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Pharmacological Potentials of the Genus *Coccoloba* and Its Phytochemical Constituents: An Updated Review Since 2020

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Abstract

Genus *Coccoloba* is the third largest genus among Family Polygonaceae(Knotweed family), comprising about 120 species. Pursuing to our exploration of various *Coccoloba* species, an updated mini-review about the phytochemical and biological profiling of its species is presented. It covers the scientific literature from 2020 - till now. Various biological activities were verified and assessed, e.g., antiprotozoal, smooth muscle relaxation, immunomodulatory and hepatoprotective. Phytochemical studies disclosed the presence of lignin oligomers, alkaloids and new methylated flavonoids. Some extracts were incorporated in modern pharmaceutical formulations for enhanced stability and permeability. Finally, the review explores the pitfalls of scientific literature investigating *Coccoloba* species.

Keywords: *Coccoloba*; Phytochemical constituents; Polygonaceae; Hepatoprotective; Smooth muscle relaxant; Immunomodulatory activity.

1. Introduction

Plants can provide bioactive lead compounds for the development of safer and more effective alternatives for the currently available harmful commercial medications [1]. Family Polygonaceae(Knotweed family) is a globally distributed family, of mostly herbaceous plants that include over 1200 species and 49 genera. Members of this family flourish in Europe,temperate North America, and Southeast Asia than inAfrica, South America, the Caribbean, or Australasia [2]. The genus *Coccoloba*is the third largest genus in this family comprising about 120 species [1].

In tropical and subtropical regions of the Americas, few species of *Coccoloba*were used in traditional medicine. It was used for the remedy of various disorders, including fever, diarrhea, menstrual disturbances, haemorrhoids,uterine hemorrhages,and gonorrhea[3-5]. In other reports, *C. mollis* was used in folk medicine for ameliorationof conditions like sleeplessness, memory loss, stress, anaemia, impaired vision, and impotence [6].

The phytochemistry of the genus has not been thoroughly investigated. The majority of the researches have focused on the more prevalent species, *C. uvifera* (sea grape, indigenous to tropical America's and the Caribbean's coastal beaches). A great chemical diversity of metabolite groups were isolated or detected from a small number of species of this genus, including flavonoids and tannins [7-9], terpenoids and sterols [7, 10, 11], anthraquinones [12, 7], and volatile constituents [13].

Several activities were previously reported for different species of the genus such as antioxidant, anti-inflammatory, antidiabetic, cytotoxicity and antimicrobial activities [14-18, 6].

The purpose of this review is to emphasize the importance of the genus *Coccoloba*as well as to highlight its biological investigations, pharmacological potentials and isolated and

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identified phytoconstituents from various classes. The data presented in this review were gathered and collected from 2020 up till 2024 in accordance to the last review [19].

1. Methodology

Several literature sources were used to gather the data, i.e., Google Scholar, PubMed, Egyptian Knowledge Bank and Scopus with time limitation from 2020 to 2024 using the following keywords alone or in combination: *Coccoloba*, plant extract, phytochemical constituents, isolated compounds, and *in-vivo, in-vitro*, biological activities. The structures of compounds were drawn using ChemDraw Ultra 8.0 program (Revvity signals, Waltham, Massachusetts, USA).

3. Different phytochemical constituents in *Coccoloba*species

Various compounds from different classes were identified/isolated from the genus *Coccoloba*such as phenolic acids, flavonoids, lignin oligomers, triterpenes and alkaloids illustrated in Fig. 1. Compared to our last review [19], three anthocyanins, three alkaloids, one coumarin, one chalcone and 4 lignin oligomers were newly identified through high-performance liquid chromatography alone or coupled with mass spectrometry HPLC/HPLC-MS. Three flavonoids *C*-glycosides were identified in *C. alnifolia*for the first time. In addition, methoxyflavonoids were newly isolated and identified in the species *C. cowellii*. The new reports would be discussed in the following sections. Different phytochemicals identified in *Coccoloba* species are summarized in Table 1 and Fig. 1.

Phenolic acids

Phenolic acids (PAs) are among the most prevalent bioactive substances in the plant world. They are, involved in growth, reproduction, and defense against microbes and environmental stress. Medicinally, they have significant roles in numerous crucial biological processes like anti-aging and prevention or alleviation of serious illnesses like HIV, diabetes, cardiovascular disease, and cancer [20].

Several phenolic acids were identified in *Coccoloba* species. They were rich in simple phenolic acids, e.g., gallic, vanillic and protocatechuic acids as well as phenyl propanoids, e.g., coumaric acid, caffeic acids along with their esters and/or derivatives [21, 22]. Two unusual acids, piscidic and eucomic acids were identified from hexane extract of *C. uvifera* leaves [22]. Both acids had promising activities. Fraction containing piscidic and eucomic acids obtained from *Opuntia ficus-indica* was reported to have the lowest IC_{50}0.03 ± 0.01mg/mL in ABTS colorimetric assay in comparison with total extract and other fractions of the plant. In addition, UV-protective property of the fraction was evaluated through its sun protection factor with value 2.23 ± 0.41 which is corresponding to 50% UV protection. Furthermore, piscidic/eucomic acids fraction was able to counteract the damaging effects of UVA irradiation on GSH and on UVA-induced cell death [23]. In another study, piscidic acid obtained from cladodes decoctions was investigated for anti-hypercholesterolemic activity *via* 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) inhibition assay and molecular docking. Piscidic acid revealed a result with IC_{50} of 149.6 µg/mL indicating a promising anti-hypercholesterolemic agent [24].

