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Research Article

Evaluation of the Phenolic and Flavonoid Contents, Antimicrobial and Cytotoxic Activities of Some Plants Growing in Al Jabal Al-Akhdar in Libya

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ABSTRACT
The methanolic extract of the aerial part of two Libyan medicinal plants *Arbutus pavarii*. Pampan (Ericaceae) and *Sarcopoterium spinosium*. L. (Rosaceae) growing in El-Jabal Al Akhdar area were studied for their phenolic and flavonoid content, antimicrobial and cytotoxic activitie. Total polyphenol contents ranged from 61.7±2.7to 163.6±0.85 µg gallic acid equivalent / g (*A. pavarii* Pampan and *S. spinosium*. L.) and total flavonoid contents ranged from 126.9±2.98 to 206.1±1.09 µg rutin equivalent (*A. pavarii* Pampan and *S. spinosium* L.). Qualitative and quantitative analysis of major phenolic and flavonoids in the extracts were conducted by high-performance liquid chromatography (HPLC). Finally, antimicrobial activities of the two plants were measured using the disc diffusion method. While, cytotoxic properties were tested against the HEPG2 and T47D cell lines. *Arbutus pavarii* extract proved to be the most cytotoxic extract in this study with IC_{50} 19.7±2.8 and 19±0.65 (μg/ml) on HEPG2 and T47D respectively.

Keywords: phenolics, flavonoids, antimicrobial, cytotoxic.

INTRODUCTION

Libya has tremendous wealth of medicinal plants scattered in a vast area. These plants are used in Libyan folklore medicine for their medical as well as nutritive values. *Arbutus pavarii*. Pampan and *Sarcopoterium spinosium*. L. are two widely distributed plants in Al -Jabal Al - Akhdar region. Plant tannins and flavonoids have been reported to have antimicrobial effects in addition to many biological activities[1-3]. They are linked to reduce the risk of cancer by delaying or reversing the carcinogenesis process by blocking or suppressing COX1, COX2 and DNA topoisomerase 1 enzymes[4]. *Arbutus pavarii*. Pampan is an evergreen shrub or a small tree that belongs to the Ericaceae family and endemic to El-Jabal El-Akhdar, Libya. It is used in honey production, as food due, as ornament trees and in medicine for treatment of gastritis, renal infections and cancer ailments[5]. Only one previous study indicated a good antioxidant activity of *Arbutus pavarii*. Pampan among other tested medicinal plants. Few reports were traced concerned with *Arbutus pavarii*. Pampan[3], the most available literature revealed the presence of different phyto-constituents in the leaves and fruits of *Arbutu unedo* L. [American strawberry tree] viz.: triterpenes and irridoid glycosides, organic acids, tannins, flavonoids, sterols, phenolic compounds and amino acid[6]. *Sarcopoterium spinosum* L. is one of the thorn plants, it is widely grown in the Mediterranean region, belong to Rosaceae family[7] and its roots are widely used as an antidiabetic drug by Bedouin healers and the roots are used for the treatment of diabetes (major medicinal use!), toothaches, digestive problems, inflammation, and pain. The findings are reviewed and compared with the current literature[8]. Chemical constituens of *Sarcopoterium spinosum* L. and their bioactivity have not been fully identified yet. The recent studies revealed that catechin and epicatechin were detected in *Sarcopoterium spinosum* L. extract using hyphenated LC-MS/MS, and provide the basis for antidiabetic activity of the extract[9]. Therefore, this study was designed to further insight into these plants and to prove medical traditional uses of the plants for infections and as cancer remedies.

MATERIALS AND METHODS

Plant materials

The whole aerial part of the plants under investigation were collected from Al –Jabal Al Akhdar- El – Bieda city – Libya during 2013, identified and authenticated by Department of Botany, Faculty of Sciences, Benghazi University, Libya. Separately air-dried, powdered and kept in tightly closed amber colored containers.

Extracts preparation

50gm of the air dried aerial parts of each studied plant were separately extracted with methanol 90% using soxhlet apparatus till exhaustion. Each of the resulted extract was

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concentrated under vacuum by rotary evaporator. The residues left after distillation of the solvent were weighed and kept in a desiccators.

Assesment of the total phenolic content

The content of total phenolic compounds in methanolic extract was determined by the Folin-Ciaculteau's method[10]. The intensity of the of blue color was measured at λ_{max} 725 nm against a blank (distilled water). Gallic acid was used to compute the standard curve[11]. Determinations were carried out in triplicates and expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g).

