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Artículo original

Actividad antioxidante de compuestos bioactivos aislados de la hoja y corteza de *Gymnanthes lucida* Sw

Antioxidant activity of bioactive compounds isolated from leaves and bark of *Gymnanthes lucida* Sw

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RESUMEN

Estudios fitoquímicos sugieren que *Gymnanthes lucida* Sw. (aité) es un candidato con potencial antioxidante. Se cuantificó el contenido de fenoles y flavonoides totales, y se evaluó la actividad antioxidante de extractos, elagitaninos y cumarinas aisladas de la hoja y la corteza mediante los métodos de secuestro de los radicales 2,2-difenil-1-pricrilhidrazil (DPPH) y ácido 2,2´-azino-bis(3-etilbenzotiazolín)-6-sulfónico (ABTS) y del poder reductor. El extracto de la hoja mostró el mayor contenido de flavonoides y fenoles y la mejor actividad de los extractos DPPH (IC_{50}=12,82 ± 0,12 μg/mL), ABTS (IC_{50}=13,83 ± 3,45 μg/mL) y poder reductor (104% respecto al ácido ascórbico). El ácido elágico (AE) mostró la mayor actividad de las muestras DPPH (IC_{50}=11,75 ± 0,53 μg/mL), ABTS (IC_{50}=11,28 ± 0,28 μg/mL) y poder reductor (107% respecto al ácido ascórbico). Los resultados sugieren que *G. lucida* es una fuente de antioxidantes donde el AE es el principal responsable de la actividad de la hoja.

Palabras clave: gymnanthes lucida Sw.; elagitaninos; cumarinas; actividad antioxidante.

ABSTRACT

Phytochemical investigations suggest that *Gymnanthes lucida* Sw. (aité) is a potential candidate for obtaining antioxidant principles. Total phenolic and flavonoid content were quantified and *in vitro* antioxidant capacity was assessed in extracts, ellagitannins, and coumarins isolated from the leaf and bark by three methods: 2,2-diphenyl-1-pricrylhydrazyl (DPPH) and 2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radicals scavenging activities and ferric reducing power. The highest phenolic and flavonoid content was observed in the leaf extract which also exhibited the highest activity among the extracts DPPH (IC_{50}=12,82 ± 0,12 μg/mL), ABTS (IC_{50}=13,83 ± 3,45 μg/mL), and reducing power (104% compared to ascorbic acid). Ellagic acid (EA) showed the highest activity, with DPPH (IC_{50}=11,75 ± 0,53 μg/mL), ABTS (IC_{50}=11,28 ± 0,28 μg/mL), and reducing power (107 % compared to ascorbic acid). Results suggest that *G. lucida* is a natural source of antioxidants where EA is the main secondary metabolite of the leaf responsible for the activity.

Keywords: gymnanthes lucida Sw.; ellagitannins; coumarins; antioxidant activity.

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Introduction

Oxidative stress plays a crucial role as main causal event in the ethiopathogenesis of chronic processes, which are a leading cause of morbidity and mortality. The global recognition of this problem has led to the development of new substances that provide protection against oxidation. As a result, synthetic antioxidants have been obtained, but their use is restricted because many of them are suspected to be carcinogenetic. [1] Therefore, there is a growing interest in natural and safer antioxidants as therapeutic alternative for the treatment of diseases related to oxidative stress. This supports and enhances the research of plants with high antioxidant power and low toxicity.

Gymnanthes lucida Sw. (*Excoecaria lucida* Sw. sin.),[2] known as yaití or aité belongs to the Euphorbiaceae family. It is commonly distributed in the tropics. In Cuba, it grows in various regions such as Sierra Maestra and Nipe-Sagua-Baracoa mountain ranges and also Las Vacas and Venado keys of Ciénaga de Zapata. [3] The decoction of the bark is traditionally used for relieving toothache and the latex for destroying callus; [3] while ethno pharmacological data revealed the use of leaves as antimicrobial and antiasthmatic. However, there are not sufficient national and international scientific studies to support its therapeutic use with efficacy, safety and quality.

Phytochemical investigations reported for the first time several bioactive compounds from the leaf and bark of this species such as hemiterpenes,[4] tannins derived from ellagic acid,[5,6] coumarins, diterpenes, triterpenes, steroids,[6] alkanes and derivatives of fatty acids.[7] The presence of several phenolic compounds could be an indicator of the potential antioxidant activity of this plant. It is well known that polyphenolic tannins have the ability to scavenge free radicals,[8] while coumarins are capable of capturing radicals and protecting tissue from damage caused by reactive oxygen species (ROS).[9]

Given the need to identify new compounds with antioxidant activity for the treatment of diseases caused by oxidative stress, *G. lucida* could be a phytotherapeutic alternative. Consequently, preclinical pharmacological studies are needed to evaluate its antioxidant capacity, which will provide the necessary experimental evidence to support its medicinal use and possible medical-pharmaceutical application. According to this, the aim of the present study was to evaluate the *in vitro* antioxidant activity of total extracts, ellagitannins and coumarins isolated from the leaf and bark of the species *Gymnanthes lucida* Sw.