Flavonoids

A widely distributed class of bioactive substances classified as polyphenolic compounds found in fruits, vegetables, and drinks derived from plants characterized by the flavan nucleus [25]. Flavonoids can lower the risk of a number of chronic illnesses, such as cardiovascular disease (CVD), cancer, and neurological problems when taken daily [26]. Many activities were reported for flavonoids such as anti-inflammatory, antiviral, antimicrobial, antiulcerative, antidiabetic, hepatoprotective and many others. As a result, flavonoids are a unique compounds which could be used to control various human diseases [27]. Several classes of flavonoids were detected in *Coccoloba*species. They were categorized in (flavonols, flavanols, flavones, methoxyflavonoids, chalcones and anthocyanins). Most identified flavonoids were *O*-glycosides while only three compounds were C-glycosides (vitexin, isovitexin and isoorientin). The majority of the flavonoids were flavonols and methoxyflavonoids identified in *C. cowellii* and *C. uvifera.*

Lignin oligomer

One of the primary components of lignocellulosic biomass is lignin. lignin utilization indicate promising potentials considering green chemistry, energy, chemical engineering, physical chemistry, materials and polymer science, and biochemistry [28]. Coniferyl, sinapyl, and *p*-coumaryl alcohols are the three main types of monolignols that form this three-dimensional, highly cross-linked polymer [29]. This polyphenolic compoundpossesses many significant biological activities such as antioxidant, antimicrobial, antidiabetic cytotoxic and antitumor activities [30]. Only one report has identified in 90% methanolic fraction of *C. cowellii l*eaves extract four isomers of lignols three trilignols and one tetralignol which differ at site of monomers' arrangement [31].

Triterpenes

Triterpenes are derivatives of isoprenoid with 30 carbon atoms that are arranged into various classes according to their squalene precursor cyclization and rearrange reactions. Several triterpenes demonstrated significant pharmacological effects, as was previously reported, including anti-inflammatory,

anticancer, immunomodulatory effects [32-34]. Three triterpenes were identified in the hexane leaves extract of *C. uvifera*[35].

Alkaloids

Alkaloids are group of compounds derived from amino acids characterized by a nitrogen atom in their heterocyclic ring with a broad range of pharmacological activities [36]. Three alkaloids were newly identified in the genus *Coccoloba*. 4-Methylquinazoline-2-carboxamide and tryptamine from the butanol fraction of *C. alnifolia* leaves extract as well as caffeine from *C. uvifera* leaves extract [21, 37]. Caffeine is a xanthine alkaloid and the most often consumed stimulant worldwide with psychological activity [38]. Caffeine demonstrated a synergistic additive benefit with medications in the treatment of asthma, low blood pressure, attention deficit hyperactivity disorder, gall bladder diseases, infant shortness of breath and weight loss [39-45].

3.1. Isolated compounds from different *Coccoloba* species

A bioassay-guided fractionation resulted in the isolation of 5 flavonoids from theleaves methanolic extract (ME)of *C. cowellii*. The isolated flavonoids were quercetin and four methoxyflavonoids, namely, 3-*O*-methylquercetin, 6-methoxymyricetin-3,4`-dimethyl ether,myricetin-3,3`,4`-trimethyl ether and 6-methoxymyricetin-3,3`,4`-trimethyl ether [31]. These compounds were reported for the first time for the plant and the genus. However, it was shown that the total extract had a higher antifungal activity against *C. albicans* when compared to the active fraction or the isolated compounds.

Nine compounds were isolated from the leaves of *C. uvifera*ME these compounds were identified as protocatechuic acid and its methyl ester, gallic acid and its methyl ester, kaempferol 3-*O*-*β*-D-neohesperidoside, quercetin 3-*O*-*β*-D-glucopyranoside (isoquercitrin), myricitrin 4″-*O*-gallate, myricetin 3-*O*-arabinopyranoside and myricetin 3-*O*-*β*-D-glucopyranoside [46].

In addition, lupeol was isolated fromthe hexane extract of *C. uvifera* leaves. It was identified using different methods against standard lupeol. It was further encapsulated into electrospun nanofibers to improve its permeability [47].

3.2. Identified compounds through HPLC Analysis

Different compounds were identified through high-performance liquid chromatography- mass spectrometry from various species of *Coccoloba* all are illustrated in Table (1). *C. cowellii* 90% methanolic fraction resulted from the leaves ME was chemically profiled using UHPLC-HRMS.The results showed flavonoids, methoxy flavonoids, lignin oligomers [31].

In another study, applying dereplication of the UHPLC-HRMS data, using combination of Global Natural Products Social Molecular Network (GNPS) feature-based molecular networking (FBMN) analysis along with the data interpretation of the MS/MS and comparison with the literature, thirteen compounds in all were found in ME of leaves of *C. cowellii*. Moreover, 80% ME of leaves of *C. cowellii*showed flavonoids, phenolic acids as a major compounds Table (1) [48].

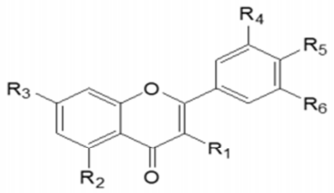
Three phenolics were identified by HPLC-DAD analysis of the water extract (WE)of *C. alnifolia*, and they were assessed at various UV spectra. Gallic acid, *p*-coumaric acid, and vitexin were identified when compared to authentic standards. The TLC findings confirmed the presence of vitexin in HPLC analysis. However, vitexin and isovitexin were identified using standards in co-TLC analysis for water and ethanolic extracts of *C. alnifolia*[49].

