Original Research Article

Synergistic antimicrobial and antioxidant activity of rich saponin extracts from *Paronychia argentea* and *Spergularia marginata*

ABSTRACT

Aims: The crude saponins extracted from the aerial parts of *Paronychia argentea* and the roots of *Spergularia marginata* were tested for their antioxidant, antimicrobial and synergistic effects with antibiotics.

Methodology: Antioxidant activity was evaluated using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical, β-carotene-linoleic acid and reducing power assays. However, the antibacterial activity was assessed by the agar disc diffusion method, whereas the MIC determination and the synergistic interaction with antibiotics were evaluated using microdilution method.

Results: Saponin extract from *Paronychia argentea* showed a higher antioxidant activity than that from *Spergularia marginata*. Using DPPH assay, the IC50 values for saponin extracts from *P. argentea* and *S. marginata* were 19.08 and 29.65 µg/ml, respectively. For β-carotene-linoleic acid assay, IC50 values were 98.24 and 614 µg/ml respectively for *P. argentea* and *S. marginata*. However, for reducing power assays, the IC50 values for saponins extracts were respectively 27.22 and 61.44 µg/ml. The result of MIC assay showed that both saponin extracts was found to be active against the majority of *Candida* strains and Gram-positive bacteria. However, crude saponin extracted from *S. marginata* was more active on microorganisms than that of *P. argentea*. In fact, the *in vitro* association of saponin extracts and some commercial antibiotics showed a synergistic effect. For bacteria strains, 30 combinations were studied, 17 (56.66%) combinations had total synergism, 7 (23.33%) had partial synergism, 4 (13.33%) had no effect and 2 (6.66%) had antagonism effect. For *Candida* strains, 8 combinations of saponins extracts and fluconazol are tested. All of these combinations (100%) exhibited a total synergism with FICi ranging from 0,31 to 0,50.

Conclusion: The results founded suggested that further work should be performed on the isolation and identification of the antioxidative and antimicrobial components of these saponin extracts.

Keywords: antimicrobial, antioxidant activity, medicinal plants, *Paronychia argentea, Spergularia marginata, synergistic activity,* saponins

1. INTRODUCTION

Paronychia argentea and *Spergularia marginata* are two indigenous plants belonging to the family of Caryophyllaceae which includes a large number of species rich in saponins with various pharmacological properties. *P. argentea* is a perennial plant, locally known as "hiddourtRaii". Infusion of *P. argentea* is commonly used in Moroccan popular medicine as aperitif and diuretic [1]. The uses of this plant in folk medicine differ from country to another. In Algeria, the plant is used as diuretic, hypoglycemic and as antiurolithiasis plant [2]. In Portugal, infusion of *P. argentea* is used as gastric analgesic, bladder; prostate ailments, abdominal ailments, and stomach ulcers [3]. In Jordany, it is used as diuretic, in treatment of kidney stones, diabetes and heart pains [4]. *S. marginata* is an annual plant native to Mediterranean area, usually used in traditional medicine for the treatment of female infertility as well as aphrodisiac [1].

Saponins have many pharmacological properties such as haemolytic, molluscicidal, anti-inflammatory, antifungal, antibacterial, antiparasitic and immunostimulant [5-7], anti-cancer [6,8] and antiproliferative [9]. Many studies have been dealing with the antimicrobial and antioxidant activities of saponins extracted from several plants [10-11]. However, no studies have been conducted to investigate the antioxidant and antimicrobial activities of saponins extracted from aerial part of *Paronychia argentea* and root part of *Spergularia marginata*.

The present study was undertaken to evaluate the antioxidant and antimicrobial activities of these saponins. Furthermore, the synergistic interaction using combination between saponin extracts and some usual antibiotics was also evaluated.

2.MATERIAL AND METHODS

2.1 Extraction of crude saponins

Aerial parts of *P. argentea* and roots of *S. marginata* were collected respectively from Oukaimeden (near to Marrakech) and Essaouira (Morocco) and identified by prof. Abbad, Laboratory of Ecology Faculty of Sciences Semlalia, Marrakesh and voucher specimens are deposited at the Herbarium of the Faculty. Plants were dried at room temperature and ground

into fine powder. The plant powders were extracted 3 times with methanol-water (v/v) and placed in an orbital shaker, set at 200 rpm for 24 h. The extracts were filtered and evaporated under reduced pressure to yield residues. The residues were suspended in hot water and extracted with water-saturated n-butanol. Crude saponin extracts were obtained by precipitation with Petroleum ether.

