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Chemistry characterization and antioxidant activity of mangosteen (*Garcinia mangostana* L., Clusiaceae) cultivated in Colombia

[Caracterización química y actividad antioxidante del mangostino (*Garcinia mangostana* L., Clusiaceae) cultivado en Colombia]

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Abstract: The objective of this work was to evaluate the antioxidant and inhibitory activities of the ethanolic extracts of the mangosteen (*Garcinia mangostana* L.) grown in Montenegro, Quindío, Colombia, in three stages of maturation, including the edible (pulp) and inedible parts (pericarp and peduncle). The alcoholic samples were phytochemically characterized by Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and by Fourier Transformation Infrared Spectroscopy (FT-IR); the antioxidant capacities were also evaluated by the diphenyl-picrylhydrazyl (DPPH•) radical method and Oxygen Radical Absorbance Capacity (ORAC), in addition to the inhibitory activity of acetylcholinesterase (AchE) and the total content of phenols and flavonoids. The tests detected phytochemical compounds such as phenols, flavonoids, alkaloids, quinones and xanthones, to which the antioxidant activity and the inhibition of AChE presented, can be attributed. In conclusion, the inedible parts of mangosteen contain higher proportions of secondary metabolites, these being the most promising sources for industrial use.

Keywords: *Garcinia mangostana***;** Antioxidant capacity; Industrial approach; Secondary metabolites; States of maturity

Resumen: El objetivo de este trabajo fue el de evaluar las actividades antioxidantes e inhibitoria de acetilcolinesterasa de los extractos etanólicos del mangostino (*Garcinia mangostana* L.) de Montenegro, Quíndio, Colombia, en tres estados de maduración, incluyendo las partes comestibles (pulpa) y no comestibles (pericarpio y pedúnculo). Las muestras alcohólicas fueron caracterizadas fitoquímicamente por Cromatografía de Capa Delgada (CCD), Cromatografía Líquida de Alta Eficiencia (HPLC) y Espectroscopía Infrarroja por Transformada de Fourier (FT-IR); la capacidad antioxidante fue evaluada también por el método de captación del radical libre 2,2-difenil-1-picrilhidracilo (DPPH• dejar el radical en superíndice) y la Capacidad de Absorción de Radicales de Oxígeno (ORAC), adicionalmente la actividad inhibitoria de la acetilcolinesterasa (AchE) y el contenido total de fenoles y flavonoides. Se detectaron compuestos fitoquímicos como fenoles, flavonoides, alcaloides, quinonas y xantonas, a quienes se les puede atribuir las actividades antioxidantes y de inhibición de la acetilcolinesterasa. En conclusión, las partes no comestibles del mangostino contienen una mayor proporción de metabolitos secundarios, siendo las fuentes más promisorias para uso industrial.

Palabras clave: *Garcinia mangostana*; Capacidad antioxidante; Aprovechamiento industrial; Metabolitos secundarios; Estados de madurez.

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INTRODUCTION

Natural products have been a source of most of the active ingredients due to the large variety of secondary metabolites that they biosynthesize (Harvey, 2008), many of these products have commercial importance, used in industries such as pharmaceutical, agricultural, food industry, dye industry, among others (Omena *et al*., 2012). In recent years the research on natural products has increased, bioactive compounds isolated from plants are promising to counteract the effect of free radicals by their antioxidant activity among other benefits, because they can reduce the incidence of diseases like cancer, inflammation, premature aging, cardiovascular problems, diabetes and neurodegenerative disorders, among others (Nualkaew *et al*., 2012).

Mangosteen is a native fruit of Southeast Asia, member of the Clusiaceae's family; its scientific name Garcinia comes from the French naturalist Laurent Garcin due to the work performed and the detailed description that he gave to this fruit in the decade of the 80's (Chen *et al*., 2008). Mangosteen or Mangostan is a tree original to Indonesia, known as the "Queen of Fruits", currently cultivated in Colombia in the areas of Mariquita (Tolima) and Montenegro (Quindío), a potential crop as a source for the production of food, pharmaceuticals and cosmetics because its metabolites; whit biological properties as: antibacterial, anticancer, antioxidant, antiplasmodial and neuroprotective (Jung *et al*., 2006).

