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(RESEARCH ARTICLE)

Antibacterial and antifungal activities of hexane and acetone extracts of sheets and fruits of *Feijoa sellowiana* O.

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Abstract

In these last decades, recent pharmacopeia was focused on the search of bioactive molecules, which were extracted from medicinal plants. In this study, our choice related to Moroccan variety *Feijoa sellowiana* (*F. sellowiana*). Because, this variety had not been scientifically evaluated yet. Meanwhile, its fruits were ignored by moroccan population, which could economically constitute an exploitable source. Thus, the objective of this study was to evaluate the microbial activities of *F. sellowiana*. Extraction was carried out by successive exhaustion of plant materials, by using two organic solvents, hexane and acetone. Therefore, the separation of each extract was done using chromatography on column. Thereafter, the screening of secondary metabolites were done by the method of screening. According to the different results obtained during this study. All extracts have showed the presence of secondary metabolites such as saponosids, tannins, steroids and terpens. Thus, they showed antimicrobial activities except acetone extract of fruits, almost all fractions revealed as inactives on *Candida albicans*. However, more studies are needed to valorize the moroccan variety *F. sellowiana* and could be used to overcome the microbial aggressions.

Keywords: *Feijoa sellowiana* O.; Antibacterial; Antifungal; Hexane extract; Acetone extract

1. Introduction

In order to deal with the drug resistance of microorganisms in patients, scientific researchers directed their research toward the traditional pharmacopeia in the objective of discovering other molecules having a biological activity, being able to precisely face the development of the infectious diseases in the world in general and countries in the process of development like Morocco.

Since antiquity, man seeks to overcome the microbial aggressions by using innumerable mixtures of extract of plants. However with the arrival of technology, these mixtures were transformed into composed purely chemical having adverse effects which were harmful for man's health. This had made a strong return back to the traditional pharmacopeia, considering its accessibility and its lower cost. Moreover, the World Health Organization (WHO) estimated in 2007 that approximately 80 % of the population of countries in the process of development could be neat starting from the plants [1-3]. In 2001, several tests carried out on Spleens, revealed that 25 % of regulation in the world of drugs and, 60 to 70 % of substances which had antibacterial and anti-cancer effects, had been extracted from plants [4-5]. In this study, our choice related to the exotic fruit moroccan *Feijoa sellowiana* (*F. sellowiana*) because, this moroccan variety had not been scientifically evaluated yet. Therefore, its fruit were ignored by moroccan population,

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which could economically constitute an exploitable source. This plant pertaining to Myrtaceae, it could be found in subtropical countries with moderate climate. *F. sellowiana* could resist dryness, but in culture, it required to be irrigated for best production of hight quality of fruits. A clay-sand ground, rich in humus, proved to be the best ground for this plant. It could be however satisfied in a poor ground, which was well-drained [6].

This shrub measured 5 to 8 meters, sometimes a buissonnant shrub, with a bark pale brown gray, gray branches and a silver plated foliage with gray reflection. Its fruits were in ovoid form with an average of weight between 25 to 60 grams and length between 5 to 8 cm. [7]. Several studies were conducted to evaluate the biological activities of this plant. Until now, published papers revealed several biological effects of a great interest among which antimicrobial activities.

Vuotto and its collaborators published several articles on component bioactifs contained in the extract of this plant, which showed an antibacterial activity. The aqueous extract of fruits of this plant could be a drug with multiple facets [8–10]. Meanwhile, other studies were also revealed some activities such as anti-inflammatory drug, anti-cancer and anti-depressive. Thus, this work will be focused on antimicrobial and antifungal activities of *F. sellowiana*.

2. Material and methods

2.1. Plant material

Sheets and fruits of *F. sellowiana* were collected in September and November 2017 in Casablanca.

2.2. Microorganism used

The provided Strains used in this study were: *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Escherichia coli* (*E. coli*) CIP 54127, *Pseudomonas aeruginosa* (*P. aeruginosa*) CIP A 22, *Bacillus cereus* (*B. cereus*) IPL141, *Candida albicans* (*C. albicans*) ATCC 90028.

