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GC-MS Analysis and Antibacterial Activities of *Feretia apodanthera* Del. (Rubiaceae) and *Ozoroa insignis* Del. (Anacardiaceae)

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Abstract: Two medicinal plants, *Feretia apodanthera* and *Ozoroa insignis,* used in west African folk medicine to treat infectious diseases, were investigated for their antibacterial potential and their biocidal components. Two extractions were processed in water and aqueous-acetone (70%) and further fractionated by column-chromatography. Both extracts and fractions exhibited selective activity against human pathogenic bacteria as assayed by disc diffusion and microdilution methods. The aqueous-acetone extract of *F. apodanthera* was active against all the Gram-negative and Gram-positive bacteria ($d \geq 8$ mm; MIC ≤ 2.5 mg/ml) while the same extract from *O. insignis* got markedly activity on Gram-negative bacteria *E. coli* and *K. pneumonia* ($d \geq 11$ mm). However, the fractions (20µg) of both two plant species were selectively more active on Gram-negative bacteria ($d \geq 11$ mm). Tetramethyl silicate, trifluoroamine oxide and neophytadiene were identified by GC-MS as the main volatile compounds present that enhance the antibacterial effects in synergy with others.

Keywords: Biocidal Compound, Column Fractionation, *Feretia apodanthera*, Medicinal Plant, *Ozoroa insignis*, *Pseudomonas aeruginosa*, Resistant Bacteria

1. Introduction

Microorganisms including bacteria are responsible for various infections in humans, animals and plants. Since now, a lot of efforts were made to fight these microorganisms and a number of antibiotics have been approved, so that morbidity and mortality from microbial diseases have been drastically reduced by modern chemotherapy. However, because of mutations of bacterial genome due to multiple factors (misuse, over-prescription and abuse of antibiotics), bacteria have developed resistance to number of available antibiotics. Then, most of current antibiotics are no longer efficient to cure bacterial infections and infectious diseases are still the world's leading cause of premature deaths [1].

Bacillus licheniformis, Escherichia coli, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphyloccocus aureus* are among the most widespread bacteria and well-known to develop resistances. The current rates of resistance and cross-resistance development to all available classes of antibiotics agents urged the search for new antimicrobial substances with different mechanisms of action. For this purpose, natural products are getting greater interest and a lot of research are targeting medicinal plants as sources of antimicrobial drugs [2].

Feretia apodanthera and *Ozoroa insignis* are two medicinal plants well known for their use in folk medicine to treat infectious diseases. Ethnopharmacological surveys indicated that *Feretia apodanthera* is used for the treatment of chronic infective wounds [3]. The stem charcoal of *O. insignis* is traditionally used as milk preservative by pastoralists in West Africa [4]. The use of *O. insignis* as herbal remedy for the treatment of urinary schistosomiasis in Zimbabwe was reported [5, 6]. Few studies reported the activity of *O. insignis* against mollusk [7] and marine crustaceans [8]. Other studies isolated from roots of *O. insignis* different compounds as orsellinic acid and anacardic acid methyl ester, eight tirucallane triterpenes, one oleaneane, three lupane type triterpenoids, four anacardic acid derivatives and one flavone [9].

The present study aims to determine the antibacterial effect of *Feretia apodanthera* and *Ozoroa insignis* extracts and fractions along with the identification of their volatile components involved in the antibacterial activity.

2. Material and Methods

2.1. Plant Material

Aerial parts of two herbaceous plants species, *Feretia apodanthera* Del. (Rubiaceae), and *Ozoroa insignis* Del. (Anacardiaceae) were freshly collected at Gampela (25 km east of Ouagadougou, Burkina Faso) on June 2013. Taxonomic identification was verified by Prof. Jeanne F. Millogo (Laboratoire de Biologie et Ecologie Vegetales, University of Ouagadougou, Burkina Faso) and a voucher specimen was deposited for each plant under the following numbers: *Feretia apodanthera* (FA_rca 001) and *Ozoroa insignis* (OI_dca 001). The plants were air-dried in the laboratory and then reduced into powder for future use.