In recent times, cleaner chemical extraction processes have been proposed, with the aim of converting them into more sustainable methods; this is why the use of some techniques has been diminished due to their high grade of contamination, because of the not reusable and toxic solvents used. The study of natural products has been of vital importance in the medicinal and industrial field, which is why the optimization of the extraction of them has been an indispensable part; finding that as the technology evolves, the extraction processes do it simultaneously (Claramunt *et al*., 2013). Currently it is common to find extraction processes using a systematized instrumentation, such as extraction by supercritical fluids, microwave extraction (MAE) or ultrasound extraction (UAE); this type of methodologies reduce working time, in addition to achieve higher yields of the isolated compounds and mainly the contribution of the use of less polluting solvents (Fang *et al*., 2011; Cheok *et al*., 2013).

This research covers extraction methods of phenolic compounds in different sections (pericarp, peduncle and pulp) and states of maturity of mangosteen, through passive maceration, microwave and ultrasound extraction. Also, it is showed antioxidant activities and inhibition of acetylcholinesterase enzyme (AchE) of the ethanolic extracts of mangosteen (*G. mangostana* L.).

MATERIALS AND METHODS

Plant Material

The plant material was harvested in the Castilla farm localized in Montenegro, Quindío municipality, Colombia, coordinates 4°32'28.2"N 75°51'36.0"W. The fruit was classified according to its maturation degree (Palapol *et al*., 2009) and was separated in three sections: pericarp, pulp and peduncle, see Figure No. 1, preserved by previous maceration with liquid nitrogen. The material was stocked and labeled in Ziploc® bags, taking into account two conditions: the mangosteen section and the maturation degree of every section.

Figure No. 1
Sections of the mangosteen (*Garcinia mangostana* L.).

Extraction

The extraction methods employed were carried out until exhaustion, in order to obtain more reliable data by enssuring the complete extraction of metabolites. The extraction was carried out with an ethanol-water mixture (70:30) with a 1:10 ratio plant-solvent material. Three methods of extraction were performed: passive maceration, microwave (Fang *et al*., 2011) and ultrasound (Cheok *et al*., 2013) with some modifications.

Preliminary Phytochemical Characterization

Infrared Spectroscopy

The infrared spectra of the extracts were taken in solid state using a Fourier Transform Infrared Spectrometer (FT-IR) Agilent Cary 630 supplied with Agilent MicroLab PC software, each spectrum was acquired in the range of 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1}. Analyses of main components where performed to reduce the data bank obtained by infrared spectrophotometry, wavelengths and phytochemical nucleus were the limiting variables for this study.

Chromatographic Methods

Thin Layer Chromatographic (TLC)

Silica gel chromatoplates $60F_{254}$ fabricated by Merck and with the following specifications: 480-540 m^2/g specific surface area, 0,72-0,84 ml/g pore volume, 9.5-11.5 µm d_{50}, 175-225 µm layer thickness, ≤ 30 µm deviation of layer thickness per plate which was used as an adsorbent. The extracts were eluates with an ethyl acetate-toluene-water system (6:3:1). For the identification of phytochemical nucleus different developers such as those showed in the Table No. 1 were used, which depended on the compounds to be analyzed (Wagner & Bladt, 1996).

Table No. 1
Phytochemical Nucleus used in TLC test

Phytochemical nucleus	Reagent/ test	Positive control
Alkaloids	Dragendorff	Papaverine Scopolamine
Flavonoids	$AlCl_3$	Kaempferol
Phenols	$FeCl_3$	Gallic acid Catechol
Xanthones	NH_3	Xanthone α-γ mangosteen α-mangosteen
Quinones	Borntrager	Anthraquinone

High Performance Liquid Chromatographic (HPLC)

The extracts with some flavonoids and xanthones standards, were analyzed in order to confirm the presence of these compounds in the extracts. The HPLC analysis was performed using a liquid chromatograph (Hitachi VWR modular), software Elite Lachrom, detector UV-VIS L-2420 and pump-2300; with a RESTEK column C18 15 cm x 3.5 mm x 3 µm, flow 0.2 ml/min, at 20°C and a wavelength of 280 nm. The mobile phase used for this method was isopropanol:methanol:formic acid (0.8:1:0.8).

