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Biological activity and chemical composition of organic extracts from three Guatemalan mangrove trees

Sully M. Cruz[1]*, María N. Marroquín[1], Armando Cáceres[1,2]

[1]Facultad de Ciencias Químicas y Farmacia, Guatemala
[2]Laboratorios de Productos Naturales Farmaya, Guatemala

*Correspondence to
Sully M. Cruz
Email:
smargotcv@gmail.com

Abstract
Introduction: Mangroves are trees or shrubs that are able to grow in brackish water along tropical and sub-tropical coasts around the world, representing an important ecological conservation system. They have a long tradition of medicinal use and are rich in secondary metabolites. The aim of this research was to evaluate the biological activity and chemical composition of three mangrove species, *Avicennia germinans, Conocarpus erectus* and *Laguncularia racemose*, from a natural reserve from Guatemala.
Methods: Leaf, bark and root were organically extracted; secondary metabolites were identified by macro and semi-micro tests and TLC, and evaluated for flavonoid and tannin content. Antioxidant activity was evaluated by DPPH, FRAP, TPC and ABTS methods, and antimicrobial activity against seven bacteria. By established protocols it was investigated the larvicidal activity against *Anopheles* and *Aedes*, cytotoxicity against *Artemia salina* and anti-tyrosinase activity by TLC.
Results: The best yields were obtained with ethanol in leaves (5.43-26.65%); the highest essential oil yields also in leaves (0.02-0.13%), in both cases *A. germinans*. Main secondary metabolites were flavonoids, tannins, coumarins, saponins and alkaloids, except C. erectus leaf and bark and *A. germinans* root. The highest amount of chlorogenic acid was found in *A. germinans* bark (15.6%), the highest percentage of tannins in *L. racemosa* root (7.2%), the highest antioxidant activity by DPPH (IC$_{50}$ 0.2-5.6 mg/mL) in *C. erectus* leaves, and FRAP (1.2-4.5 g Fe^{+2}/g of extract) and ABTS (IC50 0.2-11.6 mg/mL) in the bark. Antioxidant activity correlated with TPC, the highest amount in *A. germinans* bark (149.40-291.39 μg of gallic acid/g extract). None of the species showed larvicidal activity, nor cytotoxicity. Only *L. racemosa* root showed antibacterial activity against all strains (IC$_{50}$ 0.62 mg/mL). The ethyl acetate and ethanol extracts exhibited mild anti-tyrosinase activity.
Conclusion: Mangroves are a promising potential source of antioxidants and antibacterial compounds.
Keywords: *Laguncularia racemose, Avicennia germinans, Conocarpus erectus,* Anti-tyrosinase, Antioxidants activity

Introduction

Mangroves are a diverse group of halophytic plant species, which form highly productive forests in the area between mean sea level and the highest spring tide mark along tropical and sub-tropical coastlines and estuaries,[1] providing ecosystem services to marine and terrestrial environments, and human societies.[2,3] The most important mangrove ecosystem services include: coastline protection (in particular storm, hurricane and tsunami protection); waste water treatment; production of extractable materials; and provision of cultural sites.[4,5] Despite the known value of these forests, mangroves are highly threatened.

Mangroves are trees or shrubs that are able to grow in saline water along tropical and sub-tropical coasts around the world.[6] These plants are able to withstand a number of environmental stress factors: high salt concentrations, tidal flooding, strong winds, solar radiation and heat.[7]

Their ability to grow under these circumstances is linked to various morphological, physiological and biochemical adaptations, such as stilt and air roots, salt excretion systems and secondary metabolites. They also have a long tradition of medicinal use and are rich in secondary metabolites.[8] Some of these compounds have antimicrobial and antioxidant effects, among others, which are mainly based on ethnobotanical reports.[9]

The Multiple Use Natural Reserve of Monterrico (Reserva Natural de Usos Múltiples de Monterrico, henceforth RNUMM) in Guatemala is a wetland crucial to the natural functioning of the hydrographic basins and the neighboring coastal systems. The area is dominated by estuarine and coastal marine ecosystems, habitats of great ecological value and high diversity of animal and plant species, depending on this ecosystem for their life cycle.[10] Most of the territory under study is occupied by mangrove formations which is not surprising because up to 65% of

the RNUMM is made up of water bodies.

Mangrove swamps are coastal marine ecosystems typical to the tropical and subtropical belt of the world.[11] They tend to occur on the foreshore of gulfs, coves, marshes, estuaries or river mouths, particularly on soft, only occasionally rocky, sea floors periodically supplied with a regular run-off of fresh water.[12]

For years, mangroves have been utilized by various ethnic groups for the treatment of diseases. Ethnopharmacological uses of mangrove species is very diverse and have included treatment for: eye problems, skin diseases, rheumatism, blisters, arthritis, hemorrhage, asthma, throat and stomach ache, infections, and diabetes.[13,14]

Some species from Avicenniaceae, Meliaceae, Rhizophoraceae and Euphorbiaceae families have shown antimicrobial activity, while others belonging to the Meliaceae and Rhizophoraceae families possessed antimalarial properties.[15] Species as *Conocarpus erectus* from Combretaceae family, known as buttonwood, is one of two species in the genus *Conocarpus*. It is a folk remedy for anemia, cold, conjunctivitis, diabetes, diarrhea, and fever.[16] The leaves are eaten, and their decoction is drunk to treat fevers. The bark and the fruit of this species are used as infusion in the treatment of diarrhea, wounds, hemorrhoids and diabetes.[17,18]

Some of the proven biological properties of mangrove species include antioxidant,[8,19-21-25] anticancer,[20] hepatoprotective,[22] antiulcer[23] and antimicrobial activities.[20,24] It was suggested that mangrove extracts could be a potent source of therapeutic agents in preventing or slowing the ageing process and the oxidative stresses associated to degenerative diseases.[15,16,26] Due to the interest in the biological activity as well as the diverse bioactive metabolites, an investigation of different parts of mangrove species from RNUMM was carried out.

Materials and Methods
Plant Material
One kilogram of leaves, root and bark of three mangrove species were collected from the RNUMM. They were processed according to the chosen techniques. Voucher samples of the specimens (40,260-40,262) were deposited at the Herbarium of USCG-CECON-USAC.

The fractionated extraction started from dry plant materials using hexane, dichloromethane, ethyl acetate and ethanol in a polarity sequence. The solvent extractor was added to 200-500 g of plant material, it was percolated and repeated five consecutive days by continuing replacement of the solvent. The extraction was concentrated by reduced pressure at a temperature below 45°C using a rotary evaporator. The secondary metabolites present in the extracts were determined by phytochemical screening.[27]

Total ash was determinate in different parts of the evaluated species.

Essential Oil
The essential oil was extracted by hydro-distillation for 3 hours from 50 g of leaves in an all glass Clevenger-type apparatus using demineralized water (500 mL). At least three replicate extractions were performed for each sample. The extraction yield of the oil was calculated on the basis of dry weight of the plant material.