2.2. Chemicals

All the chemicals were at analytical grade. Acetone, methanol and dimethylsulfoxide were purchased from Fisher, nutrient agar medium and nutrient broth medium from Oxoid LTD England, p-iodonitrotetrazolium (INT) and sodium chloride from Sigma, Glycyrrhizin Acid Dipotassium Salt from WAKO Tokyo, malt extract from Becton Dickinson France, potato dextrose agar from Merck Germany, tetracycline and chloramphenicol from Sigma-Aldrich.

2.3. Extraction

An amount of 25g of powder from each plant were boiled for 45 minutes to make a decoction which was air-dried at 50°C to give the water extract. Another 25g of powder from each plant were soaked in 250ml of a mixture of acetone/water (70/30 v/v) for 48h and after filtration, the solvent was evaporated under reduced pressure (132 hPa, 40°C) and then air-dried at 50°C to give a dried extract (aqueous-acetone extract). Then, both of the two extraction processes were repeated three times.

2.4. Fractionation

The most biocidal aqueous-acetone extracts of *Feretia apodanthera* and *Ozoroa insignis* were chromatographed on a silica gel column (60-120 mesh) and successively eluted with increasing polarity gradients of a mixture (60 ml) of hexane/acetone/water (5/2/0.2, 5/3/0.2, 5/4/0.2, 5/5/0.2, 5/6/0.2, 5/6/0.4, 5/6/0.6, 5/6/0.8, 5/6/1, 5/6/1.2 v/v) and then isocratic elution with acetone/water (7/3 v/v).

Thirty-seven and Thirty-three initial fractions of about 20 ml were respectively collected from *Feretia apodanthera* and *Ozoroa insignis*. Then, each of these fractions were separated on silica gel GF254 Thin Layer Chromatography (TLC) developed with eluted hexane/acetone/water (5/6/0.4 v/v) and identified by ultra violet (UV) light at 254 nm and 365 nm. After merger of the same reference frontal (Rf) values, nine (9) fractions were finally obtained from *Feretia apodanthera* (F1 to F9) and ten (10) fractions from *Ozoroa insignis* (O1 to O10).

2.5. Antibacterial Activity

The antibacterial activity was assayed as described by Sharma *et al*. [10] with slight modifications.

2.5.1. Preparation of Bacterial Inoculum

Five bacterial strains (*Bacillus licheniformis* ATCC 12759, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853 and *Staphyloccocus aureus* ATCC 12600) were obtained from School of Biological Sciences, Universiti Sains Malaysia. Each strain was grown separately on nutrient agar at 37°C for 24 hours, and then an aliquot was suspended in nutrient broth and stand overnight at 37°C. The bacterial inoculum was then prepared by diluting this bacterial suspension in a sterile saline solution (0.9% NaCl) to get approximately 10^6 colony-forming units/ml as compared to the turbidity of 0.5 MacFarland standard.

2.5.2. Preparation of Culture Medium

Twenty-eight (28) grams of nutrient agar were mixed with 1000 ml of sterile distilled water. The mixture was then sterilized by autoclaving at 121°C for 20 minutes. Under aseptic conditions in the laminar flow hood, 20 ml of agar medium were uniformly dispensed into sterile Petri dishes. They were then covered and allowed to cool down at room temperature until the culture medium hardened. The inoculation of the bacterial culture on the agar surface was done by the spread plating technique.