Antioxidant activity

The antioxidant activity was measured through the diphenyl-picrylhydrazyl (DPPH•) radical method described by Brand-Williams *et al*. (1995) in a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer. The antioxidant activity was determined by reacting 100 µl of a DPPH• solution purchased from Sigma-Aldrich at 20 mg/l and 25 µl of the extract solution at 1000 mg/l for half an hour under dark conditions and at room temperature. As a negative control, the solvent in which the extract had been solubilized and as a positive control hydroquinone at 1000 mg/l. Radical reduction was measured at 517 nm.

Oxygen radical activity capacity (ORAC) is described in Dominguez *et al.* (2005), the activity was measured in a Thermo Scientific™ Fluoroskan Ascent™ FL Microplate Fluorometer and Luminometer. The reaction was carried out with a 75 mM phosphate buffer (pH 7.4) prepared before the test with deionized water, a solution of 120 nM fluorescein and 40 mM 2,2-azo-bis, 2-amidinopropane hydrochloride (AAPH) both

reactives purchased from Sigma-Aldrich. 20 µl of the solutions of the extracts and 120 µl of fluorescein were added. It was incubated for 15 minutes at 37°C, after which 60 µl of AAPH were added, the intensity of fluorescein was read every minute for 120 minutes, using emission and excitation filters of 538 and 485 nm.

Inhibitory Activity of Acetylcholinesterase Enzyme (AchE)

The inhibitory activity of the acetylcholinesterase enzyme was performed with the assay described by Ellman *et al*. (1961). The solutions of the extracts were made at 1000 mg/l, 25 µl of this solution was added with 25µl of Acetylcholine Iodate Solution (ATCI), 125 µl DTNB and 50 µl of Buffer B. The absorbance was measured at 405 nm every 45 s. After this time, acetylcholinesterase (25 µl) was added and the absorbance was read every 45 s at 405 nm. Galantamine was used as a positive control of the assay.

Determination of the Total Content of Phenol Compounds

Total Phenolic Content

The phenolic content was performed with the methodology described by Magalhães *et al*. (2010), 50 µl of the extract solution was added to 50 µl of the Folin-Ciocalteu reagent (1:50, v/v) and 100 µl of a NaOH solution (0.35 M). This mixture was allowed to react for three minutes in the dark and the absorbance at 760 nm was measured. The results were analyzed using GraphPad Prism 6.01V software, quantifying the phenol content by linear regression using a standard of gallic acid.

Total flavonoid content

The total flavonoid content was performed following the methodology of Kim *et al*. (2003) with modifications. The extract mixture consisting of 20 µL of extract (50 mg/l) was prepared, 115 µl of distilled H_2O and 7.5 µl of $NaNO_2$ (5%) were added, allowed to react for 5 minutes. 30 µl of $AlCl_3$ (2.5%) was added, allowed to react for 6 minutes. Finally, 50 µl of NaOH (1 M) and 50 µl of H_2O were added, after 5 minutes the absorbance at 500 nm was measured. The results were analyzed using GraphPad Prism 6.01 V software, quantifying the total flavonoid content using a quercetin standard.

RESULTS AND DISCUSSION

Yield of ethanolic extracts

A weight control of vials containing the extracts was performed to obtain yield percentages of each section of the fruit, Figure No. 2 shows that the peduncle's samples presented a greater yield placed in the numerals nineteen (19) to twenty-four (24), also the best extraction in the peduncle was provided by microwave in the state two (E2) which also corresponded to sixty percent of the maturation grade of the mangosteen.

The peduncle´s samples were physically different from those got it from pulp and pericarp, the extracts are described like viscous liquids. As described in Peredo-Luna *et al*. (2009), the oils are heterogeneous components of a series of metabolites, among them the phenolic compounds, a strong presence of these it is evidenced throughout the investigation, the results it is showed in the total fenolic section; giving a reason to the viscous behavior of these samples and the high yield found in this part of the fruit. A previous scientometric analysis shows that few studies have been carried out on the non-edible part (peduncle), so little comparative material has been found about it.

Phytochemical characterization

FT-IR/ATR characterization

A correspondence analysis, Figure No. 3 and Figure No. 4, was made to the data obtained from infrared spectrophotometry corresponding to the second derivative of each ethanolic extracts and to some flavonoids and xanthones standards. It was performed at the most representative wavelengths of the basic structure of each compound, frequencies ranging from 1800-1660 cm-1 were taken, these correspond to bands of the ether C-O-C bond and bands of C = O bond.