2.2 Detection of saponins in crude extracts

2.1.1 Thin Layer Chromatography

20 µl of samples was applied by capillary on the line marked on silica gel 60 F254 TLC plates. Then, TLC plate was placed into a separation chamber in presence of small amount of eluent. Subsequently, the plate was sprayed with the Godin reagent. Sprayed TLC plates were then heated at 100C°. Different spots were observed.

2.1.2 Determination of Foaming index

2 mg of plants powder was transferred into an Erlenmeyer flask containing 100 ml of water. The mixture was boiled for 30 min, then cooled, filtered and transferred to 100 ml volumetric flask, sufficient water was added to make up the volume. The decoction was poured into the 10 test-tubes. After 15 min, the height of foam was measure [12].

2.2. Antioxidant activity

2.2.1. DPPH radical assay

DPPH free radical-scavenging activity of saponins extracts was assessed using a stable radical DPPH: 2,2-diphenyl-1-picrylhydrazyl [13-14]. Briefly, fifty microliters of saponins extracts at different concentrations ranging from 10 to 1000 µg/ml were mixed with 2 ml of DPPH methanol solution (60 µM). After 20 min of incubation in darkness, at room temperature, the absorbance was measured at 517 nm. A blank sample containing the same amount of methanol and DPPH solution was used as negative control. Synthetic antioxidant reagent Butylated hydroxytoluene (BHT) and quercetin were used as positive controls. All tests were carried out in triplicate and the results were expressed as mean ± SD. The percentage inhibition of the DPPH radical was calculated according to the formula:

DPPH scavenging effect (%) = [(A0- A1)*100]/A0

Where: A0 is the absorbance of the control at 20 min, and A1 is the absorbance of the sample at 20 min. IC50 is the concentration of antioxidant required for 50% scavenging of DPPH

radicals and was calculated by plotting inhibition percentages against concentrations of the sample.

2.2.2. ß-Carotene/linoleic acid bleaching assay

The β-carotene/linoleic acid test evaluates the capacity of the extract to reduce the oxidative loss of β-carotene in a β-carotene–linoleic acid emulsion. This test was assessed as described by Miraliakbari and Shahidi (2008) with slight modifications [15]. A solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 µl of linoleic acid and 200 mg of Tween 40 was added. The chloroform was evaporated under vacuum using a rotary evaporator at 40 °C. Then, 100 ml of distilled water was added and the mixture was shaking vigorously to form a clear yellowish emulsion (A). 2500 µl of this emulsion was dispersed in test tubes and 350 µl of various concentrations ranging from 10 to 1000 µg/ml of saponins extracts prepared previously in water was added. The test tubes were incubated for 2 h in a water bath at 50 °C. The same procedure was repeated with positive control BHT and quercetine and with negative control (blank). A second emulsion (B) consisting of 25 µl of linoleic acid, 200 mg of Tween 40, 1 ml of chloroform and 100 ml of distilled water was prepared to adjust the zero of spectrophotometer. The absorbance values were measured at 470 nm immediately at the beginning of the experiment (t=0) and after 2h of incubation. Antioxidant activities (percentage inhibition I %) of the samples was calculated using the following equation:

$$I\% = (A\ \beta\text{-carotene after 2 h assay} / A\ \text{initial}\ \beta\text{-carotene}) \times 100$$

Where A β-carotene after 2 h assay is the absorbance values of β-carotene after 2 h assay remaining in the samples and A initial β-carotene is the absorbance value of β-carotene at the beginning of the experiment.

The antioxidant activity was expressed as 50% inhibition concentration (IC50). The IC50 was calculated by plotting inhibition percentage against the extract concentration. All tests were carried out in triplicate and IC50 values were reported as means ±SD of triplicates.

