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Artigo

PHYTOCHEMICAL INVESTIGATION OF *Wissadula periplocifolia* (L.) C. Presl AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

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A phytochemical study on the aerial parts of *Wissadula periplocifolia* using chromatographic techniques has led to the isolation of sitosterol (1a), stigmasterol (1b), sitosterol 3-*O*-β-D-glucopyranoside (2a), stigmasterol 3-*O*-β-D-glucopyranoside (2b), phaeophytin A (3), 13^2-hydroxy-(13^2-*S*)-phaeophytin A (4), phaeophytin B (5), 17^3-ethoxyphaeophorbide (6), 3,4-*seco*-urs-4(23),20(30)-dien-3-oic acid (7), 3-oxo-21β-*H*-hop-22(29)-ene (8), dammaradienone (9a), and taraxastenone (9b). The isolated compounds were characterised by spectroscopic analysis. A preliminary assay to evaluate the antibacterial activity of *W. periplocifolia* extracts and fractions showed that the dichloromethane, ethyl acetate, and n-butanol fractions were active against *Enterococcus faecalis.*

Keywords: Malvaceae; *Wissadula periplocifolia*; *Enterococcus faecalis.*

INTRODUCTION

Natural products are an important source of drug leads. The plant kingdom, with its diversity of compounds, has merited special interest in the search for new active substances due to the millennial use of several species to treat diseases[1]. Chemical constituents of plants such as salicylic acid, morphine, and quinine have contributed enormously to the development of drugs with important therapeutic applications used in modern medicine.[2] However, the use of higher plants as potential sources of new drugs is still largely unexplored, thus requiring research to shed light on our biodiversity and to discover new medicines.[3]

Previous studies on the Malvaceae family has led to the isolation of fatty acids,[4] steroids,[5] terpenoids,[6] phaeophytins,[7] flavonoids,[8] and alkaloids[9] from several species. Many Malvaceae species are traditionally used to treat diseases, e.g., the tea prepared with leaves of *Sidastrum micranthum*, a species rich in phenolic compounds, is used to treat asthma and bronchitis. The fruits of *Hibiscus esculentus*, popularly known in Brazil as "quiabo," are used to treat peptic ulcers.[10] The leaves of *Wissadula amplissima* demonstrated potential anti-inflammatory activity and are used by traditional African healers in the treatment of inflammation from spider bites and bee stings.[11] Many other studies have reported on the anti-inflammatory, antioxidant, and antimicrobial activities of the Malvaceae species.[5-12]

Aiming to contribute to the chemotaxonomic knowledge of the Malvaceae family and considering the absence of data describing the constituents of the *Wissadula* genus, *Wissadula periplocifolia* (L.) C. Presl was subjected to a phytochemical study to isolate and identify its constituents. In addition to that, an evaluation of the antibacterial activity of its extract and phases was carried out.

EXPERIMENTAL

General experimental procedures

Chromatographic separations were performed using a glass column filled with silica gel (ASTM, 230-400 mesh, Merck). TLC was performed on silica gel PF_{254} plates and the spots were visualized under UV light (254 and 366 nm) by exposure to iodine vapor and heating with vanillin–sulfuric acid reagent. The isolated compounds were identified by infrared (IR) spectroscopy (Perkin-Elmer, FT-IR-1750, and Shimadzu–Prestige 21) using KBr discs, and 1D and 2D NMR analysis (^{1}H 200 MHz, ^{13}C 50 MHz - Varian-Mercury and ^{1}H 400 MHz, ^{13}C 100 MHz–Bruker) using deuterated chloroform ($CDCl_3$) or pyridine (C_5D_5N). Melting points were measured using an MQAPF-302 apparatus (Microquimica Equipamentos Ltda).

Plant material

The aerial parts of *W. periplocifolia* were collected in the Pedra da Boca Park, located in Araruna City, Paraiba/Brazil, in August 2005. A voucher specimen (JPB 6498) was authenticated and deposited at the Lauro Pires Xavier Herbarium (JPB/UFPB).