2.5.3. Disc Application

The sample solution (10 µl, 2, 20, 40 and 60 mg/ml in water

containing 10% DMSO) was sterilized by filtration through microfilter (0.2 µm) and impregnated on sterilized paper discs (6 mm in diameter, Advantec Tokyo Inc). After drying in aseptic conditions, the discs were placed on the inoculated nutrient agar surface with flamed forceps and then gently pressed down to ensure contact with the agar surface. The Petri dishes were then incubated for 18 hours at 37°C. Then, the diameter (in mm) of the inhibition zone around each disc was measured. Antibacterial activities were indicated by a clear zone of growth inhibition around the paper disc. Each test was repeated three times. Tetracycline and chloramphenicol (10 µl, 1mg/ml in water containing 10% DMSO) were used as positive controls while a water solution containing 10% DMSO was used as negative control.

2.5.4. Microdilution Method

The microdilution method was used to determine the minimal inhibitory concentration (MIC) along the five bacterial strains. 100 µl of nutrient broth medium (21 mg/ml) were placed into each 96 wells of the microplates. The extract solutions (100 µl, 20 mg/ml) were added into first rows of microplates and two-fold dilutions (10 to 0.0097 mg/ml) were made by dispensing the solutions to the remaining wells. Then 100 µl of nutrient broth and 10 µl of bacterial inoculum were added into all the wells. For each dilution, a negative control without inoculum (100 µl of medium, 100 µl of extract solution and 10 µl of saline solution 0.9%) was prepared for optical comparison. A control without sample solution (100 µl of medium, 100 µl of DMSO 10% and 10 µl of inoculum) was prepared to ensure bacterial growth. Another well was filled with 100 µl of medium, 100 µl of DMSO 10% and 10 µl of saline solution 0.9% as control to ensure their sterility. Each test was carried out in triplicate. The sealed microplates were incubated at 37°C for 18h. Then, p-iodonitrotetrazolium violet (10 µl, 2 mg/ml in water) was added. The lowest concentration of the extract that completely inhibited macroscopic growth was determined by optical comparison with the corresponding negative control and was noted as minimum inhibitory concentration (MIC).

2.6. Gas Chromatography-Mass Spectrometer Analysis

Shimadzu-GC-9A gas chromatograph, FID at 220, N 2 at 1.0 ml/min, SPB-5 capillary column (30 m × 0.53 mm ID; 0.3 µmdf), split ratio 1:30 injector temperature 240°C, column temperature maintained at 50°C for the first five minutes and then raised to 235°C (5°C per minute) followed by five minutes at 235°C. GC-MS: Hewlett-Packard 5890 gas chromatograph, combined with a Jeol JMS-HX 110 mass spectrometer with source at 270°C at 70 eV. Injector was set at 270°C with splitting ratio 1:30. The analysis was performed on the aforementioned program on equivalent column HP-5 (25 m × 0.22 mm and 0.25 µmdf). A mass spectral survey was performed using the NIST mass spectral search program 2008 with similarity indices more than 90%.

2.7. Statistical Analysis

All the tests were run in triplicate and data are presented as mean ± standard deviation. Data were analyzed by one-way analysis of variance followed by the Tukey multiple-comparison test with XLSTAT 2013.4.08. A p-value less than 0.05 was used as the criterion for statistical significance.

3. Results and Discussion

The antimicrobial potential of the two selected plant species was assayed on five bacterial strains. The antibacterial activity was evaluated by disc diffusion method and the diameter of inhibition zone (d) was measured around the paper disc loaded with different amount of sample solution (200 µg, 400 µg and 600 µg) as indicated in Table 1. In general, the aqueous-acetone extract of *F. apodanthera* showed antibacterial activity on all the five bacterial strains while its water extract is active only on *E. coli* and the Gram-positive strains. The water extract of *O. insignis* got antibacterial activity on all the selected bacterial strains and its aqueous-acetone extract exhibited markedly activity against Gram negative bacteria *E. coli* and K. pneumonia ($d \geq 11$ mm) while it was not active at any dose on Gram positive bacteria *B. licheniformis*.

