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Research Article

The Antioxidant and Hypoglycemic Properties and Phytochemical Profile of *Clusia latipes* Extracts

Ronald Silva-Rivas[1], Natalia Bailon-Moscoso[2], Luis Cartuche[1], Juan Carlos Romero-Benavides[1,*]

ABSTRACT

Introduction: The prevalence of diabetes has increased more rapidly in low and middle-income countries than in high-income countries. Type 2 diabetes mellitus (DM2), which is the most common form of diabetes, is caused by the inefficient use of insulin in the body and is characterized by disrupted insulin action or secretion. Also, oxidative stress plays an important role in the development of disease. The goal of this study is to identify the antioxidant and hypoglycemic properties of *Clusia latipes*, an endemic species of Central and South America. **Methods:** The antioxidant and hypoglycemic capacity of the extracts (hexane, ethyl acetate, and methanol) of the leaves and stems of *Clusia latipes* were evaluated. From the most potent extract, the phytochemical study was carried out and fractionated. Antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), while hypoglycemic capacity was measured by alpha-glucosidase inhibition. **Results:** The extracts with the highest antioxidant capacity are the extracts with the highest α-glucosidase inhibition activity. Inhibitory activity increased in samples extracted with medium polar (ethyl acetate) and polar (methanol) solvents. Phytochemical screening of these extracts revealed the presence of alkaloids, carbohydrates, flavonoids/xanthones, quinones, saponins, and tannins. The highest α-glucosidase inhibitory activity was detected in the ethyl acetate fraction obtained from leaf methanol extract, with a half-maximal inhibitory concentration (IC_{50}) value of 0.90 µg/ml. The major constituent isolated from the same fraction was isoquercitrin.

Key words: α-glucosidase inhibitory activity, DPPH, Phytochemical screening, Type 2 diabetes mellitus.

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INTRODUCTION

Diabetes mellitus has become one of the primary threats to human health, with its rapidly increasing prevalence, and gravely debilitating clinical complications[1]. Over the past decade, diabetes prevalence has risen faster in low and middle-income countries than in high-income countries. The World Health Organization (WHO) has projected that DM will be the 7th leading cause of death in 2030. Diabetes is a chronic disease that occurs when the pancreas does not produce sufficient insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycemia or high blood sugar is a common effect of uncontrolled diabetes, which might lead to serious heart, blood vessel, eye, kidney, and nerve damage over time [2,3]. Type 2 diabetes mellitus (T2DM), which is the most common form of diabetes, is produced by the ineffective use of insulin in the body and is characterized by disorders of insulin action and secretion. Either one of these mechanisms could be the predominant feature, but generally, when diagnosed, both are manifested[1]. In T2DM, particularly, it is believed that oxidative stress caused by overproduction of reactive oxygen species (ROS) is the cause of vascular complication development[4]. Hyperglycemic condition of DM patients will produce tissue damage via the formation of ROS through five major mechanisms that have been shown to be activated by mitochondrial ROS overproduction: increased flux of glucose and other sugars through the polyol pathway; increased formation of advanced glycation end products; increased expression of the receptor for advanced glycation endproducts (RAGE) and its activating ligands; activation of protein kinase C isoforms; and over-activity of the hexosamine pathway[5-7].

Alpha-glucosidase is a critical enzyme that catalyzes the cleavage of absorbable monosaccharides starting with disaccharides and oligosaccharides[8]. In this manner, α-glucosidase inhibitors reduce postprandial hyperglycemia by slowing intestinal carbohydrate digestion[9]. Alpha-glucosidase inhibitors are capable of suppressing postprandial hyperglycemia; they are generally used to prevent or treat type II diabetes[10].

Plants play an important role in health care and are an important source of potentially bioactive substances[11]. The genus *Clusia* is widely distributed in the tropical and subtropical regions of Central and South America[12]. In the species of this genus, a great variety of biological activities have been found: broad-spectrum antimicrobial activity; chemopreventive cancer effects and antioxidant activity[13]; anti-inflammatory and anti-hepatotoxic activity and inhibitory action of the human immunodeficiency virus (HIV)[14–16]; and cytotoxic

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activity[17,18]. In the present study, both the antioxidant and anti-glycemic activity of leaf and stem extracts of *C. latipes* were evaluated.