Phytochemical Screening
Standard phytochemical screening was performed in tubes at semi-micro scale for identification of flavonoids, anthocyanins, anthraquinones, coumarins, essential oils, tannins and polyphenols,[27] followed by thin layer chromatography (TLC) analysis using vanillin-H_2SO_4-anisaldehyde and specific reagents for each of the functional groups.[28]

Quantification of Flavonoids and Tannins
Plant material (1 g) was added to 50 mL of hot water in a 100 mL volumetric flask in a water bath for 60 minutes. The solution was cooled to room temperature and volume adjusted with distilled water, filtered and diluted 1:10. Dilutions were prepared (10, 20, 30, 40, 50, 60 and 70 ppm) of chlorogenic acid standard and a curve was prepared at wavelength 324 nm. The chlorogenic acid curve was used to determine metabolite concentration.[29]

Total tannin content was determined by phosphomolybdium tungstic acid. The solution was prepared with 10 g of sample and 500 mL of 50% ethanol, shaken for 6 hours, stand for 8 hours, shaken again for 30 minutes and filtered. The filtrate was transferred to a 50 mL volumetric flask and diluted with distilled water to the total volume, using tannic acid as the standard, and read at 700 nm wavelength.[30]

Determination of Antioxidant Activity
Total phenolic compounds (TPC) were determined by a standard macrometric method using the Folin-Ciocalteu reagent according to Phipps et al.[31] read in a Thermo Genesys 10 Spectrophotometer at 765 nm, and the concentration was estimated by a regression curve expressed in µg of gallic acid equivalent/mg of dry extract.

1,1-diphenyl-2-picrylhidrazyl (DPPH). Qualitative evaluation was done by a standard TLC method in $60F_{254}$ silica gel plates and sprayed with DPPH. Macrometric method was performed in tubes using acetate buffer, methanol, DPPH (0.0219%), and extract; after agitation and incubation for 30 minutes at room temperature, the results were read in a Thermo Genesys 10 Spectrophotometer at 517 nm against blank, and the IC_{50} was calculated. Micrometric determination was performed in a similar setting, but taking into consideration the scaling down needed to maintain the system in a 96-well plate, which was evaluated in an Elisa reader (Bio-Tek ELx-800) at 490 nm, followed by IC_{50} calculation in mg of dry extract from the regression line or TDAC.[32]

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Discoloration of ABTS was evaluated according to Re et al.[33] The ABTS radical cation was produced by mixing ABTS solution (7 mM) with potassium persulfate (2.45 mM), kept in the dark at room temperature for 16-18 hours. For analysis, the reagent was diluted in ethanol until the absorbance at 734 nm was 0.70 ± 0.02 at 30°C. Extract dilutions were added to the diluted reagent and read at 1, 4 and 6 min. For each dilution, a curve was prepared in 60%-70% inhibition, and the IC_{50} was calculated.

Ferric reducing antioxidant power (FRAP). The methanol extract (50-500 µg of dry extract/mL) was mixed with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide 1%, and incubated at 50°C for 20 min. Trichloroacetic acid was added and then centrifuged at 3000 rpm for 10 minutes. The upper layer was mixed with water and ferric chloride (0.1%), and the absorbance measured at 700 nm. The same procedure was followed using standards (gallic and ascorbic acid). Increased absorbance of the reaction mixture indicates increased reducing power.[34]

Determination of Biological Activity

Antimicrobial. Activity against bacteria and yeast was determined by the agar plate dilution method according to Mitscher et al.[35] Antibacterial screening was performed in agar, by preparing Muller-Hinton Agar (MHA) with 1.0 mg/mL of the extract (MHA-E). Bacteria were inoculated in tubes with broth for 24 hours at 36°C, and a 1:100 dilution in sterile distilled water was prepared. Each strain was streaked in quadruplicate (error <0.05) on the MHA-E surface and incubated at 36°C for 24 hours. Bacterial growth was evaluated, and the minimal inhibitory concentration (MIC) was performed by microdilution method using MTT (3-(4,5-dimethylthiazol-2-11)-2-5-diphenyltetrazolium) according to the National Committee for Clinical Laboratory Standards guidelines.[36] The activity were assayed against six bacteria (Staphylococcus aureus, Mycobacterium smegmatis, Pseudomonas aeruginosa, Bacillus subtilis, B. subtilis subsp spizizenii; and Escherichia coli) and one yeast (Candida albicans).

Larvicidal activity: using extract dilutions of 1000, 500 and 250 µg/mL; LC_{50} was calculated by non-parametric regression analysis using a Finney program for Basic. Four instars larvae (Aedes aegypti and Anopheles albimanus) were used against dilutions, after 24 hours, death larvae were evaluated visually.[37]

Brine shrimp lethality assay: In vitro lethality assay of A. salina was used for detecting toxicity from the organic extracts. Serial dilutions (1000, 500, 250 and 125 mg/L) were prepared in 96-well microplate. Nauplii (10-15) were pipetted in each well. Each concentration was assessed by triplicate. The lethality percentage was determined by comparing the mean surviving larvae of the test with the control wells. Lethal concentration values were obtained from the best-fit line plotted concentration versus percentage lethality.[38]

Anti-tyrosinase activity: Kojic acid was prepared at a weight ratio of 1:1 using methanol as a solvent. About 0.5 µg of the mixture was then dropped onto a commercial TLC plate and the separation was carried out using 1:1 (v/v) hexane:ethyl acetate as mobile phase. After drying at room temperature, the plate was then sprayed with tyrosinase and L-tyrosine solutions. Only spots with tyrosinase inhibitors appeared white against a brownish-purple background.[39]

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. We evaluated whether differences were statistically significant ($P \leq 0.05$).

Results

Three mangroves species were collected in RNUMM, Santa Rosa, Guatemala (Table 1). L. racemosa leaves showed the major total ash (11.76%), while bark showed the less content (3.00%); the major content of acid ash was presented by L. racemosa bark (2.00%), and less content was in C. erectus bark (0.65%) (Table 2).