For acetone and butanol fractions of *C. alnifolia*leaves extract, the HPLC-DAD analysis revealed the presence of phenolic components such *p*-coumaric, gallic acids and vitexin. While the GNPS identified the presence of tryptamine,4-methylquinazoline-2-carboxamide, haploperoside E, isoorientin, vitexin, and kanakugiol in the butanol fraction analyzed by HPLC MS/MS [37].

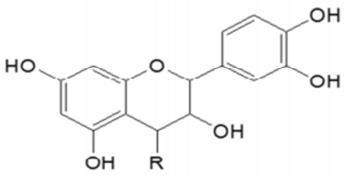
Furthermore, in another experiment seventeen compounds were identified in *C. uvifera*ethanol,aqueousand acetone extracts against standards using HPLC; these compounds are illustrated in Table (1) [21]. Hexane extract fractions of *C. uvifera*leaveswere subjected to HPLC-MS, fourteen compounds were identified. These compounds were five organic acids (piscidic, eucomic, ferulic, caffeic and quinic acid), lupeol, acacetin, myricetin-3-*O*-hexoside, digalloyl-glucose, N-caffeoyl agmatine, 1,8,10-trihydroxy-9-anthrone, benzyl-*O*-galloyl glucose and β-sitosterol [22]. In addition, lupeol, *α*-amyrin and *β*-amyrin were identified in hexane leaves extract by HPLC-MS [35].

Three anthocyanins were identified in the sea grape fruits extract, namely, malvidin-3-glucoside, cyanidin-3-glucoside, and petunidin-3-glucoside as analyzed by HPLC–MS. This extract was incorporated in submicron emulsion which improved the stability of the extract [50].

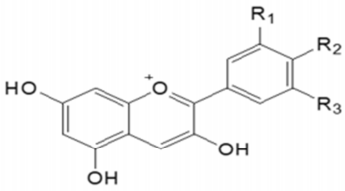
In additions, thirty secondary metabolites were identified by GC-MS analysis of *C. peltata* Schott leaves extract that was grown in Egypt. The major constituents were α-tocospiro A (17.20%), α-tocospiro B (12.02%), squalene (7.78%), 13-epi-manoyl oxide (6.80%), nonacosane (5.12%), pentatriacontane (4.17%), and hexadecanoic acid methyl ester (3.78%) [51].



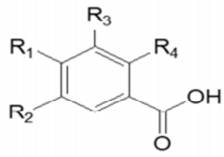
	R_1	R_2	R_3	R_4	R_5	R_6
Quercetin	OH	OH	OH	H	OH	OH
Myricetin	OH	OH	OH	OH	OH	OH
Kaempferol	OH	OH	OH	H	OH	H
Apigenin	H	OH	OH	H	OH	H
Luteolin	H	OH	OH	H	OH	OH
3-*O*-methylquercetin	OCH_3	OH	OH	H	OH	OH



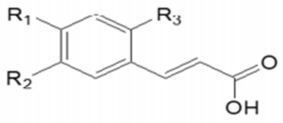
	R
Epi/catechin	H
Procyanidin B1	Epi/catechin



	R_1	R_2	R_3
Malvidin	OCH_3	OH	OCH_3
Cyanidin	OH	OH	H
Petunidin	OCH_3	OH	OH

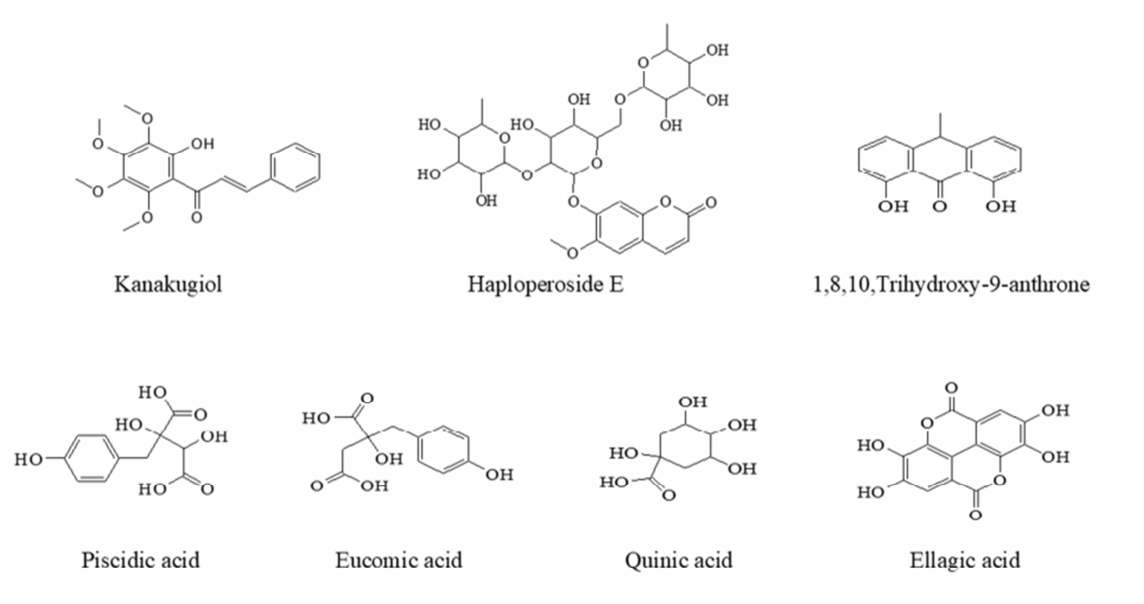


	R_1	R_2	R_3	R_4
Gallic acid	OH	OH	OH	H
Vanillic acid	OH	OCH_3	H	H
Syringic acid	OH	OCH_3	OCH_3	H
Salicylic acid	H	H	H	OH
P-Hydroxy benzoic acid	OH	H	H	H