Material and methods

Samples and equipment

Samples were obtained as result of investigations [4-7] of the program CAPES/MES 144/11 carried out by the Group of Natural Products of the Pharmacy Department of Universidad de Oriente and the Universidad de Paraiba, consisting in twelve samples from the leaf and bark of *G. lucida*.

- Sample 1: Total ethanol leaf extract (ELE).
- Sample 2: Total ethanol bark extract (EBE).
- Sample 3: Ellagic acid (EA).
- Sample 4: 3,3'-di-O-methylellagic-4'-O-β-D-xylopyranoside acid (DXA).
- Sample 5: 3,3´, 4´-tri-O-methylellagic acid (TMA).
- Sample 6: 3,3´, 4´-tri-O-methylellagic-4-O- β-D-glucopyranoside acid (TGA).
- Sample 7: 3,3'-di-O-methylellagic acid (DMA).
- Sample 8: Dichloromethane Bark Extract (DBE).
- Sample 9: 6,7-dimethoxycoumarin or scoparone (SCP).
- Sample 10: Scopoletin (SCT).
- Sample 11: 6,7-methylenedioxycoumarin or ayapine (AYA).
- Sample 12: 6,7,8-trimethoxycoumarin or dimethylfraxetine (TMC).

Samples 1 and 2 were obtained by maceration with ethanol 95% from the leaf and bark, respectively. The corresponding extracts were pooled and concentrated to dryness (40 °C) using a German IKA-Werke rotary evaporator. [4-7] Samples 3, 5 and 6 were isolated from the total ethanol leaf extract, while samples [4, 7, 9-12] were isolated from the total ethanol bark extract. Sample 8 was obtained by liquid-liquid fractionation of sample 2 using solvents in increasing order of polarity (hexane, dichloromethane, ethyl acetate and butanol). Chromatographic and spectroscopic techniques were used for isolation and structural identification, such as medium pressure liquid chromatography and column chromatography, infrared spectroscopy, one- and two-dimensional nuclear magnetic resonance, and mass spectrometry. [4-6]

Samples 1 and 2 were solubilized in pure absolute ethanol for analysis, while samples 3-7 in dimethylsulfoxide (DMSO) and 8-12 in pure methanol for analysis, according to bibliographic references. [10]

Spectrophotometric readings were determined using a Genesys 10S® UV spectrophotometer. All analyses were carried out in the MEDICUBA/SWITZERLAND laboratory from the Pharmacy Department of Oriente University and the Center of Toxicology and Biomedicine (TOXIMED) of Santiago de Cuba.

Qualitative chemical characterization

Solutions of total dry leaf and bark extracts were prepared in 95 % ethanol (1 mg/mL). The phytochemical characterization was carried out through qualitative chemical reactions for the following classes of secondary metabolites: alkaloids (Dragendorff, Mayer, Wagner), triterpenes and steroids (Liebermann-Burchard, Solkowski and Rosemheim), quinones (Borntrager and variant with benzene), coumarins (Baljet and Legal), saponins (foam), reducing sugars (Fehling and Benedict), phenols and tannins (Ferric chloride), carbohydrates (Molish), amino acids and free amines (Ninhydrin), polyuronides (Ethanol), flavonoids (Shinoda, concentrated sulfuric acid and alkalis), resins (resin test) and essential oils and fatty substances (Sudan III). [11]

Quantitative chemical characterization

Total phenolic content

Total phenolic content of ethanol leaf and bark extracts were determined by Folin-Ciocalteu method, according to Arumugam *et al.*, [1] with some modifications. A sample aliquot of 150 µL was added to 700 µL of distilled water and 100 µL of 50 % Folin-Ciocalteu reagent (Sigma, USA). Samples and blank were homogenize and 50 µL of 20 % sodium carbonate solution (Sigma, USA) were added. The absorbance was measured at 760 nm after 1 hour in the dark at room temperature. Total phenol concentration was calculated from the calibration curve using gallic acid (80-240 mg/mL) (Sigma, USA) as standard. (Equation 1: y = 0,004 425x – 0,092; R^2 = 99,9 %, p <0,05) Results were expressed as milligrams of gallic acid equivalent per grams of dry weight of extract (GAE/g).

Total flavonoid content

Total flavonoid content of ethanol leaf and bark extracts was determined using the methodology of Kumazawa *et al.*[12] This method consisted of mixing an aliquot of 0,5 mL of sample solution (1mg/mL in 95 % ethanol) with 0,5 mL of 2% ethanol aluminum chloride solution ($AlCl_3$, Riedel-de Haën, 99,9 % pure, Germany). After one hour of incubation at room temperature, the absorbance was measured at 420 nm. Quercetin solutions (Sigma, 95 % pure, USA-Aldrich®) between 5–25 µg/mL were used to construct a calibration curve. Total flavonoid content was calculated as milligrams of quercetin equivalent per grams of dry weight of extract (QE/g) using equation 2: y = 3,578x + 0, 313 9; R2 = 99,1 %, p < 0,05.