2.3. Preparation of extracts

The extraction was carried out by successive exhaustion of plant materials, by using two organic solvents, hexane and acetone, with increasing polarity. 116 g of fruits of *F. sellowiana* were crushed and piled up in a cartridge which was deposited in the Soxhlet device with 200 ml of solvent for each extraction. Once the extraction was finished solvents were eliminated by evaporation using a rotary evaporator under reduced pressure to avoid any degradation of existing compounds in the extract. Obtained extracts were kept at 4° C before analysis. The same procedure was applied using 55 g of sheets of the plant.

2.4. Phytochemical screening

Secondary metabolites of each extract were screened by the method of screening according to the standard procedures [16].

2.5. Separation using chromatography on column

Extracts of fruits and sheets of *F. sellowiana* were the object of separation by chromatography on column by using silica gel in the stationary phase (2 cm x 20 cm). Elution was carried out by using hexane and acetone. Therefore, obtained fractions underwent an evaporation to eliminate organic solvents, there after they were kept at 4 °C before analysis.

2.6. Antibacterial and antifungal activities

Antimicrobial activities were evaluated by the method of diffusion on agar and microdilution on microplate, in order to calculate minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of fractions of various extracts obtained.

2.6.1. Liquid medium

Liquid Mueller Hinton Broth (MHB): contained, beef infusion solids, casein hydrolysate and starch, their quantities were 2.0 g, 17.5 g and 1.5 g in 1L of distilled water. Final pH, 7.4 ± 0.2 (25° C).

Liquid Extract-Peptone-Glycerol (YPG): contained, bacto yeast extract, bacto peptone, glycerol, bacto agar, their quantities were 10 g, 20 g, 30 ml, 20 g in 1L of distilled water. Final pH, 7.4 ± 0.2 (25 °C). All products were purchased from Biokar Diagnostics [11–15].

2.6.2. Innoculums preparation

All stains used in this study were kept at 4 °C prior analysis. Liquid cultures were prepared and incubated at 37 °C for 24 h and solid culture sown on Petri limp and incubated at 37 °C for 24 h, for making sure the purity of cultures. These limps were used to prepare inoculums of bacteria. Meanwhile, liquid YPG medium was prepared and incubated at 30 °C for 24 h and added agar, before sown on Petri limp and incubated at 30 °C for 24 h. These limps were used to prepare the inoculums of *C. albicans*.

2.6.3. Disc diffusion method

The inhibition of microbial growth by different extracts of fruits and sheets, was evaluated by the method of diffusion on discs.

In this method, filter paper discs 6 mms in diameter which impregnated by a known volume of the substance to test, were deposited on the surface of an agar medium sown before hand surfaces by 0.1% of overnight microbial suspension. After incubation, the reading of the results was done by measurement of the diameter of zones of inhibition (ZI) in millimeters (mm) [17–19].

2.6.4. Determination of MIC using micro-dilution method

MIC was given by the method of microdilution. Into 96 wells of microplaque, 50 µl of MHB medium were introduced, except the wells of the first column, where 100 µl of each extract to be tested were deposited, then of successive dilutions of a factor of 2 were realized to the wells of the 11th column. The last column was regarded as positive witness of growth (absence of extract). The inoculation was made by 50 µl of the inoculum prepared before hand. The microplates ones were incubated with 37 °C for 24 h. Thereafter, the first concentration of which no bacterial growth was noted and counted its MIC [20, 21]. Each test was reproduced 3 times.

2.6.5. Determination of MBC

MBC was the lowest concentration of an extract which leaving at most 0.01% survivor germs. MBC of each extract was analyzed by taking 3 µl from wells which were shown as negative (no growth), started from wells of MIC, and sowed using spot on MHA medium, thereafter limps were incubated at 37 °C for 24 h.

3. Results and discussion

3.1. Extraction using Soxhlet method

Various raw organic extracts of air parts quoted above, were prepared by exhaustion hot vegetable materials using hexane and acetone according to their increasing polarity. Outputs obtained were illustrated in Fig. 1.

