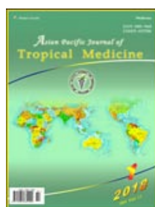

IF: 0.925

Asian Pacific Journal of Tropical Medicine

journal homepage: www.apjtm.org

doi: 10.4103/1995-7645.223531 ©2018 by the Asian Pacific Journal of Tropical Medicine. All rights reserved.

Evaluation of antiparasitic, anticancer, antimicrobial and hypoglycemic properties of organic extracts from Panamanian mangrove plants

Dioxelis Lopez[1,2], Lilia Cherigo[3], Alejandro de Sedas[1,2], Carmenza Spadafora[4], Sergio Martinez-Luis[1]✉

[1]Centro de Biodiversidad y Descubrimiento de Drogas, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), Edificio 219, Ciudad del Saber, Apartado 0843-01103, Panama, Republica de Panama

[2]Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur 522510, India

[3]Departamento de Química Orgânica, Facultad de Ciencias Exactas y Tecnología, Universidad de Panamá, P.O. Box 3366, Panama, Republica de Panama

[4]Centro de Biología Celular y Molecular de Enfermedades, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP) Edificio 219, Ciudad del Saber, Apartado 0843-01103, Panama, Republica de Panama

ARTICLE INFO

Article history:
Received 10 October 2017
Received in revised form 8 November 2017
Accepted 4 December 2017
Available online 2 January 2018

Keywords:
Mangrove plants
Anticancer activity
Antibacterial activity
Antiparasitic activity
Alpha-glucosidase inhibition

ABSTRACT

Objective: To investigate 33 organic extracts of mangrove plants for: antiparasitic, anticancer, and antibacterial activities, as well as their ability to inhibit the activity of the α-glucosidase enzyme. **Methods:** Leaves from all different plant mangrove species located in five mangrove zones of the Pacific coast of Panama were collected according to standard procedures. Qualitative phytochemical analysis of the organic extracts was performed by thin layer chromatography. The antiparasitic activity against *Plasmodium falciparum*, *Trypanosoma cruzi* and *Leishmania donovani*, toxicity against *Artemia salina*, anticancer activity in MCF-7 cell line, and antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* of all organic extract were investigated according protocols stablished in our institution. Finally, the ability to inhibit the enzymatic activity of α-glucosidase was evaluated by monitoring the hydrolysis of *p*-nitrophenyl α-*D*-glucopyranoside. **Results:** Thirty-three different samples belonging to nine different species of vascular plants with seeds of true mangroves were collected. Triterpenoids, phenolics, and tannins were the main groups of compounds found in the sampled mangroves. Saponins, quinones, and coumarins were found in less than 50% of the samples. *Laguncularia racemosa* showed moderate activity against *Plasmodium falciparum*. None of the extracts presented anticancer activity. *Rhizophora mangle* exhibited potent activity against *Staphylococcus aureus* and *Bacillus subtilis* [(90.41±7.33)% and (96.02±6.14)% of inhibition]; *Avicennia germinans* and *Conocarpus erectus* had activity against *Escherichia coli* [(71.17±6.15)% and (60.60±5.13)% of inhibition, respectively]. About 60% of the mangroves showed α-glucosidase inhibitory activity. In particular, extracts from *Laguncularia racemosa*, *Pelliciera rhizophorae*, *Conocarpus erectus*, *Mora oleifera*, and *Tabebuia palustris* species showed α-glucosidase inhibitory potential, with IC_{50} values of (29.45±0.29), (20.60±0.70), (730.06±3.74), (25.59±0.37), and (853.39±5.30) μg/mL, respectively. **Conclusions:** Panamanian mangroves are mainly a promising potential source of hypoglycemic compounds, specifically α-glucosidase inhibitors. These results highlight the therapeutic virtues of extracts from American mangrove plants.

1. Introduction

Mangroves consist of a community of halophytic plants characteristic of intertidal areas of the tropical and subtropical coastlines. Mangroves around the world can be divided into two major geographic regions: those from the east area (spanning from

First author: Dioxelis López, Centro de Biodiversidad y Descubrimiento de Drogas, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), Edificio 219, Ciudad del Saber, Apartado 0843-01103, Panamá, República de Panamá.
E-mail: dlopez@indicasat.org.pa
✉Corresponding author: Dr. Sergio Martínez-Luis, Centro de Biodiversidad y Descubrimiento de Drogas, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICSASAT AIP), Edificio 219, Ciudad del Saber, Apartado 0843-01103, Panamá, República de Panamá.
Tel: (507) 517-0700
Fax: (507) 507-0020
E-mail: smartinez@indicasat.org.pa
Fund project: This work was partially supported by the National Secretariat for Science and Technology of Panama (SENACYT) [grant number FID14-063]. Dioxelis Lopez was supported by funds from the National Secretariat of Science, Technology and Innovation (SENACYT) doctoral [grant number 270-2011-154]. Lilia Cherigo and Sergio Martinez-Luis were supported by funds from the National Research System of SENACYT [SNI1472016 and SNI1452016, respectively].

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-Share Alike 3.0 License, which allows others to remix, tweak and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2018 Asian Pacific Journal of Tropical Medicine Produced by Wolters Kluwer- Medknow

How to cite this article: Dioxelis Lopez, Lilia Cherigo, Alejandro de Sedas, Carmenza Spadafora, Sergio Martinez-Luis. Evaluation of antiparasitic, anticancer, antimicrobial and hypoglycemic properties of organic extracts from Panamanian mangrove plants. Asian Pac J Trop Med 2018; 11(1): 32-39.

west and central Pacific to the southern end of Africa) and those from the west region (covering the west coast of Africa and both coasts of the Americas)[1,2]. According to the World Atlas of Mangroves, the eastern area has a greater number of species (62) than the western area (12), with only two species in common between both zones[1]. For many years, mangroves have been utilized by various ethnic groups for the treatment of several diseases. Ethnopharmacological uses of different mangrove species have been very diverse and have included treatment for the following conditions and symptoms: eye problems, skin diseases, rheumatism, blisters, arthritis, hemorrhage, asthma, throat and stomach ache, infections, and diabetes[1,3]. Some species from Avicenniaceae, Meliaceae, Rhizophoraceae and Euphorbiaceae families have shown antimicrobial activity, while others belonging Meliaceae and Rhizophorae families possessed antimalarial properties. In turn, species from Acanthaceae and Myrsinaceae families demonstrated anticancer activity. Most existing studies that have previously evaluated the biological properties of mangrove plants have been carried out on eastern area species. In that part of the world, several scientific studies have supported the ethnomedical use of mangrove plants. On the other hand, there are very few reports, which have demonstrated the medicinal properties attributed to the mangrove plants from the western area species. This is a peculiar finding due to the extensive medicinal uses of this resource by various ethnic groups of several countries in the American continent[1,3-5].