Antioxidant activity

DPPH free radical scavenging activity

The methodology followed by Patra *et al.*, [13] was used with slight modifications in order to evaluate the 2, 2 diphenyl-1-picrylhydracil (DPPH) (Sigma-Aldrich®) radical scavenging of samples. Briefly, a 0.1 mM solution of DPPH was prepared in absolute ethanol (EMSURE®), and 1,5 mL of this solution was added to 0,25 mL of the samples´ solutions at different concentrations (samples 1, 2, 4 and 6: 50-800 µg/mL; sample 3: 5-800 µg/mL; samples 5 and 7:

10-800 µg/mL and samples 8-12: 10-200 µg/mL). These solutions were vortexed thoroughly and kept in dark for 30 min. The absorbance was measured at 517 nm against a blank. As a positive control, ascorbic acid was used at concentrations of 5-800 µg/mL in 95% ethanol. The percent of DPPH radical scavenging capacity was calculated using equation 3: DPPH scavenging effect (%) = (A_B-A_S) / A_B * 100. (A_B: Blank Absorbance; $A_{S:}$ Sample Absorbance). The antioxidant capacity against this radical was expressed as the half-maximal inhibitory concentration (IC_{50}) which is the sample´s concentration required for 50% radical inhibition.

ABTS$^{\bullet+}$ scavenging activity

The ABTS scavenging activity of the samples was measured according to Kilic *et al.*,[14] with slight modifications. ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical was produced by the reaction between 7 mM ABTS and 2,45 mM potassium persulfate in distilled water, leaving the mixture in the dark overnight at room temperature. Then, 10 mL of the mixture were taken and diluted with 840 mL of distilled water. Next 3 mL of ABTS solution was added to 1 mL of each sample at different concentrations (samples 1-7 were 10-1000 µg/mL, sample 8 was 10-250 µg/mL and samples 9-12 were 350-700 µg/mL). After 90 min, the absorbance was measured at 734 nm. A solution of distilled water and 3 mL of ABTS solution were used as blank. Ascorbic acid was used as positive control (50-1000 µg/mL in 95% ethanol). The ABTS scavenging activity was calculated using equation 4: ABTS radical scavenging (%) = (A_B-A_s) / AB * 100. (A_B: Blank Absorbance; $A_{S:}$ Sample Absorbance). The antioxidant capacity against this radical was expressed as the half-maximal inhibitory concentration (IC_{50}).

Fe^{3+} reducing power assay

The ability of samples to reduce iron (III) was assessed by the reducing power assay according to Deepa *et al*.[15] First, 2,5 mL of samples (10-500 µg/mL) were mixed with 2,5 mL of phosphate buffer (0,2 M, pH 6,6) and 2,5 ml of potassium ferricyanide (Reachim®) ($K_3Fe(CN)_6$; 10 g/l). Then, the mixture was incubated at 50 °C for 30 min and 2,5 ml of 10 % trichloroacetic acid (UNI-CHEM®) (100 g/L) was added. Then, the mixture was centrifuged at 2000 rpm during 10 min. Finally, 2, 5 ml of the supernatant solution was mixed with 2,5 ml of distilled water and

0,5 mL of $FeCl_3$ (Sigma-Aldrich®) (1g/L) and was measured at 700 nm against a blank prepared with distilled water. Ascorbic acid (10-500 µg/mL in absolute ethanol) was used as the reference standard. The transformation of Fe^{3+}/ Fe^{2+} was measured through the absorbance generated by the Blue Prusian complex formed. The reducing power of samples was expressed as the percentage of the absorbance reached respect to ascorbic acid (100 %) at the evaluated concentrations.

Statistical analysis and processing of the results

Microsoft Excel 2016 and STATGRAPHICS Plus Version 5.1. softwares were used for mathematical processing and statistical analysis of data. All data were collected from independent triplicates experiments and expressed as mean ± standard error (± SD). Means were compared using the Student's T-statistic at ($P < 0,01$) and one-way analysis of variance (ANOVA) at ($P < 0,05$). Turkey's Least Significant Differences Test (LSD) determined differences between data means. A 95% confidence level was considered in all cases. Relation among the antioxidant variables, as well as between these variables and total phenolic and flavonoid content were analyzed using correlation and regression analysis ($P < 0,05$).

Results and discussion

Qualitative chemical characterization of total ethanol leaf and bark extracts

Results according to qualitative determination of secondary metabolites of total ethanol leaf (ELE) and bark (EBE) extracts are shown in table 1.