Extraction and isolation

The plant material was dried in an oven at 40 °C for 72 h and then ground with a mechanical mill, yielding 8.9 kg of powder, which was submitted to maceration with ethanol for 3 d. This process was repeated to maximize the extraction. The ethanolic extract was concentrated using a rotary evaporator yielding 705 g of crude ethanol extract (CEE). The CEE (200 g) was solubilized in ethanol:water (9:1) and submitted to liquid–liquid extraction using hexane, dicloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and n-buthanol in a separation funnel. From this process we obtained 48 g of hexane fraction (HF), 33 g of CH_2Cl_2 fraction (DF), 28 g of EtOAc fraction (EAF), 9 g of n-buthanol fraction (BF), and 72 g of hydroalcohol fraction (HAF). Each fraction was analyzed by ^{1}H NMR to determine the compounds present in the samples.

HF (10 g) was subjected to column chromatography with silica gel and eluted with hexane, CH_2Cl_2, and methanol resulting in 56 fractions (100 mL), which were analyzed by thin-layer chromatography (TLC). The fraction 12/14 (90 mg) yielded a precipitate that, after washing with hexane, yielded 65 mg of colorless crystals

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(1). Fraction 55 (120 mg) was rechromatographed in the silica gel column and eluted with hexane, CH_2Cl_2, and methanol resulting in 30 fractions (30 mL). Fraction 20/27 from this procedure yielded 68 mg of a white powder (2), which was analyzed by NMR. Fraction 33/39 (400 mg) rechromatographed on the silica gel column afforded compound **3**, an amorphous dark green solid (25 mg). The silica gel chromatography of fraction 40/51 (355 mg) using hexane, CH_2Cl_2, and methanol led to the isolation of compounds **4** (17 mg), **5** (15 mg), and **6** (26 mg).

To isolate terpenes (indicated by ^{1}H NMR), HF (3.5 g) was submitted to chromatographic column from which it was eluted with hexane and CH_2Cl_2 to obtain 125 fractions of 20 mL. The resulting fractions were analyzed and combined by TLC and analyzed by ^{1}H NMR, which confirmed the presence of triterpenes in the fractions 48–65 (180 mg). In order to purify the individual compounds, preparative TLC was performed using Merk silica plates and Hex:EtOAc (9:1) as the mobile phase. After spraying vanillin–sulfuric acid reagent on the edges, five bands were detected, which were cut separately, washed with CH_2Cl_2:MeOH (1:1), and filtered to recover the isolated compounds. The separated compounds were analyzed by ^{1}H NMR, and the bands numbered as II (more polar), IV, and V (less polar) were identified as the compounds **7** (42 mg), **8** (6 mg), and **9** (25 mg), respectively.

The isolated substances were analyzed by IR and NMR, including two-dimensional NMR experiments.

3, 4-*Seco*-urs-4(23),20(30)-dien-3-oic acid (**7**): White amorphous solid; m.p. 268–270 °C; ^{1}H-NMR (pyridine-*d*5, 400 MHz): see Table 1; ^{13}C-NMR (pyridine-*d*5, 100 MHz): see Table 1. The ^{1}H- and ^{13}C-NMR spectral data are consistent with the published data.[13]

3-Oxo-21β-H-hop-22(29)-ene (**8**): Colorless needles; m.p. 249–251 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz): see Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1. The ^{1}H- and ^{13}C-NMR spectral data are consistent with the published data.[14]

Dammara-20(21),24-diene-3-one (dammaradienone) (9a): Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz): see Table 1. ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1. The ^{1}H- and ^{13}C-NMR spectral data are consistent with the published data.[15]

Taraxast-20(30)-en-3-one (taraxasterone) (9b): Colourless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz): see Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): see Table 1. The ^{1}H- and ^{13}C-NMR spectral data are consistent with the published data.[16]

