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Evaluation of the anti-inflammatory activity of aqueous and methanolic extracts of *Hibiscus sabdariffa* and *Camellia sinensis*

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Abstract: *Hibiscus sabdariffa* and *Camellia sinensis* are attributed with various properties or health benefits, among which stands out is its anti-inflammatory capacity associated with the presence of bioactive compounds such as flavonoids. The aim of the research was to evaluate the anti-inflammatory effect of the extracts aqueous and methanolic of *H. sabdariffa* and *C. sinensis* by inhibition of the enzyme cyclooxygenase-2. Vegetal material corresponding to calyxes of *H. sabdariffa* and leaves of *C. sinensis* were used to obtain the extracts. The Folin-Ciocalteu and Marinova method was used for the determination of total phenolic compounds and flavonoids. On the other hand, anti-inflammatory activity was carried out using a commercial kit and a gallic acid standard as a control.The results showed a higher percentage of inhibition in the methanolic extracts of each vegetable material. Statistical difference was observed when buying the aqueous and methanolic of *H. sabdariffa* (p=0.025), aqueous and methanolic of *C. sinensis* (p=0.035) extracts. These findings allow us to conclude that the anti-inflammatory activity of the extracts is related to the content of phenolic compounds present in the plant material and to the extraction method.

Keywords: *Hibiscus flower, Green Tea, Phenolic compounds, COX-2 inhibition*

I. Introduction

Hibiscus sabdariffa and *Camellia sinensis* , are considered among the top 10 most important plants in the world, and their popularity has grown because of their diverse potentialities and health effects [1,2] Among the properties attributed to them are their effects, antimicrobial, anticarcinogenic, antioxidant, hypoglycemic and especially its anti-inflammatory capacity[3-5]. The effects mentioned above are due to its high content of bioactive compounds such as polyphenols, which are a group of about 8,000 substances and can be classified according to their structure into flavonoids with a basic structure C6-C3-C6, anthocyanins, catechins and epicatechins, the latter predominant in *C.sinensis* [6-8].

On the other hand, inflammation is a dynamic process that starts in response to mechanical damages, burns, microbial infections and other stimulations that can affect individual's wellbeing [9-

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11]. Likewise, the inflammatory process involves the synthesis of local mediators such as prostaglandins (PG) induced by the enzyme cyclooxygenase-2 (COX-2). On the other hand, there is evidence that shows that flavonoids are capable of inhibiting *in vitro* triggered or induced inflammation in various biological models. [12,13]. Such compounds have shown antioxidant activity and radicals' removal, as well as capacity to rule several cell activities, like COX enzymatic activity. By this reason, the flavonoids inhibitory effect on COX-2 is considered one of the most important anti-inflammatory cell mechanisms [14]. Therefore, the aim of this study was to evaluate the inhibitory capacity of COX-2 of these two plants, in order to expand the study of natural products for therapeutic purposes.

II. Materials and Methods

II.1. Origin of plant material (PM)

The plant material was obtained from a popular market in the city of Maracay, Aragua State, Venezuela (Geographical coordinates: 10° 14′ 49″ N, 67° 35′ 45″ W).

Figure .1: a) H. sabdariffa dehydrated calyxes b) Organic green tea

II.2. Sample preparation for extraction

For the aqueous extract 2 grams of plant material were weighed. This was poured into a 400 mL Beaker, to which 200 mL of distilled water previously heated to the boiling point was added. The sample was slightly stirred for 4 min and filtered using Whatman No. 4 paper [15].

To obtain the alcoholics extracts, 2 g of plant material were weighed, placing them in a 400 mL beaker with 200 mL of 70% v/v methanol/water. It was macerated and left to rest for 24 h, and then, it was subjected to microwave radiation for 15 s with a power of 125 MHz. The extracts were filtered, using Whatman filter paper number 4 [16].

II.3. Determination of total phenolics

For the determination of total phenolics, 50 µL of the alcoholic extract were mixed with 250 µL of the Folin-Ciocalteu 1 N reagent (Analytical grade, Merck). It was left to stand for 8 min and then, 750 µL of 20% Na_2CO_3 and 950 µL of distilled water were added. Was incubated for 30 min at room temperature and the absorbance was read on a Genesis 20 UV/VIS spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). A calibration curve for Gallic Acid (Sigma-Aldrich, Germany) was prepared with concentrations of 50, 100, 200, 300, 400, 500 and 1000 ppm. The results were expressed in mg of Gallic Acid Equivalents (GAE) / g of PM [17].

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II.4. Determination of flavonoids

A volume of 100 µL of sample was mixed with 30 µL of 5% w/v $NaNO_2$, 30 µL of 10% w/v $AlCl_3$, 200 µL of 1 M NaOH and adjusted with distilled water to a final volume of 1 mL. The reading was performed at 510 nm in a Genesis 20 UV / VIS spectrophotometer and was compared with a standard curve with standard (+)-catechin. The results were expressed in mg of Catechin Equivalents (CE) / g of PM [18].

II.5. Determination of inhibition percentage of COX-2 enzyme

The anti-inflammatory activity of the extracts was determined using a commercial inhibition kit of COX-2 (Cayman Chemical Company, Ann Arbor, MI, USA). The results were processed according to Cayman. A Gallic Acid control solution with a concentration of 75 µg/mL was [19].

II.6. Statistical analysis

Experimental values are shown like mean of data ± mean's standard error (SE) of analysis by triplicate. For all determinations, data were analyzed by ANOVA method in one way with significant level $p< 0.05$, followed by means comparison using Tukey test (Statistic 9.0 program for Windows).