Assesment of the total flavonoid content

The spectrophotometric method, based on measuring the intensity of the color developed when flavonoids are complexed with aluminum chloride was adopted[12]. The intensity of the developed yellow color directly measured, at λ=420 nm in a UV/Vis spectrophotometer against a blank experiment prepared in the same way, using 1 mL of methanol instead of the standard solution. Determinations were carried out in triplicates and expressed as mg rutin equivalents per gram dry weight (mg Rut/g).

HPLC for phenolics and flavonoids

Flavonoid and phenolic compounds were determined by HPLC according to the method of [13,14] as follows: Air dried aerial parts of the plants under investigation (5g) was mixed with 62.5% aqueous methanol (40 ml) and centrifuged at 1000 rpm for 10 minutes and the supernatant was filtered through a 0.2 µm Milipore membrane filter and the filterate was made up to 100 ml with methanol then 1-3 ml was collected in a vial for injection into HPLC system (Hewlett Packeard 1050) using a lichrosorb RP 18 column (4.0 mm i.d.×250 mm; particle size 5µm) (Merck, Dramastdt). Gradient separation was carried out using methanol and acetonitrile (2:1) as a mobile phase at flow rate of 1ml/min. Authentic phenolics and flavonoids were dissolved in mobile phase and injected into HPLC. Identification of the individual components was performed by comparison of their retention times with those of available authentic samples similarly analyzed.

Cytotoxicity Assay[15]

Potential cytotoxicity of the methanolic extract from the aerial parts of the samples were tested in vitro using the method of skehan,*et al* (1990) against two human cell lines, (liver carcinoma cell line) HEPG2 and (Breast carcinoma cell line) T47D. The cells were plated in 96-multi well plate (10^4 cell / well) for 24 hrs before treatment with the tested extracts to allow the attachment of cells to the wall of the plate. Different concentrations of each extract under test (0, 1, 2.5, 5, 10 mg / well) were added to the cell monolayer. Triplicate wells were prepared from each individual dose. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration. Is platted to get the survival curve of each tumor cell line after the specified concentration– By fitting the curves to the straight line equation, IC_{50} (the concentration that cause 50% of death of the cancer cells) could be calculated.

Antimicrobial activity

The antimicrobial activity was carried out using the disk diffusion method[16,17] Here a number of paper disks containing different antibiotic and tested methanolic extract of the plants are placed on the surface of an agar plate that has been uniformly spread with isolated pathogen. After overnight incubation the plate is examined for zones of inhibition of growth around the vanes disc. A zone of inhibition indicated that the organimisms are sensitive to the extracts contained in the disc (this method is called primary sensitivity testing). Samples measuring 50 ul of the ehtanolic extracts were added to the cups. The same volume of DMSO was used as a negative control as well as discs (50 mg /disc) of ampecillin (g+ve bacteria), gentamycine (g-ve bacteria) and amphotericin β (antifungal agent) as a positive control. The plates were incubated inverted at 37Cº for 24 hr in case of bacteria and at 25 Cº for 48 hr in case of fungi. After incubation, the diameters of the inhibition zones were recorded in mm Diameter less than 5mm indicate no effect. Triplicate wells were prepared for each extract.

Sources of fine chemicals

All chemicals and solvents used were analytical grade, and purchased from Sigma Aldrich (St. Louis, MO, USA).

Statistical Analysis

The values obtained from each group were expressed as Mean ± Standard deviation. One way Anova was done to compare the statistical significant changes between control, Paracetamol induced hepatotoxicity, Silymarin treatment rats and with Foeniculum Vulgare extracts treatment. The p-value was computed to detect the significant ($p<0.05$) changes within the group. The significant levels between the groups was compared using DMRT.

RESULTS

Results are illustrated in the tables (1-5)

DISCUSSION

Plants provide a large range of natural compounds belonging to different molecular families offering various medicinal properties. Ethno-botanical information revealed that the plant selected in this study is traditionally used for various medicinal purposes. Few scientific research has provided evidence supporting the traditional medicinal importance with respect to *Arbutus pavarii* Pampan. There is only one study talked about antioxidant, antimicrobial and antiproliferative activities of *Arbutus pavarii*. Pampan[1]. Malheiro *et al*[18] worked to initiate the study of antioxidant and antimicrobial activities, and total

Table 1: Total phenols and flavonoid contents* in the plants under investigation.