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of triterpenes from *W. periplocifolia*

C	**7**[x]		**8**[y]		**9a**[y]		**9b**[y]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.70 (*m,* 2H)	29.73	2.07 (*m*)	31.77	1.43-1.90 (*m*, 2H)	39.97	1.47-1.96 (*m*, 2H)	39.66
2	2.53 (*m,* 2H)	35.55	2.28 (*m*)	33.96	1.94-2.44 (*m*, 2H)	34.10	1.94-2.44 (*m*, 2H)	34.15
3	-	176.9	-	218.29	-	218.00	-	218.06
4	-	148.42	-	47.09	-	47.4	-	47.39
5	2.16 (*m,* 1H)	50.51	2.06 (*m*, 1H)	47.60	1.34 (*m*, 1H)	55.37	1.34 (*m*, 1H)	54.93
6	1.46 (*m,* 2H)	26.26	1.45 (*m*)	20.74	1.45 (*m*)	19.67	1.45 (*m*)	19.67
7	1.04 (*m,* 2H)	33.11	1.10 (*m*)	34.35	1.31 (*m*)	34.76	1.42 (*m*)	33.36
8	-	41.09	-	42.58	-	40.37	-	40.84
9	1.00 (*m,* 1H)	41.16	1.44 (*m*, 1H)	43.58	1.37 (*m*, 1H)	50.30	1.41 (*m*, 1H)	49.85
10	-	42.96	-	36.59	-	36.93	-	36.85
11	1.13 (*m,* 2H)	22.59	1.44-1.28 (*m*)	21.54	1.54 (*m*)	21.92	1.54 (*m*)	21.99
12	1.46 (*m,* 2H)	27.28	1.46 (*m*)	24.26	1.91(*m*)	28.80	1.14 (*m*)	26.20
13	1.60 (*m,* 1H)	39.82	1.36 (*m*, 1H)	49.99	1.57 (*m*, 1H)	45.4	1.60 (*m*, 1H)	39.30
14	-	42.96	-	43.15	-	49.40	-	42.11
15	1.33 (*m,* 2H)	26.66	1.39 (*m*)	33.73	1.10-1.57 (*m*, 2H)	31.36	1.13 (*m*)	26.65
16	1.07 (*m,* 2H)	38.80	1.44-1.28 (*m*)	21.54	1.54-1.60 (*m*, 2H)	24.98	1.17-1.25 (*m*)	38.25
17	-	35.03	1.45 (*m*, 1H)	55.32	2.18 (*m*, 1H)	47.75	-	34.14
18	1.04 (*m,* 1H)	49.02	-	44.55	0.99 (*s*, 3H)	15.35	0.96 (*m*, 1H)	48.63
19	2.15 (*m*)	39.89	1.63 (*m*)	41.81	0.92 (*s*, 3H)	16.06	-	39.36
20	-	155.15	1.85 (*m*)	27.48	-	152.51	-	154.46
21	2.02 (*m,* 2H)	25.30	2.70 (*m*, 1H)	46.51	Ha: 4.70 (*br s*) Hb: 4.73 (*br s*)	107.59	1.57 (*m*)	25.61
22	1.01 (*m,* 2H)	39.59	-	149.35	1.97 (*m*)	34.10	1.39 (*m*)	38.88
23	Ha: 4.91(*br s*) Hb:4.99 (*br s*)	114.09	1.05 (*s*, 3H)	29.53	2.10 (*m*, 2H)	27.07	1.01 (*s*, 3H)	26.71
24	1.82 (*s,* 3H)	24.05	1.03 (*s*, 3H)	19.74	5.12 (*t*, 1H)	124.44	1.06 (*s*, 3H)	21.02
25	0.91 (*s,* 3H)	20.96	0.76 (*s*, 3H)	23.43	-	131.41	0.92 (*s*, 3H)	16.11
26	1.04 (*s,* 3H)	15.84	1.17 (*s*, 3H)	22.26	1.67 (*s*, 3H)	25.71	1.04 (*s*, 3H)	15.73
27	0.98 (*s,* 3H)	14.64	0.89 (*s*, 3H)	17.22	1.60 (*s*, 3H)	17.72	0.92 (*s*, 3H)	14.70
28	0.95 (*s,* 3H)	19.59	0.71 (*s*, 3H)	16.13	1.06 (*s*, 3H)	21.02	0.84 (*s*, 3H)	19.51
29	1.06 (*d,* 3H)	25.91	4.78 (br *s*, 2H)	110.22	1.01 (*s*, 3H)	26.76	0.99 (*d*, 3H)	25.48
30	Ha: 4.75 (*br s*) Hb: 4.80 (*br s*)	107.93	1.75 (*s*, 3H)	25.20	0.86 (*s*, 3H)	15.83	Ha: 4.59 (*br s*) Hb: 4.61 (*br s*)	107.34