Table 1- Qualitative chemical composition of the total extracts of the leaf and bark

Metabolites	Essays	Results	
		Total ethanol leaf extract	Total etanol bark extract
Alkaloids	Dragendorff	(++)	(-)

	Mayer	(+)	(-)
	Wagner	(++)	(-)
Triterpenes and steroids	Lieberman-Burchard	(+++)	(+)
	Solkowski	(++)	(++)
	Rosemheim	(-)	(-)
Quinones	Borntrager	(+++)	(+++)
	Bencene variant	(-)	(-)
Coumarins	Baljet	(+++)	(+++)
	Legal	(+)	(+)
Saponins	Foam	(-)	(-)
Resins	Resins	(-)	(-)
Essential oils and fatty substances	Sudan III	(++)	(++)
Reducing sugars	Fehling	(+)	(+)
	Benedict	(++)	(++)
Phenols and tannins	Ferric chloride	(+)	(+)
Free amino acids and amines	Ninhydrin.	(-)	(-)
Carbohydrates	Molisch	(-)	(-)
Polyuronides	Ethanol	(+)	(-)
Flavonoids	Concentrated sulfuric acid	(+)	(+)
	Shinoda	(+)	(+)
	Álcalis	(+)	(+)

Legend: (+++) indicates clear positive evidence; (++) indicates positive evidence, (+) indicates positive evidence with possibilities of interference or not defined; (-) indicates negative result.

Both extracts, exhibit positive evidences for triterpenes and steroids, quinones, coumarins, reducing sugars, essential oils, flavonoids, phenols and tannins. Additionally, alkaloids and polyuronides were detected on the leaf. The highest number of positive results was obtained for ELE, where test evidences were better appreciated. This may be related to the photosynthesizing function of this organ in which several biosynthetic pathways converge to produce secondary metabolites. Ochoa *et al.* obtained similar results in 2017.[(6)]

Quantitative chemical characterization of the total ethanol leaf and bark extracts

The ELE and EBE were evaluated in order to determinate the concentrations of some secondary metabolites, previously identified in the qualitative chemical composition analysis, which could confer antioxidant activity, such as phenols and flavonoids. Table 2 reflects the results obtained.

Table 2- Total of phenols and flavonoids content in the ethanol leaf and bark extracts

Extracts	Total phenolic content (GAE/g of dry weight extract) Mean ± SD	Total flavonoids content (QE/g of dry weight extract) Mean ± SD
ELE	$687,9 \pm 0,8^{a}$	$113,82 \pm 0,001^{c}$
EBE	$621,2 \pm 2,2^{b}$	$33,56 \pm 0,001^{d}$

Legend: ELE: Total ethanol leaf extract, EBE: Total ethanol bark extract. Different superscript letters indicate statistically significant differences between the means of the evaluated samples (Student's t-test; $p < 0.05$)

Results shows a high concentration of phenolic and flavonoid compounds compared to bibliographic reports of other *Excoecaria* species.[1,16-18] It were found statistic differences between both extracts at $p < 0,05$. The ELE shows higher concentration of total phenols and flavonoids compared EBE. Phytochemical studies on *G. lucida* reports compounds with polyphenolic structures.[6] Other studies have shown higher yield of polyphenolic compounds in the leaf compared to those identified in the bark [1 168,9 mg vs. 91,1 mg (0,25 % of the total weight of the dry leaf vs. 0,002 6 % of the dry bark)].[4,5]

In addition, similar behavior of total flavonoid concentration has been observed for *E. agallocha* where higher values were obtained in the leaf (2,74 ± 0,000 4 mg of quercetin/g of dry extract) than in the bark (1,24 ± 0,000 62 mg of quercetin/g of dry extract).[19]

Total phenol content of plants has been associated with their antioxidant activity due to its redox properties, acting as hydrogen donors and oxygen unpaired electron acceptors. Flavonoids has the ability to transfer electrons to free radicals, chelation of metal catalysts, activation of antioxidant enzymes, and mitigation of oxidative stress caused by nitric oxide.[8]

Evaluation of antioxidant activity

There is no single method able to describe the "total antioxidant capacity of a sample" since this parameter should express the capacity of lipophilic and hydrophilic compounds, reflect the antioxidant mechanisms and evaluate its reactivity against different reactive species.[8] For that reason, ellagitannins and coumarins isolated from both organs of the plant were evaluated using different methodologies to determine their possible contribution to the total antioxidant capacity of the extracts.

Anti-radical activity against DPPH and ABTS

Some of the tested samples exhibited high significant radical scavenging activity in a concentration-dependent manner when compared to the standard ascorbic acid. The IC_{50} values for both radical scavenging are represented in the table 3.