The lack of scientific evidence on the efficacy of the ethnomedical properties and toxicity attributed to mangrove plants could be a major health problem. Therefore, there is an urgent need to carry out scientific studies that will validate or refute the ethnomedical uses of mangrove plant species on the American continent. Additionally, these plants could be of utmost importance to global bioprospecting programs dedicated to searching new (chemical) alternatives to combat diseases of high-impact on humanity, especially if potential activities are found in the studies mentioned above, their ancestral/past use increases their possible application.

We consider that Panama is an ideal country to evaluate the bioactivity from west plant mangroves because 92% of the continental species (eleven of the twelve species for the whole continent) are in this country. Therefore, information generated from studies conducted in Panama could serve to provide an overview of the biological activities of mangrove plants in America. In this context, our research group decided to carry out a systematic evaluation of extracts from leaves of vascular plants belonging to Panamanian mangroves. We conducted antiparasitic [*Plasmodium falciparum* (*P. falciparum*), *Trypanosoma cruzi* (*T. cruzi*), and *Leishmania donovani* (*L. donovani*)], anticancer (MCF-7 cell lines), hypoglycemic (α-glucosidase inhibition), and antibacterial bioassays with the purpose of generating scientific information that helps to promote the safe use of mangrove plants. As we have seen before, these studies are urgent and necessary, even more so if we take into account that mangrove forests are considered among the most vulnerable ecosystems on the planet, and they are more and more at risk of disappearing. In recent years, Panama has experienced an unprecedented and vast expansion in urbanization, as well as tourism infrastructure that have significantly affected mangrove areas in the country[6]. About 20% of the country's mangroves are in the Bay of Panama (central urban area), and the country has a lot of beautiful beaches (all with extensive mangrove areas). All of this suggests that the country’s most productive activities are located in the main area of mangrove coverage, and because of this situation, many hectares of Panamanian mangroves could disappear in a few years due to the great pressures of human activity on the environment[7,8].

2. Materials and methods

2.1. Plant material

The leaves of nine different plant mangrove species were collected in five mangrove areas from the Pacific coast of Panama. Zones 1 (Chame Bay) and two (Juan Diaz and Veracruz) were located in Panama Province, meanwhile zone 3 (Horconcito and Hermosa Beach) and 4 (la Barqueta beach and Pedregal Port) were visited in Chiriqui province. Finally, zone 5 (El salado beach) collected in Cocle province. This study focused on the Pacific region, due to 96.5% of the country's mangrove forests being located in this area.

Samples were stored in black plastic bags and transported to the laboratory. The samples were rinsed with water and placed to dry at room temperature. Once dried, samples were pulverized and stored for further processing. In addition, plants were taxonomically identified, and voucher specimens were deposited at the Herbarium of the University of Panama (PMA) in Panama City, Panama. The accession numbers of the species are as follows: *Avicennia bicolor (A. bicolor)* 105807, *Avicennia germinans (A. germinans)* 107546, *Conocarpus erectus (C. erectus)* 105836, *Laguncularia racemosa (L. racemosa)* 107547, *Mora oleifera (M. oleifera)* 110761, *Pelliciera rhizophorae (P. rhizophorae)* 105838, *Rhizophora mangle (R. mangle)* 105837, *Rhizophora racemosa (R. racemosa)* 107712, and *Tabebuia palustris (T. palustris)* 107539.

2.2. Preparation of extracts

The extract from each plant was prepared by maceration with a mixture of chloroform: methanol (1:1). Extracts were concentrated using a rotary evaporator (Laborota 4010, Germany) and stored at - 4 ℃ until further usage.

2.3. Phytochemical analysis

The crude extracts from mangrove leaves were subjected to qualitative tests to identify the families of secondary metabolites contained in each species through a visualized thin layer chromatography (TLC) profile employing several specific stain reagents. TLC plates with Silica gel 60 F254 (Sigma Aldrich, USA), as stationary phase, were used for this analysis. Extracts were drawn

with capillary tubes and applied as spots on TLC plates about 1 cm from the base. Then, a spot of the successive samples were placed with 1 cm away from the previous sample to avoid cross-contamination and interference as they move up during the elution process. TLC plates were placed into a chromatographic tank, which contained the appropriate mobile phase (Chloroform) at room temperature for the elution process. At the end of the elution process, plates were taken out from the chromatographic tank and dried. Spots in each extract were visualized under ultra-violet light at two wavelengths (UV 254 nm and UV 365 nm), and then plates were impregnated with chromogenic reagents.

Presence of the different types of compounds that each organic extract contained, were qualitatively determined using the following specific reagents for plate visualization[9]: Borntrager reagent for the presence of quinones and coumarins.; *p*–anisaldehyde for the presence of steroids; vanillin–sulfuric acid for the presence of saponins and triterpenoids compounds; Liebbermann Buchard reagent for the presence of triterpenoids; Ferric chloride for the presence of phenolics compounds; Vanillin–chloridic acid for the presence of tannins.

2.4. In vitro antiparasitic assays

Culture procedure: *L. donovani* parasites used in the assays were transformed from promastigote transformation into amastigote forms transferring 1 mL of promatigotes log phase culture into 5 mL of Medium 199 Modified (SIGMA-Cat M3769), and maintained at 35 ℃ until its use in bioassays. Meanwhile, *P. falciparum* parasite, chloroquine-resistant strain (Indochina W2), was kept in synchrony by thermal cycling incubation. Additionally, *P. falciparum* parasite were maintained in continuous log phase growth in RPMI-1640 medium (SIGMA) at 37 ℃ under an atmosphere of 5% CO_2 and 5% O_2, with a balance of N_2 (90%). *T. cruzi* parasite, (Tulahuen) C4 strain lactosidase (Lac Z) gene was maintained on VERO Cells (African Green Monkey cell line)[10]. *T. cruzi* parasite was grown in RPMI-1640 medium at 37 ℃ under an atmosphere of 5% CO_2/95% air mixture. Medium was supplemented with 10% heat inactivated FBS.