Four solvents were used for extraction (hexane, dichloromethane, ethyl acetate and ethanol) of leaves, bark and root from three mangrove species. Extract yield

Table 1. General Information on Mangrove Samples Collected in Monterrico Reserve, Guatemala

	Languncularia racemosa (White Mangrove)(LR)	Conacarpus erectus (Button Mangrove) (CE)	Avicenia germinans (Black Mangrove)(AG)
Geographical coordinates	13°53'10,88" N 90°27'46,11 W	13°53'8,4624"N 90°27'46,476" W	14°5'53.484" N 90°27'41.082" W
Altitude (masl)	14	13	8
Botanic sample	4	3	4
Voucher Herbario USCG-Cecon-USAC	40,260	40,262	40,263
Accompanying flora	Palm, mango, jocote, mangrove, grass, algae	Nymphs, grass, red mangrove, white mangrove.	Red mangrove, nymphs, water lilies
Accompanying fauna	Herons, lizards, insects, fish	Fish, shrimp, herons, tadpoles	Fish, shrimps, herons, insects, ants

Table 2. Total and Acid Ash in Mangrove Tree Organs

Species	Part used	% Total Ash	% Acid ash
LR	Leaves	11.76 (0.15)	1.55 (0.08)
	Bark	3.00 (0.04)	2.00 (0.05)
	Root	5.34 (0.63)	0.69 (0.11)
CE	Leaves	8.16 (0.03)	1.20 (0.16)
	Bark	4.49 (0.06)	0.65 (0.04)
	Root	5.11 (0.20)	0.68 (0.04)
AG	Leaves	8.73 (0.22)	1.72 (0.44)
	Bark	8.68 (0.38)	1.00 (0.09)
	Root	4.84 (0.15)	0.31 (0.07)

varied widely in the case of hexane extracts *L. racemosa* root showed 0.30% while leaves (1.72%), dichloromethane extracts *L. racemosa* bark showed 0.20% and *C. erectus* leaves 1.45%, ethyl acetate extracts *C. erectus* bark showed 0.04% and *A. germinans* root 1.67% and ethanol extracts *C. erectus* root showed 3.78% and *A. germinans* leaves 26.65%. Essential oil showed in *L. racemosa* root was 0.02% and *A. germinans* leaves 0.13% (Table 3).

Preliminary phytochemical TLC screening of the extracts of different species from the collected mangroves revealed the presence of various groups of compounds (flavonoids, tannins, coumarins, alkaloids, and saponins). This data was obtained according to the colors observed on the spot extracts when each specific reagent was added. Results are summarized in Table 4. *L. racemosa* root showed the major content of tannins (7.29%) and *A. germinans* bark showed the major content of chlorogenic

acid (15.59 ppm) (Table 5).

Important antioxidant activity was found in three species, especially in ethanol extracts, by the three methods assayed. By DPPH, *L. racemosa* showed activity in leaves (IC_{50} 0.42 mg/mL), bark (IC_{50} 0.45 mg/mL), and root (IC_{50} 0.40 mg/mL), *C. erectus* showed activity in leaves (IC_{50} 0.21 mg/mL), bark (IC_{50} 0.38 mg/mL), and root (IC_{50} 0.39 mg/mL), and *A. germinans* in leaves (IC_{50} 5.63 mg/mL), bark (IC_{50} 0.59 mg/mL), and root (IC_{50} 2.37 mg/mL), but less than the standard evaluated as quercetin, vitamin C, trolox and TBHQ. The same extracts gave significant total phenolics content in *L. racemosa* root (188.40 µg/g), *A. germinans* bark (291.39 µg/g), except *C. erectus* in which the ethyl acetate leaves extracts (247.62 µg/g) showed better activity. By FRAP assay *C. erectus* bark ethanol extract showed better activity (4.52 gFe^{+2}/g) (Table 6).

None of the extracts tested at 1 mg/mL showed activity against the different instars of *A. albimanus* and *A. salina*. Among the extracts, *L. racemosa* leaves exhibited activity against *S. aureus*, *M. smegmatis*, *P. aeruginosa* and *E. coli*, the root showed activity against all microorganisms evaluated and bark did not exhibit any activity, while *C. erectus* leaves exhibited activity against six microorganisms except *E. coli*, bark against five bacteria and root showed no activity. For *A. germinans* only bark showed activity against two bacteria *P. aeruginosa* and *B. subtilis* (Table 7).

Ethyl acetate and ethanol extracts showed inhibition of tyrosinase with moderate effects in particular from *L. racemosa* leaves and bark, *C. erectus* bark and *A. germinans* bark (Table 8).

Table 3. Essential Oil and Extract Yield (%) of Organs Mangrove Species

Species	Part Used	Essential Oil	Extracts			
			Hex	DCM	EA	EtOH
LR	Leaves	0.05 (0.003)	1.72	1.00	0.38	11.60
	Bark	0.04 (0.021)	0.11	0.20	0.48	11.34
	Root	0.02 (0.001)	0.30	0.30	0.47	3.97
CE	Leaves	0.09 (0.001)	1.03	1.45	0.30	5.43
	Bark	0.02 (0.008)	0.67	0.86	0.04	11.49
	Root	0.03 (0.01)	0.74	0.13	0.35	3.78
AG	Leaves	0.13 (0.033)	0.95	0.87	1.29	26.65
	Bark	0.04 (0.018)	1.05	0.70	1.30	4.71
	Root	0.06 (0.002)	0.85	0.90	1.67	8.51

Table 4. Phytochemical Analysis of Organic Extracts of Mangrove Samples

Species	Part Used	Flavonoids	Tannins	Coumarin	Alkaloids	Saponins
LR	Leaves	+ (2)	+	+	+ (1)	+ (2)
	Bark	+ (3)	+	+	+ (2)	+(2)
	Root	+ (2)	+	+	+ (2)	+(2)
CE	Leaves	+ (2)	+	-	-	+(3)
	Bark	+ (2)	+	-	-	+(4)
	Root	+ (2)	+	+	+(1)	+(2)
AG	Leaves	+ (2)	+	+	+ (1)	+(4)
	Bark	+ (2)	+	+	+ (1)	+(3)
	Root	+ (1)	+	+	-	+(2)

Table 5. Quantification of Total Tannins and Flavonoids as Chlorogenic Acid

Species	Part used	Tannins (%)	Chlorogenic acid (ppm)
LR	Leaves	3.52 (0.24)	4.29 (0.04)
	Bark	2.43 (0.11)	2.36 (0.09)
	Root	7.29 (0.19)	0.63 (0.064)
CE	Leaves	4.44 (0.58)	4.93 (0.09)
	Bark	5.09 (0.20)	2.19 (0.07)
	Root	4.42 (0.15)	0.44 (0.03)
AG	Leaves	0.21(0.03)	1.41 (0.05)
	Bark	0.24 (0.07)	15.69 (0.30)
	Root	0.12 (0.02)	0.64 (0.040)

Discussion

Mangrove populations were selected in two transects of RNUMM and the description of the accompanying flora and fauna was made, observing a great diversity of species, which denotes the ecosystemic, environmental and touristic importance which makes it one of the five most important protected areas of Guatemala. Many biological activities are attributed to mangrove species in folk medicine. Species of plants with therapeutic purposes have been used in several scientific investigations around the world.