2.2.3. Reducing power determination

The determination of reducing power was evaluated as described by Oyaizu (1986) [16]. Diffrent concentrations ranging from 10 to 1000 µg/ml of saponins extracts were mixed with

phosphate buffer (2.5 ml, 0.2 mol/L, pH 6.6) and potassium ferricyanide ($K3Fe(CN)_6$) (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of trichloroacetic acid (10%, w/v) was added to the mixture to stop the reaction, and the mixture was centrifuged at 650 × g for 10 min. At last, 2.5 mL of the supernatant was mixed with distilled water (2.5 mL) and ferric chloride $FeCl_3$ (0.5 mL, 1%) and the absorbance was measured at 700 nm against a blank. BHT and quercetin were used as reference compound. The extract concentration providing 0.5 of absorbance (IC50) was calculated by plotting absorbance at 700 nm against the corresponding extract concentration. The test was carried out in triplicate and IC50 values were reported as means ± SD.

2.3. Antimicrobial activity

The crude saponins were screened against two Gram-negative bacteria: *Escherichia coli* (ATCC 25922), and a clinically isolated strain *Klebsiella pneumoniae*, three Gram-positive bacteria: *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (CCMM B3) and *Bacillus cereus* (ATCC 14579) and four candida: *Candida albicans* (CCMM L5), *Candida glabrata* (CCMM L7), *Candida krusei* (CCMM L10) and *Candida parapsilosis* (CCMM L18).

2.3.1. Disc diffusion method:

The disc diffusion method is carried out to evaluate the antimicrobial activity of saponins extracted from *P. argentea* and *S. marginata* [17]. Suspensions prepared from overnight cultures of each microorganism were uniformly spread on a Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (DSA) for yeasts. Sterile paper discs (6mm in diameter) impregnated with 20 µl of saponins extracts corresponding to 6 mg/disc of saponins extracts, were placed on the surface of each inoculated agar plates. Plates were placed at 4 °C for 4 h and incubated for 24 h at 37 °C for bacteria strains and 28 °C for *Candida* strains. After incubation, the diameters of inhibition zones were measured in millimeter. Disc impregnated with sterile distilled water served as negative control and disc impregnated with antibiotics (Ciprofloxacin 5 µg/disc, Kanamycin 30 µg/disc, and Cefixime 10 µg/disc and Fluconazole 40 µg/disc) served as positive control. Each assay was performed in triplicates.

2.3.2. Determination of the Minimum Inhibitory Concentration

MIC assay of saponin extracts were assessed using micro-well dilution method [18]. Two-fold serial dilutions of saponin extracts ranging from 1mg/ml to 128 mg/ml were prepared in sterile distilled water. Then, three to four colonies of overnight cultures of each microorganism was inoculated into 5 ml of sterile nutrient broth and incubated for 3 to 5 h. 50 µl of this suspension was diluted in twice concentrated Mueller-Hinton Broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for yeasts to adjusted the culture to 10^6 CFU/ml for bacteria and 1–2 $\times 10^3$ cells/ml for candida. Microwells containing 100 µl of saponin dilution were, separately, inoculated with 100 µl of bacterial or yeasts suspensions initially prepared. The inoculated microplates were incubated at 37 °C for 18–24 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of saponins extracts inhibiting visible growth of the tested strains. The Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by sub-culturing 10 µl of the MIC test solutions on nutrient agar plate for bacteria or PDA for yeasts and the plates were incubated in the same conditions. The MBC and MFC were defined as the lowest bactericidal and fungicidal concentration. Ciprofloxacine, cefixime, kanamycine and fluconazole were served as conventional antibiotics. All tests were performed in duplicate and repeated three times at least and the results were averaged.

2.3.3. Synergetic interaction

The synergistic interactions between conventional antibiotics (ciprofloxacin, kanamycin, and cefixime), and a classical antifungal (fluconazole), and saponin extracts from *P. argentea* and *S. marginata* were tested using the checkerboard essay method [19]. Synergy between antibiotics and saponins extracts at low concentration (fraction of the MIC: MIC/4) was studied using microdilution assay. 50 µl of the saponins extracts at MIC/4 were added to microwells containing, separately, 50 µl of antibiotics or antifungal dilutions, and inoculated with 100 µl of cell suspension of approximately 10^6CFU/ml for bacteria and 1–2 × 10^3 cells/ml for yeasts. Then, the microwells were incubated in the same condition. All assays were performed in duplicate and repeated thrice.

-The gain was determined as MIC of antibiotic alone/MIC of antibiotic in combination with saponins extracts.