Table 1. *Inhibition zone diameters (mm) of bacterial growth by water and aqueous-acetone extracts of F. apodanthera and O. insignis.*

Bacteria		Extract amount (µg/disc)	*F. apodanthera*		*O. insignis*	
			Water	H_2O-acetone	Water	H_2O-acetone
Gram negative	*E. coli*	200	8 ± 1^b	9 ± 1^b	R^a	11 ± 1^c
		400	8 ± 0^b	11 ± 0^c	8 ± 1^b	11 ± 0^c
		600	8 ± 0^b	11 ± 0^c	8 ± 1^b	12 ± 0^c
	K. pneumoniae	200	R^a	R^a	7 ± 1^b	11 ± 1^c
		400	R^a	9 ± 0^b	9 ± 1^c	11 ± 1^c
		600	R^a	10 ± 1^c	9 ± 1^c	11 ± 1^c
	P. aeruginosa	200	R^a	8 ± 0^b	7 ± 1^b	9 ± 0^b
		400	R^a	8 ± 0^b	7 ± 1^b	9 ± 0^b
		600	R^a	9 ± 1^b	9 ± 1^c	9 ± 0^b
Gram positive	*Bacillus licheniformis*	200	8 ± 0^b	9 ± 0^b	R^a	R^a
		400	8 ± 0^b	10 ± 1^c	9 ± 1^c	R^a
		600	9 ± 1^b	10 ± 1^c	10 ± 1^c	R^a
	S. aureus	200	R^a	8 ± 0^b	8 ± 0^b	9 ± 1^b
		400	R^a	8 ± 0^b	8 ± 0^b	9 ± 1^b
		600	9 ± 1^b	8 ± 0^b	8 ± 0^b	9 ± 1^b

Values are expressed as mean of 3 replicates ± standard deviation. Values (a, b, c) within each column with different superscripted letters differ significantly ($P \leq 0.05$). R = no inhibition ($d \leq 6$ mm).

The minimal inhibitory concentrations (MIC) as determined by the microdilution method (Table 2) indicate that *K. pneumoniae* is less sensitive (MIC ≥ 5 mg/ml) to all the extracts as compared to the other four strains; along these four strains, aqueous-acetone extracts of both *F. apodanthera* and *O. insignis* are more active (MIC ≤ 2.5 mg/ml) than their respective water extracts. *F. apodanthera* (aqueous-acetone extract) is more active on *S. aureus* (MIC = 0.31 mg/ml) while *O. insignis* (aqueous-acetone extract) is more active on *S. aureus* and *E. coli* (MIC = 0.62 mg/ml).

The aqueous-acetone extracts of both *F. apodanthera* and *O. insignis* were then fractionated by column chromatography and the antibacterial activities of the obtained fractions (20 µg) were tested by disc diffusion method (Table 3) and compare to those of the standards chloramphenicol and tetracycline (10 µg). Gram positive bacteria (*B. licheniformis* and *S. aureus*) are generally less sensitive (d ≤ 10 mm) to the fractions of both *F. apodanthera* and *O. insignis* except the O2 fraction of *O. insignis* which exhibited significant activity on *S. aureus* (d = 13 ± 2 mm). The different fractions of both *F. apodanthera* and *O. insignis* are selectively active on the different Gram-negative bacteria and most of them exhibited significant activity on *P. aeruginosa* (d ≥ 11 mm) as compare to the two standards chloramphenicol (no inhibition) and tetracycline (d = 16 mm).

Table 2. *Minimal inhibitory concentrations (mg/ml) of water and aqueous-acetone extracts on bacterial strains.*

Plant species	Extracts	Yield (%)	Gram negative			Gram positive	
			E. coli	*K. p*	*P. a*	*B. l*	*S. aureus*
F. apodanthera	Water	16,44 ± 4.41^{b}	5^{c}	10^{b}	2.5^{a}	10^{c}	2.5^{c}
	H_2O-acetone	28,06 ± 4.04^{c}	1.25^{b}	5^{a}	2.5^{a}	1.25^{a}	0.31^{a}
O. insignis	Water	13,48 ± 1.01^{a}	1.25^{b}	10^{b}	2.5^{a}	5^{b}	2.5^{c}
	H_2O-acetone	18,58 ± 5.28^{b}	0.62^{a}	5^{a}	2.5^{a}	1.25^{a}	0.62^{b}