Table 3- The IC_{50} values of DPPH and ABTS radical scavenging essays for samples and positive control

Samples	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
AA	12,27 ± 0,28 [a]	86,35 ± 0,02[b]
ELE	12,82 ± 0,12 [a]	13,83 ± 3,45 [a]
EBE	69,8 ± 1,17 [c]	603,44 ± 0,64[f]
EA	11,75 ± 0,53 [a]	11,28 ± 0,28 [a]
DXA	NA	522,35 ± 2,21[e]
TMA	NA	NA
TGA	NA	543,30 ± 9,97[e]
DMA	180,97 ± 0,80 [g]	395,03 ± 3,94[d]
DBE	44.7 ± 1.25 [b]	128,16 ± 1,09[c]
SCP	126,8 ± 2,24 [d]	624,98 ± 3,55[f]
SCT	70,06 ± 2,19 [c]	593,62 ± 7,94[f]
AYA	148,98 ± 5,83 [f]	NA
TMC	135,89 ± 2,89 [e]	NA

Legend: AA: ascorbic acid (positive control), ELE: total ethanol leaf extract, EBE: total ethanol bark extract, EA: ellagic acid, DXA: 3,3'-di-O-methylellagic-4'-O-β-D-xylopyranoside acid, TMA: 3,3´, 4´-tri-O-methylellagic acid, TGA: 3,3´, 4´-tri-O-methylellagic-4-O-β-D-

glucopyranoside acid, DMA: 3,3'-di-O-methylellagic acid, DBE: Dichloromethane Bark Extract (DBE), SCP: scoparone, SCT: scopoletin, AYA: ayapine, TMC: 6,7,8-trimethoxycoumarin. NA: Not Active.

Means values with different superscripts in the same column are significantly different (p <0, 05).

Data shows that ellagic acid and the total ethanol leaf extract displays potent radical scavenging in both tests, with an IC_{50} statistically similar to ascorbic acid value against the radical DPPH (11,75 ± 0,53 µg/mL and 12,82 ± 0,12 µg/mL vs. 12,27 ± 0,28 µg/mL, respectively); while the IC_{50} values of ABTS assay is lower than ascorbic acid with 11,28 ± 0,28 µg/mL; 13,83 ± 3,45 µg/mL vs. 86,35 ± 0,02 µg/mL, respectively.

The dichloromethane bark extract and the total ethanol bark extract also show good activity against the DPPH radical, with an IC_{50} values of 44,7 ± 0,13 µg/mL and 69,8 ± 1,17 µg/mL respectively, although statistically less active than the positive control. The dichloromethane phase also shows good ABTS scavenging activity compared to the rest of the evaluated samples, (IC_{50} value of 128,16 ± 1,09 µg/mL), unlike the total ethanol bark extract with an IC_{50} value of 603,44 ± 0,64 µg/mL.

The four coumarins are active in the DPPH assay, however, scopoletin has the higher activity with IC_{50} value of 70,06 ± 2,19 µg/mL, showing no significant statistical differences in relation to the total ethanol bark extract (69,8 ± 1,17µg/mL) at a level of 95 % confidence. This behavior was also observed when facing ABTS radical, although with less significant results than in the DPPH test (IC_{50} 593, 62 ± 7, 94 µg/mL). Therefore, this compound could be the main responsible of the antioxidant activity of this extract and its dichloromethane phase. Results indicate that isolated coumarins individually have no superior activity than their origin extracts (ethanol and dichloromethane) suggesting a synergistic effect, although other compounds may also contribute to the activity. The antioxidant activity of scopoletin has been reported by several authors.[19,20]

Results confirm that total ethanol leaf and bark extracts of *G. lucida* have active compounds able to donate a hydrogen atom/ transferring an electron to a free radical, thus showing antiradical activity. However, the total ethanol leaf extract shows higher activity than the bark extract in both tests, with statistical significance for 95 % confidence.

Ellagic acid proved to be the best antioxidant of evaluated samples in both tests, including the standard reference, which agrees with previous reports.[14, 21] This compound, isolated in a high

concentration from the ethyl acetate phase of the leaf, may be responsible of the greater anti-radical activity of the total extract of this organ.

It is well known that the antioxidant activity of polyphenols is structurally related with the degree of free phenolic hydroxyl groups present in the molecule, especially if they are adjacent to each other, forming catechols and galloils.[8]According to this, ellagic acid is the only with this chemical characteristic and the highest activity. On the other hand 3,3'-di-O-methylelagic acid, 3,3 'acid, 4'-tri-O-methylelagic acid, 3,3'-di-O-methylelagic acid-4'-O-β-D-xylopyranoside and 3,3', 4'-tri-O-methylelagic acid-4-O-β-D-glucopyranoside having occupied those phenols positions shows the lowest activity.

In the case of coumarins, free radical scavenging activity depends mainly on the number and position of hydrogen donating hydroxyl groups in the aromatic ring of phenolic molecules, It can be affected by other factors, such as glycosylation of aglycones and groups that donate hydrogens (-NH, -SH).[22] According to these approaches, scopoletin is the only one that possesses free phenolic hydroxyl and the highest activity.