-Fraction inhibitory concentration index (FICI) was calculated by the method reported by Didry et al. (1993) [20], according to the following formula:

FICI = MICA/B /MICA+MICB/A/ MICB

where MICA = MIC of the compound A alone and MICA/B is the MIC of compound A in combination with compound B. MICB and MICB/A are defined in the same way as for compound A.

Total synergism (FICI ≤ 0.5), partial synergism (0.5<FICI ≤0.75), no effect (0.75 < FICI ≤ 2) or antagonism (FICI > 2) were evaluated.

3. RESULTS AND DISCUSSION

3.1 Detection of saponins in crude extracts:

As shown in table 1, the yield of saponins crude extract of *P. argentea* was higher than that of *S. marginata*. However, the presence of persistent foam in aqueous extracts of both plants, calculated using the foaming index which was higher than 100%, indicated the presence of saponins.

The first indication of the presence of saponins in crude saponin extracts can be obtained by spraying Godin reagent on the TLC chromatogram. As can be seen from the TLC chromatogram (figure 1), crude saponins of *P. argentea* and *S. marginata* showed different spots with different Rf and colors. Blue and purple blue spots indicated the presence of triterpenoids saponins [5]. This is in accordance with the result obtained with foaming index assay, which showed the presence of foam in aqueous extracts of the studied plants (foaming index higher than 100).

Table 1. Percentage yield and Foaming index of crude saponins

	Yield of crude extract (%)	**Foaming index (%)**
P. argentea	3.2±0,41	275
S. marginata	2.5±0,15	200

Figure 1. TLC chromatogram of crude saponins extract on silica gel sprayed with the Godin reagent. Chloroform/ Ethyl acetate/ Methanol/ Water (60: 30: 8: 1) used as solvent. Par: crude saponin extract of *P. argentea*; Sper: saponin extract of *S. marginata*.

Table 2. Rf values for crude saponin extracts on TLC Silica Gel.

***P. argentea* crude saponins**		***S. marginata* crude saponins**	
Rf values	**Spot color**	**Rf values**	**Spot color**
0.13	Blue	0.11	Blue
0.30	Blue	0.34	Blue
0.44	Yellow	0.68	Blue
0.58	Blue	0.69	Blue
0.69	Blue	0.75	Blue
0.77	Yellow	0.85	Blue
0.90	Purple blue	0.90	Blue

3.2. Antioxidant activity

The antioxidant activity of saponins extracted from *P. argentea* and *S. marginata* was evaluated by three complementary tests: DPPH free radical scavenging, β-carotene-linoleic acid and reducing power activities [21]. DPPH was used to test the Free radical scavenging ability of tested saponins. As shown in Table 3, both saponin extracts had a potent antioxidant activity, exhibiting ability to reduce the stable radical DPPH. Saponin extract of *P. argentea* (IC50=19.08 µg/ml ±0.62) have a higher antioxidant activity than that from *S. marginata* (IC50=29.65 µg/ml ±0.40). Comparing IC50 values of saponins with those of synthetic antioxidant, the tested extracts were less potent than BHT and quercetin (4.21 µg/ml ±0.08 and 1.07 µg/ml ±0.01 respectively).

However, the results for β-carotene/linoleic acid bleaching test showed that saponin extract from *P. argentea* exhibited ability to inhibit conjugated dienehydroperoxides formation than that obtained from *S. marginata* with IC50 values of 98.24±0.48 µg/ml and 614±0.17 µg/ml respectively. Saponin extracts were found to possess less antioxidant activity in comparison with BHT and quercetin (7.09 µg/ml ± 0.10 and 2.29 µg/ml ± 0.10 respectively).

Besides, the ability of saponin extracts to donate an electron or hydrogen to Fe^{3+} to reduce Fe^{2+} was investigated. It can be seen from table 3 that saponins extracted from *P. argentea* (27.22 µg/ml ±0.57) and those from *S. marginata* (61.44 µg/ml ±0.19) exhibited a potent capacity to reduce Fe^{3+}. Although, these saponins was less effective than BHT and quercetin (7.09 µg/ml ± 0.10 and 2.29 µg/ml ± 0.10 respectively).

To our knowledge, no results from experiments with antioxidant activity of saponins extracted from *P. argentea* and *S. marginata* have been reported. While previous studies were conducted only on the antioxidant activity of *P. argentea* and *S. rubia* (same genus as *S. marginata*) methanolic and water extracts and demonstrated great activity for these extracts [3-22].