Yield values are expressed as mean of 3 replicates ±standard deviation. Values (a, b, c) within each column with different superscripted letters differ significantly (P ≤ 0.05). K. p = *Klebsiella pneumonia*; P. a= *Pseudomonas aeruginosa*; B. l= *Bacillus licheniformis.*

Table 3. *Diameter of inhibition (mm) of column fractions from F. apodanthera and O. insignis.*

Fractions (20 µg/disc)	Yield (%)	Gram negative			Gram positive	
		E. coli	*K. pneumoniae*	*P. aeruginosa*	*B. licheniformis*	*S. aureus*
F1	1.46^{c}	R^{a}	14 ± 3^{b}	R^{a}	R^{a}	7 ± 0^{b}
F2	0.38^{b}	11±0^{c}	8 ± 0^{a}	R^{a}	8 ± 0^{b}	R^{a}
F3	1.58^{c}	R^{a}	8 ± 1^{a}	15 ± 1^{e}	7 ± 0^{b}	R^{a}
F4	2.02^{c}	11±0^{c}	11 ± 3^{a,b}	14 ± 0^{d}	R^{a}	9 ± 0^{b}
F5	23.02^{d}	R^{a}	12 ± 2^{b}	14 ± 1^{d}	8 ± 0^{b}	9 ± 1^{b}
F6	0.43^{b}	R^{a}	10 ± 1^{a,b}	13 ± 1^{d}	R^{a}	R^{a}
F7	39.65^{e}	R^{a}	7 ± 1^{a}	13 ± 0^{d}	9 ± 1^{b}	R^{a}
F8	17.67^{d}	8 ± 0^{b}	11 ± 2$^{a, b}$	R^{a}	R^{a}	8 ± 0^{b}
F9	5.48^{c}	R^{a}	10 ± 1^{a,b}	16 ± 1^{e}	R^{a}	8 ± 1^{b}
O1	5.71^{c}	R^{a}	12 ± 0^{b}	R^{a}	R^{a}	10 ± 0^{b}
O2	0.11^{b}	10 ± 1^{c}	8 ± 0^{a}	9 ± 1^{b}	8 ± 0^{b}	13 ± 2^{c}
O3	0.61^{b}	R^{a}	8 ± 0^{a}	14 ± 0^{d}	10 ± 1^{b}	R^{a}
O4	0.32^{b}	13 ± 0^{c}	9 ± 0^{a}	13 ± 1^{d}	8 ± 0^{b}	10 ± 1^{b}
O5	0.65^{b}	12 ± 1^{c}	9 ± 1^{a}	13 ± 1^{d}	R^{a}	8 ± 0^{b}
O6	0.54^{b}	R^{a}	9 ± 1^{a}	8 ± 0^{b}	8 ± 0^{b}	10 ± 2^{b}
O7	43.87^{e}	10 ± 0^{c}	10 ± 2^{a,b}	12 ± 0^{c}	8 ± 1^{b}	8 ± 0^{b}
O8	0.08^{a}	8 ± 0^{b}	8 ± 0^{a}	11 ± 0^{c}	8 ± 0^{b}	R^{a}
O9	17.65^{d}	R^{a}	9 ± 1^{a}	8 ± 1^{b}	R^{a}	7 ± 0^{b}
O10	4.00^{c}	R^{a}	11 ± 1^{a,b}	12 ± 0^{c}	R^{a}	7 ± 0^{b}
Chloram (10µg/disc)		29 ± 1^{d}	29 ± 1^{c}	R^{a}	19 ± 1^{c}	34 ± 1^{d}
Tetra (10µg/disc)		42 ± 1^{e}	30 ± 0^{c}	16 ± 1^{e}	30 ± 0^{d}	33 ± 1^{d}

Values are expressed as mean of 3 replicates ± standard deviation. Values (a, b, c, d, e) within each column with different superscripted letters differ significantly (P ≤ 0.05). R= no inhibition (d ≤ 6mm). F1-F9= *F. apodanthera fractions*; O1-O10= *O. insignis* fractions. Chloram = chloramphenicol; Tetra = tetracycline.