The pure samples showed ABTS radical scavenging activity in a different order than DPPH assay, except for ellagic acid. This behavior conditioned the statistical analysis showing a weak correlation between both methods (R^2 = 0, 17; p ≥ 0, 05). This may be related to different reaction conditions, the kinetics of both radicals and molecular interactions in the reaction medium. Another important difference is that ABTS activity can be measured in organic or aqueous medium according to the hydrophilic or lipophilic nature of the sample. On the contrary, DPPH can only be measured in an organic medium, which limits the interpretation of the antioxidant capacity of the most hydrophilic evaluated compounds[8] (3,3'-di-O-methylelagic-4'-O-β-D-xylopyranoside acid and 3,3´, 4´-tri-O-methylelagic-4-O-β-D-glucopyranoside acid).

The quantitative analysis shows a direct correlation between the DPPH antioxidant activity and total phenols and flavonoids content. Correlation coefficients of 0,839 8 (p <0, 05) and 0,689 7 (p > 0,05) respectively, confirms that the phenolic compounds extracted from *G. lucida* are responsible of the 83 % of the measured antioxidant activity. Flavonoids do not show any statistic significant relationship. Same analysis was performed for ABTS radical, obtaining ar value of 0,873 4 (p < 0,05) and 0,858 3 (p < 0,05) for total phenols and flavonoids respectively.

According to these results, it is inferred that the antioxidant activity of the ethanol extracts is not only limited to phenolic or flavonoid compounds, but may also be related with other secondary antioxidant metabolites such as coumarins, alkaloids, terpenes and others, which in this case they contribute in a minor percentage to the activity.

Determination of the reducing power

The reducing capacity of test substances could be an indicator of its antioxidant potential. Figure 1 shows the samples that exhibited the most effective reducing power compared to the standard showing a dependent absorbance-concentration relationship.

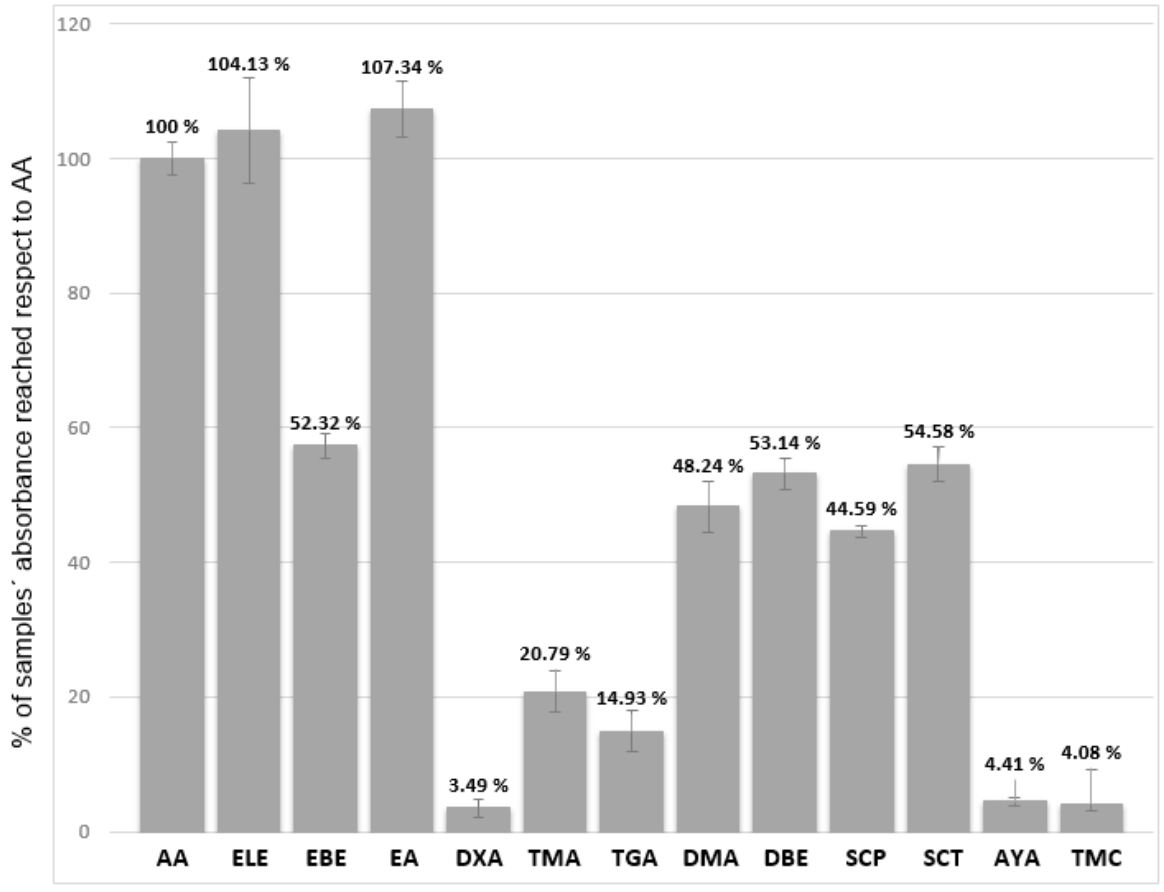

Fig. 1- Percentage of the samples´ reducing power respect the reference antioxidant ascorbic acid (100 %)