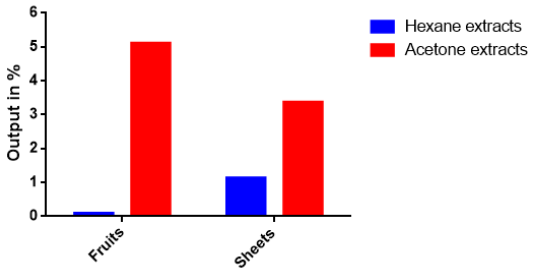

Figure 1 Output of hexane and acetone extracts

The highest output obtained was, acetone extracts which were 5.11% (5,11 g) and 3.37% (3,37 g) for sheets and fruits respectively compared to hexane extracts which were given 1.14% (0,63 g) and 0.09% (O,104 g) for sheets and fruits respectively. Meanwhile, results of hexane extracts were comparable with those obtained by Nakashima (2001), but the value of hexane extract of fruits was 0.1% [22]. whereas, values of acetone extract were different from other values published by Motohashi et al. (2000), which was around 1.96% [23].

From polarity point of view, highest outputs were polar extracts (acetone) compared to non-polar extracts (hexane). these results reflected low content of lipids in sheets and fruits.

3.2. Separation using chromatography on column

Extracts obtained by soxhlet underwent a chromatography on column, and followed-up by Thin layer chromatography, to get fractions had same chemical compounds. Thus several fractions were recovered for each extract. Chromatography on column separation, fractions were visually given by color change. Raw hexane extract of fruits was given three fractions H_1-H_3, whereas that of sheets was given four fractions H_1-H_4. Meanwhile, acetone extracts of fruits and sheets were given three fractions A_1-A_4.

3.3. Phytochemical screening

To determine secondary metabolites of each extract, which were responsible of biological activities, screening method was used according to the standard procedures [24]. Results were indexed in Table 1.

Table 1 The presence and absence of secondary metabolites in extracts

Phytoconstituents	Sheets		Fruits	
	Hexane extract	Acetone extract	Hexane extract	Acetone extract
Saponoside	–	+	–	–
Tanin	–	+	–	+
Steroid	+	+	+	–
Terpene	+	+	–	+
Flavonoid	–	–	–	–
Coumarine	+	–	+	+

'+' indicates presence and '-' indicates absence of secondary metabolites.

In the case of sheets extracts, both secondary metabolites steroid and terpene were present in acetone and hexane extracts, whereas tannin and saponoside were just present in hexane extract, and coumarine was just in acetone extract. In the case of fruits extracts, coumarine was present in both hexane and acetone extracts, meanwhile tannin and terpene were just present in acetone extract, and steroid was just in hexane extract. Therefore, steroid, terpene and coumarine were highlighted in almost all extracts except, in acetone and hexane extract of fruits, and in acetone extract of sheets respectively. Thereafter, tannin was present in both of acetone exracts and saponoside was just in extract of sheets. These results were comparable with those published earlier, indeed, Monforte et al. (2014) and Weston (2010) identified saponins, tannins, steroids,terpenes, flavonoids and coumarines among other components in extracts of *F. sellowiana* [25, 26].

3.4. Antibacterial and Antifungal activities

Antibacterial activities of fractions of all extracts of *F. sellowianaw* were carried out on referenced microbial strains. Results were represented in Table 2 and Table 3.

Raw hexane extract of fruit (H_0) was active on four bacterial strains tested. Indeed, ZIs were 13.5, 14, 12 and 13 mm on *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus* respectively.

Whereas the fraction H_1 of hexane extract of fruits showed an average activity with ZI of 13 mm on *E. coli*, and a weak activity on *S. aureus* was (9 mm). However the 2nd fraction was slightly active on three strains *E. coli*, *P. aeruginosa*, and *S. aureus* with some 12, 10 and 10.5 mm respectively. Meanwhile, fraction H_3 of hexane extract of fruits didn't show an activity on all strains and on *C. albicans*.