[x]Pyridine-*d*5; [y]$CDCl_3$.

Antibacterial study

Test bacteria

Antibacterial activity was evaluated against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumonia*, and *Acinetobacter* spp. The bacterial species were obtained from the Hospital Universitário Lauro Wanderley and Microbiology Laboratory of Physiology and Pathology Department (Universidade Federal da Paraíba).

Agar diffusion well–variant

The plate hole diffusion assay (modified Kirby–Bauer method) was used to determine the growth inhibition of bacteria by *W. periplocifolia* crude ethanolic extract (CEE) and its hexane (HF), dicloromethane (DF), ethyl acetate (EAF), n-butanol (BF), and hydroalcoholic fractions (HAF). The microorganisms were grown in Müller–Hinton growth medium and incubated for 24 h at 36 °C.

The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile MH agar Petri dish. Then, using sterile tubes, wells (50-µL capacity) were made, and one was filled with 50 µL of the test sample. The concentrations used were 25, 50, 75, and 100 mg/mL and sterile distilled water was used as the negative control. After incubation of the cells for 24 h at 36 °C, bacterial growth was observed. Inhibition of the bacterial growth was measured in mm and the experiments were performed in triplicate. The sample was considered active when the inhibition zone was bigger than 10 mm.[17]

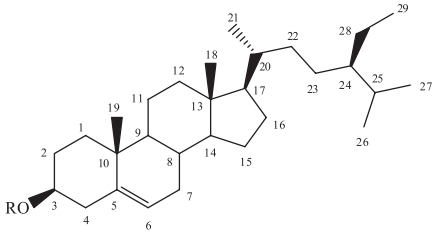
RESULTS AND DISCUSSION

Structural elucidation of the isolated compounds

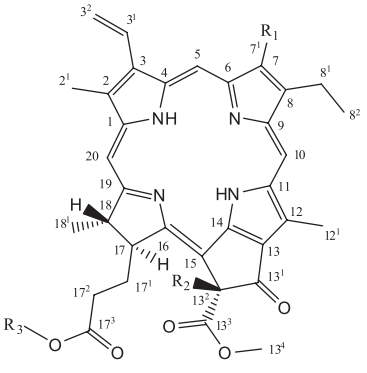
Twelve substances were identified from the hexane fraction of *W. periplocifolia* (Figure 1). The structural assignments of compounds 1 and 2 were performed based on spectral analysis and the data agreed well with those reported in the literature. Thus, they were identified as two steroid mixtures: sitosterol/stigmasterol (1)[18] and their glucopyranosides sitosterol 3-*O*-β-D-glucopyranoside/stigmasterol 3-*O*-β-D-glucopyranoside (2).[19]

The compounds **3**, **4**, **5,** and **6** had the same appearance (dark green amorphous solids) showing similar IR bands at 3446 cm^{-1}, which suggested the presence of hydroxyl or amino groups. Bands at 2926 cm^{-1} and 2854 cm^{-1} were observed and characteristic bands for ester and ketone carbonyl groups were found at 1739 cm^{-1} and 1701 cm^{-1}.