Legend: AA: ascorbic acid (positive control), ELE: total ethanol leaf extract, EBE: total ethanol bark extract, EA: ellagic acid, DXA: 3,3'-di-O-methylellagic-4'-O-β-D-xylopyranoside acid, TMA: 3,3´, 4´-tri-

O-methylellagic acid, TGA: 3,3´, 4´-tri-O-methylellagic-4-O-β-D-glucopyranoside acid, DMA: 3,3'-di-O-methylellagic acid, DBE: Dichloromethane Bark Extract (DBE), SCP: scoparone, SCT: scopoletin, AYA: ayapine, TMC: 6,7,8-trimethoxycoumarin.

These results behave statistically similar to those obtained by the DPPH method, so the analysis shows a high correlation between both tests (r = 0,983 5). The ABTS method shows a weak correlation (r = -0,676 3) and a lack of statistic significant relationship with reducing power assay (p > 0,05). According to Apak (2018), the complex nature of the nitro-radical ABTS can cause a lack of correlation with other tests that measure the antioxidant capacity, as in the case of the reducing power test, by slowing down the reaction with polyphenols. [8]

From the twelve evaluated samples, ellagic acid and total ethanol leaf extract exhibit the higher reducing power (statistically similar at a 95 % confidence level), even higher than ascorbic acid at the tested concentrations. The rest of the samples were less active than the reference antioxidant standard.

Other authors had reported similar observations for ellagic acid, like Kilic and collaborators in 2014 who revealed that the reducing power of ellagic acid exceeded the reference antioxidants such as α-tocopherol, ascorbic acid, BHT (butyl-hydroxytoluene) and BHA (butyl-hydroxyanisole).[14] The strategic position of hydroxyl and carbonyls groups around this molecule facilitates its activity in chelating metals.[23]

The number of free phenolic hydroxyl groups in the structure of the evaluated compounds seems to be directly related to a greater reducing power. This observation has also been raised by other researchers who stated that the reducing power of a sample is apparently related to an extension in the conjugation of phenols, as well as the amount of hydroxyls constituents.[8]

The correlation coefficients between the reducing power and total phenols and flavonoids content were 0,686 2 ($p>0,05$) and 0,869 1 ($p<0,05$) respectively, indicating flavonoid type substances as the main responsible for this activity. Several studies have shown that there is a direct relationship between the content of total flavonoids and the reducing power of many plant species.[8] However, a low correlation between the total phenols content and the reducing power

could be related to the fact that extracts can contain phenolic and non-phenolic compounds that can influence their antioxidant potential.[8]

Conclusions

The experimental results obtained revealed that *G. lucida´*s leaf and bark has significant radical scavenging activity and ferric reducing power. However, leaf ethanol extract exhibited higher activity that can be related to its higher polyphenolic content and the presence of ellagic acid and other metabolites with synergistic effect. Ellagic acid is suggested as the main responsible metabolite of the antioxidant activity of the leaf.

References bibliographical

1. ARUMUGAM, M. *et al.* "Antibacterial and antioxidant activity between micropropagated and field grown plants of *Excoecaria agallocha* L. *International Research" Journal of Pharmacy.* 2012, **3**, 235-240. ISSN 2230-8407

2. DÍAZ, J.; CELIA, C.; BISSE, J. "Estudio comparativo de *Gymnanthes lucida* Sw. en Cuba". *Revista del Jardín Botánico Nacional Universidad de La Habana*. 1983, **4** (2), 19-36. ISSN 0253-5696

3. ROIG, J.T. *Plantas Medicinales, Aromáticas o Venenosas de Cuba*. 1st ed. Ciencia y Técnica, Editorial. La Habana: Cuba; 1974. ISBN 978-959-05-0809-7

4. OCHOA, A.P. *et al*. "Evaluación fitoquímica de hojas de *Excoecaria lucida* Sw.(Aité) (Euphorbiaceae) y Aislamiento e identificacion de un hemiterpenoide". *Revista Cubana de Plantas Medicinales.* 2015, **20** (1), 117-130. ISSN 1028-4796

5. OCHOA, A.P. *et al*. "Bioassay-guided *in vitro* study of the antimicrobial and cytotoxic properties of the leaves from *Excoecaria lucida* Sw". *Pharmacognosy Research.* 2017, **9** (4), 396-400. ISSN 0974-8490