III. Results and Discussion

III.1. Total phenolics and flavonoids of the extracts

There are several internal and external factors that affect the quality and/or quantity of total phenolic compounds in plants, such as genetic diversity (variety and origin of the sample), stage of maturity, environmental variables (light intensity, climate, temperature, fertilizer use) [20-21]. Prior to the evaluation of the antioxidant activity of the extracts, the concentration of total phenolics and flavonoids was determined in triplicate, observing a statistical difference aqueous-methanolic of *Hibiscus sabdariffa* (p=0.035) and aqueous-methanolic of *Camellia sinensis*. The difference observed is similar to that reported in several studies carried out with *H. sabdariffa* and *C. sinensis*, which indicate that the extraction of phenolic compounds is influenced by the type of solvent used [22-24].

Table 1. Total phenolic compounds (GAE / g of PM) *and flavonoids*(CE) / g of PM) *in the aqueous and methanolic extracts* (*means ± standard deviation*)

Bioactive compounds	***Hibiscus sabdariffa***		***Camellia sinensis***	
	Aqueous	Methanolic	Aqueous	Methanolic
Total Phenolics	12.3±0.23	19.3±0.23	1421.3±0.23	2420.3±0.23
Flavonoids	8.3±0.23	14.3±0.23	342.3±0.23	1029.3±0.23

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III.2. Anti-inflammatory activity (COX-2 inhibition)

The results of COX-2 inhibition, showed a greater effect on the methanolic extract of C. sinensis, compared to other extracts, the latter being very similar the to gallic acid control. Regardless of the method used to obtain the extracts, it is evident that *C. sinensis* compared to *H. sabdariffa* has a higher concentration of biaxial compounds such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG).

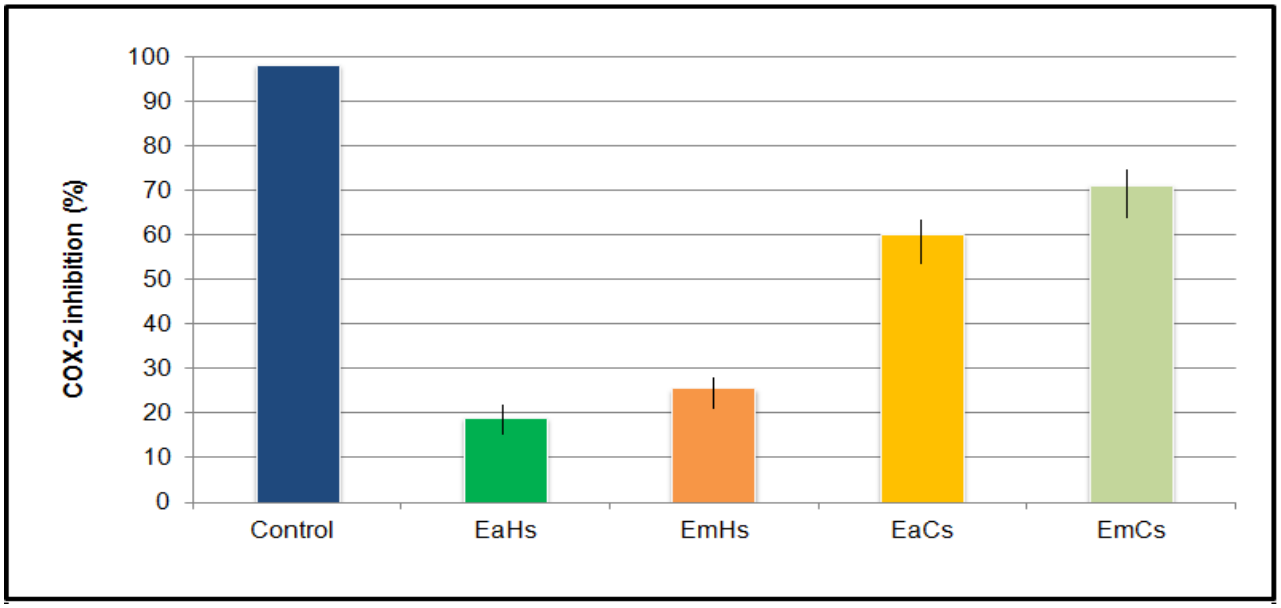

***Figure 2**. Anti-inflammatory activity by COX-2 inhibition.*

EaHs=aqueous extract of H. sabdariffa, EmHs=methanolic extract of H. sabdariffa, EaCs=aqueous extract of C. sinensis, EmCs=methanolic extract of C. sinensis

The anti-inflammatory activity of *H. sabdariffa* polyphenols has been previously reported in diverse studies, this sense ameliorating the oxidative status might be one of the strategies by which *H.sabdariffa* polyphenols exert their anti-inflammatory effects, since oxidation and inflammation are closely associated [25]. It has been suggested that antioxidant capacity of polyphenols existing in *C.sinensis* play an important role in their anti-inflammatory activity [26]. In this regard, it has been detected that compound EGCG, main component of green tea, is related with the decrease of expression of COX-2 enzymes and nitric oxide synthase (NOS) through blocking activation of nuclear factor NF-κB. Other studies proved that green tea polyphenols inhibit survival of carcinogen cells by suggesting that inhibition of COX-2 activity is the main process involved in this property [27-29].

IV. Conclusion

In conclusion, it was evident that organic green tea extracts presented not only the highest concentration of bioactive compounds, but also the greatest inhibitory effect on cyclooxygenase. Finally, it is important to note that both *Hibiscus sabdariffa* and *Camellia sinensis* provide micronutrients that can represent a real alternative for the treatment of inflammatory processes.

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V. References

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