Table 6. Antioxidant Activity of 3 Organs From Mangrove Species

Species	Part used	Extracts	DPPH, IC$_{50}$ (mg/mL)	Total phenols (µg Gallic Acid/g Extract)	gFe^{+2}/g extract	ABTS, IC$_{50}$ (mg/mL)
LR	Leaves	Hex	18.57 (0.38)	16.61 (0.80)	0.39 (0.04)	
		DCM	3.80 (0.01)	58.40 (1.07)	0.23 (0.06)	
		EA	2.67 (0.01)	28.77 (2.90)	0.53 (0.01)	
		EtOH	0.42 (0.01)	182.77 (4.90)	1.20 (0.19)	0.50 (0.01)
	Bark	Hex	17.87 (0.28)	6.61 (0.76)	0.07 (0.08)	
		DCM	13.52 (0.28)	18.27 (1.77)	1.26 (0.06)	
		EA	5.89 (0.20)	48.57 (2.34)	3.16 (0.34)	
		EtOH	0.45 (0.02)	179.04 (4.79)	1.31 (0.22)	0.38 (0.06)
	Root	Hex	> 20	3.61 (0.16)	0.69 (0.09)	
		DCM	> 20	8.47 (0.48)	0.65 (0.04)	
		EA	4.29 (0.17)	56.61 (0.80)	2.39 (0.12)	
		EtOH	0.40 (0.02)	188.40 (5.07)	3.74 (0.78)	0.46 (0.02)
CE	Leaves	Hex	5.58 (0.06)	16.61 (1.56)	0.13 (0.09)	
		DCM	7.31 (0.25)	16.87 (1.39)	0.43 (0.03)	
		EA	7.29 (0.20)	247.62 (6.67)	1.06 (0.11)	
		EtOH	0.21 (0.02)	54.89 (1.77)	2.10 (0.01)	0.48 (0.01)
	Bark	Hex	> 20	2.61 (0.26)	0.10 (0.04)	
		DCM	15.52 (0.31)	15.40 (0.63)	0.30 (0.04)	
		EA	6.29 (0.20)	60.61(1.80)	1.42 (0.15)	
		EtOH	0.38 (0.02)	191.39 (5.38)	4.52 (0.17)	0.21 (0.02)
	Root	Hex	18.52 (0.31)	3.61 (0.12)	0.46 (0.07)	
		DCM	14.52 (0.28)	5.39 (0.98)	0.63 (0.04)	
		EA	4.29 (0.17)	78.21 (2.28)	1.39 (0.13)	
		EtOH	0.39 (0.02)	149.40 (3.07)	2.28 (0.03)	0.64 (0.01)
AG	Leaves	Hex	> 20	6.21 (0.56)	0.20 (0.02)	
		DCM	16.72 (0.29)	26.29 (1.39)	0.89 (0.03)	
		EA	8.45 (0.20)	147.32 (2.67)	0.70 (0.02)	
		EtOH	5.63 (0.35)	154.29 (3.77)	3.38 (0.02)	11.63 (0.24)
	Bark	Hex	18.42 (0.29)	3.91 (0.76)	0.07 (0.01)	
		DCM	16.52 (0.31)	8.29 (0.83)	0.42 (0.02)	
		EA	4.69 (0.20)	53.91 (1.99)	3.17 (0.34)	
		EtOH	0.59 (0.01)	291.39 (6.38)	4.31 (0.22)	10.91 (0.47)
	Root	Hex	17.22 (0.25)	5.29 (0.29)	0.47 (0.09)	
		DCM	13.52 (0.31)	15.89 (2.18)	0.39 (0.02)	
		EA	9.79 (0.20)	135.71 (3.28)	2.39 (0.12)	
		EtOH	2.37 (0.10)	186.90 (5.72)	2.25 (0.20)	4.86 (0.24)
Standars		Rutin	0.1671 (0.0062)			
		Quercetin	0.0749 (0.0004)			0.1136 (0.0008)
		Vitamin C	0.0876 (0.0105)			0.0201 (0.0002)
		Trolox	0.1147 (0.0008)			0.2726 (0.0006)
		TBHQ	0.1147 (0.0007)			0.1992 (0.0008)

Table 7. Antibacterial Activity by Agar Dilution Screening and Microdilution Confirmation

Species	Part Used	A	B	C	D	E	F	G
LR	Leaves	0.62	0.62	0.62	> 1	> 1	> 1	0.62
	Bark	> 1	> 1	> 1	> 1	> 1	> 1	> 1
	Root	0.62	0.62	0.62	0.62	0.62	0.62	0.62
CE	Leaves	0.62	0.62	0.62	0.62	0.62	0.62	> 1
	Bark	> 1	0.62	0.62	0.62	0.62	0.62	> 1
	Root	> 1	> 1	> 1	> 1	> 1	> 1	> 1
AG	Leaves	> 1	> 1	> 1	> 1	> 1	> 1	> 1
	Bark	> 1	> 1	0.62	> 1	> 1	0.62	> 1
	Root	> 1	> 1	> 1	> 1	> 1	> 1	> 1
Ampiciline sulbactan		0.16	0.16	0.32	0.32	0.02	0.32	0.02

A = *Staphylococcus aureus*, B = *Mycobacterium smegmatis*, C = *Pseudomonas aeruginosa,* D = *Candida albicans*, E= *Bacillus subtilis*; F= *Bacillus subtilis subsp Spizizenii*; G = *Escherichia coli.*

Table 8. Anti-tyrosinase Activity of Mangrove Species by Thin Layer Chromatography

Species	Part used	Hexane	Dichloromethane	Ethyl acetate	Ethanol
LR	Leaves	-	-	+	+
	Bark	-	-	+	+
	Root	-	-	-	-
CE	Leaves	-	-	-	+
	Bark	-	-	+	+
	Root	-	-	-	-
AG	Leaves	-	-	+	-
	Bark	-	-	+	+
	Root	-	-	-	-
Standard	Kojic acid	+++*	+++	+++	+++

(-) negative, (+) moderate activity, (+++) high activity.

The total ash method is designed to measure the total amount of material remaining after ignition, including physiological and non-physiological ash. Ash values are important quantitative standards and criteria to analyze the identity and purity of crude drugs especially in powder form. Moreover, the total ash of a crude drug also reflects the intensity of care taken in drug preservation, and purity of the crude drug. According to the results obtained, the highest amount of ash was observed in the *L. racemosa* leaves (11.76%), while the lowest content was found in the bark. Studies conducted in India reported the total ash value content of *Avicennia alba* Blume and *L. racemosa* being 14.0% and 16.22% respectively,[40] which shows values higher that those reported in Guatemalan species.