Sample name	Flavonoids mg Rut/ g**	phenolic mg GA/g***
Arbutus pavarii. Pampan	206.1±1.09	163.6±0.85
Sarcopoterium spinosium. L	126.9±2.98	61.71±2.7

*Significance difference from standard $P<0.05$ n=3
**Rutin (Rut) equivalent
*** Gallic acid (GA) equivalent

Table 2: Identification and assessment of the major flavonoid constituents in the plants under investigation using HPLC.

S. No	Flavonoid	Flavonoid conetnts (ppm)	
		A. pavarii	*S. spinosium*
1.	Quercetin	17.03	9.85
2.	Quercetrin	163.9	122.43
3.	Hesperidin	909.44	136.37
4.	Hesperitin	23.31	17.84
5.	Narengin	175.91	173.09
6.	Kaempferol	2.20	2.63
7.	Apegenin	3.23	7.05
8.	Rutin	5096.13	95.05
9.	7-OH flavone	8.73	0.31
10.	Catetchin	476.67	182.17
11.	Epicatetchin	1197.81	35.52
	Total identified flavonoids	8074.36	472.85

ppm= part per million

Table 3: Identification and assessment of the major phenolic compounds in the plants under investigation using HPLC.

S. No	Phenolics	Phenolic conetnts (ppm)	
		A. pavarii	*S.spinosium*
1.	Gallic acid	169.87	12.40
2.	Protocatechuic acid	871.65	78.36
3.	Catechol	270.99	22.67
4.	*Trans*-Cinnamic acid	86.0	29.0
5.	Isoferulic acid	158.73	40.0
6.	3-OH-Tyrosol	244.93	633.8
7.	Pyrogallol	1104.19	218.07
8.	*p*-hydoxy benzoic acid	30.15	168.47
9.	3,4,5-trimethoxy cinnamic	43.2	43.4
10.	Vanillic acid	24.36	25.8
11.	4-aminobenzoic acid	54.89	20.0
12.	Benzoic acid	50.75	97.8
13.	Chlorogenic acid	694.92	248.9
14.	Ferulic acid	22.14	89.3
15.	Salicylic acid	109.26	55.2
16.	Caffeic acid	21.06	55.0
17.	Ellagic acid	15.62	147.9
18.	Reversetrol	31.87	65.6
19.	E-vanillic acid	153.07	884.3
20.	Rosmarinic	471.99	30.05
21.	Cinnamic acid	8.60	2.90
Total identified phenolics		4638.24	2968.93

ppm= part per million

phenolic content in 19 different genotypes of wide spread Arbutus species *(Arbutus unedo* L.) leaves from the Tras-os-Montes region of Portugal. The results suggest that *Arbutus unedo* L. leaves are a potential source of natural compounds with valuable bioactive properties that could be explored by the pharmaceutical, chemical and food industries. The presence of a close association between the chemical composition of *Sarcopoterium spinosium* L. and their antiproliferative activity against *in-vitro* cytotoxic activity was also reported by[19]. Total phenolic and flavonoid contents of the methanolic extracts of the aerial parts of *Arbutus pavarii* Pampan and *Sarcopoterium spinosium* L are showen in the tables 1. Total polyphenol contents ranged from 61.71 to 163.6 µg gallic acid equivalent / g and total flavonoid contents ranged from 126.9 to 206.1 µg rutin equivalent. *Arbutus pavarii* Pampan extract showed the highest total phenolic and flavonoid contents (163.6 µg and 126.9 µg respectively). Identification and assessment of the phenolic and flavonoid constituents in the studied extracts (tables 2 and 3), which conducted through HPLC revealed that the pyrogallo and E-vanillic acid were the most abundant phenolic compounds in the *Arbutus pavarii* Pampan and *Sarcopoterium spinosium* L extracts (11041.9 and 884.3 ppm respectively). While the least abundant phenolic was cinnamic acid ranging between (8.6 and 2.9 ppm respectively). HPLC analysis of the flavonoid contents in the extract of the plants under investigation revealed that rutin was the most abundant in *Arbutus pavarii* Pampan extract (5096.13ppm) and catetchin in *S.spinosium* L (182.17 ppm). Kaempferol was the least abundant flavonoid in *Arbutus pavarii* Pampan extract (2.20 ppm) and 7-OH flavone was the least in *S. spinosium* L (0.31ppm). Cytotoxicity effects on both HepG2 and T47D expressed as IC_{50} values, which calculated from the dose-survival curves obtained from skehan,*et al* assay. According to table 4. The extract of *Arbutus pavarii* Pampan showed significant cytotoxicity on HepG2 and T47D with IC_{50} (19.7±2.8and 19±0.65) respectively, this study was found a similsr profile to ones reported by[19] for *Arbutus unedo* L., followed by *Sarcopterium spinosium*.L 23±1.87 and 31.9±4.82. The antimicrobial activities of the methanol extract of the plants under invetigation were determined against different bacterial and fungal strains and recorded in (Tables 5) The results showed variation in the antimicrobial properties of plant extracts. E. coli was found to be the most inhibited pathogen for both plants. Concerning Gram positive bacteria, methanol extract of the aerial part *Arbutus pavarii.* Pampan had higher inhibitory activity than *Sarcopoterium spinosium* L. extract specially against *Staphylococcus epidermidis* and on other hand both plants showed no effect against Streptococcus pyogenes. Concerning Gram negative