Raw hexane extract of sheets revealed antibacterial effects on all four strains. Diameters of ZIs were 12.5, 14, 12 and 13 mm on *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus* respectively. H_1 fraction showed a weak activity against *B. cereus* (10 mm) whereas it was inactive on other three strains. For H_2 fraction ZIs were 10, 14, 11 et 11.5 mm on *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus* respectively. Whereas the 3rd fraction proved to be very active on all strains, with diameters of ZIs 26, 15, 18, and 24 mm on *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus* respectively. With regard to H_4 fraction didn't show an activity on all strains.

For the antifungal effect, in the case of hexane extract of sheets. H_2 and H_3 fractions were active on *C. albicans*, with diameters of ZIs 13, 10.5 and 25 mm respectively.

Table 2 Diameter of zones of inhibition, obtained by fractions of hexane extracts of fruits and sheets, and raw hexane extract

Fractions	*E. coli*	*P. aeruginosa*	*B. cereus*	*S. aureus*	*C. albicans*
Fractions of hexane extract of fruits					
H_0	13.5	13	12	12	-
H_1	13	-	-	9	-
H_2	12	10	-	10.5	-
H_3	-	-	-	-	-
Fractions of hexane extract of sheets					
H_0	12.5	14	12	13	13
H_1	-	-	10	-	-
H_2	10	14	11	11.5	10.5
H_3	26	15	18	24	25
H_4	-	-	-	-	-

Results expressed in millimeter (mm). H_0: Raw hexane extract of fruits and sheets. H_1, H_2, H_3, H_4: Fractions of hexane extract of fruits and sheets.

These antimicrobial effects could be allotted to chemical compositions of hexane extracts which would be riched in terpenes, steroids and coumarines, whose antimicrobial capacity was shown considerable, Nakashima (2001). The evaluation of antimicrobial activities of fractions of hexane extract of fruits of *F. sellowianaw* was carried out by Nakashima (2001). Its results agree with our antibacterial results, whereas they were different compared to antifungal results. Indeed, results of Nakashima showed antibacterial activities of hexane fractions on *E. coli* and *S. epidermidis*, and an important activity of the fraction H_2 on *C. albicans*.[22].

Table 3 Diameter of zones of inhibition, obtained by fractions of acetone extracts of fruits and sheets, and raw acetone extract

Fractions	*E. coli*	*P. aeruginosa*	*B. cereus*	*S. aureus*	*C. albicans*
Fractions of acetone extract of fruits					
A0	20	19	13	23	-
A1	22	18	13	19	-
A2	16.5	13	16	20	10
A3	25	17	26	22	-
Fractions of acetone extract of sheets					
A0	19	25	21	15	20
A1	17.5	12	13	-	-
A2	-	-	14	-	23
A3	18	16	-	19	13.5

Results expressed in millimeter (mm). A_0: Raw acetone extract of fruits and sheets. A_1, A_2, A_3 fractions of acetone extract of fruits and sheets.

In the case of raw acetone extract of fruits (A_0), it was active on four bacterial strains. Diameter of ZIs were 20, 19, 13 and 23 mm respectively on *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus*; A_1 fraction of the same extract showed an important activity with diameters of 22, 18, 13 and 19 mm on *E. coli*, *P. aeroginosa*, *B. cereus* and *S. aureus* respectively. Whereas, A_2 fraction was more active on *S. aureus* (20 mm), followed by *E. coli* (16.5 mm), *B. cereus* (16 mm) and *P. aeruginosa* (13 mm). A_3 fraction of acetone extract of fruits was the most active compared to three other fractions. Thus, it was active on all strains, diameter of ZIs were 25, 17, 26 and 22 mm on *E. coli*, *P. aeruginosa*, *B. cereus* et *S. aureus* respectively.

For the antifungal effects, all fractions revealed as inactives, unless A_2 fraction recorded a weak activity with the diameter of 10 mm.

In the case of raw acetone extract of sheets (A_0), it was largely active on all bacterial strains. Diameter of ZIs 19, 25, 21 and 15 mm on *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus* respectively.