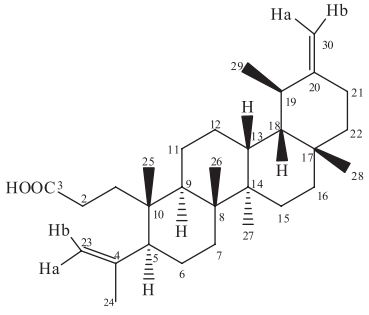
^{1}H NMR indicated the presence of porphyrin rings in the structures, indicating that the compounds could be structurally similar. The presence of a methyl proton at δ_H 3.2 indicated that the compounds 3, 4, and 6 contained phaeophytin A rings. Unlikely, the compound 5 showed an aldehyde proton at δ_H 11.15, which suggested that this substance possessed a phaeophytin B ring. The ^{1}H NMR of 4 showed the absence of a singlet at δ_H 6.27, the characteristic chemical shift of protons at the 13^2 position (H-13^2), which suggested that this position could be substituted. The lack of a set of methylene signals in the ^{1}H NMR spectrum of 6 indicated that this compound might be a pheophorbide. ^{13}C NMR spectra showed an oxygenated carbon at δc 89.0 (C-13^2) in 4, an aldehyde carbonyl group at δc 187.8 (C-7^1) in 5, and the presence of only 37 carbons in 6, confirming the previously proposed structures. The data were compared with those reported in the literature and the compounds were identified as chlorophyll-derived molecules: phaeophytin A (3),[20] 13^2-hydroxy-(13^2-*S*)- phaeophytin a (4),[21] phaeophytin b (5),[22] and 17^3-ethoxyphaeophorbide (6).[23] Studies have revealed that chlorophyll degradation results in the formation of phaeophytins and phaeophorbides by the action of enzymes such as Mg-dechelatase and chlorophyllase.[24] However, phaeophytins and phaeophorbides can suffer additional structural modifications



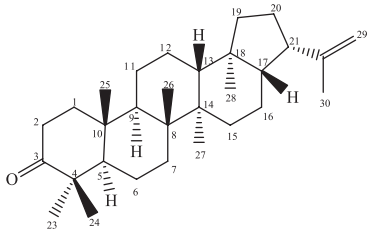
1a; R= H
1b: R= H; $\Delta^{22,23}$
2a: R= β-D-Glucopyranosyl
2b: R= β-D-Glucopyranosyl; $\Delta^{22,23}$



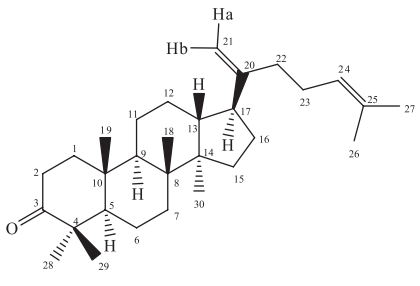
3: R_1= CH_3, R_2=H, R_3= phytol
4: R_1= CH_3, R_2=OH, R_3= phytol
5: R_1= CHO, R_2=H, R_3= phytol
6: R_1= CH_3, R_2=OH, R_3= CH_3



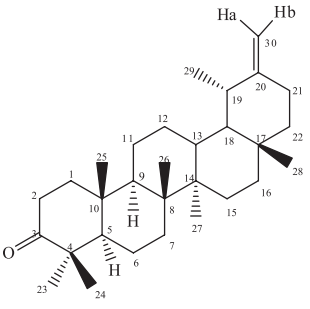
7: 3,4-seco-urs-4(23),20(30)-dien-3-oic acid