Table 3. Antioxidant activity of saponin extracts, BHT and quercetin.

Tests	**IC50 µg/ml**			
	Saponin extract of *P. argentea*	**Saponin extract of *S. marginata***	**BHT**	**Quercetin**
DPPH	19.08 ±0.62	29.65±0.4	4.21±0.08	1.07±0.01

β-carotene-linoleic acid	98.24 ±0.48	614±0.17	4.30±0.33	0.95±0.02
Reducing power	27.22 ±0.57	61.44±0.19	7.09± 0.1	2.29± 0.10

Values represent means ± standard deviations for triplicate experiments.

Many saponins are known by their antioxidant activity which can be related to the structure of their aglycones and the number of attached sugar residue [23]. As discussed above, saponins extracts of *P. argentea* and *S. marginata* exhibited high antioxidant activity, this is could be due to their structure and their surface-active. Huong et al. (1998) [24] have investigated the antioxidant activity of saponins from Vietnamese ginseng; and found that saponins exerted protective action against free radical-induced tissue injury. Another study reported that Triterpene saponins from *Butyrospermum parkii* of Cameroon showed an antioxidant activity using DPPH method [25].

3.3. Antimicrobial activity

The antimicrobial and synergistic interactions with antibiotics were assayed *in vitro*. The crude saponins were screened against Gram-negative bacteria (*Escherichia coli*, and *Klebsiella pneumoniae*), Gram-positive bacteria (*Micrococcus luteus*, *Staphylococcus aureus* and *Bacillus cereus*) and candida (*Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida parapsilosis*). The results of disc diffusion method (Table 4) demonstrated that Gram-positive bacteria were the most sensitive being inhibited by the tested saponins extracts. *M. luteus* was the most sensitive with inhibition zones of 11.10±0.35 mm and 14.50±0.76 mm respectively for saponin extract of *P. argentea* and *S. marginata*. The studied saponin extracts had a weak effect on *E. coli* and no effect on *K. pneumoniae*. The inhibition zones of *Candida* strains showed an inhibition zone ranging from 10.37 to 15.73 mm and from 9.40 to 13.07 mm for extracts of *S. marginata* and *P. argentea* respectively.

Moreover, the results obtained for micro-well dilution method (Table 5), showed that both saponin extracts were found to be active against all *Candida* strains except *C. krusei*. In fact, saponin extract from *P. argentea* exhibited higher activity against *Candida* strains than those from *S. marginata*. The highest MIC value (4 mg/mL) was observed from *S. marginata* against *C. albicans*, followed by *C. glabrata* and *C. parapsilosis*.

Both saponin extracts showed antimicrobial activity against tested Gram-positive bacteria. The MIC values for *P. argentea* were 8 and 16 mg/mL respectively for *M. luteus*, *B. cereus* and *S. aureus*. While the MIC values for those of *S. marginata* were 8mg/mL for all Gram-positive bacteria. Furthermore, the crude saponins extracts had no effect on gram-negative bacteria

The MIC was equivalent to the MBC and MFC for *S. marginata* extract principally against *M. luteus*, *C. albicans* and *C. parapsilosis*, indicating a bactericidal action.

Synergistic interaction between antibiotics and saponin extracts from *P. argentea* and *S. marginata* was also evaluated in this study. The results were presented in Table 6. For bacteria strains, 30 combinations were studied, 17 (56.66%) combinations had total synergism, 7 (23.33%) had partial synergism, 4 (13.33%) had no effect and 2 (6.66%) had antagonism effect. For Gram positive-bacteria, the best synergistic antibacterial action were obtained with the combination saponins-cefixime with total synergistic effect (FICi ranging from 0.38 to 0.49), followed by the combination saponins-kanamycin.