	R1	R2	R3
p-Coumaric acid	OH	H	H
o-Coumaric acid	H	H	OH
Caffeic acid	OH	OH	H
Ferulic acid	OH	OCH3	H

Figure 1: Main identified compounds from different classes in the genus *Coccoloba*



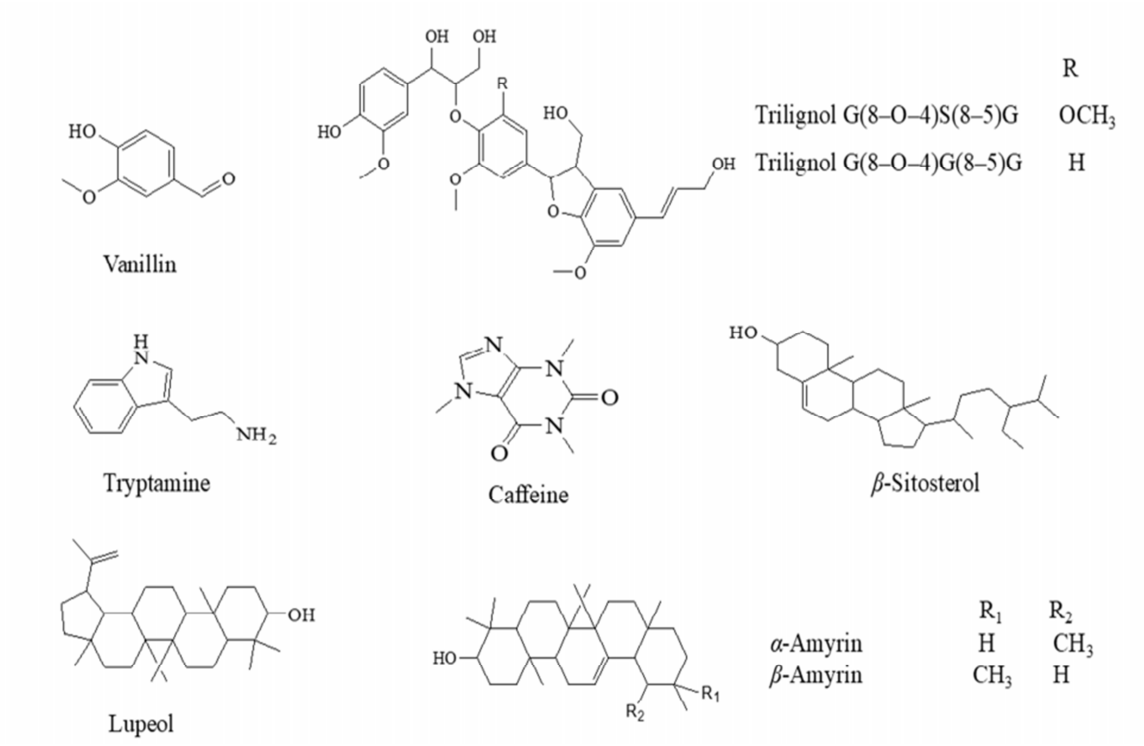


Figure 1: Continued.

Table 1: **Identified phytochemical compounds from different species of *Coccoloba* genus**

Compound	Species	Part used/solvent	Reference
Flavonols			
Quercetin Myricetin	*C. uvifera*	Aqueous, acetone, ethanol leaves extracts	[21]
Myricetin-3-*O*-galactoside	*C. cowellii*	80% methanol leaves extract	[48]
Myricetin-*O*-glucuronide	*C. cowellii* *C. cowellii*	80% methanol leaves extract 90% methanolic fraction of leaves extract	[48] [31]
Myricetin-3-*O*-deoxyhexoside Quercetin-*O*-hexoside Quercetin-3-*O*-glucuronide	*C. cowellii*	80% methanol leaves extract	[48]
Quercetin-O-pentoside	*C. cowellii* *C. cowellii*	80% methanol leaves extract 90% methanolic fraction of leaves extract	[48] [31]
Rutin	*C. uvifera*	Aqueous, acetone, ethanol leaves extracts	[21]
Flavanol			
Catechin Epicatechin Epicatechin-3-*O*-gallate Procyanidin B1 monogallate	*C. cowellii*	80% methanol leaves extract	[48]
Flavone			
Vitexin	*C. alnifolia* *C. alnifolia*	Water leaves extract Acetone and butanol fractions from aqueous leaves extract	[49] [37]
Isovitexin	*C. alnifolia*	Water leaves extract	[49]
Isoorientin	*C. alnifolia*	Butanol fraction from aqueous leaves extract	[37]
Methoxyflavonoids			
3-O-Methylquercetin 6-Methoxymyricetin 3,4' -dimethyl ether 6-Methoxymyricetin 3,3',4' -trimethyl ether Myricetin 3,3' ,4' -trimethyl ether Methoxyquercetin dimethyl ether Methoxymyricetin tetramethyl ether Myricetin tetramethyl ether	*C. cowellii*	90% methanolic fraction of leaves extract	[31]
Acacetin	*C. uvifera*	Hexane extract of leaves	[22]
Anthocyanins			
Cyanidin-3-glucoside Malvidin-3-glucoside Petunidin-3-glucoside	*C. uvifera*	Fruits extract	[50]
Chalcone			
Kanakugiol	*C. alnifolia*	Butanol fraction from aqueous extract	[37]
Coumarins			
Haploperoside E	*C. alnifolia*	Butanol fraction from aqueous extract	[37]
Anthraquinones			
1,8,10-Trihydroxy-9-anthrone	*C. uvifera*	Hexane extract of leaves	[22]
Phenolic acids			
Gallic acid	*C. cowellii* *C. alnifolia*	80% methanol leaves extract Water leaves extract	[48] [49]