8: 3-oxo-21β-*H*-hop-22(29)-ene



9a: dammaradienone



9b: taraxastenone

Figure 1. *Compounds isolated from W. periplocifolia.*

and have been shown to possess many biological activities. Thus, researchers are interested in understanding if they belong to the secondary metabolism of plants.[25]

In its IR spectrum, compound 7 exhibited bands at 3435, 2941, 890, and 1709 cm^{-1}, indicating the presence of hydroxyl, aliphatic sp^3 carbons, double bonds, and carbonyl groups. The ^1H NMR spectra of this compound showed four singlet methyls (δ_H 0.91, δ_H 0.95, δ_H 0.98, δ_H 1.04), a secondary methyl (δ_H 1.06), a vinyl methyl (δ_H 1.82), and two exomethylenes (δ_H 4.75, δ_H 4.80, δ_H 4.91, δ_H 4.99). The ^{13}C NMR spectra exhibited 6 methyls, 12 methylenes, 5 methines, and 7 quaternary carbons, confirming a triterpenoid skeleton. A carbonyl group was found at δc 176.91 and two terminal double bonds were confirmed by the presence of the signals at δc 155.15, δc 107.93, δc 148.42, and δc 114.09. The location of the double bonds was determined by performing HMQC and HMBC experiments. Correlations were observed between H-23 and C-24 and C-5; between the methyl protons H-24 and C-5 and C-23; H-30 and C-19 and C-21; and H-29 and C-20 and C-18. The 2D correlations were carefully analyzed and 7 was identified as 3,4-*seco*-urs-4(23),20(30)-dien-3-oic acid, which is a 3,4-*seco*-ursane-type triterpene originally reported from *Betula platyphylla.*[13]

The IR spectrum of 8 showed bands at 2943, 2862, and 1708 cm^{-1}. The ^1H NMR spectra showed seven methyl singlets at δ_H 0.71, δ_H 0.76, δ_H 0.89, δ_H 1.03, δ_H 1.05, δ_H 1.17, and δ_H 1.75, with the last one being the characteristic chemical shift of a methyl group attached to an sp^2 carbon. In addition, olefinic protons were found as a broad singlet at δ_H 4.78, indicating the presence of an isopropenyl group. The ^{13}C NMR spectrum showed 30 carbons, and the isopropenyl group was confirmed by the signals at δc 149.35 and δc 110.22, suggesting that the compound possesses a lupene or hopene skeleton. Correlations exhibited in the HMQC and HMBC spectra showed that the carbons C-17, C-18, C-19, and C-21 in 8 are consistent with a hopene-type skeleton containing a carbonyl carbon at C-3. Since all the NMR data are in agreement with the literature,[21] the compound 8 was identified as 3-oxo-21β-*H*-hop-22(29)-ene, which was previously reported from *Maytenus robusta*.[14] The chemical shift of C-21 (δc 46.5) indicated a pseudo-axial configuration of the isopropenyl group (21β-*H* series). When this group has a pseudo-equatorial configuration, C-21 is deshielded at δc 48.00.[14]

The compound 9 was isolated as a colorless oil. Its ^1H NMR spectra showed a busy triterpene profile with two exomethylene doublets at δ_H 4.71 and δ_H 4.79. Their integration values (1.0 and 0.61) and the presence of 60 carbons in the ^{13}C NMR spectrum indicated that the compound 9 is a mixture of two triterpenes.

The major compound (9a) showed two deshielded methyl protons (δ_H 1.67 and δ_H 1.60) and a methynic proton at δ_H 5.11, which are the characteristic chemical shifts of prenyl groups. Moreover, in the ^{13}C NMR spectrum, evidence for a carbonyl at δc 218.0 (C-3), prenyl group signals (δc 124.44 and δc 131.41), and a terminal double bond (δc 152.51 and δc 107.59) was seen.

