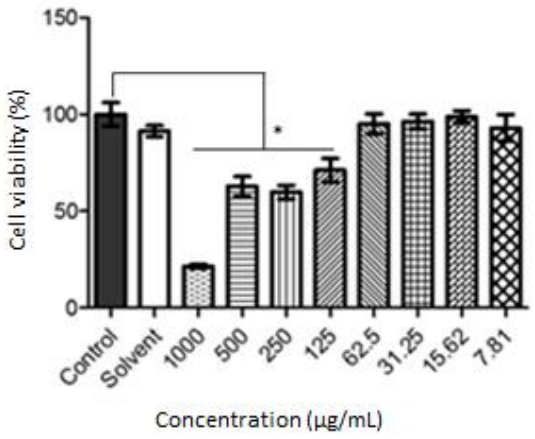

Fig. 1: *Effect of different concentrations of C. tayuya aqueous extract on murine fibroblast L929 viability after 48 h of exposing. Data are expressed as mean ± standard errors of the mean versus control. Analyses of variance (ANOVA) followed by Bonferroni's test *p< 0.05.*

Studies with natural compounds for neurodegenerative diseases search for, and then focus on those ligands that offer effective inhibition of the enzymes monoamine oxidase and acetylcholinesterase (AChE). Vinutha et al [37] classified the aqueous extracts capacity of inhibiting AchE as potent (>50% inhibition), moderate (30-50% inhibition) or low activity (<30% inhibition). For our *C. tayuya* extract, the *in vitro* percentage of inhibition

found was 22.1% (IC_{50} of 4.56 mg/mL), which means a low ability to suppress AchE action.

IV. Conclusion

In conclusion, the present data highlight the potential pharmacological activities *Cayaponia tayuya*. It was demonstrated an antioxidant capacity even being low when compared to the scientific literature, probably due the extracted fraction utilized. The antioxidant capacity opens up possibilities to apply *C. tayaya* extract in prevention and treatment of inflammatory and oxidative diseases, such as cancer. On the other hand, a non-toxicity on *A. salina* opens up the possibility of a safe natural product, but it is still needed more investigations in this direction once there are controversial results previously reported. In concentrations below 125 µg/mL, the extract was non-toxic to the L929 cell line. Finally, no promising data were found on AchE inhibition.

Acknowledgment

The authors gratefully acknowledge FAPEMIG, PROPESQ/UFJF, CNPq and CAPES for financial support.

Conflicts of Interest

The authors declare no conflict of interest.

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