Table 1: **Continued.**

	C. alnifolia *C. uvifera*	Acetone fraction from aqueous leaves extract Aqueous, acetone, ethanol leaves extracts	[37] [21]
p-coumaric acid	*C. alnifolia* *C. alnifolia* *C. uvifera*	Water leaves extract Acetone fraction from aqueous leaves extract Aqueous, acetone, ethanol leaves extracts	[49] [37] [21]
o-Coumaric acid	*C. uvifera*	Aqueous, acetone, ethanol leaves extracts	[21]
Eucomic acid Piscidic acid	*C. uvifera*	Hexane extract of leaves	[22]
Caffeic acid	*C. uvifera* *C. uvifera*	Hexane extract of leaves Aqueous leaves extracts	[22] [21]
Ferulic acid	*C. uvifera*	Aqueous, acetone, ethanol leaves extracts	[21]
Ferulic acid 4-*O*-glucoside Quinic acid Digalloyl-glucose Benzyl-O-galloyl glucose	*C. uvifera*	Hexane extract of leaves	[22]
N-caffeoyl agmatine	*C. uvifera*	Hexane extract of leaves	[22]
p-Hydroxy benzoic acid Salicylic acid Ellagic acid Syringic acid Vanillic acid	*C. uvifera*	Aqueous, acetone, ethanol leaves extracts Aqueous, ethanol leaves extracts	[21]
Phenolic aldehyde			
Vanillin	*C. uvifera*	Aqueous, acetone, ethanol leaves extracts	[21]
Lignin oligomer			
Trilignol G(8–O–4)G(8–5)G Trilignol G(8–O–4)X(8–8)X Trilignol G(8–O–4)S(8–5)G Tetralignol G(8–O–4)G(8– O–4)S(8–8)S	*C. cowellii*	90% methanolic fraction of leaves extract	[31]
Alkaloids			
4-methylquinazoline-2-carboxamide tryptamine	*C. alnifolia*	Butanol fraction from aqueous leaves extract	[37]
Caffeine	*C. uvifera*	Aqueous, acetone, ethanol leaves extracts	[21]
Sterols			
β-sitosterol	*C. uvifera*	Hexane extract of leaves	[22]
Triterpenes			
Lupeol	*C. uvifera*	Hexane extract of leaves	[35, 22]
α-amyrin *β*-amyrin	*C. uvifera*	Hexane extract of leaves	[35] [35]

4. Pharmacological potentials of *Coccoloba* species

Compared to our last review [19], since 2020, various biological activities were further propagated for various *Coccoloba* species, e.g., such as antimicrobial, antioxidant, anti-inflammatory, anticholinesterase, mutagenic, and cytotoxic activities. However, four newly investigated biological potentials were evaluated, namely, antiprotozoal, smooth muscle relaxant, immunomodulatory and hepatoprotective activities. As will be discussed in the following sections, the investigated species were *C. cowellii, C. peltata, C. uvifera* and for the first time *C. alnifolia.* Several *in vitro* assays were done to evaluate the antioxidant activity. In addition, *in vivo* antioxidant assay using *Caenorhabditis elegans* model was performed for two *C. alnifolia* extracts [49]. Enzymes inhibition assays against COX-1 and COX-2 were performed for the assessment of *C. cowellii* anti-inflammatory activity [31]. Nitric oxide reduction assay was done for determining *C. alnifolia* extracts immunomodulatory effect [37]. In addition, an *ex vivo* assay was done for different *C. uvifera* leaves extracts to investigate their vasorelaxant and spasmolytic activities [52].

4.1. Antifungal/antibacterial/ Antiprotozoal

The antimicrobial activity of 80% ME of *C. cowellii* leaves was screened and examined against a wide range of microorganisms, including yeast, mold, protozoa, and both Gram-positive and Gram-negative bacteria. At the studied concentrations (0.25 to 128

µg/mL), the extract was generally inactive against *Aspergillus fumigatus, Escherichia coli, and Staphylococcus aureus*. Conversely, the extract exhibits modest efficacy against the parasites *Trypanosoma brucei, T. cruzi*and a potent antifungal impact against *Candida albicans* and *C. neoformans* with IC_{50} values of 1.7 ± 0.6 and 2.7 ± 2.0 µg/mL, respectively [48].

Similar to the last study, at doses of 64 µg/mL and less, *C. cowellii* 80% ME of leaves and its fractions were generally inactive against *Aspergillus fumigatus*. A robust antifungal action was demonstrated by the total extract against several strains (*C. neoformans*, *C.albicans, C. parapsilosis, C. glabrata*) except for *C. tropicalis*. With higher IC_{50} values, only the fraction 90% MeOH exhibited behavior that was comparable to the original extract. IC_{50} values against the investigated *Candida* strains ranged from 2.4 ± 0.4 to 13.3 ± 1.1µg/mL for the 90% MeOH fraction while for the total extract it ranged from 0.4± 0.0 to 21.2 ± 1.8 µg/mL for the *Candida* strains and *A. fumigatus* [31].

When compared to ciprofloxacin and clotrimazole, the DCM/MeOH(1:1) leaves extract of *C. peltata* had strong antibacterial/antifungal activities against *E. coli*, *Bacillus subtilis*, and *C. albicans*, with activity indices of 46.1, 73.9, and 81.5%, respectively. %Activity Index is calculated as zone of inhibition by tested compound (diameter)/ zone of inhibition by standard (diameter) x 100. Meanwhile, its fractions they showed lower antibacterial/antifungal activities compared to the total extract [51].