The minor compound (**9b**) showed a carbonyl at δc 218.06 (C-3) and a terminal double bond (δc 154.46 and δc 107.34). The HMBC spectrum exhibited correlation between the secondary methyl at δ_H 0.99 and the carbon at δc 154.46 (J^3), indicating that the minor compound possessed an ursane-type skeleton. Observation of the difference of intensities in the ^{13}C NMR spectra and careful analysis of the HMQC, HMBC, and COSY correlations led to the identification of a mixture of the triterpenes dammara-20(21),24-diene-3-one (**9a**) and taraxast-20(30)-en-3-one (**9b**). These compounds have been previously isolated from *Chisocheton penduliflorus*[15] and *Melaleuca leucadendron.*[16] The integration values allowed to determine that dammara-20(21),24-diene-3-one represented 60% of the mixture.

Antibacterial study

The CEE and all the tested phases did not show any antibacterial activity against *Escherichia E. coli, S. aureus, P. aeruginosa, K. pneumonia*, and *Acinetobacter* spp. However, the dicloromethane (DF), ethyl acetate (EAF), and n-butanol (BF) phases were active against *Enterococcus faecalis*. Table 2 lists the samples tested against *Enterococcus faecalis* and the respective growth inhibition zones.

Table 2. Evaluation of the antibacterial activity of the *W. periplocifolia* crude extract and fractions against *E. faecalis*

Concentrations (mg mL^{-1})	Inhibition zone (mm)					
	CEE	HF	DF	EAF	BF	HAF
0	-	-	-	-	-	-
25	-	-	-	-	-	-
50	-	-	12.0	-	-	-
75	-	-	16.7	12.3	-	-
100	-	-	17.0	14.3	11.7	-

DF was the most active sample in demonstrating antibacterial activity from 50 mg/mL, followed by EAF and BF. *Enterococcus faecalis* is known to be one of the most antibiotic-resistant bacteria. It is the dominant *Enterococcus* species related to urinary tract infections, endocarditis, and meningitis, being responsible for 80–90% of human enterococcal infections.[26] As observed in the ^1H NMR spectra of the tested phases (Figures 1S to 5S), the antibacterial activity of DF, EAF, and BF can be related to the phenol compounds present in these samples. The results are in agreement with the results reported by Konaté et al., who have studied the antimicrobial activity of *Sida alba* L. phenol fractions and have reported that the phenol-rich fractions are active especially against *Enterococcus faecalis.*[27]

CONCLUSION

From the aerial parts of *W. periplocifolia* (L.) C. Presl, we identified two mixtures of steroids: sitosterol/stigmasterol and sitosterol 3-*O*-β-D-glucopyranoside/stigmasterol 3-*O*-β-D-glucopyranoside; four chlorophyll derivatives: phaeophytin a, 13^2-hydroxy-(13^2-*S*)-phaeophytin a, phaeophytin b, and 17^3-ethoxyphaeophorbide; and four triterpenes: 3,4-*seco*-urs-4(23),20(30)-dien-3-oic acid, 3-oxo-21α-*H*-hop-22(29)-ene, dammaradienone, and taraxastenone. The compounds 3,4-*seco*-urs-4(23),20(30)-dien-3-oic acid, 3-oxo-21α-*H*-hop-22(29)-ene, and dammaradienone have been reported for the first time from the Malvaceae species.

Furthermore, the dicloromethane, ethyl acetate, and n-butanol fractions of *W. periplocifolia* showed antibacterial activity against *Enterococcus faecalis,* a pathogenic microorganism known to be one of the most antibiotic-resistant bacteria. The antimicrobial activity of the above mentioned fractions seemed to be related to the phenol constituents present in the active samples. The activity shown by these extracts could be a viable clue in the search for new antibacterial agents from natural sources as well as speak for the use of the plant as an antiseptic in Brazil.

SUPPLEMENTARY MATERIAL

NMR spectra available as supplementary material free of charge as PDF file at quimicanova.sbq.org.br.

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