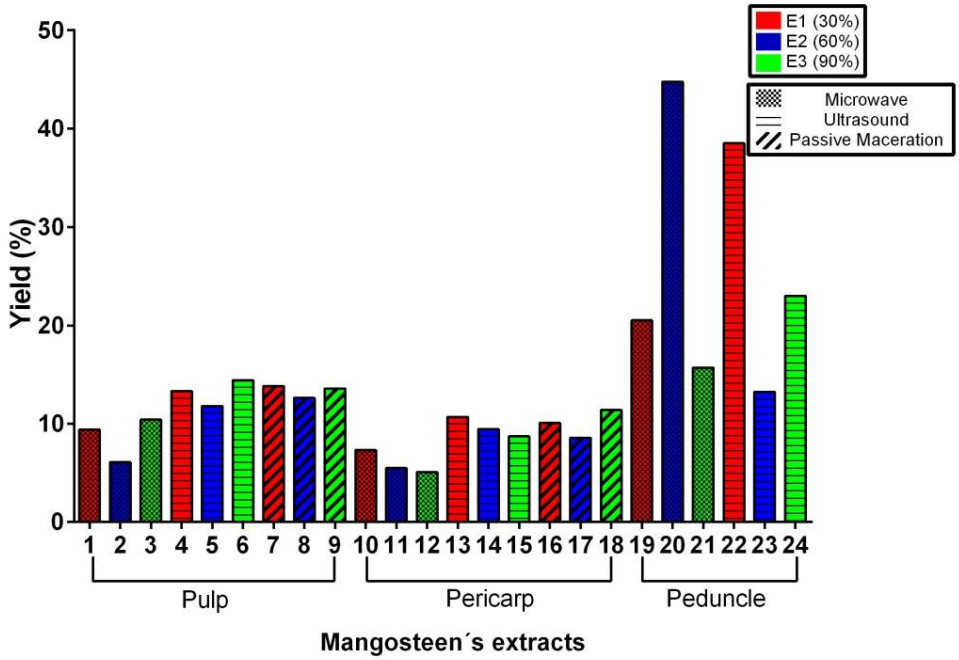

Figure No. 2
Yield of mangosteen extracts

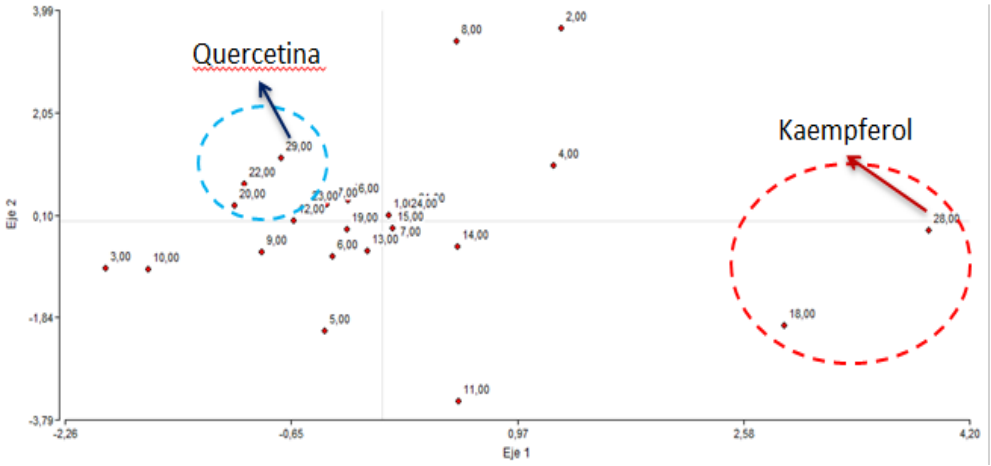

Figure No. 3
Correspondence analysis of the bands 1800-1660 cm^{-1} of mangosteen extracts compared with flavonoids standards