Different *C. uvifera* extracts were examined for their antibacterial and antifungal activities. It was active against three fungal pathogens obtained from strawberry plants, namely, *Fusarium culmorum, Rhizoctonia solani,* and *Botrytis cinerea.* A wood treated with 3% ethanolic leaves extract demonstrated the greatest growth inhibition against *R. solani, B. cinerea, and F. culmorum*, with mycelial growth being inhibited by 64.4%, 100%, and 38.5%, respectively. On the other hand, *C. uvifera* extracts revealed moderate activities against the growth of six phytopathogenic bacteria, namely, *Agrobacterium tumefaciens*, *Ralstonia solanacearum, Erwinia amylovora*, *Pectobacteriumatrosepticum*, *P. carotovorum* subsp. *carotovorum* and *Dickeyasolani* [21].

It could be concluded that *Coccoloba* species had moderate to potent antifungal activity and weak to moderate antibacterial activity.

4.2. Antimutagenic activity

The Fractions of *C. uvifera* hexane leaves extract were assessed for their antimutagenic activity using Ames test. Four fractions were able to reduce the mutagenicity of sodium azide on the TA100 test strain of *Salmonella typhimurium*. The activity was attributed to lupeol, acacetin and *β*-sitosterol as disclosed by HPLC [22]. In another study, *C. uvifera*hexane extract loaded in nanofibers elaborated by electrospinning with the biopolymers gelatin (G)/high-grade polymerization agave fructans (HDPAF) revealed a significant effect on the mutagenicity induced by sodium azide on the TA100 strain at 1 µg/mL[35].

Phenolic extract of *C. uvifera* fruits extract was microencapsulated with maltodextrin using spray drying. Both the extract and its microencapsulated form displayed a potent antimutagenic activity, more than 85% protection by 1mg/ml sample. However, microencapsulation provided the extract with thermo- and photoprotection against UV radiation while maintaining its antimutagenic action against sodium azide [53].

4.3. Cytotoxic activity

No cytotoxic activity for 80% ME of *C. cowellii* leaves was detected when tested against MRC-5 SV2 (human fetal lung fibroblasts) cells (IC_{50} > 64.0 µg/mL) [48]. However, only the hexane fraction had activity (IC_{50} 29.3 ± 1.5 µg/mL) [31].

The DCM/MeOH (1:1) leaves extract of *C. peltata* had promising anticancer activity against the cancer cell lines HepG-2, HCT-116, MCF-7, HeLa and PC3 (IC_{50} 7.56-14.26 µg/mL). In contrast, the tested fractions showed lowered activity in comparison to the total extract [51].

Six extracts were prepared from *C. alnifolia* extracts, namely, hexane, chloroform, ethanol, methanol, water end extract and total aqueous extract. All extracts were generally not cytotoxic to six tumor and normal cell lines[49].

ME (70%) of *C. uvifera* leaves and its isolated compounds had anticancer activity against Ehrlich ascites carcinoma cells (EACC) when tested at 100 µg/mL. Protocatechuic acid revealed the highest cytotoxic activity (78.71 ± 0.21% dead cells), followed by gallic acid and isoquercitrin (> 70% inhibition) [46].

Using the MTT assay, three fractions from hexane extract of *C. uvifera* leaves were examined for the antiproliferative activity in HeLa, HCT 116, and ARPE-19 cells. In HeLa cells, IC_{50} of the fractions were comparatively high (147.12 to 178.04 µg/mL) compared to cisplatin. One fraction showed the highest antiproliferative activity against the HCT 116 cells with IC_{50} value 24.2 ± 9.5 µg/mL demonstrating no significant difference to cisplatin. This fraction revealed IC_{50} on ARPE-19 normal cell line of 112.2 ± 3.9 µg/mL classifying it as a bioselective compounds [22]. In another study, nanofibers loaded with 50 µg of extract/mL of encapsulated *C. uvifera* hexane leaves extract significantly reduced the rate of

proliferation of (M12.C3.F6), with a dose dependent activity and had antimutagenic activity in Amos test [35].

Using resazurin assay multiple concentrations of lupeol rich fraction from *C. uvifera* extract were examined for their cytotoxicity potential on human colon adenocarcinoma cell line (Caco-2). Concentrations from 10-50 µg/mL didn't result in a reduction in cell viability. Even yet, there was a 10% reduction in cell viability at 50 µg/mL, even if this decrease was not significantly different. In comparison, applying 100 µg/mL of the fraction resulted in a significant 39% decrease in the percentage of cell viability [47].

Ultimately, it can be concluded that cytotoxic activity of *Coccoloba* species should be examined at the compound level. Different fractions from the same plant have contradictory results.

4.4. Anti-inflammatory

Using a cell-free experiment, the *C. cowellii* 80% leaves ME effectively reduced the activity of COX-1 and COX-2 enzymes in a dose-dependent manner. on the extract had higher inhibition against COX-1 (IC_{50} 4.9 µg/mL) than on COX-2 (IC_{50} 10.4 µg/mL) [48]. This is disappointing as the extract would not be used as anti-inflammatory because of its very poor selectivity.

4.5. Antioxidant activity

Antioxidants play a critical role in maintainingaerobic species survival by preventing oxidative stress. Antioxidants work as electron donors, reducing highly reactive species to theirmoe stable reduced forms [54].

The leaves extract of *C. peltata* was assessed for 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity; it showed a comparable activity (IC_{50} 32.86 µg/mL) with that of ascorbic acid (IC_{50} 29.70 µg/mL). Meanwhile, the fractions exhibited lower radical scavenging activity; the chloroform, hexane, and butanol fractions had IC_{50} values of 46.05, 57.42 and 50.06 µg/mL, respectively[51].