Table 4: Cytotoxic Activity of the extracts of the plants under investigation (IC_{50} µg/ml)*.

Plant name	HepG2 IC_{50}**	T47D IC_{50}**
Arbutus pavarii. Pampan	19.7±2.8	19±0.65
Sarcopoterium spinosium.L	23±1.87	31.9±4.82
Doxorubicin	15±0.87	16.7±0.89

*Significance different from standard (Doxorubicin) P<0.05 n=3 **(mean±S.D)

Table 5: Antimicrobial activity of the methanol extract of the aerial parts of *the* plants under investigation expressed as diameter of zone of inhibition in mm*.

Tested microorganisms	Samples		
	Zone of inhibition mm (mean±S.D)		
	A. pavarii	*S. spinosium*	Standard
Fungi			Amphotericin B
Aspergillus funigatus (RCMB02564)	33.3 ± 1.2	22.1±1.2	23.7±0.63
Aspergillus niger (RCMB02542)	21.1± 1.5	20.6±0.63	21.9±0.58
Candida albicans (RCMB05035)	NA	18.3±1.2	26.4±0.72
Candida tropicalis (RCMB 05084)	19.2±0.58	NA	25.4±1.5
Gram Positive Bacteria			Ampicillin
Staphylococcus aureas (RCMB010027)	24.3±0.63	16.3±1.5	28.9±1.2
Staphylococcus epidermidis (RCMB010024)	22.1±0.58	20.3±2.1	25.4±0.63
Streptococcus pyogenes (RCBM010015)	NA	NA	26.4±0.34
Bacillis subtilis (RCBM010067)	25.3±1.5	23.3±0.63	32.4±1.2
Gram Negative Bacteria			Gentamycin
Proteous vulgaris (RCMB 010085)	NA	NA	23.4±0.58
Pseudomonus aeruginosa (RCMB 010043)	NA	19.3±1.2	17.3±0.63
Salmonella Typhimurium (RCMB010315)	26.2±1.2	NA	24.8±0.63
Escherichia coli (RCMB010056)	26.9±2.1	26.3±0.58	25.3±0.18

Significance different from control P<0.05 n=3

bacteria, the strongest antimicrobial activities were observed against *Escherichia coli* with zone of inhibition 26.9±2.1 and 26.3±0.58 for *Arbutus pavarii*. Pampan and *Sarcopoterium spinosium* L respectively. On other hand did not exhibit any antimicrobial activities against *Proteous vulgaris*. *Sarcopoterium spinosium* L. had a moderate activity against *Pseudomonus aeruginosa* and *Arbutus pavarii*. Pampan had a good effect against *Salmonella Typhimurium*. The tested samples showed considerable antifungal effect specially *Arbutus pavarii*. Pampan which had higher activity against *Aspergillus funigatus* and *Aspergillus niger* than Amphotericin β. Followed by *Sarcopoterium spinosium* L. These findings are also supported by earlier reports that plant metabolites such as flavonoids, tannins, catechins and other phenolic compounds possess[1,5,22,23].

CONCLUSION

The results of the present study indicated that ethanolic extract of *Arbutus pavarii*. Pampan and *Sarcopoterium spinosium*. L. have potent cytotoxic and antimicrobial effecs. *Arbutus pavarii*. Pampan has been found highly effective. This study indicated that these plants could be a potential source of effective antimicrobial agents. further investigations are needed to be done on a wide range of bacteria and fungi to assess the spectrum of such plant extracts. Moreover, other parts of the examined plants are also needed to be assessed for their antimicrobial activity.

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