MATERIALS AND METHODS

Plant material

The leaves and stems of *Clusia latipes* Planch. & Triana, *Clusiaceae* were collected in Gonzanama-Quilanga in the Loja province of Ecuador, and species identification was made by Fani Tinitana, PhD. A voucher specimen (PPN-CI 002) was deposited at the Herbarium of the Universidad Técnica Particular de Loja, Ecuador.

Extraction and partitions of Clusia latipes

For 7 days at 30°C, the plant material was dried in a tray dryer with airflow and then manually pulverized. Leaves and stems were processed separately. The pulverized leaves (1540 g) and stems (1608 g) were extracted by static maceration at ambient temperature with hexane (Hex), ethyl acetate (EtOAc), and methanol (MeOH) sequentially during three days with each solvent[18]. The procedure was repeated three times and concentrated on a rotary evaporator (Buchi R210; Switzerland, Flawil) at 50 mbar and 35°C, to yield a total of six extracts: leaf extracts, 71.00 g (Hex-L), 28.52 g (EtOAc-L), and 283.87 g (MeOH-L); and stem extracts, 39.44 g (Hex-S), 44.23 g (EtOAc-S), and 117.23 g (MeOH-S).

The most active extracts were partitioned. A portion of the dried methanolic leaf (20 g) and stem (20 g) extracts were then dissolved in methanol:water 9:1 v/v and sequentially partitioned three times with 400 mL of each solvent [(Hexano (Hex), dichloromethane (DCM), and ethyl acetate (EtOAc)] using a separatory funnel at room temperature (Figure 1). The solvents were removed using a rotary evaporator (Buchi R210; Switzerland, Flawil) at 35°C under vacuum. From the Hex leaves fraction (F-Hex-L), 0.59 g was obtained. The Hex stems fraction (F-Hex-S) yielded 0.19 g, the DCM leaves fraction (F-DCM-L) 5.21 g, the DCM stem fraction (F-DCM-S) 10.28 g, the EtOAc leaves fraction (F-EtOAc-L) 1.82 g, the EtOAc stems fraction (F-EtOAc-S) 2.02 g, the aqueous leaves fraction (F-Aq-L) 11.15 g, and the aqueous stems fraction (F-Aq-S) 6.37 g.

Phytochemical screening

Using the most active extracts and their partitions phytochemical tests were done. Phytochemical screening to test for the presence of secondary metabolites (alkaloids, flavonoids, quinones, saponins, tannins, and terpenoids-steroids), carbohydrates, and fats in the extracts and fractions was carried out using standard procedures. Phytochemical screening results from tests on extracts and fractions revealed the presence or absence of the main secondary metabolites and other phytochemicals based on the presence (+) or absence (-) of expected color changes. The tests performed were based on those reported in the literature [19,20].

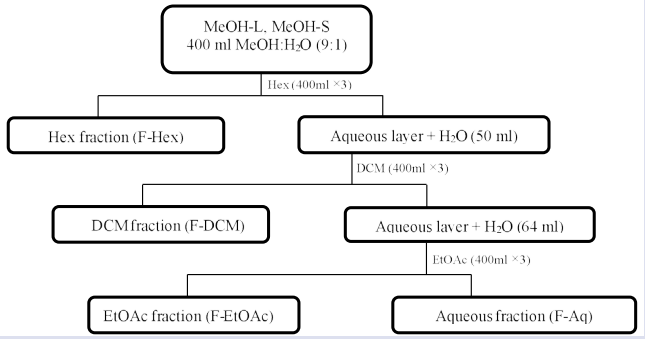

Figure 1: Fractionation scheme of the methanolic extracts from leaves and stems of *Clusia latipes*.

Isolation of secondary metabolites

The most active sample, the EtOAc leaves fraction (1.82 g), was separated by column chromatography with C18-reversed-phase silica gel (40–63 µm; Merck) with extract:silica (g/g) proportion of 1:20, eluting with a gradient solvent system of MeOH-H_2O in v/v ratios of 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0 to obtain 200 eluates; according to the chromatographic profile in Thin-layer chromatography (TLC) analysis, 20 fractions were obtained (F1–F20).