The Gas Chromatography/Mass Spectrometer (GC/MS) analysis of the aqueous-acetone extracts and fractions of both plants was then conducted to identify their volatile compounds that could justify the observed antibacterial effects. The list of the identified constituents is shown in Table 4.

Table 4. *List of the identified compounds in the aqueous-acetone extracts and fractions of F. apodanthera and O. insignis.*

No	RT (min)	Name	F. a	O. i	F1	O1
			Area (%)			
Benzene derivatives						
1	3.77	2-Thiobenzoylamino-benzo [d] thiazole	-	-	-	4.1
2	3.79	Benzene, 2-methoxy-1-(2-nitroethenyl)-3-(phenylmethoxy)-	-	-	3.52	-
3	19.61	Benzaldehyde, 3,5-dimethyl-	-	-	-	0.87
4	26.08	o-Hydroxyacetophenonylidene-4,5-dimethyl-O-phenylenediamine	53.14	52.04	-	-
Silicates						
5	3.94	Tetramethyl silicate	29.81	30.6	22.54	39.06
6	29.39	Silane, methoxytrimethyl-	-	-	1.3	-
Hydrocarbons derivatives						
7	11.83	Decane < n->	11.25	11.36	-	-
8	14.93	Pyrolo [3,2-d]pyrimidin-2,4(1H,3H)-dione	-	-	1.09	0.93
9	15.31	Undecane < n- >	5.8	6	1.03	1.71
10	33.52	3-Eicosene, (E)-	-	-	-	1.02
11	35.32	Neophytadiene	-	-	29.29	6.37
Fatty acids						
12	31.5	2-Propenoic acid, tridecyl ester	-	-	1.82	-
13	36.37	Hexadecanoic acid, methyl ester	-	-	-	1.49
14	37.22	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl) ethyl] pentyl ester	-	-	-	1.46
15	37.69	Octadecanoic acid, ethyl ester	-	-	-	1.24
16	38.91	Tetrahydropyran Z-10-dodecenoate	-	-	2.05	-
17	39.65	9,15-Octadecadienoic acid, methyl ester, (Z, Z)-	-	-	0.76	-
18	39.78	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	-	-	0.79	-
Others						
19	34.42	Trifluoroamine oxide	-	-	14.67	3.24
20	35.79	1,2,4,5-Tetrazine	-	-	1.14	-

F. a = *Feretia apodanthera*, O. i = *Ozoroa insignis*, F1 = Fraction F1; O1 = Fraction O1; RT = Retention Time;- = not identified.

It is noted that 20 compounds were detected and identified with typical library search match exceeding 90% and most of these compounds are present in the F1 (14 compounds) and O1 (13 compounds) fractions of *F. apodanthera* and *O. insignis* respectively while their crude aqueous-acetone extracts contain less compounds (4 and 5 compounds respectively). This is related to the fact that the fractions F1 and O1 are oils and contain more volatile compounds than their crude extracts which are not oils. The major volatile compounds identified in the crude aqueous-acetone extracts of both plant species were found to be tetramethyl silicate (29.81% and 30.6%) and o-hydroxyacetophenonylidene-4,5-dimethyl-O-phenylenediamine (53.14% and 52.04%). Tetramethyl silicate is also a major compound common to both F1 (22.54%) and O1 (39.06%) fractions while neophytadiene (29.29%) and trifluoroamine oxide (14.67%) were identified in significant amounts in F1 fraction. Some other compounds were also identified in smaller amounts in the extracts and fractions of the two plants species, such as benzene derivatives, hydrocarbon derivatives and fatty acids. Most of the identified compounds were reported to have biocidal activity that could justify the antimicrobial activity of the extracts and fractions in the present study.