Assays: Dry samples were diluted in 50 µL of DMSO (dimethylsulfoxide) to obtain a concentration of 4 mg/mL and screening in sterile 96-well microtitre culture plates (Costar Cat 3595). Primary screenings was performed in duplicate wells, at a final concentration of 6.25 µg/mL.

L. donovani and *P. falciparum*: Samples and parasite were incubated for 48 h. The amount of parasite in culture was determined using a DNA cross linking agent. PicoGreen® solution (1%) was added to wells, in a dark condition, after 48 and 72 h of incubation. Plate were shaken and read in a microplate reader set up to 485/20 nm for excitation step and to 528/20 nm for emission. Positive controls were Amphotericin B (IC_{50} = 70–120 ng/µL) for *L. donovani* and chloroquine (IC_{50} = 80–100 nmol/L) for *P. falciparum*[11].

T. cruzi: Trypomastigotes (intracellular form) were exposed to test sample, in three different concentrations (50, 10 and 2 µg/mL) during 120 h in 96-wells plate. The resulting colour from the cleavage of chlorophenol red- β -*D*–galactoside (CPRG) by β -Gal expressed by the parasite was used to quantify the inhibition of parasite growth. Plates were read at 570 nm. Positive control was Nifurtimox (IC_{50} = 0.15–13.4 µmol/L)[11,12].

2.5. Brine shrimp lethality assay

In vitro lethality assay of *Artemia salina* (*A. salina)* was used for detecting toxicity from the organic extracts[13]. Brine shrimp eggs (2.5 g) were placed in artificial seawater solution (3.8% of instant ocean salt w/v in sterile water) and incubated at 28 ℃. Solution was saturated with oxygen bubbling air with a fish tank pump. Eggs were hatched within 48 h providing a large number of larvae (nauplii). Test samples and positive control were prepared by dissolving 0.6 mg in a solution containing 50 µL of DMSO and 550 µL of artificial seawater solution. Serial dilutions (1 000, 500, 250 and 125 mg/L) were made in separated wells of 96-well microplate. Nauplii were placed in each well by pipetting them until deposited 10–15 organisms. Each concentration was assessed by triplicate. Positive control was potassium dichromate, while negative control was the solvent for sample dissolution. Plate was incubated at 28 ℃. The percentage lethality was determined by comparing the mean surviving larvae of the test and control wells. Lethal concentrations values were obtained from the best-fit line plotted concentration versus percentage lethality.

2.6. Cancer bioassay

In a 96-well plate, 100 µL of RPMI buffer containing MCF-7 cells were added to each well and incubated for 24 h at 37 ℃. Samples were diluted in DMSO to final concentration of 6.25 µg/mL and analyzed by duplicate. After the incubation period, 100 µL of sample was added to each well. Colour control was RPMI without cells and positive control was Adriamycin. The 96-well-plate was incubated for 48 h at 37 ℃ under an atmosphere of 5% CO_2/95% air mixture. After the incubation period, cells were fixed with 50% trichloroacetic acid for one hour, air dried and stained with sulforhodamine B. Washing the 96-well-plate with 1% acetic acid removed excess of sulforhodamine B. Bound sulforhodamine B was dissolved with 10 mmol/L Tris. Plate was shaken and read in a microplate reader set up to 515 nm[14].

2.7. Antimicrobial bioassay

The antimicrobial assay against Gram-positive Bacteria *Staphylococcus aureus* (*S. aureus*) (ATCC® 43300™) and *Bacillus subtilis* (*B. subtilis*) (ATCC® 6051™); Gram-negative *Escherichia coli* (*E. coli*) (ATCC® 10536™) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC® 10145™) was performed according to Kumar *et al.*, with some modifications[15]. Sterile 96-well microplates (Greiner bio-one 655101) were used for each assay. Samples solutions were tested by duplicate at 10 µg/mL. The bacterial solution was prepared

with turbidity comparable to the standard of 0.5 McFarland. Wells were filled with 20 μL of the bacterial solution, 5 μL of the sample solution, 95 μL of sterile water and 80 μL of Muller Hinton culture medium and incubated at 37 ℃ for 24 h. Chloramphenicol instead of sample solution, DMSO instead of sample solution, sterile water instead of bacterial solution and non-bacterial solution instead of sample were used as positive, negative, color and sterility controls, respectively. After incubation period, bacteria growth inhibition was evaluated in a microplate reader at 750 nm. The activity of the sample was calculated as percentage of bacterial growth inhibition (% *GI*) according to the following equation:

%*GI* = [Negative correction – Sample correction]/Negative correction ×100.

Where, Negative correction: Abs of negative control – Abs of sterility control; Sample correction: Abs of the samples – Abs of color control.

2.8. α –glucosidase inhibitory assay

The inhibition of α-glucosidase, from Saccharomyces cerevisiae (Sigma Chemical Co), function was measured spectrophotometrically[16], with modifications. The absorbance (A) of 4-nitrophenol released from the hydrolysis of *p*–nitrophenyl α-*D*–glucopyranoside (PNP-G), 2 mmol/L, by enzyme solution (32 mU/mL) in potassium phosphate buffer (100 mmol/L), was measured at 400 nm by Synergy HT Bio Tek microplate spectrophotometer. Samples, dissolved in DMSO or MeOH, were incubated for 7 min with 150 μL of enzyme solution at 37 ℃ in 96-well microplates (Greiner bio-one 655101) by duplicate. After incubation period, 150 μL of substrate (PNP-G) was added and incubated for another period of 20 min at 37 ℃. Acarbose was use as positive control. The percentage of α-glucosidase inhibition function was calculated in comparison to a control (DMSO or MeOH instead of sample solution) according to the equation:

$\%\text{Inhibition} = (\Delta A_{control} - \Delta A_{sample})/\Delta A_{control} \times 100\%$.

Where, $\Delta A_{control}$: absorbance of the control – absorbance of the blank; ΔA_{sample}: absorbance of the sample – absorbance of the sample blank.

Specific concentration of α-glucosidase enzyme solution (32 mU/mL) were incubated with different concentrations of substrates (PNP-G) and inhibitors (active mangrove concentration equivalent to IC_{50}) with the same conditions of α-glucosidase inhibitory assay. The results were plotted according to standard procedure for Lineweaver–Burk or double reciprocal. All the determinations were performed in triplicate.