Fractions with a good chromatographic profile were separated by column chromatography with silica gel 60 (63-200 µm: Merck) in proportion de 1:100 (extract:silica, g/g), eluting with mixtures of three solvents EtOAc-MeOH-H_2O in v/v ratios of 90:6:4, 85:10:5, 81:11:8, 77:13:10, and 74:16:10. Finally, these are purified on a column of Sephadex LH-20 (Merck) with MeOH-H_2O 1:1 (v/v), obtaining a yellow amorphous solid, which was identified as isoquercitrin.

Characterization and identification of secondary metabolites

The melting point was determined using a Fisher Johns apparatus (Fisher Scientific Company, USA), and the temperature was not corrected. The ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Varian 400 MHz-Premium Schelded equipment (Agilent Technologies, USA). CD_3OD was used as the solvent, and chemical shifts were expressed in parts per million (ppm). Coupling constants (*J*) were reported in Hz.

Assays for antioxidant capacity

All extracts and fractions were evaluated for antioxidant capacity using stable free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS).

DPPH radical scavenging assay

DPPH free radical scavenging capacity was evaluated with a microplate analytical assay according to the literature[21,22], with slight modifications. A 30-µl aliquot of the different sample concentrations and standard were mixed with 270 µl of DPPH$^{\bullet}$ in methanol solution 100 µM (O.D. adjusted to 1.1 at 515 nm). After incubation at 20°C for 60 min, the absorbance of each solution was measured using a microplate reader (EPOCH 2 BioTek; USA, Vermont) at 515 nm. The percentage of scavenging activity was determined by following the equation described by Cheng *et al.* (2006):

$$[1 - (A_{sample} - A_{blank})/(A_{control} - A_{blank})]x100 \quad (1)$$

The SC_{50} value was obtained through interpolation from linear or logarithmic regression analyses according to the behavior of the data, and the sample concentrations required to scavenge 50% of the DPPH radical were determined. Trolox was used as the reference compound.

ABTS radical scavenging assay

For the ABTS assay, the procedure followed the method of Thaipong *et al.* (2006) with slight modifications. From the different concentrations, a 30-µl aliquot was taken, and a 270 µl of ABTS$^{\bullet+}$ solution (ABTS 7.4 mM and 2.6 mM persulfate, 1:1 ratio) was added to a 96-well microplate assay. This was incubated at 20°C for 60 min, and the absorbance of each solution was recorded at 715 nm in a microplate reader (EPOCH 2 BioTek; USA, Vermont). The percentage of ABTS$^{\bullet+}$ scavenging by the sample was calculated using the Eq. (1). Trolox was used as the reference compound.

α-glucosidase inhibitory activity

Ten milligrams of each sample was dissolved in 1 ml of methanol:H_2O (1:1 ratio). In cases of complete inhibition, dilutions of the sample solution were made in phosphate-buffered saline (PBS; SIGMA).

The α-glucosidase enzyme inhibitory effect was determined using a 96-well microtiter plate with *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG; SIGMA) as substrate according to the methods described by Tao *et al.* (2013), with slight modifications[23]. First, 5 µl of the sample was mixed with 75 µl of PBS (SIGMA) and 20 µl of enzyme solution (0.15 U/ml in PBS pH 7.4; SIGMA). This mixture was then pre-incubated at 37°C for 5 min. After preincubation, 20 µl of *p*NPG (5 mM in phosphate buffer, pH 7.4) was added and then incubated at 37 °C. Acarbose (5 mg/ml) was used as a positive control. The amount of *p*-nitrophenol (*p*-NP) released was measured at 405 nm for 60 min, recording the absorbance every 5 min on a spectrophotometer microplate reader (EPOCH 2 BioTek; USA, Vermont). The results were expressed as percentage inhibition using the formula previously described[24]:

$$Inhibition\ (\%) = [(Ao - As)/Ao]x100 \quad (2)$$

in which *A*o is the absorbance recorded for the enzymatic activity without inhibitor (control) and *A*s is the absorbance recorded for the enzymatic activity in the presence of the inhibitor (sample test). The IC_{50} was calculated using GraphPad Prism v 5.0 software.

RESULTS

Regarding antioxidant capacity, both in the DPPH free radical elimination activity test and ABTS free radical test, we found that the greater the polarity of the extracts, the greater the antioxidant capacity. Thus, leaf and stem methanol extracts had SC_{50} values of 6.44 µg/ml and 6.77 µg/ml, respectively. In the ABTS free radical test, the methanolic extracts present SC_{50} values of 5.43 µg/ml for leaves and 4.59 µg/ml for stems (Table 1).