Acid insoluble ashes are a part of total ash and measure the amount of silica present, especially as sand and siliceous earth. In the case of acid ash none was greater than 2% and the highest content was present in the leaves of *A. germinans*. The percentages values of acid insoluble ash reported in previous studies were 3.10% in *A. alba* and 3.40% in *L. racemosa*. Acid insoluble ash value is frequently necessary to evaluate the purities of crude drug content. This ash value indicates contamination with siliceous material. The comparison of this with the total ash value of the sample will differentiate between contaminating minerals and variations of the natural ash of the drug.[40,41]

Regarding the extraction yields in all the species, a higher yield was observed in the ethanol extracts (3.97-26.6%), the highest being *A. germinans* leaves, while hexane extract yields were highest in *L. racemosa* leaves (1.7%), dichloromethane in *C. erectus* leaves (1.5%) and ethyl acetate in *A. germinans* root (1.7%), which confirms the presence of polar compounds since the largest amount of extract was obtained in polar solvents. Studies carried out on leaves of *A. alba* reported yields of 5.0% and *for L. racemosa* of 8.5%, those obtained in Guatemalan species were higher. According to Jacoeb et al[42] *Avicennia marina* (Rossk) Vierh leaves showed an extraction yield of 9.6% using methanol while ethyl acetate and hexane was 1.28 and 0.6% respectively. Sulmartiwi et al[21] reported the yields of bark extraction of *A. rumphiana* with hexane of 0.39%, in ethyl acetate 2.8% and in ethanol 3.4%, while in the leaves the reported yield was 3.1% in hexane, 1.4% in ethyl acetate and 6.2% in ethanol. This is consistent with the results obtained that the highest yields are obtained in ethanolic extracts.

The essential oil presented low yields (0.02-0.13%), the highest being *A. germinans* leaves. Studies conducted on the essential oil of *A. marina* leaves report the presence of cryptomeridiol (7.82%), cedrondyol (7.13%), nonadecane (3.90%), ecosane (3.47%) and octen-2-ol (3.19%), which is related to its antioxidant effect.[43] Studies on *Rhizophora mucronata* Lam. essential oil leaf are also reported, mainly identifying fatty acids and α-pinene as one of the main components (35.87%), demonstrating larvicidal and repellent activity against *Anopheles stephensi* and *Culex*

quinquefasciatus.[44] Studies of the flowers of three species of *Rhizophora* demonstrate the presence of alkanes, fatty acids, phenylpropanes and monoterpenes with eugenol one of the major components.[45] In general, there are few studies about essential oils in mangrove species.

Phytochemical screening showed the presence of flavonoids in all samples, mainly chlorogenic acid, tannins, volatile oils, and saponins; coumarins were detected in seven of the nine analyzed samples, except for the leaves and bark of *C. erectus*, alkaloids were not evidenced in leaves and bark of button mangrove and *A. germinans* root.

A previous phytochemical study identified flavonoids, saponins and tannins in an aqueous extract of *C. erectus* leaves.[46] High-performance liquid chromatography analysis of ethyl acetate and n-butanol extracts of leaves, stem, flowers and fruits of *C. erectus* revealed the presence of gallic acid, catechin, apigenin, quercetin, quercetin-3-O-glucoside, kaempferol-3-O-glucoside, rutin and quercetin-3-O-glucoside-6-O-gallic acid.[47]

In a recent study, 33 organic extracts of mangrove species were analyzed, detecting triterpenoids, phenolic compounds and tannins as the main phytochemical groups found in the samples; saponins, quinones and coumarins were found in at least 50% of the samples.[15] The phytochemical screening of three species of mangrove leaf (*R. mucronata*, *Sonneratia alba* J. Smith and *Excoecaria agallocha* L) revealed the presence of saponins, glycosides, tannins, flavonoids, volatile oils,[48] which agrees with the results in the species under study.

Phytochemical studies of leaves of *Conocarpus erectus* L indicated the presence of gallic acid, ellagic acid, 3,3´-Dimethoxyellagic acid,[49] brevifolin carboxylic acid,[50] quercetin 3-O-glucuronide[51] myricetin 3-O-glucuronide,[52] syringetin 3-O-glucuronide[53] triterpenes in n-hexane extract and absence of saponins in the methanol extract.[46,54]

This fact reinforces the need for studies that assess the differences in chemical composition between the organs of the same plant, different times of collection, different cultivation environments and even different forms of plant nutrition.

The secondary metabolites represent a chemical interface between plants and the surrounding environment, so their synthesis are often affected by environmental conditions such as rainfall, UV radiation, atmospheric composition, circadian rhythm, plant age and temperature.[46]

The quantification of flavonoids based on chlorogenic acid revealed that in *A. germinans* bark the highest amount (15.59 ± 0.30 ppm) occurs and in the case of the leaf, *C. erectus* species is the one that outperforms the other species (4.93 ± 0.09 ppm), while the roots showed the lowest amount in all the samples evaluated (0.44-0.64 ppm). No studies were found in the literature that reports the quantity of flavonoids in the mangrove species under study, these results were the first quantitative findings on these metabolites.

Sulmartiwi et al[21] reported flavonoids expressed in meq of quercetin/g of extract using 3 solvents in bark, fruits and leaves of *Avicennia rumphiana* Hallier f. finding values of 2.3-13.8 meq/g, polar extracts, (ethanolic and ethyl acetate) of leaves and fruit presented the highest quercetin content.

Quantification of tannins showed the highest percentage in *L. racemosa* root (7.29 ± 0.19%), while in the leaf and bark the highest percentage was presented by *C. erectus* (4.44 ± 0.58% and 5.09 ± 0.20 respectively). Sulmartiwi et al43 reported tannins equivalent to tannic acid/g extract in *A. rumphiana*, with the highest values in ethyl acetate extracts of fruit (74.63 meq/g) and leaf (21.29 meq/g).

Antioxidant activity was presented by ethanolic extracts and the highest activity was showed in *C. erectus* leaf (CI50 0.21±0.02 mg/mL). The FRAP method showed that ethanolic extract leaf and root of *L. racemosa* had the highest amount of Fe+2 g/g of extract (1.20±0.19 g/g), while in the bark it was the ethyl acetate extract (3.16 ± 0.34 g/g), in *C. erectus* in the 3 organs, the greatest amount of iron formed was in the ethanolic extracts, with the bark exhibiting the highest concentration (4.52 ± 0.17 g/g), in the same *A. germinans* ethanolic extract bark had the highest concentration (4.31 ± 0.22 g/g).

Ayoub,[56] isolated and characterized a trimethoxyellagic acid glucuronide compound from the leaves of *C. erectus*, which showed important antioxidant activity using the xanthine/hypoxanthine oxidase assay. Several flavonoids and phenolic compounds were isolated such as gallic acid, quercetin 3-O-glucuronide, myricetin 3-O-glucuronide, siringetin 3-O-glucuronide, ellagitannins, castalagin, quercetin, myricetin, siringetin, 3,4,3'-trimethoxylagic acid, ellagic acid and 3,3'-dimethoxylagic acid.