Table 4. Antimicrobial activity of saponin extracts and reference antibiotics against bacteria and *Candida* strains using disc diffusion method

Test bacteria	Inhibition zone diameter (mm)					
	saponin extracts		Antibiotics			
	***P. argentea* (6mg/disc)**	***S. marginata* (6mg/disc)**	**Cefixime (10 µg/disc)**	**Ciprofloxacin (5 µg/disc))**	**Kanamycin (30 µg/disc))**	**Fluconazole (40 µg/disc))**
E. coli ATCC25922	7.00±0.10	8.17±0.17	19.33±0.80	30±0.58	22.00±1.15	NT
K .pneumoniae	NI	NI	10.33±0.30	7.83±0.93	NI	
M. luteus ATCC10240	11.10±0.35	14.50±0.76	20.17±0.44	29.67±0.88	25.00±1.15	
S. aureus CCMM B3	9.83±0.20	10.17±0.60	15.17±0.10	27.67±0.67	23.33±1.66	
B. cereus ATCC14579	7.90±0.46	9.33±0.33	26.33±0.30	35.67±0.88	37.00±2.19	

C. albicans CCMM L5	13.07±0.12	15.73±0.43	NT		23.33±1.76
C. glabrata CCMM L7	11.17±0.17	13.25±0.20			19.00±0.58
C. krusei CCMM L10	7.73±0.15	10.73±0.15			28.00±0.76
C. parapsilosis CCMM L18	9.40±0.10	12.20±0.12			32.17±0.73

-Values represent averages ±standard deviations for triplicate, -Inhibition zone including disc diameter (6 mm),- NI: No inhibition, NT: not tested

Table 5. Antimicrobial activity of antibiotics and saponins extracted from *P. argentea* and *S. marginata* using micro-well dilution method.

	Saponin extracts				**Antibiotics**							
	***P. argentea* mg/ml**		***S. marginata* mg/ml**		**Ciprofloxacin mg/ml**		**Cefixime mg/ml**		**Kanamycin mg/ml**		**Fluconazole mg/ml**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MFC**
E. coli ATCC25922	32	64	32	64	0.008	0.015	0.015	0.062	0.015	0.031		
K. pneumonia	64	>64	64	>64	0.250	>0.250	1	>1	0.500	>0.500	NT	
S. aureus CCMM B3	16	32	8	16	0.031	0.125	0.062	0.125	0.125	>0.125		
M. luteus ATCC10240	8	16	8	8	0.015	0.062	0.031	0.062	0.062	0.125		
B. cereus	16	32	8	32	0.015	0.031	0.015	0.06	0.06	0.125		

ATCC14579					2	2		
C. albicans CCMM L5	8	8	4	4			0.031	0.031
C. glabrata CCMM L7	8	32	8	16			0.500	>0.500
C. parapsilosis CCMM L18	16	32	8	8			0.062	0.125
C. krusei CCMM L10	>64	>64	>64	>64			0.250	0.500

NT: not tested. MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, MFC: Minimum Fungicidal Concentration.

Table 6. Synergistic interaction between *Paronychia argentea and Spergularia marginata* saponin extracts and antimicrobial agents against selected bacteria.

strains	PA+ C		PA+CP		PA+K		SM+C		SM+CP		SM+K	
	FICi	Gain	FICi	Gain	FICi	Gain	FICi	Gain	FICi	Gain	FICi	Gain
E. coli	0.52b	4	0.50a	4	0.78b	2	0.78b	2	0.50 a	4	0.78b	2
K. pneumoniae	1.25c	1	2.30d	1	1.25c	1	1.25c	1	2.30 d	1	1.25c	1
S. aureus	0.49a	4	0.50a	4	0.75b	2	0.38 a	8	0.50 a	4	0.75b	2
M. luteus	0.38a	8	0.50a	4	0.38a	8	0.38 a	8	0.40 a	8	0.38a	8
B. cereus	0.38a	8	0.40a	8	0.49a	4	0.52b	4	0.50 a	4	0.49a	4

PA:saponins of *P. argentea*; SM: saponins of *S. marginata*; C: Cefixime ; CP: Ciprofloxacin; K: Kanamycin; FICi: Fractional inhibitory concentration. [a] Total synergism / [b] Partial synergism/ [c] No effect/. [d]Antagonism index, NT: not tested

Table 7. Synergistic interaction between *Paronychia argentea and Spergularia marginata* saponin extracts with fluconazole antimicrobial agent against selected yeasts

fungi	PA+F		SM+F	
	FICi	Gain	FICi	Gain
C. albicans	0.50a	4	0.38a	8
C.glabrata	0.31a	16	0.31a	16
C. parapsilosis	0.50a	4	0.38a	8
C. krusei	0.49a	4	0.50a	4

PA:saponins of *P. argentea*; SM: saponins of *S. marginata*; ; F: Fluconazole. FICi: Fractional inhibitory concentration. [a] Total synergism / [b] Partial synergism/ [c] No effect/. [d]Antagonism index, NT: not tested

For *E. coli*, the best combination was saponins-ciprofloxacin with partial synergism (FICi=0.5). However, the three combinations of the both saponin extracts and antibiotics had no effect on *K. pneumoniae*. For *Candida* strains, 8 combinations of saponins extracts and fluconazole are tested. All of these combinations (100%) exhibited a total synergism with FICi ranging from 0.31 to 0.50.