The ferric reducing power test and the DPPH free radical scavenging activity were used to assess the antioxidant activity of the ethanol extract (EE) of *C. cowellii*leaves. The extract showed 34.01 ± 4% inhibition at 50 µg/ml in both assays which was comparable to the ascorbic acid employed as standard in both assays. The extract's total phenolic, tannin, and flavonoid levels were in line with its antioxidant activity [55].

The antioxidant activity of the ME of *C. uvifera* leaves and the isolated compounds were assessed using four assays: radical cation, DPPH,ferric-reducing power (FRAP) and 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The total extract and isolated compounds showed significant antioxidant activities in the four assays [46].

Furthermore, *C. uvifera* fruits extract demonstrated antioxidant capacity for ABTS and DPPH assays by 128.95 ± 1.00 and 26.18 ± 0.60 µg Equivalent Trolox/mL, respectively [50]. The fractions of *C. uvifera* hexane extract exhibited low inhibitory activity against DPPH (< 10.78%) than against ABTS (< 32%) at 5 mg/mL. On the other hand, the fractions showed low capacity for the chelation of ferrous ions assay also at 5mg/mL [22]. Furthermore, the hexane extract of *C. uvifera* demonstrated a 24.6% ± 0.6% inhibition for the DPPH radical [47]. These results indicated a weak antioxidant activity for the *C. uvifera* hexane extract and its fractions.

Various *C. alnifolia* leaves extracts were assessed for the antioxidant activity through different assays. The *C. alnifolia* ME revealed the highest antioxidant activity along with its EE in total antioxidant capacity, reducing power and superoxide radicle scavenging assays. Chloroform extract illustrated the highest inhibition for DPPH assay at 250 μg/mL. In additions, polar extracts of *C. alnifolia* ethanol, methanol, water end and water extracts showed the highest comparable activities for the reducing power assay in reducing potassium ferricyanide into potassium ferrocyanide at 250 μg/mL with similar result for the superoxide radicle scavenging assay [49].

Furthermore, *in vivo* antioxidant assay was assessed for the EE and WE of *C. alnifolia* leaves using *Caenorhabditis elegans* modelwith 2 different concentrations 1 and 10 mg/mL. These worms were then examined to determine whether they could survive the oxidative stress treatment with tert-butyl hydroperoxide (t-BOOH). With EE and WE treatments, there was an increased resistance to oxidative stress conditions. The average survival time for the control animals in the EE treatments was 7.5 hours, while the average survival time for the animals treated with EE (1 mg/mL and 10 mg/mL) was 10.5 hours, signifying a 28.6% increase. On the other hand, WE showed 7.9 hours survival time with 39.2% increase than control at 1mg/mL and 8.2 hours survival time with 41.3% increase than control at 10 mg/mL [49].

The RAW 264.7 macrophage cell line was used to investigate the intracellular ROS levels stimulated by a stress inducer LPS and treated with EE and WE of *C. alnifolia.* At all the three tested concentrations (100, 250, and 500 µg/mL), there was a reduction in ROS level ≥50% for both the EE and WE [37].

Total antioxidant capacity, DPPH, FRAP, superoxide radicle scavenging, and copper chelating activities were determined for the ethyl acetate (AF) and butanol fractions (BF) partitioned from aqueous extract of *C. alnifolia*. The BF revealed the highest activity in the five assays when tested using three

concentrations (100, 250, and 500 µg/mL) [37]. The production of reactive oxygen species (ROS) in zebrafish was traced using the oxidation-sensitive fluorescent probe, Dichloro-dihydro-fluorescein diacetate (DCFH-DA). The embryos were treated first with samples at 500 µg/mL then the stressor agent (H_2O_2) was added. It was found that these animals were protected from the presence of H_2O_2 by the EE, AE, BF, and AF which had a ROS protective effect [37].

4.6. Immunomodulatory activity

Immunomodulatory activity for two *C. alnifolia* extracts was evaluated through nitric oxide reduction assay using RAW 264.7 macrophage cell cultures. EE reduced nitric oxide (NO) production of about 50% while WE caused 30% reduction at the same concentration (500 µg/mL). Furthermore, both extracts EE, WE showed about 30% and 50% inhibition of phagocytosis activity, respectively. On the other hand, BF and AF fractions of *C. alnifolia* reduced NO production effectively by approximately 40–50% at the same concentration, indicating better activity than WE [37].

Moreover, incomparison to the positive control (LPS), the EE of *C. alnifolia* leaves induced a little suppression of TNF-α and encouraged the production of IL-17. Analysis of additional cytokines revealed no significant difference to the positive control. On the other hand, the WE had IL-17 levels that were comparable to the positive control and a minor increase in TNF-*α* production in comparison to the positive control. Both extracts showed inhibition for the mRNA expression of IL-10 and iNOS. The authors concluded that the total extracts and fractions had potential immunomodulatory activity [37].

4.7. Smooth muscle relaxation

Different WE of *C. uvifera* leaves were examined for their vasorelaxant and spasmolytic activities using *ex-vivo* assay; meanwhile, the *in vivo* assay was used to investigate the antiperistaltic and diuretic effects. *Ex vivo* experiment was performed on segments of ileum tissues. Infusion, maceration, decoction and digestion aqueous extracts demonstrated a moderate vasorelaxant and spasmolytic in a dose-dependent manner. Percolation aqueous extract showed the least activity while the higher activity was for the decoction, overall activities of the four extracts were lower than those of papaverine and carbachol. Furthermore, *in vivo* assay showed that *C. uvifera* decoction showed the best antiperistaltic and diuretic effects [52].