Huseein,[16] reported antioxidant activity in the butanolic fraction of *C. erectus* leaves by DPPH and FRAP, extract presented activity comparable to ascorbic acid and butylhydroxytoluene (BHT). Aerial parts of *C. erectus* demonstrated antioxidant and acetylcholinesterase activity in ethanolic and butanol extracts.[57] Previous studies with *A. marina* leaf extracts showed a phenolic content of 18.72 mg of gallic acid/g and an average effective concentration to inhibit the DPPH radical of 9650 ppm, in the iron reduction test a value of 69.1 mg/mL was obtained.[43]

Sulmartiwi et al[21] reported the antioxidant activity of *A. rumphiana* demonstrating the highest activity in the ethanolic extract of leaves (CI$_{50}$ 492.22 ppm), which agrees with our results. Total phenolics in extracts of *A. rumphiana* was reported, with 0.9-23.86 mg equivalents of gallic acid/g of extract, the highest values were detected in the fruits of ethyl acetate extracts. The phenolic compounds commonly found in plants along with the flavonoids are chemical groups with antioxidant activities, so the results show a correlation in the chemical composition and antioxidant activity presented in the polar extracts.

None of the extracts showed cytotoxic activity against *A. salina*, and no insecticidal activity was evidenced in

the mangrove species studied. One study reported the acute toxicity of aqueous extracts of *C. erectus,* estimating the LD$_{50}$ above 2000 mg/kg, showing a low acute toxicity classified as category 5. The main compounds identified were flavonoids, tannins and saponins.[46]

Antibacterial activity was evaluated against nine strains of pathogenic bacteria, inhibitory activity of *L. racemosa* leaves and roots ethanolic extracts was demonstrated against *S. aureus, M. smegmatis, P. aeruginosa* and *E. coli* (MIC 0.62 mg/mL), while the bark did not present any activity. *C. erectus* presented activity mainly in leaves and bark, in the roots no activity was evidenced. *A. germinans* did no showed activity in leaves nor root, only the bark inhibited two bacteria *P. aeruginosa* and *B. subtilis* (MIC 0.62 mg/mL).

Phytochemical composition and pharmacological properties of *C. erectus* have been studied throughout history,[20,58,59] phenolic compounds, especially tannins, are the major components of this species[56] and many studies have investigated antimicrobial and antioxidant properties promoted by these compounds.[6,8,11,13]

Shohayeb et al[18] suggested that tannins of *C. erectus* are largely responsible for antimicrobial activity of this plant. These authors, studying alcoholic extracts of leaves, stem, fruit and flower of *C. erectus* collected in Saudi Arabia, showed antibacterial activity for S. aureus, B. subtilis (gram-positive), acid-fast *Mycobacterium phlei* and gram-negative bacteria *E. coli, Salmonella typhimurium, Klebsiella pneumoniae* and *P. aeruginosa.*

This is probably related to variations in the kind of compounds present in the extracts that may arise from differences between individual plants, different collection moments, different cultivation environments, different extraction solvents and different extraction methods, as well as possibly intrinsic factors.[46]

Studies on extracts of leaves of *C. erectus* showed antimicrobial,[18] antioxidant,[55] anticancer,[20,58] and hepatoprotective activity.[22,58] However, pharmacological properties can be associated with phytochemical compounds found in this study such as flavonoids which have antioxidant, anti-inflammatory and hepatoprotective properties, and saponins related to antimicrobial and anti-inflammatory activities. Santos et al,[48] reported that the aqueous extract of *C. erectus* leaves showed moderate bacteriostatic activity and immunomodulatory activity promoter, identified flavonoids, phenylpropanoglycosides, saponins, proanthocyanidins and hydrolysable tannins.[18] Lopez et al[15] reported that leaf ethanolic extracts of mangrove species *R. mucronata*, *S. alba* and *E. agallocha* showed antibacterial activity while phytochemical screening revealed the presence of saponins, glycosides, tannins, flavonoids, and volatile oils.

A study with *A. germinans* and *C. erectus* showed activity against *E. coli,*[15] which agrees with the data obtained that show inhibitory activity of the extracts against certain bacterial strains. *C. erectus* extracts were found to possess antioxidant and anticancer capacities. The higher susceptibility of the tested gram-positive as compared to gram-negative bacteria to *C. erectus* extracts is consistent with previous studies on the antibacterial activity of natural products.[60-62] Tannins are water-soluble polyphenols that are commonly found in higher herbaceous and woody plants.[63] They have been reported to possess both bacteriostatic and bactericidal activities,[64,65] because *C. erectus* contains large amounts of tannins.[22] Extracts of different mangrove species investigated in this study possessed broad-spectrum antimicrobial activity against gram-positive, and gram-negative bacteria. The broad-spectrum antibacterial activity of the plant extracts, confirms its use as a health remedy in folklore medicine.

Anti-tyrosinase activity was evaluated by TLC in the mangrove species, in which a moderate activity was evidenced for ethyl acetate and ethanol extracts. This is an assay that allows us to determine the ability to inhibit the enzyme tyrosinase, an enzyme related to hyperpigmentation of the skin, because it limits the speed of melanin biosynthesis and is important in the coloration of the skin, eyes and hair; several compounds of both synthetic and natural origin have been studied and reported as inhibitors of tyrosinase activity, some have been included in cosmetic formulas with skin lightening properties.[66] The literature review does not report that this test has been performed in mangrove species, only inhibitory activity against another type of enzyme, like α-glucosidase, is reported mainly in *L. racemosa* and *C. erectus.*[15]

Based on the results reported here, it can be concluded that mangroves are a promising potential source of antioxidants compounds. Due to the evident antibacterial and antioxidant potential, mangrove studies will be initiated to investigate the bioactive metabolites and the detailed mechanisms of action of these bioactive metabolites in the obtained extracts.

Competing Interests
None.

References
1. Tomlinson PB. The Botany of Mangroves. Cambridge: Cambridge University Press; 1994.
2. Gilman EL, Ellison J, Duke NC, Field C. Threats to mangroves from climate change and adaptation options: a review. Aquat Bot. 2008;89(2):237-250. doi:10.1016/j.aquabot.2007.12.009

3. Nagelkerken I, Blaber SJM, Bouillon S, et al. The habitat function of mangroves for terrestrial and marine fauna: a review. Aquat Bot. 2008;89(2):155-185. doi:10.1016/j.aquabot.2007.12.007

4. Rönnbäck P, Crona B, Ingwall L. The return of ecosystem goods and services in replanted mangrove forests: perspectives from local communities in Kenya. Environ Conserv. 2007;34(4):313-324.doi:10.1017/S0376892907004225

5. Warren-Rhodes K, Schwarz AM, Boyle LN, et al. Mangrove ecosystem services and the potential for carbon revenue programmes in Solomon Islands. Environ Conserv. 2011;38(4):485-496.　　doi:10.1017/S0376892911000373

6. Kathiresan K, Bingham BL. Biology of mangroves and mangrove Ecosystems. Adv Mar Biol. 2001;40:81-251. doi:10.1016/S0065-2881(01)40003-4

7. Spalding M, Kainuma M, Collins L. World Atlas of Mangroves. London: Routledge; 2010.

8. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl Ecol Manag. 2002;10(6):421-452. doi:10.1023/a:1021397624349

9. Patra JK, Thatoi HN. Metabolic diversity and bioactivity screening of mangrove plants: a review. Acta Physiol Plant. 2011;33(4):1051-1061. doi:10.1007/s11738-010-0667-7

10. Sigüenza de Micheo R. Ruíz-Ordoñez J. Plan Maestro de la Reserva Natural de Usos Múltiples Monterrico. Centro de Estudios Conservacionistas, Consejo Nacional de Áreas Protegidas, Proyecto "Aprovechamiento sostenible de los recursos asociados a los manglares del Pacífico de Guatemala" (INAB-UICN-UE). Guatemala; 1999.