On the other hand, the α-glucosidase inhibitory activity was observed *in vitro* in all samples. The positive acarbose control had an IC_{50} of 377 µM (243.39 µg/ml), which was in good agreement with the results reported by Feng *et al.* (2011)[25]. Similar to the α-glucosidase inhibitory activity, the samples that showed high activities were of methanolic extracts of leaves and stems with IC_{50} values of 5.01 µg/ml and 2.30 µg/ml, respectively (Table 1).

Given the activity of the extracts, the most effective extracts were fractionated according to the scheme presented in Figure 1. The results obtained from the phytochemical examination revealed the presence of alkaloids, carbohydrates, flavonoids/xanthones, quinones, saponins, and tannins (Table 2).

The antioxidant capacity and α-glucosidase inhibitory activity were evaluated from the fractions of methanol extracts obtained from leaves and stem (Table 3). The antioxidant activities of the DCM, EtOAc, and aqueous leaf and stem fractions increased significantly relative to the Hex fractions. The fractions with the highest biological activity are those obtained in EtOAc (F-EtOAc), both in leaves and stems. The SC_{50} of F-EtOAc from leaves was 4.70 µg/ml, and for DPPH and 3.29 µg/ml for ABTS, thus exhibiting higher antioxidant activity for ABTS. The F-EtOAc stems had a DPPH antioxidant capacity of SC_{50}: 3.58 µg/ml and ABTS of 2.27 µg/ml (Table 3).

Similar to the extracts, the fractions with the highest antioxidant capacity also have the highest α-glucosidase inhibitory activity. Thus, the F-EtOAc leaves and F-EtOAc stems exhibited potency, with IC_{50} values ranging from 0.90 to 3.88 µg/ml (Table 3).

Based on these results, the F-EtOAc leaf fraction was separated by column chromatography to obtain a flavonoid glycoside, isoquercitrin (7.3 mg); the structure is shown in Figure 2. The structural characterization of this compound was carried out by spectroscopic and spectrometric analyses and by comparison with published data[26,27].

Physical and spectroscopic characteristics of isoquercitrin: m.p: 239–242 °C. ESI-MS *m/z* 465 $[M + H]^+$ ($C_{21}H_{20}O_{12}$). ^{1}H-NMR (400 MHz; CD_3OD): δ 3.21 (1H, d, *J* = 3.2 Hz, H-5″), 3.31 (1H, s, H-4″), 3.42 (1H, m, H-3″), 3.48 (1H, d, *J* = 2.3 Hz, H-2″), 3.57 (1H, d, *J* = 5.5 Hz, H-6b), 3.70 (1H, d, *J* = 1.9 Hz, H-6a), 5.49 (1H, s, H-1″), 6.19 (1H, d, *J* = 2.0 Hz,

Table 1: DPPH and ABTS free radical-scavenging activity and α-glucosidase inhibitory activity of *Clusia latipes* extracts.

Morphological structure	Extracts	DPPH	ABTS	α-glucosidase inhibitory
		SC_{50} (µg/ml)	SC_{50} (µg/ml)	IC_{50} (µg /ml)
LEAVES	Hex	>100	64.80 ± 0.21	231.00 ± 0.75
	EtOAc	85.53 ± 0.88	42.45 ± 0.52	33.96 ± 1.15
	MeOH	6.44 ± 0.52	5.43 ± 0.30	5.01 ± 0.75
STEMS	Hex	>100	52.06 ± 0.44	177.40 ± 0.48
	EtOAc	46.93 ± 0.69	25.81 ± 0.45	5.80 ± 0.02
	MeOH	6.77 ± 0.59	4.59 ± 0.34	1. 30 ± 0.15

Hex = hexane; EtOAc = ethyl acetate; MeOH = methanol. All values were expressed as means ± standard error (n = 3).

Table 2: Phytochemical screening of methanolic extracts of *C. latipes* leaves and stems and their fractions.