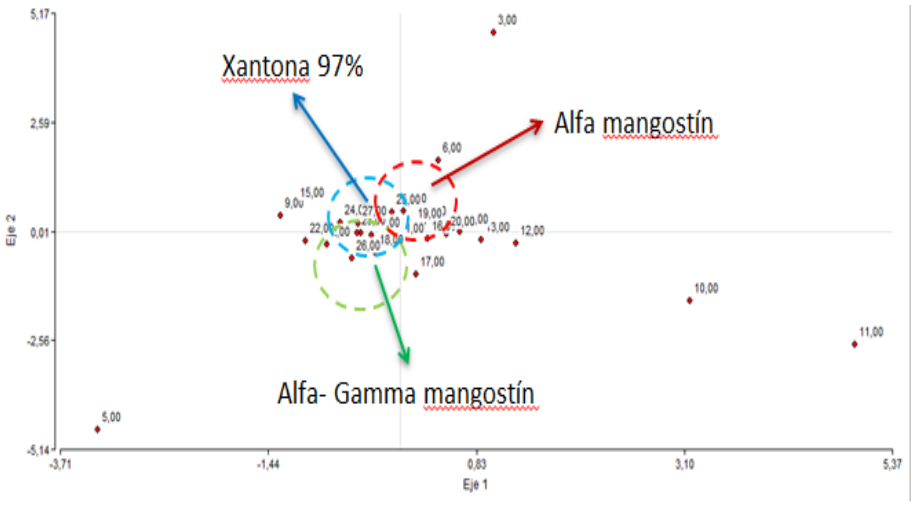

Figure No. 4
Correspondence analysis of the bands 1800-1660 cm^{-1} of mangosteen extracts compared with xanthones standards

A greater relationship of the different extracts with the xanthone patterns, due to the closeness in the correspondence analysis, it is detailed in Figure No. 3. For flavonoids (Figure No. 4) the comparison of extracts with patterns of kaepmferol (28) and quercetin (29) is performed it is observed that five extracts of the mangosteen have a relationship with flavonoids, the extract 18 corresponding to the pericarp state 3 and the extract 22 corresponding to the peduncle state 1, both with ultrasound extraction, have a greater degree of association that is confirmed by be closer to patterns in both frequency lengths.

Determination of the total phenol content

Total phenolic content

Plant materials may contain a range of chemically diverse phenolics varying from simple (phenolic acids, anthocyanins) to highly polymerized substances (tannins) in different quantities (Lallianrawna *et al*., 2013), it is common to observe the behavior shown in Figure 5 where each of the sections presents a large amount reported in µg of gallic acid/mg of extract, this provides an overview of general information about the presence of phenolic compounds in the fruit. Also in the graphic it could be seen that the edible part had a lower quantity of this metabolites than the nonedible parts, this results are supported by another reposts such as Zadernowski *et al*. (2009). Phenolic compounds were reported such as procyanidin, prodelphinidin, sterioisomers of afzekechin/epiafzelechin, catechin/epichatechin, and gallocatechin/epigallo-catechin were also found in mangosteen pericarps (Chaovanalikit *et al*., 2012), this ones may be present in the samples obtained.

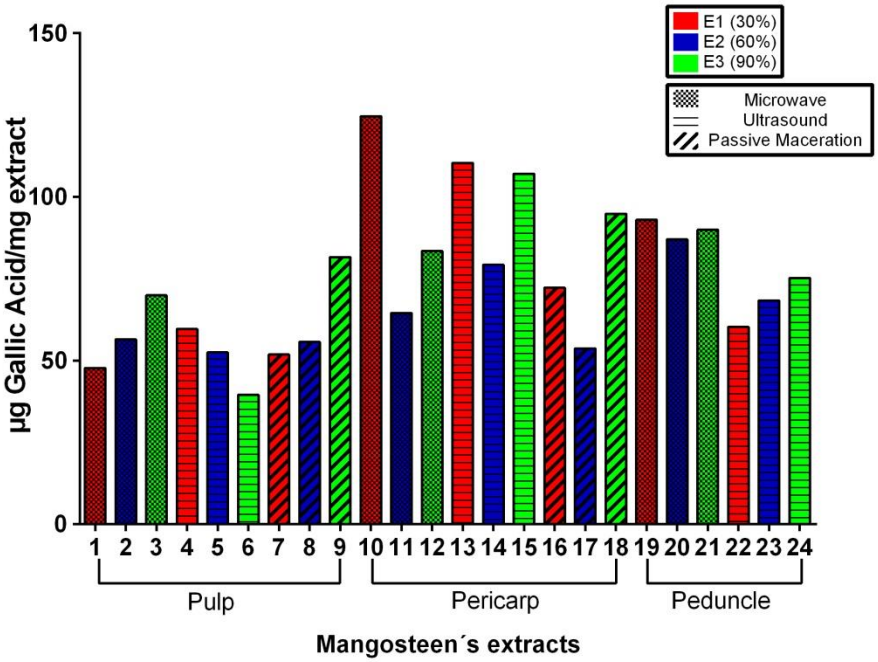

Figure No. 5
Total phenols of alcoholics extracts of mangosteen (*G. mangostana* L) by Folin-Ciocalteu method