Across the five bacterial strains, the crude extracts and fractions exhibited selective antibacterial effect. The crude extracts tested at higher amounts (200, 400 and 600 µg) globally exhibited weaker inhibition on Gram negative bacteria as compared to most of their respective fractions tested at an amount 10-fold smaller (20 µg), suggesting that the most antibacterial compounds are contained in these fractions. For example, the diameter of inhibition zone of the most active extract of *F. apodanthera* (the aqueous-acetone extract) do not exceed 11 mm across the five bacteria while some of its fractions got more than 11 mm: fraction F1 got 14 mm on *K. pneumonia*, fractions F3, F4, F5, F6, F7 and F9 showed inhibition diameters more than 13 mm ($d \geq 13$ mm) on *P. aeruginosa*. In the same scheme, the aqueous-acetone extract of *O. insignis* mostly exert its antibacterial effect on Gram negative bacteria *E. coli* and *K. pneumonia* but with an inhibition diameter not exceeding 12 mm; therefore, some of its fractions at lower amounts got at least the same inhibition diameter like the fraction O1 on *K. pneumonia* (d = 12 mm), O4 on *E. coli* (d = 13 mm), O3, O4 and O5 on *P. aeruginosa* ($d \geq 13$ mm). The range of the extracts concentrations that were tested (200, 400 and 600 µg/disc) does not showed significant changes in the antibacterial effects and greater amounts might involve noticeable variations. Previous screening of medicinal plants from South Africa [11] indicated that the water-extract of *Ozoroa insignis* was active on all the tested bacterial strains [12, 13] and it displayed higher activity at a higher dose (1000 µg/disc) on both *E. coli* (15 mm) and *S. aureus* (25.3 mm) as compare to the results from the present study (8 mm for both strains at 600 µg/disc). Moreover, the results found by Koohsari *et al*. [14] showed that the Gram-positive bacterium (*S. aureus*) is more sensitive to the extracts of plant extracts as compared to all the Gram-negative bacteria including *E. coli*. This last finding is apart from our result which show that globally both crude extracts and fractions of the two plant species (*F. apodanthera* and *O. insignis*) got higher inhibition on Gram-negative bacteria as compared to Gram-positive bacteria; these plant samples may have high diffusion potential to reach the external double-bond membrane of Gram-negative

bacteria and inhibit their growth, since this membrane make them impermeable and resistant to antibiotics [15]. Indeed, the exterior of the gram-negative outer membrane presents a strong negative charge conferred by lipopolysaccharide and forms a hydrophilic permeability barrier that provides protection against the effects of highly hydrophobic drugs [16]. Then, these diffusible compounds in the two plant samples could be represented by their fatty acids contents (Octadecanoic acid, ethyl ester; Tetrahydropyran Z-10-dodecenoate; Octadecanoic acid, ethyl ester etc) as identified by the GC-MS analysis. Free fatty acids were reported as antibacterial against several micro-organisms, including Gram-negative and Gram-positive bacteria [17]. It was hypothesized that fatty acids act as anionic surfactants that can penetrate the extensive meshwork of peptidoglycan in the cell wall without significant changes, and reach the bacterial membrane leading to its disintegration, causing damage to bacteria as well as fungi and yeasts [18]. However, the permeability was not only dependent to the compounds but it was also related to the structure and composition of bacterial membrane [16].