2.9. Statistical analysis

All collected data was reported as the mean±SD of three replicates. Analysis was carried out using Excel 2013. One-way analysis of variance (ANOVA) and Tukey posttest were used to evaluate the possible differences among the means. There were significant differences with *P* values ≤0.05.

3. Results

3.1. Diversity of mangrove plants

In total, 33 mangrove's samples from nine different species were collected from different places along the Pacific coast of the Republic of Panama, from 2011 to 2013 (Table 1).

Table 1

Species of mangrove plants collected and identified from the Pacific Coast of Panama.

Code	Plant species	Family	Coordinates
ML-M-001	*Conocarpus erectus*	Combretaceae	8°39'29.2"N 79°48'10.8"W
ML-M-002	*Laguncularia racemosa*	Combretaceae	8°39'29.2"N 79°48'10.8"W
ML-M-003	*Pelliciera rhizophorae*	Pellicieraceae	8°39'29.2"N 79°48'10.8"W
ML-M-004	*Rhizophora mangle*	Rhizophoraceae	8°39'29.2"N 79°48'10.8"W
ML-M-005	*Laguncularia racemosa*	Combretaceae	9°01'04.1"N 79°26'14.0"W
ML-M-006	*Avicennia germinans*	Avicenniaceae	9°01'04.1"N 79°26'14.0"W
ML-M-007	*Conocarpus erectus*	Combretaceae	8°55'34.5"N 79°33'56.0"W
ML-M-008	*Rhizophora mangle*	Rhizophoraceae	8°55'34.5"N 79°33'56.0"W
ML-M-009	*Laguncularia racemosa*	Combretaceae	8°19'17.8"N 82°09'31.7"W
ML-M-010	*Avicennia germinans*	Avicenniaceae	8°19'17.8"N 82°09'31.7"W
ML-M-011	*Conocarpus erectus*	Combretaceae	8°13'15.8"N 82°09'42.9"W
ML-M-012	*Rhizophora mangle*	Rhizophoraceae	8°13'15.8"N 82°09'42.9"W
ML-M-013	*Avicennia germinans*	Avicenniaceae	8°13'15.8"N 82°09'42.9"W
ML-M-014	*Pelliciera rhizophorae*	Pellicieraceae	8°17'37.0"N 82°30'32.9"W
ML-M-015	*Rhizophora mangle*	Rhizophoraceae	8°17'37.0"N 82°30'32.9"W
ML-M-016	*Conocarpus erectus*	Combretaceae	8°17'37.0"N 82°30'32.9"W
ML-M-017	*Avicennia bicolor*	Avicenniaceae	8°17'37.0"N 82°30'32.9"W
ML-M-018	*Avicennia bicolor*	Avicenniaceae	8°12'00.4"N 80°29'00.7"W
ML-M-019	*Conocarpus erectus*	Combretaceae	8°12'00.4"N 80°29'00.7"W
ML-M-020	*Rhizophora racemosa*	Rhizophoraceae	8°12'00.4"N 80°29'00.7"W
ML-M-021	*Rhizophora mangle*	Rhizophoraceae	8°12'00.4"N 80°29'00.7"W
ML-M-022	*Laguncularia racemosa*	Combretaceae	8°12'00.4"N 80°29'00.7"W
ML-M-023	*Avicennia germinans*	Avicenniaceae	8°12'00.4"N 80°29'00.7"W
ML-M-024	*Mora oleifera*	Caesalponiaceae	8°12'00.4"N 80°29'00.7"W
ML-M-025	*Tabebuia palustris*	Bignoniaceae	8°12'00.4"N 80°29'00.7"W
ML-M-026	*Pelliciera rhizophorae*	Pellicieraceae	8°12'00.4"N 80°29'00.7"W
ML-M-027	*Rhizophora mangle*	Rhizophoraceae	8°12'00.4"N 80°29'00.7"W
ML-M-028	*Mora oleifera*	Caesalponiaceae	8°21'34.4"N 82°25'59.8"W
ML-M-029	*Pelliciera rhizophorae*	Pellicieraceae	8°21'34.4"N 82°25'59.8"W
ML-M-030	*Avicennia germinans*	Avicenniaceae	8°21'34.4"N 82°25'59.8"W
ML-M-031	*Pelliciera rhizophorae*	Pellicieraceae	8°21'34.4"N 82°25'59.8"W
ML-M-032	*Laguncularia racemosa*	Combretaceae	8°21'34.4"N 82°25'59.8"W
ML-M-033	*Tabebuia palustris*	Bignoniaceae	8°21'34.4"N 82°25'59.8"W

3.2. Phytochemical analysis

Preliminary phytochemical TLC screening of the cloroformo: metanol (1:1) extracts of different species from the collected mangroves revealed the presence of various groups of compounds (triterpenoids, phenolics, tannins, saponins, quinones, and coumarins). This data was obtained according to the colours observed on the spot extracts when each specific reagent was added. Results are summarized in Table 2.

3.3. Antiparasitic activity of the tested plants

In the antiparasitic assays, samples were tested at 6.25 μg/mL. Only

Table 2

Phytochemical analysis of organic extracts of mangrove samples from the Pacific Coast of Panama.

Compound	Test applied	*Pelliciera rhizophorae*	*Avicennia germinans*	*Rhizophora mangle*	*Avicennia bicolor*	*Conocarpus erectus*	*Rhizophora racemosa*	*Laguncularia racemosa*	*Mora oleifera*	*Tabebuia palustris*
Triterpenoids	Ammonium Molibdate	++	+++	+++	+++	++	++	+	++	+
Triterpenoids	Lieberman–Burchard	+++	+++	+++	++	++	+++	+++	++	+
Phenols	Ferric chloride	+++	+++	+++	-	-	++	++	+	-
Tannins	Vanillin–chloridic acid	+	+	++	+	+	++	+	-	-
Saponins	Vanillin–sulfuric acid	-	-	+	-	-	+	+	+	-
Steroids	*p*–Anisaldehyde	-	-	-	-	+	-	-	+	+
Quinones and coumarins	Borntrager	-	-	+++	-	-	-	-	-	-

- = negative; + = slightly positive; ++ = moderate positive; +++ = highly positive.

one sample belonging to *L. racemosa* species showed antiparasitic activity, in particular against *P. falciparum* [(60.1±3.1)% inhibition], the rest of the samples were inactive or showed weak activity which was equal to or less than 30% of inhibition.