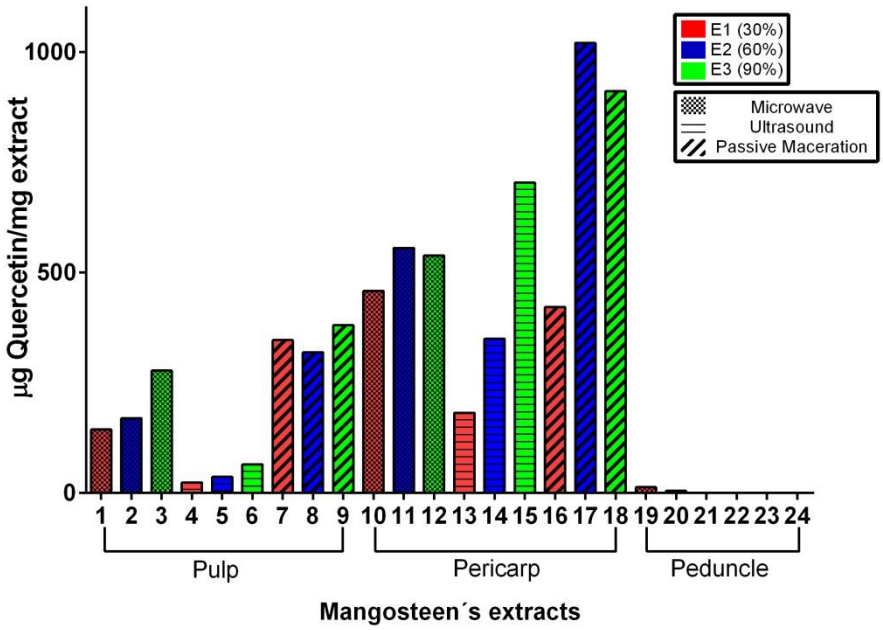

Figure No. 6
Total flavonoids of mangosteen alcoholics extracts through $AlCl_3$

Total flavonoid content

According to the data recorded in Figure No. 6, it can be observed that the highest flavonoid content corresponds to the samples of the pericarp, this behavior is due to the anthocyanins present in it (Chaovanalikit *et al*., 2012). Anthocyanins are widespread natural pigments in plants and fruits which belong to the flavonoids compound family, cyanidin 3-glucoside and cyanidin 3-sophoroside that comprise the major red color pigment in *Garcinia mangostana* (Nayak *et al*., 2010), this antecedent supports the total flavoind content.

Chromatographic characterization

Thin Layer Chromatographic (TLC)

Alkaloids, phenols, flavonoids, quinones and xanthones were characterized in the alcoholic's extracts of *G. mangostana*. It is highlighted the chromatoplates showed in Figure No. 7, for the nucleus of flavonoids and xanthones, alluding a great quantity of these two phytochemical nucleus that were analyzed with more precision in the High Perfomance Liquid Chromatographic (HPLC).

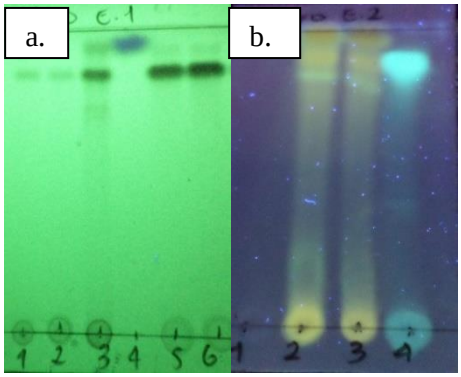

Figure No. 7
Phytochemical nucleus studied a. Xanthones b. Flavonoids

High Liquid Performance Chromatographic (HPLC)

The analysis of eight alcoholic extracts was carried out to identify flavonoids and xanthones, due to the fact that they were the ones that presented the most significant results in the phytochemical march. Every sample was compared with the retention times of injected standards, in this study, it was found that the first two parameters of samples correspond to this compound groups, it is possible then with these data, to say that there is the presence of xanthones and some flavonoids (Data not revealed), although this technique does not allow us to confirm them, studies have revealed the presence of the polyphenolic compounds (Walker, 2007).

