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Research Article

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Phytochemical screening, antioxidant, antimicrobial and anti-proliferative activities study of *Arbutus pavarii* plant

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ABSTRACT

The paper presents the results of phytochemical screening, antioxidant, antimicrobial and anti-proliferative activity studies of Arbutus pavarii (A. pavarii) plant. The whole aerial part of the plant was collected during the spring season (2010), identified and extracted with methanol, chloroform and n-Hexane. Phytochemical screening was carried out using standard procedures. Antioxidant activity was done using 2, 2, Diphenyl-1-picryhydrazyl (DPPH) assay. Antimicrobial evaluation was performed using agar well diffusion method. The anti-proliferative activity was evaluated on two breast adenocarcinoma and lung carcinoma cells. Phytochemical screening showed the presence of flavonoids, tannins, glycosides, simple phenolics, free reducing sugers, triterpenes and sterols in the plant extracts. The methanolic and chloroform extracts exhibited a potential antioxidant activity with IC_{50} of 4.55±1.90 µg/ml and 21.55±1.1 µg/ml in comparison to Quercetin. Methanolic extract exhibited antimicrobial effect against Staphylococus aureus, Escherichia coli and Candida albicans with zones of inhibition of 20mm, 18 mm and 21 mm and the minimum inhibitory concentrations (MICs) were 4.8 mg/ml, 69.30 mg/ml and 4.76 mg/ml using Ciprofloxacin and Amphotericin B as standards. The anti-proliferative activity of extracts with IC_{50} (less than 30 µg/ml) against breast adenocarcinoma (MCF7) and lung carcinoma (A549) was within the limits of the American National Cancer Institute for cytotoxic activity. A. pavarii may be exploited for the search of new antimicrobial and anticancer drugs.

Key words: *Arbutus pavarii*, antioxidant activity, antimicrobial activity, Anti-proliferative activity

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INTRODUCTION

The importance of medicinal plants as a source of active drugs emerged from the chemical profile that produces a clear physiological action on the biological system. Flavonoids, alkaloids, tannins and phenolic compounds have been established as the most important bioactive compounds of plants [1]. Plant tannins and flavonoids have been reported to have antimicrobial effects and many biological activities [2-4]. 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 [5]. *A. pavarii* is an evergreen shrub or a small tree that belongs to the *Ericaceae* family and endemic in 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 aliments [6]. The plant is recorded among the endemic medicinal species [7]. Previous study indicated a good antioxidant activity of *Arbutus pavarii* among other tested medicinal plants[8]. Few reports highlighted the importance of biological activities of *Arbutus pavarii*.

Therefore, this study was designed to further insight into the plant and to prove medical traditional uses of the plant for infections and as cancer remedies.

EXPERIMENTAL SECTION

Materials

A. Pavarii family *Ericaceae* plant was collected from El-Jabal El-Akhdar, Libya during the spring season 2010. Strain of gram-positive bacteria *S. aureus* (ATCC 29213), gram-negative bacteria E. coli (ATCC.25922), and fungal strain *C. albicans* (ATCC 10231) were procured from the American type of cell culture collection. Nutrient broth, Mueller Hinton agar and Sabouraud dextrose agar were from (Liofilchem, Italy). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was from Sigma, UK. Human Caucasian breast adenocarcinoma (MCF7) and Human Caucasian lung carcinoma (A549) were obtained from European Collection of Cell Cultures. Methanol, chloroform and *n*-Hexane were used as received.

Preparation of extracts

The whole aerial part of *A. Pavarii* family *Ericaceae* plant collected was identified and authenticated by Department of Botany, Faculty of Sciences, Tripoli University, Libya. The plant was than dried on shade, and reduced to coarse powder using a mechanical grinder. The powdered plant (1 Kg) was extracted for 72h with methanol, chloroform *and n*-Hexane using soxhlet apparatus. The crude extracts were then dried using rotary evaporator and stored at -20 °C until further use.

Phytochemical screening

The phytochemical screening of the *A. Pavarii* extracts was performed according to the standard procedures mayer's and dragendorff's tests for alkaloids, fehling's test for free reducing sugers, fehling's test for glycosides, liebermann - burchard's test for triterpenoids, liebermann-burchard's test for steroids, frothy test for saponins, shinoda's and sodium hydroxide tests for flavonoids , ferric chloride test for tannins and borntrager's test for free anthraquinones [9, 10].

Antioxidant activity

The free radical scavenging activity of *A. Pavarii* extracts was evaluated using 2,2, Diphenyl-1-picryhydrazyl (DPPH) assay [11]. Briefly, stock solution (1mg/ml) of the extract was prepared from which 1–25µg/ml dilutions were prepared in 1 ml cuvette. 400µl (0.1µM) of DPPH solution was added to each dilution followed by 600 µl of ethanol 99%. The mixture was shaken vigorously and allowed to stand at room temperature for 5 min in the dark and its absorbance was read at 517 nm using U.V-Visible-NIR spectrophotometer (Varian Cary 5000-U.S.A). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The radical scavenging activities of the tested samples percentages were calculated according to the following equation [12]:

$$\% \text{ DPPH inhibition} = [(A_A - A_B)/A_B] \times 100$$

Where A_A and A_B are the absorbance values of the test and the blank samples. From a percent inhibition concentration curve, the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value. The results are presented as means ± SEM of three independent experiments.