3.4. Anticancer activity of the tested plants

None of the extracts (tested at 6.25 µg/mL) showed considerable anticancer activity, so all extracts were discarded for further anticancer evaluations.

3.5. Toxicity of the tested plants against A. salina

Only three sample (*P. rhizophorae*, *C. erectus*, and *A. bicolor*) showed concentration that killed the 50% of nauplii (IC_{50}) below of 1 000 mg/L [IC_{50} = (883.0±14.3), (963.0±13.1) and (368.0±9.0) mg/L, respectively). In fact, the toxicity that resulted for *A. bicolor* sample was the only one that could draw our attention.

3.6. Antibacterial activity of the tested plants

The antibacterial activities of the 33 extracts were assayed *in vitro* by the agar microdilution method against four bacteria (*S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*) at 6.25 µg/mL. Among the mangroves plants, only *R. mangle* exhibited strong activity against *S. aureus* and *B. subtilis* (four samples with inhibition between 90%–96%), while an *A. germinans* extract had activity against *E. coli* [(71.1±1.9)% of inhibition] and a *C. erectus* extract showed moderate activity against *E. coli* [(60.1±1.0)% of inhibition]. Finally, none of the extracts showed activity against *P. aeruginosa*.

3.7. α –glucosidase inhibition evaluation of the tested plants

Extracts from five species from a total of nine-collected showed activity showed inhibition of α-glucosidase enzyme, with effects ranging from moderate to relatively high. In particular, extracts from *L. racemosa*, *P. rhizophorae*, *C. erectus*, *M. oleifera*, and *T. palustris* species showed α-glucosidase enzyme inhibitory potential, with IC_{50} values of (29.45±0.29), (20.60±0.70), (730.06±3.74), (25.59±0.37), and (853.39±5.30) µg/mL, respectively. Among these five-plant mangrove extracts, *L. racemosa*, *P. rhizophorae* and *M. oleifera* possessed better hypoglycaemic activity than the positive control [Acarbose, IC_{50} value of (140.55±4.30) µg/mL].

Lineweaver–Burk kinetic analyses were performed to characterize the enzymatic inhibition mode of active plant extracts against α-glucosidase (Figure 1). Michaelis–Menten constant (K_m) and Maximum reaction velocity (V_{max}) values of the mangrove plant extracts against α-glucosidase were calculated (Table 3).

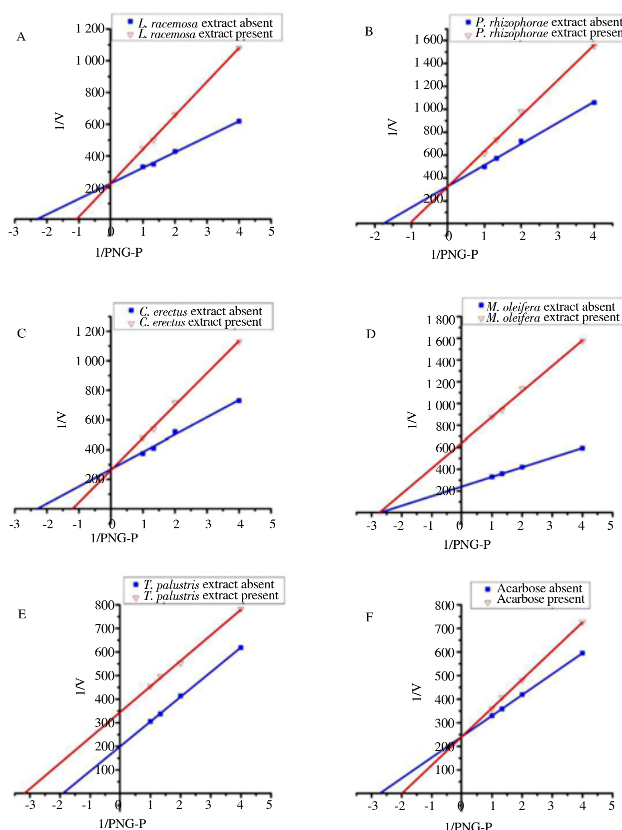

Figure 1. Lineweaver–Burk plot of α-glucosidase inhibition in the presence of active plant mangrove extracts.

Reaction velocity (V) in unit of mmol/(L min). PNP-G (*p*–nitrophenyl α-*D*–glucopyranoside) in unit of mmol/L.

Table 3

Kinetic parameters of α-glucosidase inhibition by mangrove plants extracts

Samples	K_m (mmol/L)	V_{max} [mmol/(L•min)]
α-glucosidase	0.365±0.001	0.004 1±0.000 1
L. racemosa	0.927±0.003	0.004 1±0.000 1
P. rhizophorae	0.943±0.002	0.004 1±0.000 2
C. erectus	0.833±0.001	0.004 1±0.000 1
M. oleifera	0.365±0.001	0.001 5±0.000 1
T. palustris	0.313±0.002	0.002 8±0.000 2

K_m = Michaelis–Menten constant; V_{max} = Maximum reaction velocity.

4. Discussion

4.1. Diversity of mangrove plants

In Panama, mangrove forests are relatively simple containing very few species, although this country is one of the most biodiverse in the western mangrove zone. The Word Atlas of Mangroves reports that Panama has 10 species of vascular plants with seeds belonging to true mangrove plants category[1]. In our collection trips, we collected 33 different samples belonging to nine different species of vascular plants with seeds of true mangroves. *Rhizophora harrisonii* was not located in any of the points we visited; this was the only species reported for the country that we were unable to find. On the other hand, *R. mangle*, *L. racemosa*, *P. rhizophorae* and *C. erectus* were the most abundant species.

4.2. Phytochemical analysis

The dominant groups of compounds detected in the preliminary phytochemical analysis were triterpenoids and phenolics, and they have also been identified in several mangrove species belonging to the eastern mangrove zone. This helps us to conclude that the geographic location does not seem to affect the secondary metabolism of mangrove plants[4,17-19]. Based on our results, tannins represent the second most abundant group of compounds among all the samples of mangroves collected in Panama. Tannins gave positive reactions in 77% of the samples. Several authors have reported the presence of tannins in the mangroves of the eastern mangrove zone[20,21]. Other types of compounds that were present in the mangrove samples of the Pacific Coast of Panama were saponins, steroids, quinones and coumarins. The latter group of compounds was present in less than 50% of the samples. The presence of these compounds has also been described for mangroves in the eastern mangrove zone[22-24]. Quinones and coumarins were only present in the samples of *R. mangle*, which can be considered as a group of selective compounds for this type of mangroves in the region.