Antioxidant Activity

Figure No. 8 and Figure No. 9 show the results of determinations of antioxidant capacity of *G. mangostana* fruits by $DPPH^{\cdot}$ and ORAC methods.

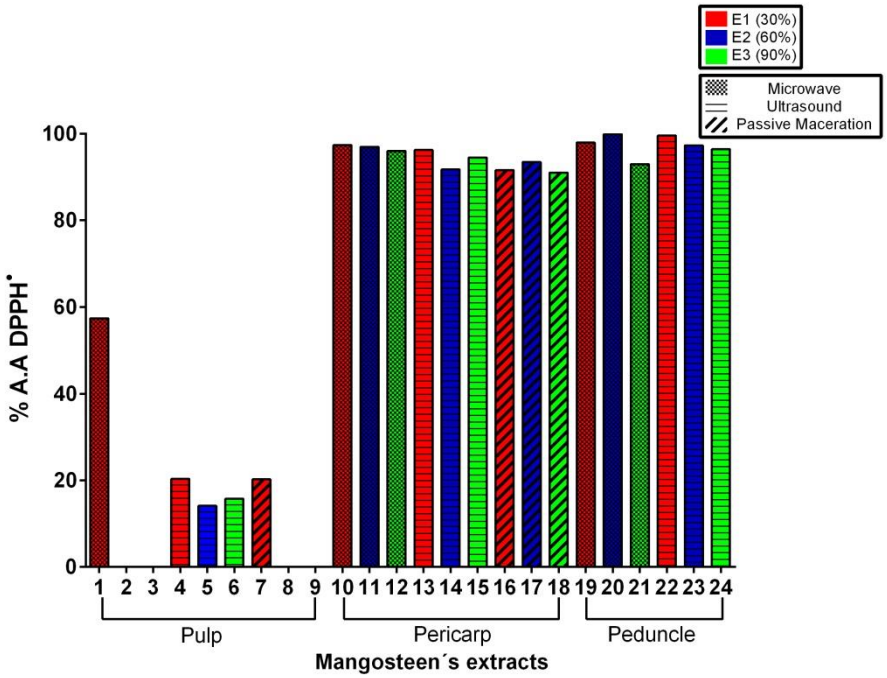

Figure No. 8
Percent of antioxidant capacity of mangosteen alcoholics extracts by DPPH• method

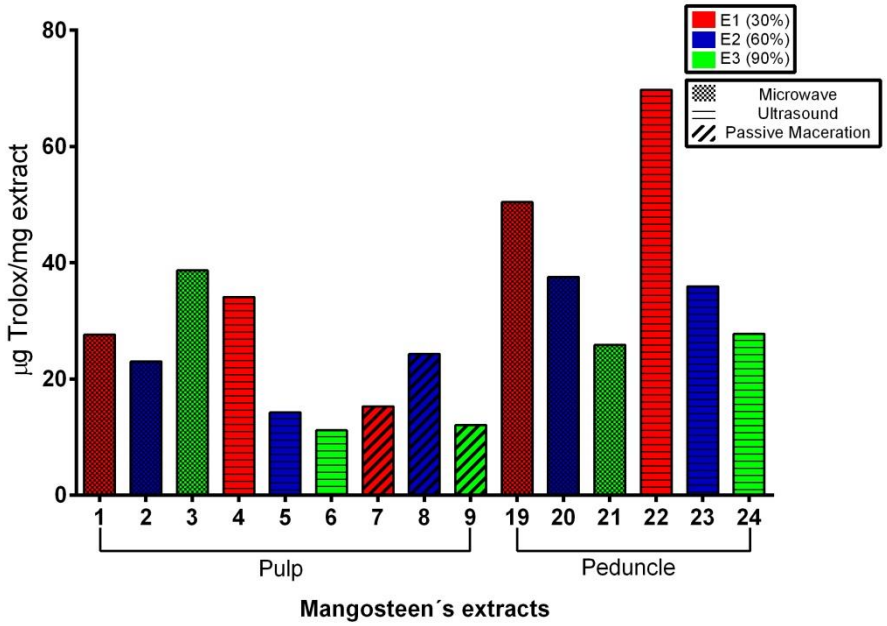

Figure No. 9
Trolox equivalents concentration in the pulp and peduncle samples of mangosteen by ORAC method

Ethanolic pulp samples show little antioxidant capacity, on the other hand the pericarp and peduncle present a high antioxidant capacity, this may be due to the high content of several secondary metabolites such as: xanthones, flavonoids, phenolic and alkaloids compounds present in these two sections of *G. mangostana* (Nilar *et al*., 2005; Chaovanalikit *et al*., 2012), these results are complemented with the characterization by thin layer chromatography made it.