The 1st fraction showed an average activity with respect on *P. aeruginosa* (12 mm) and *B. cereus* (13 mm), whereas it was very active on *E. coli* (17.5 mm). The A_2 fraction of the same extract had an activity on *B. cereus* (14 mm). Bacterial activities of the 3rd fraction were noticed on *E. coli*, *P. aeruginosa* and *S. aureus* with diameter of ZIs of 18, 16 and 19 mm respectively.

For the antifungal effects in the case of acetone extract of sheets, A_0 and A_2 fractions had a remarkable effects on *C. albicans* with diameters of 20 and 23 mm respectively, whereas the 3rd fraction was fairly active with the diameter of 13.5 mm.

According to the analysis of these results, it arose that acetone extracts have a antibacterial and antifungal activities which their effectiveness varies from a fraction to another. In addition, the phytochemical sifting of the raw acetone extracts showed the presence of secondary metabolites such as saponosids, tannins, steroids and terpens which their antimicrobial activities were already confirmed.

Our results were on the same track with that of Motohashi and Al, (2000). Indeed their study carried on the antimicrobial activities of *F. sellowiana* which revealed antimicrobial activities of fractions of acetone extracts of fruits on *E. coli*, *P. aeruginosa*, *S. epidermidis* and *C. albicans*. In the same way, another study of antibacterial effects of acetone extracts of sheets proved their capacity of inhibition on *E. coli*, *P. aeruginosa* and *S. aureus* [23].

3.5. Microdilution

Results of MIC of hexane and acetone fractions of *F. sellowiana*, obtained by microdilution were illustrated in Table 4 and 5.

Table 4 MIC of hexane fraction of fruits and sheets, and raw hexane extract

Fractions	MIC in %			
	E. coli	***P. aeruginosa***	***B. cereus***	***S. aureus***
	Fractions of hexane extract of fruits			
H_0	20	40	40	40
H_1	40	-	-	20
H_2	20	2.5	-	20
	Fractions of hexane extract of sheets			
H_0	40	20	40	10
H_1	-	-	20	-
H_2	20	40	20	20
H_3	5	10	40	40

Results expressed in %. H_0: Raw hexane extract of fruits and sheets. H_1, H_2, H_3: Fractions of hexane extract of fruits and sheets.

The result of MIC of raw (H_0) and fractions of hexane extract of fruits obtained, showed different values between 2.5% to 40% (v/v), whereas those of sheets were between 5% to 40% (v/v). H_2 fraction of fruits showed a significant inhibition on *P. aeruginosa* sight its value was 2.5% (v/v) and on *E. coli* showed the value of 20% (v/v) which was identical when used the raw extract (H_0). this last value also appeared on *S. aureus* when used H_1 and H_2 fractions of the same extract. Therefore, the raw extract (H_0) of the same extract revealed similar inhibitions on *P. aeruginosa*, *B. cereus* and *S. aureus* the value was 40% (v/v), and on *E. coli* appeared the same value when used H_1 fraction.

In the case of hexane extract of sheets, the H_3 fraction gave the most remarkable effect which was 5% (v/v) on *E. coli*, therefore, on this last stain the MIC was 20% (v/v) when used H_2 fraction. Meanwhile, H_3 fraction had showed the value of 10% (v/v) which was sufficient to inhibit *P. aeruginosa*. Whereas, on this last stain the MIC was 20% (v/v) when used H_0 fraction and 40% (v/v) when used H_2 fraction. H_3 and H_0 fractions showed identical MIC (40% (v/v)) on *B. cereus*. Therefore, on this last train revealed another similar MIC which was 20% (v/v) when used H1 and H2 fractions.

Thereafter, on the strain *S. aureus* revealed an important inhibition which was 10% (v/v), fallowed by 20% (v/v) and 40% (v/v) when used H_0, H_2 and H_3 fractions respectively.