Compounds	LEAVES					STEMS				
	MeOH	F-Hex	F-DCM	F-EtOAc	F-Aq	MeOH	F-Hex	F-DCM	F-EtOAc	F-Aq
Alkaloids	+	-	+	+	+	+	-	+	+	+
Carbohydrates	+	-	-	-	+	+	-	-	-	+
Fats	-	+	-	-	-	-	-	-	-	-
Flavonoids/ Xanthones	+	-	+	+	+	+	-	+	+	+
Quinones	+	-	+	+	+	+	-	+	+	+
Saponins	+	-	+	+	+	+	-	-	+	+
Tannins	+	-	-	-	+	+	-	-	+	+
Terpenoids- steroids	-	+	-	-	-	-	+	-	-	-

+ = presence, - = absence

Table 3: DPPH and ABTS free radical-scavenging activity and α-glucosidase inhibitory activity of fractions obtained from methanolic extracts of *C. latipes* leaves and stems.

Morphological structure	Fractions of methanol extracts	DPPH	ABTS	α-glucosidase inhibitory
		SC_{50} µg/ml	SC_{50} µg/ml	IC_{50} µg/ml
LEAVES	F-Hex	>100	63.68 ± 0.77	102.80 ± 0.43
	F-DCM	7.27 ± 0.70	5.74 ± 0.38	174.96 ± 1.02
	F-EtOAc	4.70 ± 0.80	3.29 ± 0.35	0.90 ± 0.12
	F-Aq	6.52 ± 0.53	5.03 ± 0.68	3.04 ± 0.27
STEMS	F-Hex	>100	70.16 ± 0.57	128.98 ± 0.71
	F-DCM	6.86 ± 0.48	3.97 ± 0.51	110.85 ± 0.10
	F-EtOAc	3.58 ± 0.50	2.27 ± 0.51	3.88 ± 0.81
	F-Aq	4.20 ± 0.43	2.75 ± 0.33	2.98 ± 0.13

All values were expressed as means ± standard error (n = 3).

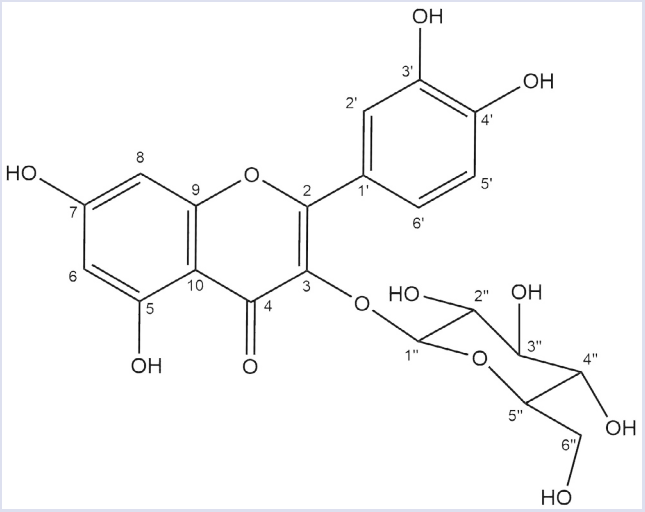

Figure 2: The chemical structure of isoquercitrin.

H-6), 6.38 (1H, d, *J* = 1.9 Hz, H-8), 6.88 (1H, d, *J* = 1.0 Hz, H-5′), 7.62 (1H, dd, *J* = 2.2 Hz, H-6′), 7.64 (1H, d, *J* = 1.1 Hz, H-2′). ^{13}C-NMR (100 MHz; CD_3OD): δ 61.0 (s, C-6″), 70.0 (s, C-4″), 75.6 (s, C-2″), 76.8 (s, C-3″), 76.9 (s, C-5″), 93.2 (s, C-8), 99.4 (s, C-6), 103.9 (s, C-1″), 104.3 (s, C-10), 114.6 (s, C-2′), 115.9 (s, C-5′), 121.8 (s, C-6′), 121.9 (s, C-1′), 133.9 (s, C-3), 144.6 (s, C-4′), 148.3 (s, C-3′), 156.8 (s, C-2), 157.0 (s, C-9), 161.7 (s, C-5), 164.5 (s, C-7), 178.1 (s, C-4).