The obtained results showed that saponins extracted from *P. argentea* and *S. marginata* had an inhibitory effect on the growth of Gram-positive bacteria and no effect on Gram-negative bacteria. While the tested extracts exhibited an anticandidal activity against most *Candida* strains used in the study except *C. krusei*.

Several studies reported that saponins have antibacterial, antifungal and antiviral activities [10-26]. Our finding is in agreement with other study conducted on the crude saponins and saponins-rich fraction from *Guar meal*, which showed that crude saponins from this plant were active only against Gram-positive bacteria and not on Gram-negative ones. In contrast, saponin-rich fractions of Guar meal were active against both Gram-positive bacteria and Gram-negative bacteria [11]. Another study proved that saponins of *Sorghum bicolor* have an inhibitory effect on gram-positive bacteria but not on gram-negative one and fungi [10]. Whereas saponins of *Anabasis articulate* exhibited a higher degree of antimicrobial activity against Gram-negative and Gram-positive bacteria and *Candida albicans* [27].

The differences on ineffectiveness may be due to the degradation of saponins by some glucosidase enzymes produced by Gram-negative bacteria or may be saponins extracts could not be able to penetrate the cell membranes of the microorganisms [28]. In fact, Gram-negative bacteria are known by their resistance for commercial antibiotics. This could be explained by the structure of cell envelope; Gram-negative bacteria possess an additional membrane, termed outer membrane, delineating the periplasmic space with the cytoplasmic membrane that restricts diffusion of hydrophobic compounds [29].

Combined antibiotic therapy has been shown to delay the emergency of bacteria resistance and may also produce favorable synergistic effects in the treatment of bacterial infection. Drug synergism between known antibiotics and bioactive plant extracts is one of the novel ways to overcome the resistance mechanisms of bacteria. In this study, synergistic effect resulting from the combination of antibiotics and the crude saponins was studied. Our results showed that saponins extracts combined with antibiotics exhibited a high synergistic effect

against most microbial strains and the MICs of antibiotic were reduced. Several studies on the interaction between plant extracts and antibiotics indicated a synergistic interaction [19, 30-31]. Synergism effect between antimicrobial agent and water extracts of some plants was occurred in both sensitive and resistant strains but the magnitude of minimum fold inhibition in resistant strains was higher than the sensitive strains [32]. In fact, plant extracts combined with antibiotics showed a decrease in MIC and this can be explained by the presence of different phytochemicals product [33], which might inhibit bacteria by different mechanisms. The double attack of both agents on different target sites of the bacteria could theoretically lead either an additive or synergistic effect [34].

The moderate activity of crude saponins extracted from *P. argentea* and *S. marginata* against some strains used in this study can be explained by the fact that the crude saponin extract is a mixture of several fractions each one could be as effective as antibiotics or even more effective than the popular antibiotics. In our laboratory fractionation of crude extracts is being undertaken in order to separate fractions more active than the crude extracts. Subsequently, fractionation may lead to pure saponins with a strong activity.

4. CONCLUSION

The results obtained in this study demonstrated that both crude saponins extracts had a remarkable antioxidant and antimicrobial activities. Saponin extract from *Paronychia argentea* were more effective than that from *Spergularia marginata* as antioxidant. Furthermore, both saponin extracts was found to be active against the majority of *Candida* strains and Gram-positive bacteria. However, crude saponin extracted from *S. marginata* was more active on microorganisms than that of *P. argentea*.

In addition, the combinations between saponins extract and classical antibiotics exhibited synergistic interactions against resistant bacteria and candida. The results founded suggested that further work should be performed on the isolation and identification of the antioxidative and antimicrobial components of these saponin extracts.

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