Only fifteen alcoholic extracts of mangosteen were analyzed in ORAC assay, this ones were selected by the DPPH• results, first essays of each sample were carried out at different concentrations, because the sensitive of the assay (Apak *et al*., 2013), the samples must be properly diluted before analysis to avoid interferences. The extracts corresponding to the peduncle presented a greater Trolox concentration equivalent. The peduncle samples in their stage one (1) maturity had the best results, besides the decrease of these concentrations with respect to their maturation state. The pulp samples shown variation, there is no trend, and the highest Trolox concentration was presented in the microwave sample in its maturation stage three (3).

Inhibitory Activity of Acetylcholinesterase enzyme (AchE)

Given the multiple properties of the acetylcholinesterase enzyme at the neurological level, inhibitory compounds of acetylcholinesterase have been implemented in pharmaceuticals and phytotherapeutic uses in degenerative diseases such as the Alzheimer (Mukherjee *et al*., 2007), it was decided to evaluate this activity in the ethanolic extracts obtained from the mangosteen. Due to the low presence of alkaloids in the ethanolic extracts obtained from mangosteen, the low activity in the inhibition of the acetylcholinesterase enzyme of the analyzed samples was observed. Making a respective comparison with galantamine, the positive control in the test, the results seen in Figure No. 10 were very low, therefore, it can be deduced that the mangosteen's ethanolic extracts do not have a high inhibitory potential against the acetylcholinesterase enzyme.

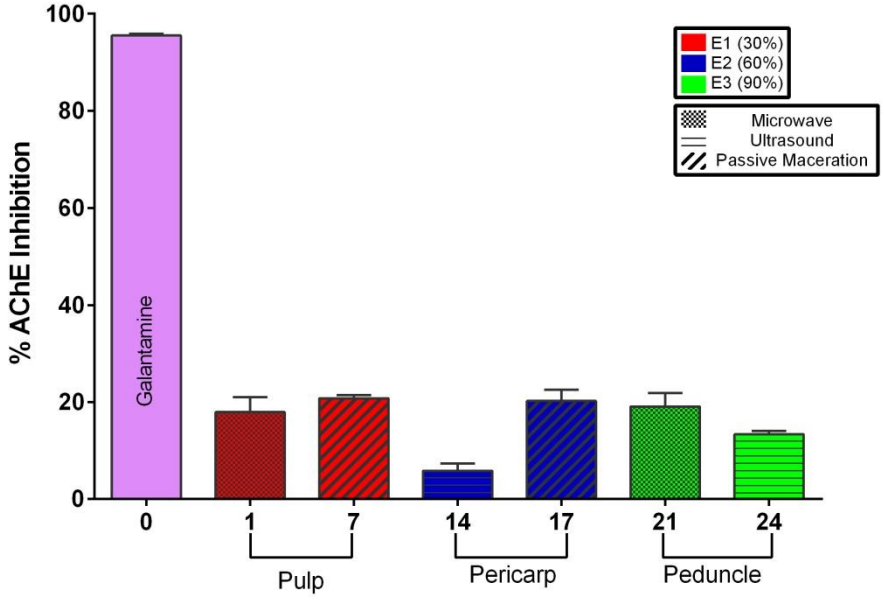

Figure No. 10
Inhibition percent of Acetylcholinesterase enzyme (AchE) for six alcoholics extracts of mangosteen (*G. mangostana* L)

CONCLUSIONS

Along experimental work, the potential of mangosteen shows the evidence that the edible part (pulp) contains a lower proportion of secondary metabolites, which are an important source for the benefits that this fruit presents; on the other side the inedible parts (pericarp and peduncle) contain higher proportions of flavonoids and xanthones, being the more promissory sources for the industrial exploitation of *G. mangostana* L.

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