It is noticeable to mention the particular inhibition of *P. aeruginosa* by most of the fractions of *F. apodanthera* and *O. insignis* as compared to the standards chloramphenicol and tetracycline tested at only 2-fold lesser extent (10 µg). At this dose, chloramphenicol has no inhibition on this bacterium while the inhibition by tetracycline is similar to the fractions. Resistance to chloramphenicol often occurs together with resistance to tetracycline [19] and this might explain the low inhibition diameter obtained with tetracycline (16 mm). *Pseudomonas aeruginosa* was cited to develop resistance to chloramphenicol through acetyltransferase enzymes that inactivate the drug or by inducing a blockage of bacterial permeability [20]. The observed antibacterial effect of these fractions and particularly the F1 fraction may be mainly dependent on its content in unsaturated fatty acids as 9,15-(Z, Z)-Octadecadienoic acid methyl ester and 9,12,15-(Z,Z,Z)-Octadecatrienoic acid methyl ester that are able to encounter these resistance factors from *Pseudomonas aeruginosa* since the activity of unsaturated fatty acids against multi-resistant bacteria was reported [21].

The relatively low activity of the targeted plant samples on Gram-positive bacteria *S. aureus* and *B. licheniformis* can be related to their ability to develop resistance to many biocidal compounds, because these bacteria are known to produce β-lactamase enzymes that can inactivate some antibiotic drugs by hydrolyzing their β-lactame structure [22]. *B. licheniformis* strain was reported to be resistant to chloramphenicol and clindamycin [23] while *S. aureus* was known as a multi-resistant bacterium [24].

The antimicrobial activities of most of the hydrocarbons detected in the extracts and fractions were reported [25] and they may have significant contribution to the activity of the plant samples. Therefore, hydrocarbon derivatives were cited to possess lower antimicrobial properties because of their limited hydrogen bound capacity and their low water solubility that limits their diffusion through the medium [26].

The presence of ketones as Pyrolo[3,2-d]pyrimidin-2,4(1H,3H)-dione and aldehydes (3, 5-dimethyl-benzaldehyde) in the two plant samples may enhance their biocidal activity but with different specificity and levels of activity depending on the functional group and also the hydrogen-bounding parameters. Previous results showed that greater antimicrobial potential could be ascribed to the oxygenated terpenes and especially phenolic compounds in relation with their hydrogen donating ability [27]. Phenolic lipids as anacardic acids earlier identified in *Ozoroa insignis* [28] and known for their antibacterial potentials [29] may also involve in the antibacterial activity of the plant even if they were not identified in the present study because of their non-volatility.

The GC-MS analysis of other plant species as *Aloe vera* revealed that eicosane and phytol were the main constituents and were responsible for the high antimicrobial activity against clinical pathogens [30]. Similarly, the respective derivatives diterpenes 3-(E)-Eicosene and neophytadiene were identified in the fractions of *F. apodanthera* and *O. insignis* and neophytadiene was the main compound in the fraction F1 that may involve greater contribution in the antimicrobial potential of the plant species. Therefore, the components present in the great proportions are not necessarily responsible for a great share of the total activity. The different antibacterial activity exhibited by the extracts and fractions can be explained by either the synergistic effect of their different components and /or by the presence of other components that may be active even in small proportions. In this scheme, the presence of silicates as main components in the aqueous-acetone extracts and the F1 fraction is noteworthy since these nanoparticles are known to increase the antibacterial effect when they are used in combination with antibiotics [31]. The nanoshells made of silica find application in the field of drug delivery since they can go inside the microorganism and increase the penetration of the antibiotic within the bacterial cell [32]. The antibacterial activity of ciprofloxacin-encapsulated silica nanoshells showed greater activity as compared to free ciprofloxacin [32].

4. Conclusion

The extracts and fractions of both plant species *F. apodanthera* and *O. insignis* exhibited selective and significant growth inhibition on the five bacterial strains. They activity against multi-resistant drugs bacteria might be partially related to their volatile components that involve different antibacterial mechanisms. The antibacterial activity of these plants can justify some of their traditional uses in folk medicine and more investigations are needed on the interaction between their chemical components and their silica nanoparticles for their exploitation as natural antimicrobial agents.

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