4.3. Anticancer and antiparasitic activities of the tested plants

The increasing of resistance from bacteria, cancer cells and parasites is common nowadays. For this reason, the human being is in a constant search for new treatments to combat all these malignant agents. Plants have been a traditional source of compounds with therapeutic potential, so it would not be a surprise that in scarcely studied plants we could found potential drugs. In the case of the plants evaluated in the present study, they lack antiparasitic and anticancer activities, which does not surprise us due to there are not many reports about similar sources with these activities. Only one sample belonging to *L. racemosa* species showed antiparasitic activity against *P. falciparum*[(60.1±3.1)% inhibition], the rest of the samples were inactive or showed weak activity which was equal to or less than 30% of inhibition.

4.4. Toxicity of the tested plants against A. salina

A. salina lethality test is perhaps the simplest cytotoxicity bioassay, and it is widely used in the evaluation of toxicity of natural plant extracts. Of all the samples tested, only one sample belonging to *A. bicolor* species shows an IC_{50} denoting potential toxicity in its plant extract, the remaining samples are lacking *in vitro* toxicity. This data suggests that Panamanian mangrove plants could have a high innocuity.

4.5. Antibacterial activity of the tested plants

With the results of the antibacterial evaluation obtained in the present study, we can highlight two important observations. First, mangroves samples collected possess activity against Gram-positive and Gram-negative bacteria. In the case of the *A. germinans* mangrove, there are studies of samples gathered in India and Iran which have activity against Gram-positive bacteria (*S. aureus* and *Bacillus* sp.) and Gram-negative bacteria (*E. coli*), respectively[25,26]. On the other hand, *C. erectus* mangrove has various reports in both the American and Asian regions. In America, studies with samples collected in Florida, demonstrate the potential of the *C. erectus* to inhibit quorum sensing in *P. aeruginosa*[27]. While samples gathered in Iraq showed activities against *Streptococcus pneumonia* and *E. coli*[28]. Second, *R. mangle* mangrove, collected in Panama, possesses specific activity against Gram-positive. The antibacterial activity of some *R. mangle* has also been reported in Brazil, where it was active against Gram-positive bacteria (*S. aureus* and *Micrococcus luteus*)[29].

4.6. α-glucosidase inhibition evaluation of the tested plants

Postprandial high blood glucose leads to the appearance of vascular complications associated with diabetes[30]. α-Glucosidase, a key enzyme related to diabetes mellitus, is an intestinal enzyme that breaks down the carbohydrates to monosaccharide. Therefore, its inhibition delays the absorption of ingested carbohydrates and thus decreasing postprandial blood glucose and insulin peaks. In the traditional medicine of different countries, there are medicinal plants preparations with reputed hypoglycaemic effect[31-33]. In the case of mangrove plants in the west zone, there are a lot of reports on its ethnomedical use for the treatment of diabetes mellitus[23,34]. In fact, *Rhizophora mucronata* and *Sonneratia apetala* have been shown to exert part of their effect by inhibiting the α-glucoside enzyme, with IC_{50} values of 9.45 and 286 μg/mL, respectively[35,36].

In our case, samples of five different mangrove species, from a total of nine, showed activity. This represents about of 55% of active species, which compared with other ecosystems is a relatively high percentage of bioactive samples. On the other hand, this also suggests that this ecosystem could be a prolific producer of α-glucosidase inhibitory compounds. Reviewing in the literature,

we found that the IC_{50} value of three evaluated species (*L. racemosa*, *P. rhizophorae* and *M. oleifera*) is very similar to those of two species of plants from the other mangrove zone, which have a wide traditional use as antidiabetics.

On the other hand, these results also support the use of species *L. racemosa*, *P. rhizophorae* and *M. oleifera* in traditional medicine as hypoglycaemic agents. This latter statement is supported by comparing the activity found in these mangrove plants with *Rhizophora mucronata* and *Sonneratia apetala* activities, and with the activity of other widely used plants in traditional medicine such as *Erythroxylum laurifolium*, *Elaeodendron orientale*, *Antidesma madagascariensis*, *Stillingia lineata* (IC_{50} values of these three plants were between 87.41 and 96.87 μg/mL)[37], *Euclea undulata* (IC_{50} value of 49.95 μg/mL)[38], and *Symplocos cochinchinensis* (IC_{50} value of 82.07 μg/mL)[39]. In the case of *C. erectus* and *T. palustris*, their IC_{50} values were not good enough to support their efficient use in humans as hypoglycemic agents, although their moderate activity could be due to the very low amount of active compounds they contain. However, both plants could still be potential sources of hypoglycemic molecules.

Finally, by correlating the results of the hypoglycemic activity test with those of the phytochemical study, we observed that all the samples that possess α-glucosidase inhibitory properties were positive for the presence of triterpenoids and phenols. These results are in agreement with previous reports on the α-glucosidase inhibitory properties of both types of chemical compounds and their presence in several plants with antidiabetic ethnomedical uses[34,40]. Therefore, it is very likely that these types of compounds are responsible for the enzymatic inhibition in the extracts evaluated.

To determine the enzymatic inhibition type we proceeded to calculate the K_m and V_{max} values using different concentrations of inhibitor and substrate, to generate Lineweaver–Burk graphic of each specie. As compared with uninhibited reaction, *L. racemosa*, *P. rhizophorae* and *C. erectus* presented an increase in K_m; meanwhile, the V_{max} remained the same, this is the characteristic feature of a competitive inhibitor. On the other hand, *M. oleifera* produced a decrease in V_{max} value, while the K_m was not affected; this feature describes a non-competitive inhibition. Finally, *T. palustris* Lineweaver–Burk plot showed two parallels lines that mean the inhibitor reduced both V_{max} and K_m values; this is a typical for an uncompetitive inhibitor. Also, these samples showed the presence of triterpenoids and phenols. There are several publications that describe competitive[41,42] and non-competitive[41,43-45] inhibition produced by this kind of chemical compounds.