Table 5 MIC of acetone fractions of fruits and sheets, and raw acetone extract

Fractions	MIC in %			
	E. coli	***P. aeruginosa***	***B. cereus***	***S. aureus***
	Fractions of acetone extracts of fruits			
A_0	40	10	5	10
A_1	5	10	40	40
A_2	10	40	40	10
A_3	40	20	20	20
	Fractions of acetone extracts of sheets			
A_0	20	40	10	20
A_1	10	20	40	-
A_2	-	-	40	-
A_3	20	10	-	40

Results expressed in %. A_0: Raw acetone extract of fruits and sheets. A_1, A_2, A_3: Fractions of acetone extracts of fruits and sheets.

The test of microdilution revealed values of MIC were between 5% (v/v) to 40% (v/v) for fractions of acetone raw extracts of fruits. Whereas MIC results of fractions of acetone raw extracts of sheets showed were between 10% (v/v) to 40% (v/v).

In the case of acetone extracts of fruits, the effect of A_1 fraction was more accentuated with MIC of 5% (v/v), A_2 fraction with 10% (v/v), the raw extract (A_0) and A_3 fractions with 40% (v/v) on *E. coli*.

The inhibition of *P. aeruginosa* was made by concentrations of 10% for A_0 and A_1 fractions, and 20% (v/v), 40% (v/v) for A_3 and A_2 fractions respectively. In the case of *B. cereus*, the inhibition of A_0 fraction with the value of 5% (v/v) was significant, followed by the value of 20% (v/v) for A_3 fraction and 40% (v/v) for A_1 and A_2 fractions. However, the inhibition of *S. aureus* was made by a concentration of 10% (v/v) for the raw extract and A_2 fraction, followed by a concentration of 20% for A_3 fraction and 40% for A_1 fraction. The test of determination of MIC of extract A_0 of fruit by Basile and al. (2010), gave values of MIC variable from 3.9 to 62.5 µg/ml. In another study published in 1997, Basile et al. deferred that concentrations going from 4 to 64 µg/ml were sufficient to inhibit the growth of *P. aeruginosa*, *E. coli* and *S. aureus* [7, 27].

3.6. Determination of MBC

MBC of acetone and hexane extracts of sheets and fruits, were illustrated in the tables below (tables 6 and 7).

Table 6 MBC of hexane fractions and raw hexane extract

Fractions	MBC in %			
	E. coli	***P. aeruginosa***	***B. cereus***	***S. aureus***
	Fractions of hexane extract of fruits			
H0	40	80	80	80
H1	80	-	-	40
H2	40	5	-	40
	Fractions of hexane extract of sheets			
H0	80	40	80	20
H1	-	-	40	-
H2	40	80	40	20
H3	10	20	80	80

Results expressed in %. H_0: Raw hexane extract of fruits and sheets. H_1, H_2, H_3: Fractions of hexane extract of fruits and sheets.

Table 7 MBC of acetone fractions and raw acetone extract

Fractions	MBC in %			
	E. coli	***P. aeruginosa***	***B. cereus***	***S. aureus***
Fractions of acetone extract of fruits				
A0	80	20	10	20
A1	10	20	80	80
A2	20	80	80	20
A3	80	40	40	40
Fractions of acetone extract of sheets				
A0	40	80	20	40
A1	20	40	80	-
A2	-	-	80	-
A3	40	20	-	80

Results expressed in %. A_0: Raw acetone extract of fruits and sheets. A_1, A_2, A_3: Fractions of acetone extract of fruits and sheets.

The values of the MBC of fractions and raw extracts tested, were higher than those of the MIC. Results showed bacterial activities of organic extracts tested against studied germs.These results are in agreement with those stated by Vutto et al. (2000), Basile et al. (2010 and 1997) [27, 8].

4. Conclusion

According to the different results obtained during this study, the highest output obtained, was the acetone extracts compared to hexane extracts. All extracts have showed the presence of secondary metabolites such as saponosids, tannins, steroids and terpens. Thus, they showed antimicrobial activities except acetone extract of fruits, almost all fractions revealed as inactives on *C. albicans*. However, more studies are needed to valorize the moroccan variety *F. sellowiana* and could be used to overcome the microbial aggressions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have declared that no competing interest exists.

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