6. OCHOA, A. *Evaluación fitoquímica de hoja y corteza de Gymnanthes lucida Sw. (Yaití) (Euphorbiaceae)*. Doctoral thesis, Universidad de La Habana: Instituto De Farmacia Y Alimentos, La Habana, Cuba, 2017. Disponible en: http://eduniv.reduniv.edu.cu/index.php?page=13&id=26&db=1 Consultado: 15/03/2019

7. OCHOA, A. *et al*. Phytochemical Study of Nonpolar Extracts from *Excoecaria lucida* Sw. Leaves (Euphorbiaceae). *Acta Chromatográfica.* 2016, **28**(3), 429–437. ISSN 2083-5736

APAK R, CAPANOGLU E, SHAHIDI F. *Measurement of antioxidant activity and capacity: Recent trends and applications.* 1st ed. Canada: Editorial John Wiley & Sons, 2018. ISBN 9781119135357

8. APAGÓN, S.M. *Cumarinas en la prevención del estrés oxidativo dependiente de la edad.* Doctoral thesis, Universidad Complutense de Madrid, Madrid, España, 1994. Disponible en: http://eprints.ucmes/id/eprint/3845/ Consultado: 20/06/2019

9. TUKIRAN, T.; WARDANA, A.P.; HIDAYATI, N.; & SHIMIZU, K. "An Ellagic Acid Derivative and Its Antioxidant Activity of Chloroform Extract of Stem Bark of *Syzygium polycephalum* Miq. (Myrtaceae)". *Indonesia Journal of Chemistry.* 2018; **18** (1), 26-34. ISSN 1411-9420-2460-1578

10. FARNSWORTH, N.R. "Biological and Phytochemical Screening of Plants. *International" Journal of Pharmaceutical Sciences.* 1966; **55** (3), 262. ISSN 0975-1491.

12. KUMAZAWA, S.; HAMASAKA, T.; NAKAYAMA, T. "Antioxidant activity of propolis of various geographic origins". *Food Chemistry*. 2004, **84** (3), 329-339. ISSN 0308-8146

13. PATRA, J.K. *et al*. "Screening of antioxidant and antifilarial activity of leaf extracts of *Excoecaria agallocha* L". *International Journal of Integrative Biology.* 2009, **7** (1), 9-15. ISSN 0973-8363

14. KILIC, I.; YEŞILOĞLU, Y.; BAYRAK, Y. Spectroscopic studies on the antioxidant activity of ellagic acid. *Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy*. 2014, **130** (2014),447-52. ISSN 13861425

15. DEEPA, M.; DARSAN, M.B.; RAMALINGA, C. "*In Vitro* Evaluation of the antioxidant, anti-inflammatory and antiproliferative activities of the leaf extracts of *Excoecaria agallocha* L". *International Journal of Pharmaceutical Sciences.* 2015, **7** (11), 346-52. ISSN 0975-1491

16. HOSSAIN, S. J.; AOSHIMA, H.; EL-SAYED, M.; & AHMED, F. Antioxidative and anti-

histamine release activities of *E. agallocha* L. *Pharmacologyonline.* 2009, **2**, 927-936. ISSN 1827-8620

17. SOFÍA, S. & TERESA, M.M. "Investigation of bioactive compounds and antioxidant activity of *Excoecaria agallocha,* L". *International Journal of Pharmaceutical Sciences Research.* 2016, **7** (12), 5062-5066. ISSN 0975-8232

18. THIRUNAVUKKARASU, P. *et al*. "Screening of antioxidant status in selected mangrove plants in Pichvaram mangrove forest (south east coast of India). *International Journal of Bioassays.* 2013, **2** (3), 537-541. ISSN 2278-778X

19. MALIK, A. *et al*. *In vitro* antioxidant properties of Scopoletin. *Journal of Chemistry and Pharmacy Research.* 2011, **3** (3), 659-665. ISSN 0975-7384

20. DELLAGRECA, M. *et al*. Antioxidant and Radical Scavenging Properties of *Malva sylvestris. Natural Products Communications*. 2009, **4** (7), 893-896. ISSN 1555-9475

21. GIL, M.I. "Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing". *Journal of Agriculture Food Chemistry.* 2000, **48**(10), 4581-9. ISSN 1520-5118

22. KOSTOVA, I. *et al.* "Coumarins as Antioxidants". *Current Medicinal Chemistry.* 2011, **18** (25), 3929-3951. ISSN 1875-533X

23. HOELZ, L.V.B. *et al*. "Quantitative structure-activity relationships of antioxidant phenolic compounds". *Journal of Chemical and Pharmaceutical Research.* 2010, **2** (5), 291-306. ISSN 0975-7384

Conflict of interests

Authors declare that there are no conflict of interests.

Authors contributions

Diana Julia Arró Díaz, Ania Ochoa Pacheco and Yuri Mangueira Do Nascimento designed the experiments. The experiments were performed and the data analysed by Diana Julia Arró Díaz, Naylan Castelnaux Ochoa and Ania Ochoa Pacheco. Ania Ochoa Pacheco supervised the experiments.