DISCUSSION

EtOAc fractions from methanolic extracts of leaves and stems had the most active DPPH antioxidant activity and were shown to be more effective than the Trolox positive control. The ranges of antioxidant capacity obtained are within similar ranges to other species of the same family, both for DPPH and ABTS[28-31]. Likewise, F-EtOAc leaf fractions had α-glucosidase inhibitory activity, similar to other species in the *Clusiaceae* family[29,32-34]. Flavonoids and xanthones have been isolated from some species of the *Clusiaceae* family[34-38]. There is a strong relationship between phenolic compounds from natural sources and α-glucosidase inhibition[39-43]. Isoquercitrin is of interest to the food and pharmaceutical industries because of its biological properties as anti-inflammatory, hypotensive, anti-mutagenesis, anti-oxidative, anti-depressant, hypolipidemic, and anti-viral effects[27,44,45]. It has been previously reported that isoquercitrin has several activities that are related to the control and prevention of diabetes[46,47]. Previously, it has been experimentally established that isoquercitrin has an α-glucosidase inhibitory activity with an IC_{50} of 0.185 mM[48].

CONCLUSION

The highest α-glucosidase inhibitory activity was detected in the ethyl acetate fraction obtained from leaf methanol extract, with a half-maximal inhibitory concentration (IC_{50}) value of 0.90 µg/ml. In this study, we observed an association between α-glucosidase content and the antioxidant activities of the *C. latipes* fractions.

We propose that the α-glucosidase inhibitory and antioxidant activities of *C. latipes* could be due to the presence of isoquercitrin.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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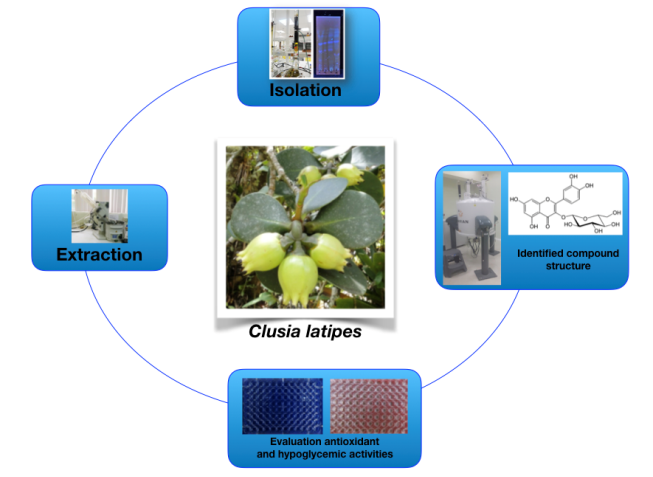
REFERENCES

1. Gregg EW, Bracco P. The Dynamics of Diabetes Prevalence, Morbidity, and Mortality. In: In The Diabetes Textbook. Cham: Springer; 2019:11-21.
2. World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications.; 1999.
3. World Health Organization. Global Report on Diabetes. 2016; 978:88.
4. Ullah A. Diabetes mellitus and oxidative stress –A concise review. Saudi Pharm J. 2016;24(5):547-53.
5. Brownlee M. The pathobiology of diabetic complications a unifying mechanism. 2005;54:1615-25.
6. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2014;107(9):1058-70.
7. Rahimi-Madiseh M, Malekpour-Tehrani A, Bahmani M, Rafieian-Kopaei M. The research and development on the antioxidants in prevention of diabetic complications. Asian Pac J Trop Med. 2016;9(9):825-31.
8. Bolen S, Feldman L, Vassy J, Wilson L, Yeh H. Review Annals of Internal Medicine Systematic Review : Comparative Effectiveness and Safety of Oral Medications for Type 2 Diabetes Mellitus. Ann Intern Med. 2007;147:386-99.
9. Luthra T, Agarwal R, Estari M, Adepally U, Sen S. A novel library of -arylketones as potential inhibitors of α -glucosidase : Their design, synthesis, *in vitro* and *in vivo*. Sci Rep. 2017;7:13246.
10. Saltos MBV, Puente BFN, Faraone I, Milella L, Tommasi N De, Braca A. Inhibitors of α-amylase and α-glucosidase from *Andromachia igniaria* Humb. & Bonpl. Phytochem Lett. 2015;14:45-50.
11. Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. Biochim Biophys Acta. 2013;1830(6):3670-95.
12. Moura A, Perazzo F, Maistro E. The mutagenic potential of *Clusia alata* (Clusiaceae) extract based on two short-term *in vivo* assays. Genet Mol Res. 2008;7(4):1360-8.
13. Ferreira RO, Junior ARDC, da Silva TMG, Castro RN, da Silva TMS, de Carvalho MG. Distribution of metabolites in galled and non-galled leaves of *Clusia lanceolata* and its antioxidant activity. Rev Bras Farmacogn. 2014;24(6):617-25.