11. Giri C, Ochieng E, Tieszen LL, et al. Status and distribution of mangrove forests of the world using earth observation satellite data. Glob Ecol Biogeogr. 2011;20(1):154-159. doi:10.1111/j.1466-8238.2010.00584.x

12. García-Fuentes A, Castillo-Cabrera FJ, Dávila-Pérez CV, et al. Study of the vegetation in the Multiple Use Natural Reserve of Monterrico (Guatemala). Plant Biosyst. 2014;148(6):1064-1074. doi:10.1080/11263504.2013.826295

13. Wu J, Xiao Q, Xu J, Li MY, Pan JY, Yang MH. Natural products from true mangrove flora: source, chemistry and bioactivities. Nat Prod Rep. 2008;25(5):955-981. doi:10.1039/b807365a

14. Xu DB, Ye WW, Han Y, Deng ZX, Hong K. Natural products from mangrove actinomycetes. Mar Drugs. 2014;12(5):2590-2613. doi:10.3390/md12052590

15. Lopez D, Cherigo L, de Sedas A, Spadafora C, Martínez-Luis S. 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. doi:10.4103/1995-7645.223531

16. 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.

17. Santos D, de Almeida VS, de Araujo DRC, et al. Evaluation of cytotoxic, immunomodulatory and antibacterial activities of aqueous extract from leaves of *Conocarpus erectus* Linnaeus (Combretaceae). J Pharm Pharmacol. 2018;70(8):1092-1101. doi:10.1111/jphp.12930

18. Shohayeb MM, Abdel-Hameed ES, Bazaid SA. Antimicrobial activity of tannins and extracts of different parts of *Conocarpus erectus* L. Int J Pharm Biol Sci. 2013;3(2):544-553.

19. Cruz SM, Marroquín N, Alvarez LE, Chang DE, Cáceres A. Evaluation of Mangrove (*Rhizophora mangle* L.) products as coloring, antimicrobial and antioxidant agents. Int J Phytocos Nat Ingred. 2015;2(1):12-12. doi:10.15171/ijpni.2015.12

20. Abdel-Hameed ES, Bazaid SA, Shohayeb MM, El-Sayed MM, El-Wakil EA. Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of

21. Sulmartiwi L, Pujiastuti DY, Tjahjaningsih W, Jariyah. Potential of mangrove *Avicennia rumphiana* extract as an antioxidant agent using multilevel extraction. IOP Conf Ser Earth Environ Sci. 2018;137:012075. doi:10.1088/1755-1315/137/1/012075

Conocarpus erectus L. growing in Taif, Saudi Arabia. European J Med Plants. 2012;2(2):93-112. doi:10.9734/EJMP/2012/1040

22. Abdel-Hameed ES, Bazaid SA, Sabra AN. Protective effect of *Conocarpus erectus* extracts on CCl4-induced chronic liver injury in mice. Glob J Pharmacol. 2013;7(1):52-60. doi:10.5829/idosi.gjp.2013.7.1.7188

23. Perera LM, Ruedas D, Gómez BC. Gastric antiulcer effect of *Rhizophora mangle* L. J Ethnopharmacol. 2001;77(1):1-3. doi:10.1016/s0378-8741(01)00277-x

24. Sánchez-Perera LM, Varcalcel L, Escobar A, Noa M. Polyphenol and phytosterol composition in an antibacterial extract from *Rhizophora mangle* L. bark. J Herb Pharmacother. 2007;7(3-4):107-128. doi:10.1080/15228940802142720

25. Sánchez J, Melchor G, Martínez G, Escobar A, Faure R. Antioxidant activity of *Rhizophora mangle* bark. Fitoterapia. 2006;77(2):141-143. doi:10.1016/j.fitote.2005.11.011

26. Sánchez J, Melchor G, Martínez G, Sánchez LM, Faure R, Vinardel P. Protective effect of *Rhizophora mangle* bark on lipid peroxidation and erythrocyte hemolysis. Pharmacogn Mag. 2005;1(3):101-104.

27. Wagner H, Bladt S. Plant Drug Analysis. Berlin: Springer-Verlag; 1996:320.

28. de Ugaz OL. Investigación fitoquímica: métodos en el estudio de productos naturales. 2nd ed. Lima: Pontificia Universidad Católica del Perú; 1994.

29. Belay A, Gholap AV. Characterization and determination of chlorogenic acids (CGA) in coffee beans by UV-Vis spectroscopy. Afr J Pure Appl Chem. 2009;3(11):234-240.

30. Adamczyk B, Kitunen V, Smolander A. Protein precipitation by tannins in soil organic horizon and vegetation in relation to tree species. Biol Fertil Soils. 2008;45(1):55-64. doi:10.1007/s00374-008-0308-0

31. Phipps SM, Sharaf MH, Butterweck V. Assessing antioxidant activity in botanicals and other dietary supplements. Pharmacopeial Forum. 2007;33(4):810-814.

32. Ravishankara MN, Shrivastava N, Padh H, Rajani M. Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* R. Br. (Anantmul). Phytomedicine. 2002;9(2):153-160. doi:10.1078/0944-7113-00104

33. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231-1237. doi:10.1016/s0891-5849(98)00315-3

34. Kosem N, Han YH, Moongkarndi P. Antioxidant and cytoprotective activities of methanolic extract from *Garcinia mangostana* hulls. Sci Asia. 2007;33:283-292. doi:10.2306/scienceasia1513-1874.2007.33.283

35. Mitscher LA, Leu RP, Bathala MS, Wu WN, Beal JL. Antimicrobial agents from higher plants. I. Introduction, rationale, and methodology. Lloydia. 1972;35(2):157-166.

36. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility testing. Ninth informational supplement NCCLS document M100-S9. Wayne, PA: NCCLS; 2008:120-126.

37. Subramonia Thangam T, Kathiresan K. Mosquito larvicidal activity of mangrove plant extracts and synergistic activity of *Rhizophora apiculata* with pyrethrum against *Culex quinquefasciatus*. Int J Pharmacogn. 1997;35(1):69-71. doi:10.1076/phbi.35.1.69.13263

38. Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson

JD. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med. 1993;59(3):250-252. doi:10.1055/s-2006-959661

39. Wangthong S, Tonsiripakdee I, Monhaphol T, Nonthabenjawan R, Wanichwecharungruang SP. Post TLC developing technique for tyrosinase inhibitor detection. Biomed Chromatogr. 2007;21(1):94-100. doi:10.1002/bmc.727

40. Reddy PHB, Audipudi AV. Physico-Phyto and Chromato Graphic Analysis of *Aviccinia alba* and *Laguncularia racemosa* Leaves. Int J Pharmacogn Phytochem Res. 2016;8(11):1819-1821.