In conclusion, at least three of the nine species (*L. racemosa*, *P. rhizophorae*, and *M. oleifera*) of Panamanian mangrove plants showed an excellent *in vitro* inhibition of the enzyme α-glucosidase, their activity was greater than that of acarbose, a hypoglycemic drug used as positive control. Extracts from *L. racemosa* and *P. rhizophorae* exhibited a competitive type of inhibition, and *M. oleifera* extracts displayed a non-competitive mode of inhibition against α-glucosidase. According to the phytochemical analysis of plant mangroves extracts and the available literature, triterpenoids and phenols seem to be the compounds responsible for the enzymatic inhibition in the extracts evaluated. Except for certain species, which showed remarkable activity, mangroves plant extracts from Panama, demonstrated a poor antiparasitic, cytotoxic and antibacterial potential. In general terms, *in vitro* inhibitory properties against α-glucosidase enzyme were far superior to the antiparasitic, antibacterial and cytotoxic properties of these mangrove plants. Based on the results reported herein, it can be concluded that Panamanian mangroves are a promising potential source of hypoglycaemic compounds, specifically α-glucosidase inhibitors. Due to the evident hypoglycemic potential, Panamanian mangroves studies will be underway to investigate the bioactive metabolites and the detailed mechanisms of action of these bioactive metabolites in extracts.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

We would like to thank the government of Panama (Ministerio de Ambiente) for granting permission to make the collections of mangrove samples. This work was partially supported by the National Secretariat for Science and Technology of Panama (SENACYT) [grant number FID14-063]. Dioxelis Lopez was supported by funds from the National Secretariat of Science, Technology and Innovation (SENACYT) doctoral [grant number 270-2011-154]. Lilia Cherigo and Sergio Martinez-Luis were supported by funds from the National Research System of SENACYT [SNI1472016 and SNI1452016, respectively]. Finally, to Alberto E. Morales (Department of Anthropology at the University of California, Irvine) for the spelling and grammar revision.

References

[1] Spalding M, Kainuma M, Collins L. *World atlas of mangroves*. 1st ed. London: Earthscan; 2010.

[2] Xu D-B, Ye W-W, Han Y, Deng Z-X, Hong K. Natural products from mangrove actinomycetes. *Mar Drugs* 2014; **12**(5): 2590-2613.

[3] Wu J, Xiao Q, Xu J, Li M-Y, Pan J-Y, Yang M. Natural products from true mangrove flora: Source, chemistry and bioactivities. *Nat Prod Rep* 2008; **25**(5): 955-981.

[4] Simlai A, Roy A. Biological activities and chemical constituents of some mangrove species from Sundarban estuary: An overview. *Pharmacogn Rev* 2013; **7**(14): 170-178.

[5] Patra JK, Gouda S, Dhal NK, Thatoi H. Comparative evaluation of antibacterial properties and phytochemical profile of selected mangrove plants from Bhitarkanika, India. *J Biol Act Prod from Nat* 2014; **4**(2): 136-148.

[6] Castellanos-Galindo GA, Kluger LC, Tompkins P. Panama's impotent mangrove laws. *Science* 2017; **355**(6328): 918-919.

[7] Polidoro BA, Carpenter KE, Collins L, Duke NC, Ellison AM, Ellison JC, et al. The loss of species: Mangrove extinction risk and geographic areas of global concern. *PLoS One* 2010; **5**(4): e10095.

[8] Atkinson SC, Jupiter SD, Adams VM, Ingram JC, Narayan S, Klein CJ, et al. Prioritising mangrove ecosystem services results in spatially variable management priorities. *PLoS One* 2016; **11**(3): e0151992.

[9] Wagner H, Bladt S. *Plant drug analysis – A thin layer chromatography atlas*. 2nd ed. Heidelberg: Springer Berlin Heidelberg; 1996.

[10]Campos FF, Sales Junior PA, Romanha AJ, Araujo MS, Siqueira EP, Resende JM, et al. Bioactive endophytic fungi isolated from *Caesalpinia echinata* Lam. (Brazilwood) and identification of beauvericin as a trypanocidal metabolite from *Fusarium* sp. *Mem Inst Oswaldo Cruz* 2015; **110**(1): 65-74.

[11]Almaliti J, Malloy KL, Glukhov E, Spadafora C, Gutierrez M, Gerwick WH. Dudawalamides A–D, antiparasitic cyclic depsipeptides from the marine cyanobacterium Moorea producens. *J Nat Prod* 2017; **80**(6): 1827-1836.

[12]Lopez D, Cherigo L, Spadafora C, Loza-Mejia MA, Martinez-Luis S. Phytochemical composition, antiparasitic and α-glucosidase inhibition activities from *Pelliciera rhizophorae*. *Chem Cent J* 2015; **9**(1): 53.

[13]Trifman YJ, Aknin M, Gauvin-Bialecki A, Benayahu Y, Carmeli S, Kashman Y. Bisdioxycalamenene: A bis-sesquiterpene from the soft coral *Rhytisma fulvum* fulvum. *Mar Drugs* 2016; **14**(2): 41.

[14]Youssef MM, Arafa RK, Ismail MA. Synthesis, antimicrobial, and antiproliferative activities of substituted phenylfuranylnicotinamidines. *Drug Des Devel Ther* 2016; **10**: 1133-1146.

[15]Kumar S, Verma N, Kumar N, Patel A, Roy P, Pruthi V, et al. Design, synthesis, molecular docking, and biological studies of novel phytoestrogen-tanaproget hybrids. *Synth Commun* 2016; **46**(5): 460-474.

[16]Girones-Vilaplana A, Moreno DA, Garcia-Viguera C. Phytochemistry and biological activity of Spanish *Citrus* fruits. *Food Funct* 2014; **5**(4): 764-772.

[17]Zou J-H, Dai J, Chen X, Yuan J-Q. Pentacyclic triterpenoids from leaves of *Excoecaria agallocha*. *Chem Pharm Bull* 2006; **54**(6): 920-921.

[18]Suh S-S, Hwang J, Park M, Park H-S, Lee T-K. Phenol content, antioxidant and tyrosinase inhibitory activity of mangrove plants in Micronesia. *Asian Pac J Trop Med* 2014; **7**(7): 531-535.