14. Camara CAG Da, Shepherd SLK, Joaquim DRG. Análise química da cultura de tecidos do híbrido *Clusia paralicola* X *Clusia weddelliana*. Rev Bras Farmacogn. 2002;12(Tabela 1):1-3.
15. Huerta-Reyes M, Basualdo MDC, Lozada L, Jimenez-Estrada M, Soler C, Reyes-Chilpa R. HIV-1 inhibition by extracts of *Clusiaceae species* from Mexico. Biol Pharm Bull. 2004;27(6):916-20.
16. Mangas Marín R, Montes de Oca Porto R, Bello Alarcón A, Nival Vázquez A. Caracterización por cromatografía de gases/espectrometría de masas del extracto apolar de las hojas de *Clusia minor* L. Lat Am J Pharm. 2008;27(5):747-51.
17. Díaz-Carballo D, Gustmann S, Acikelli AH, *et al.* 7-epi-nemorosone from *Clusia rosea* induces apoptosis, androgen receptor down-regulation and dysregulation of PSA levels in LNCaP prostate carcinoma cells. Phytomedicine. 2012;19(14):1298-1306.
18. Bailon-Moscoso N, Romero-Benavides JC, Sordo M, *et al.* Phytochemical study and evaluation of cytotoxic and genotoxic properties of extracts from *Clusia latipes* leaves. Brazilian J Pharmacogn. 2016;26(1):44-9.
19. Mandal SC, Mandal V, Das AK. Qualitative Phytochemical Screening. Essentials Bot Extr. 2015:173-15.
20. Miranda M, Cuellar A. Farmacognosia y Productos Naturales. 2001;141.
21. Cheng Z, Moore J, Yu L. High-throughput relative DPPH radical scavenging capacity assay. J Agric Food Chem. 2006;54(20):7429-36.
22. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal. 2006;19(6-7):669-75.
23. Tao Y, Zhang Y, Cheng Y, Wang Y. Rapid screening and identification of α-glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. Biomed Chromatogr. 2013;27(2):148-55.
24. Matos MA, Tannuri U, Guarniero R. The effect of zoledronate during bone healing. J Orthop Traumatol. 2010;11(1):7-12.
25. Feng J, Yang XW, Wang RF. Bio-assay guided isolation and identification of α-glucosidase inhibitors from the leaves of *Aquilaria sinensis*. Phytochemistry. 2011;72(2):242-7.
26. Kazuma K, Noda N, Suzuki M. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. Phytochemistry. 2003;62(2):229-37.
27. Sukito A, Tachibana S. Isolation of hyperoside and isoquercitrin from *Camellia sasanqua* as antioxidant agents. Pakistan J Biol Sci. 2014;17(8):999-1006.
28. Kaennakam S, Mudsing K, Rassamee K, Siripong P, Tip-pyang S. Two new xanthones and cytotoxicity from the bark of *Garcinia schomburgkiana*. J Nat Med. 2018;73(1):257-61.
29. Sarma R, Kumari S, Elancheran R, Deori M, Devi R. Polyphenol rich extract of *Garcinia pedunculata* fruit attenuates the hyperlipidemia induced by high fat diet. Front Pharmacol. 2016;7(AUG):1-11.
30. Ruma K, Sunil K, Prakash HS. Antioxidant, anti-inflammatory, antimicrobial and cytotoxic properties of fungal endophytes from *Garcinia species*. Int J Pharm Pharm Sci. 2013;5(SUPPL 3):889-97.
31. Sylvie DD, Anatole PC, Cabral BP, Veronique PB. Comparison of in vitro antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa, Garcinia lucida* and *Hymenocardia lyrata*. Asian Pac J Trop Biomed. 2014;4(Suppl 2):S625-32.
32. Ryu HW, Cho JK, Curtis-Long MJ, *et al.* α-Glucosidase inhibition and antihyperglycemic activity of prenylated xanthones from *Garcinia mangostana*. Phytochemistry. 2011;72(17):2148-54.
33. Triadisti N, Rani Sauriasari BE. Fractionation and α-glucosidase Inhibitory Activity of Fractions from *Garcinia hombroniana* Pierre Leaves Extracts. Pharmacogn J. 2017;9(4):488-92.