Muscarinic acetylcholine receptor subtype 3 (m3AChR) is involved in gastrointestinal motility in physiological and pathophysiological conditions. Diterpene royleanone and triterpenes *α*- amyrin, *β*-amyrin, lupeol and *β*-sitosterol previously identified/isolated from *C. uvifera* were assessed *in silico* against m3AChR to predict their antimuscarinic activity. Binding energies of *β*-sitosterol and royleanone to m3AChR were −9.08 and −8.59 kcal/mol, respectively which were less than scopolamine (-10.18 kcal/mol) [52].

4.8. Anti-cholinesterase activity

The ME of stems of *C. uvifera* was assessed for its anticholinesterase and butyrylcholinesterase activity. The results demonstrated that *C. uvifera* extract revealed anticholinesterase activity with IC_{50} 3.67 ± 0.160 µg/mL, and anti-butyrylcholinesterase activity with IC_{50} 5.60 ± 0.149 µg/mL. Furthermore, the extract was evaluated using behavioral tests for improving learning and memory functions on chronic cerebral hypoperfusion (CCH) rats. Oral administration of *C. uvifera* extract did not affect basic motor function or exploratory behavior of CCH rats in the automated open field test, yet it did not ameliorate the learning and memory dysfunctions in CCH rats [56].

4.9. Hepatoprotective

C. uvifera had hepatoprotective activity against thecarbon tetrachloride (CCl_4) induced hepatotoxicity in rats. The results showed that sea grapes, significantly improved serum glucose levels, lipid profiles, liver enzymes, and kidney functions. Subsequently, sea grape might be used as a complementary medicine in CCl_4 hepatotoxicity [57].

All the reported biological activities of the genus *Coccoloba*are illustrated in fig. (2). However, it could be observed that most studies were merely a screening for the therapeutic potential of *Coccoloba* extracts or fractions with no deep elucidation of the possible mechanism of action nor identification of the key bioactive metabolites. Indeed, more comprehensive pharmacological studies coupled with adequate identification of the key bioactive phytochemicals are urgently needed.

Figure 2: Reported biological activities from various species in the genus *Coccoloba*

5. Prepared formulations

Stability of *C. uvifera* fruits extract was evaluated in a submicron emulsion to protect the extract. A shear-thinning fluid was obtained from an ultrasound submicron emulsion showing a monomodal droplet size distribution of 0.424 μm, low viscosity (1.94 mPa s), and it was stable over time. The submicron emulsion containing the *C. uvifera* extract was thermostable up to 212 ˚C, as demonstrated by the thermogravimetric analysis (TGA). These emulsions can be incorporated into foods, dried for later usage as tablets, or added to beverages as nutraceuticals [50].

C. uvifera hexane leaves extract was encapsulated in nanofibers formed with the biopolymers gelatin (G)/high-grade polymerization agave fructans (HDPAF). Coupling the two polymers allowed the stabilization of the solutions' surface tension, viscosity, and electrical conductivity. The fibers containing the *C. uvifera* extract had homogenous nanometric diameters and morphology. The assessed biological activities of the *C. uvifera* extract were maintained when encapsulated in nanofibers created by electrospinning with the G/HDPAF (1:1) formulation. Moreover, it could be considered as a viable formulation for the protection and use of high biological value compounds [35].

6. Conclusion

This review highlights the recent scientific findings regarding the bioactivity and phytochemistry of the genus *Coccoloba* and its high importance globally as evidenced by its ethnobotanical, phytochemical, medicinal values. *Coccoloba* is an important genus that could be utilized in many different types of therapies, particularly for diabetes, inflammation, asthma, infection, memory loss and cancer, according to the scientific research that has been performed on different species. Numerous compounds, including flavonoids, phenolic acids and their glycosides, anthraquinones, lignins, and triterpenoids, are thought to be responsible for *Coccoloba* therapeutic potentials. The growing therapeutic significance of *Coccoloba*is encouraging researchers for more investigation of the undiscovered species in this genus to potentially uncover more phytochemicals as well as pharmacological activities. These investigations have the potential to improve the medication system by identifying novel components that promote human welfare. However, there is still a lot of questions to be answered by significant researches. First, no sufficient information is available about the safety of genus *Coccoloba* on long term administration. Moreover, the lack of studies concerned with the antiviral activity, pharmacokinetics of *Coccoloba* extracts, and clinical studies aiming at verifying the *in vivo* animal models. Further investigations are required to be done on other species and to explore new bioactive compounds and their pharmacological activities. Concurrently, detailed studies about the mechanism of action and molecular targets of *Coccoloba* phytoconstituents should be considered.

7. Conflict of interest

The authors have no conflicts of interest to declare.

8. List of abbreviations

MRC-5 SV2	human fetal lung fibroblasts
HepG-2	hepatoblastoma cell line
HCT-116	Colorectal Carcinoma
MCF-7	breast cancer cell line
PC3	human prostate cancer
EACC	Ehrlich ascites carcinoma cells
NIH/3T3	Fibroblast cell line
ARPE-19	Spontaneously arising retinal pigment epithelia cell line
Caco-2	Human colon adenocarcinoma
COX-1	Cyclooxygenase1
COX-2	Cyclooxygenase2
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation
EE	Ethanol extract
ME	Methanol extract
WE	Water extract
AF	Ethyl acetate fraction from *C. alnifolia* water leaves extract
BF	Butanol fraction from *C. alnifolia* water leaves extract
DCFH-DA	Dichloro-dihydro-fluorescein diacetate
TNF-α	Tumor necrotic factor α
IL-10/IL-17	Interleukins
CCL_4	Carbon tetrachloride

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