41. Gite VN, Pokharkar RD, ChopadeVV, Takate SB. Evaluation of physicochemical standardization parameter of *Enicostemma axillare*. J Biosci Tech. 2010;1(4):187-190.

42. Jacoeb AM, Purwaningsih S, Rinto. Anatomi, komponen bioaktif dan aktivitas antioksidan daun mangrove api-api (*Avicennia marina*). J Pengolah Has Perikan Indones. 2011;14(2):143-152. doi:10.17844/jphpi.v14i2.5323

43. Molaee M, Sahari MA, Esmaeilzadeh Kenari R, Amirkaveei S, Arbidar E. A study on the composition and antioxidant properties of *Avicennia marina* leaf extract. Curr Nutr Food Sci. 2017;13(2):131-136. doi:10.2174/1573401313666170110110131

44. Yogananth N, Anuradha V, Syed Ali MY, Muthezhilan R, Chanthuru A, Prabu MM. Chemical properties of essential oil from *Rhizophora mucronata* mangrove leaf against malarial mosquito *Anopheles stephensi* and filarial mosquito *Culex quinquefasciatus*. Asian Pac J Trop Dis. 2015;5:S67-S72. doi:10.1016/S2222-1808(15)60859-3

45. Saranya J, Pious BR, Eganathan P, Ramasubramanian R, Selvam V. Essential oil composition of whole flowers of *Rhizophora apiculata, Rhizophora mucronata* and *Rhizophora x annamalayana* from Pichavaram, India. J Essent Oil-Bear Plants. 2015;18(3):728-733. doi:10.1080/0972060X.2014.909748

46. Nascimento DK, Souza IA, Oliveira AF, et al. Phytochemical screening and acute toxicity of aqueous extract of leaves of *Conocarpus erectus* Linnaeus in Swiss albino mice. An Acad Bras Cienc. 2016;88(3):1431-1437. doi:10.1590/0001-3765201620150391

47. Abdel-Hameed ES, Bazaid SA, Shohayeb MM. RP-HPLC-UV-ESI-MS phytochemical analysis of fruits of *Conocarpus erectus* L. Chem Pap. 2014;68(10):1358-1367. doi:10.2478/s11696-014-0570-6

48. Sahoo G, Mulla NS, Ansari ZA, Mohandass C. Antibacterial activity of mangrove leaf extracts against human pathogens. Indian J Pharm Sci. 2012;74(4):348-351. doi:10.4103/0250-474x.107068

49. Nawwar MAM, Buddrus J, Bauer H. Dimeric phenolic constituents from the roots of *Tamarix nilotica*. Phytochemistry. 1982;21(7):1755-1758. doi:10.1016/S0031-9422(82)85054-1

50. Nawwar MAM, Hussein SAM, Merfort I. NMR spectral analysis of polyphenols from *Punica granatum*. Phytochemistry. 1994;36(3):793-798. doi:10.1016/S0031-9422(00)89820-9

51. Nawwar MAM, Souleman AMA, Buddrus J, Bauer H, Linscheid M. Polyphenolic constituents of the flowers of *Tamarix nilotica*: the structure of nilocitin, a new digalloylglucose. Tetrahedron Lett. 1984;25(1):49-52. doi:10.1016/S0040-4039(01)91145-1

52. Nakanishi T, Inatomi Y, Murata H, et al. Triterpenes and flavonol glucuronides from *Oenothera cheiranthifolia*. Chem Pharm Bull (Tokyo). 2007;55(2):334-336. doi:10.1248/cpb.55.334

53. Yasukawa K, Ogawa H, Takido M. Two flavonol glycosides from *Lysimachia nummularia*. Phytochemistry. 1990;29(5):1707-1708. doi:10.1016/0031-9422(90)80155-A

54. Rehman S, Azam F, Rehman S, et al. A review on botanical, phytochemical and pharmacological reports of *Conocarpus erectus*. Pak J Agric Res. 2019;32(1):212-217. doi:10.17582/journal.pjar/2019/32.1.212.217

55. Gobbo-Neto L, Lopes NP. [Medicinal plants: factors of influence on the content of secondary metabolites]. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim Nova. 2007;30(2):374-381. doi:10.1590/S0100-40422007000200026

56. Ayoub NA. A trimethoxyellagic acid glucuronide from *Conocarpus erectus* leaves: isolation, characterization and assay of antioxidant capacity. Pharm Biol. 2010;48(3):328-332. doi:10.3109/13880200903131567

57. Raza MA, Anwar F, Shahwar D, et al. Antioxidant and antiacetylcholine esterase potential of aerial parts of *Conocarpus erectus, Ficus variegata* and *Ficus maclellandii*. Pak J Pharm Sci. 2016;29(2):489-495.

58. Bashir M, Uzair M, Ch BA. A review of phytochemical and biological studies on *Conocarpus erectus* (Combretaceae). Pak J Pharm Res. 2014;1:1-8. doi:10.22200/pjpr.201511-8

59. Hayashi T, Thomson RH. New lignans in *Conocarpus erectus*. Phytochemistry. 1975;14(4):1085-1087. doi:10.1016/0031-9422(75)85192-2

60. Halawani EM, Shohayeb MM. Shaoka and Sidr honeys surpass in their antibacterial activity local and imported honeys available in Saudi markets against pathogenic and food spoilage bacteria. Aust J Basic Appl Sci. 2011;5(4):187-191.

61. Ceylan E, Fung DYC. Antimicrobial activity of spices. J Rapid Methods Autom Microbiol. 2004;12(1):1-55. doi:10.1111/j.1745-4581.2004.tb00046.x

62. López P, Sánchez C, Batlle R, Nerín C. Solid- and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. J Agric Food Chem. 2005;53(17):6939-6946. doi:10.1021/jf050709v

63. Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991;30(12):3875-3883. doi:10.1016/0031-9422(91)83426-L

64. Chung KT, Stevens SE Jr, Lin WF, Wei CI. Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. Lett Appl Microbiol. 1993;17(1):29-32. doi:10.1111/j.1472-765X.1993.tb01428.x

65. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against Staphylococcus aureus. J Antimicrob Chemother. 2001;48(4):487-491. doi:10.1093/jac/48.4.487

66. van Gelder CW, Flurkey WH, Wichers HJ. Sequence and structural features of plant and fungal tyrosinases. Phytochemistry. 1997;45(7):1309-1323. doi:10.1016/s0031-9422(97)00186-6