34. Karunanayake S, Sotheeswaran S, Uvais M, Sultanbawa S, Balasubramaniam S. Xanthones and triterpenes of *Calophyllum tomentosum*. Phytochemistry. 1981;20(6):1303-4.
35. Jamila N, Khan N, Khan AA, *et al. In vivo* Carbon Tetrachloride-Induced Hepatoprotective and *in vitro* Cytotoxic Activities of *Garcinia hombroniana* (Seashore Mangosteen). African J Tradit Complement Altern Med. 2017;14(2):374-82.
36. Elya B, He HP, Kosela S, Hanafi M, Hao XJ. A new cytotoxic xanthone from *Garcinia rigida*. Fitoterapia. 2008;79(3):182-4.
37. Gontijo VS, De Souza TC, Rosa IA, *et al.* Isolation and evaluation of the antioxidant activity of phenolic constituents of the *Garcinia brasiliensis* epicarp. Food Chem. 2012;132(3):1230-5.
38. Martha S, Elya B, Hanafi M. Comparison of inhibitory activity against the α-glucosidase enzymes in the extracts and fractions from leaves of the *Garcinia kydia* Roxburgh. Asian J Pharm Clin Res. 2017;10(7).
39. Apostolidis E, Lee CM. *In vitro* potential of ascophyllum nodosum phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition. J Food Sci. 2010;75(3).
40. Lordan S, Smyth TJ, Soler-Vila A, Stanton C, Paul Ross R. The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. Food Chem. 2013;141(3):2170-6.
41. Ferreres F, Gil-Izquierdo A, Vinholes J, Silva ST, Valentão P, Andrade PB. *Bauhinia forficata* Link authenticity using flavonoids profile: Relation with their biological properties. Food Chem. 2012;134(2):894-904.
42. Yilmazer-Musa M, Griffith AM, Michels AJ, Schneider E, Frei B. Grape Seed and Tea Extracts and Catechin 3-Gallates Are Potent Inhibitors of α-Amylase and α-Glucosidase Activity. J Agric Food Chem. 2012;60(36):8924-9.
43. Inthongkaew P, Chatsumpun N, Supasuteekul C, *et al.* α-Glucosidase and pancreatic lipase inhibitory activities and glucose uptake stimulatory effect of phenolic compounds from *Dendrobium formosum*. Rev Bras Farmacogn. 2017;27(4):1-8.
44. Valentová K, Vrba J, Bancířová M, *et al.* Isoquercitrin : Pharmacology , toxicology , and metabolism. Food Chem Toxicol. 2014;68:267-82.
45. Bailon-Moscoso N, Cevallos-Solorzano G, Romero-Benavides JC, Orellana Ramirez MI. Natural Compounds as Modulators of Cell Cycle Arrest: Application for Anticancer Chemotherapies. Curr Genomics. 2017;18(2):106-31.
46. Baba K. Antioxidant constituents in the dayflower (*Commelina communis* L.) and their a -glucosidase-inhibitory activity. 2008:349-53.
47. Zhang L, Zhang S, Yin Y, Xing S, Li W, Fu X. Hypoglycemic effect and mechanism of isoquercitrin as an inhibitor of dipeptidyl peptidase-4 in type 2 diabetic mice. R Soc Chem. 2018;8:14967-74.
48. Li YQZ, Gao FC, Bian F, Sheng JB, Shan F. Comparative Evaluation of Quercetin, Isoquercetin and Rutin as Inhibitors of r-Glucosidase. J Agric Food Chem. 2009;57:11463-8.

GRAPHICAL ABSTRACT



SUMMARY

All the data shown revealed the antioxidant and hypoglycemic capacity of the extracts of the leaves and stems of *Clusia latipes*. The highest α-glucosidase inhibitory activity was detected in the ethyl acetate fraction; with a half-maximal inhibitory concentration (IC_{50}) value of 0.90 µg/ml. Isoquercitrin was isolated from the fraction more active.

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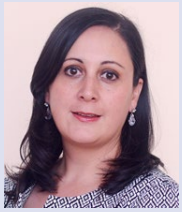

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