[19]Nurjanah N, Jacoeb AM, Hidayat T, Hazar S, Nugraha R. Antioxidant activity, total phenol content, and bioactive components of lindur leave (*Bruguierra gymnorrhiza*). *Am J Food Sci Heal* 2016; **2**(4): 65-70.

[20]Zhang L-L, Lin Y-M, Zhou H-C, Wei S-D, Chen J-H. Condensed tannins from mangrove species *Kandelia candel* and *Rhizophora mangle* and their antioxidant activity. *Molecules* 2010; **15**(1): 420-431.

[21]Edu EAB, Edwin-Wosu NL, Udensi OU. Evaluation of bioactive compounds in mangroves: A panacea towards exploiting and optimizing mangrove resources. *J Nat Sci Res* 2015; **5**(23): 1-9.

[22]Poompozhil S, Kumarasamy D. Studies on phytochemical constituents of some selected mangroves. *J Acad Ind Res* 2014; **2**(10): 590-592.

[23]Revathi P, Jeyaseelan Senthinath T, Thirumalaikolundusubramanian P, Prabhu N. An overview of antidiabetic profile of mangrove plants. *Int J Pharm Pharm Sci* 2014; **6**(3): 1-5.

[24]Sur TK, Hazra AK, Bhattacharyya D, Hazra A. Antiradical and antidiabetic properties of standardized extract of Sunderban mangrove *Rhizophora mucronata*. *Pharmacogn Mag* 2015; **11**(42): 389-394.

[25]Rajeswari, Prakash B, Gowrisankar J, Gajendiran, Ezhilarasu A. Study of preliminary phytochemical analysis and antibacterial activity of *Avicennia germinans*. *Int J Adv Interdiscip Res* 2013; **1**(1): 37-40.

[26]Alizadeh-Behbahani B, Tabatabaei-Yazdi F. *In vitro* study of antibacterial activity of mangle negro extracts against selected pathogens from Enterobacteriaceae and Bacillaceae familia. *Zahedan J Res Med Sci* 2015; **17**(X): 29-32.

[27]Adonizio A, Kong K-F, Mathee K. Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. *Antimicrob Agents Chemother* 2008; **52**(1): 198-203.

[28]Hussein RA. Evaluation antioxidant and antibacterial activities of *n*-butanol fraction of *Conocarpus erectus* L. leaves extract. *Int J Pharm Med Res* 2016; **4**(6): 394-400.

[29]Ferreira FS, Santos SC, Barros TF, Rossi-Alva JC, Fernandez LG. Atividade antibacteriana *in vitro* de extratos de *Rhizophora mangle* L extracts. *Rev Bras Plantas Med* 2011; **13**(3): 305-310.

[30]Brewer RA, Gibbs VK, Smith DL. Targeting glucose metabolism for healthy aging. *Nutr Heal Aging* 2016; **4**(1): 31-46.

[31]Prabhakar PK, Doble M. A target based therapeutic approach towards diabetes mellitus using medicinal plants. *Curr Diabetes Rev* 2008; **4**(4): 291-308.

[32]Afifi FU, Kasabri V. Pharmacological and phytochemical appraisal of selected medicinal plants from Jordan with claimed antidiabetic activities. *Sci Pharm* 2013; **81**(4): 889-932.

[33]Kumar Bhateja P, Singh R. Antidiabetic activity of *Acacia tortilis* (Forsk.) Hayne ssp. raddiana polysaccharide on streptozotocin-nicotinamide induced diabetic rats. *Biomed Res Int* 2014; **2014**: 572013.

[34]Das SK, Samantaray D, Patra JK, Samanta L, Thatoi H. Antidiabetic potential of mangrove plants: A review. *Front Life Sci* 2016; **9**(1): 75-88.

[35]Rege AA, Chowdhary AS. Evaluation of alpha-amylase and alpha-glucosidase inhibitory activities of *Rhizophora mucronata*. *Int J Pharm Sci Res* 2014; **5**(6): 2261-2265.

[36]Patra JK, Das SK, Thatoi H. Phytochemical profiling and bioactivity of a mangrove plant, *Sonneratia apetala*, from Odisha Coast of India. *Chin J Integr Med* 2015; **21**(4): 274-285.

[37]Picot CMN, Subratty AH, Mahomoodally MF. Inhibitory potential of five traditionally used native antidiabetic medicinal plants on α-amylase, α-glucosidase, glucose entrapment, and amylolysis kinetics *in vitro*. *Adv Pharmacol Sci* 2014; **2014**: 739834.

[38]Deutschlander MS, van de Venter M, Roux S, Louw J, Lall N. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *J Ethnopharmacol* 2009; **124**(3): 619-624.

[39]Antu KA, Riya MP, Mishra A, Anilkumar KS, Chandrakanth CK, Tamrakar AK, et al. Antidiabetic property of *Symplocos cochinchinensis* is mediated by inhibition of alpha glucosidase and enhanced insulin sensitivity. *PLoS One* 2014; **9**(9): e105829.

[40]Rayar A, Manivannan R. *In–vitro* alpha-amylase and alpha-glucosidase inhibition activity of umbelliferone and beta-ionone isolated from *Coriandrum sativum* Linn. *World J Pharm Pharm Sci* 2016; **5**(1): 1280-1289.

[41]Kang W, Zhang L, Song Y. α-Glucosidase inhibitors from *Luculia pinciana*. *Zhongguo Zhong Yao Za Zhi* 2009; **34**(4): 406-409.

[42]Chu Y-H, Wu S-H, Hsieh J-F. Isolation and characterization of α-glucosidase inhibitory constituents from *Rhodiola crenulata*. *Food Res Int* 2014; **57**: 8-14.

[43]Wang X, Mao Z-G, Song B-B, Chen C-H, Xiao W-W, Hu B, et al. Advances in the study of the structures and bioactivities of metabolites isolated from mangrove-derived fungi in the South China Sea. *Mar Drugs* 2013; **11**(10): 3601-3616.

[44]Liu X, Zhu L, Tan J, Zhou X, Xiao L, Yang X, et al. Glucosidase inhibitory activity and antioxidant activity of flavonoid compound and triterpenoid compound from *Agrimonia pilosa* Ledeb. *BMC Complement Altern Med* 2014; **14**: 1-10.

[45]Inthongkaew P, Chatsumpun N, Supasuteekul C, Kitisripanya T, Putalun W, Likhitwitayawuid K, 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): 480-487.