Antimicrobial activity

The antimicrobial activity of the *A. pavarii* extracts was determined using the agar well diffusion bioassay method [13]. Mueller Hinton agar plates were seeded with 0.1 ml bacterial suspension (equivalent to 10^7 –10^8 CFU/ml) and Sabouraud dextrose agar plates seeded with fungal strain. The seeded plates were incubated for12 h at 37°C, allowed to set and on each plate wells were made by sterile standard cork borer. Each well was filled with 50µl (20 mg/ml) of the extract and the plates were then re-incubated for further 24 h at 37°C. The diameters of zones of inhibition were measured and compared to Ciprofloxacin and Amphotericin B as standards. The results are presented as mean ± SEM of three independent experiments.

MIC Determination

The MIC values were determined using microdilution method [14]. A stock solution (50 mg/ml) of the plant extract was prepared in phosphate buffer. A serial of dilutions (50, 25, 12.25, 6.25 and 3.12 mg/ml) of the extracts were made. At the end of the incubation period, the MIC values were determined using ciprofloxacin and amphotericin B as positive control. The results are presented as mean of three independent experiments.

Cell Culture

Human Caucasian breast adenocarcinoma (MCF7) and Human Caucasian lung carcinoma (A549) cells were cultured in RPMI-1640 (Sigma, UK) medium with 10 % v/v fetal bovine serum (Sigma, UK), 100 IU/ml penicillin (Sigma, USA) and 100 µg/ml streptomycin (Sigma, UK) as a complete growth medium (CGM).

Anti-proliferative activity assay

The anti-proliferative activity of *A. Pavarii* extracts was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [15]. Exponentially growing cells were washed and seeded at 5000 cells/well in 200 µl of growth medium using 96 well microplates. After 24 h incubation, and when partial monolayer was formed, the media was removed and 200 µl of the medium containing the plant extract initially dissolved in dimethyl sulfoxide (DMSO) added and plates re-incubated for further 48 h. 100 µl of the medium were aspirated and replaced by 15 µl MTT solution in each well. After 4 h contact with the MTT solution, and when blue crystals were formed, 100 µl of the stop solution were added and incubated for further 1h. Reduced MTT was measured at 550 nm using a microplate reader (Multiskan Go, Thermo Scientific). 100 µg/ml of each *A. Pavarii* solvent extract was tested against MCF7 and A549 cell lines. Control groups received the same amount of DMSO (0.1%), Untreated cells were used as a negative control and cells treated with vincristine sulfate at concentrations of (0.05, 0.1, 0.5, 1, 5, 10, 25, 50 and100 nM) were used as positive control. Extracts with more than 50% inhibition were tested for IC50 measurements. 200, 100, 50, 25 and 5 µg/ml samples from each active extract were tested for IC50 values as the concentrations that show 50% inhibition of proliferation on any tested cell line. Stock solutions of the plant extract were dissolved in DMSO, diluted with the medium and sterilized using 0.2 µm membrane filters. The final dilution of extracts was used for treating the cells with no more than 0.1% DMSO. The anti-proliferative effect of the tested extracts was determined by comparing the optical density of the treated cells against the optical density of the control (untreated cells). The experiment is repeated three times and results are presented as means ± SEM.

RESULTS

The preliminary phytochemical screening of *A. Pavarii* revealed presence of the active constituents; flavonoids, tannins, glycosides, simple phenolics, free reducing sugers, triterpenes and sterols and the absence of saponines, anthraquinones and alkaloids. *A. Pavarii* showed a good antioxidant activity, where IC_{50} for methanolic extract was (4.55±1.90 µg/ml) and for chloroform extract was (21.55±1.1 µg/ml) using Quercetin with IC_{50} of 3.35±0.3µg/ml) as standard. The *n*-Hexane extract did not show any antioxidant activity**.** The antimicrobial activity results of *A. Pavarii* are shown in Table 1. The methanolic extract exhibited the highest activity against the tested bactria stains among other tested extracts with zones of inhibition of 20, 18 and 21 mm and MICs of 4.86, 9.30 and 4.76 mg/ml against *S. aureus*, *E. coli* and *C. albicans*. Chloroform extract was active only against *S. aureus* with zone of inhibition of 10 mm. The *n*-Hexane extract showed activity against *C. albicans* only with zone of inhibition of 12 mm using Ciprofloxacin and Amphotericin B as standards.

Table 1: Antimicrobial activities presented as Zone of inhibition (mm) and MICs (mg/ml) of *A. Pavarii* extracts

Extract	Zone of inhibition (mm) (MIC in mg/ml)		
	S. aurious (ATCC 29213)	*E. coli* (ATCC.25922)	*C. albicans* (ATCC 10231)
Methanol	20 4.86	18 9.30	21 4.76
Chloroform	10 ND	-ve	-ve
***n*-hexane**	-ve	-ve	12 ND
***Ciprofloxacin**	24 0.25	32 10	------
***Amphotericin B**	---------	----------	22 0.5

*The results are the mean of three values. -ve: No activity, ND: not determined. * Standard antibiotics.*

Anti-proliferative activity

The anti-proliferative activity of *A. Pavarii* extracts was tested against MFC7 and A549 cell lines. The % Viability of the plant extracts was measured by the MTT assay. The methanolic and chloroform extracts exhibited high anti-proliferative potential against the tested cell lines. The IC50 determined for extracts on MCF7 and A549 cell lines are shown in Table 2.

Table 2: Anti-proliferative activities of 100 µg/ml of *Arbutus Pavarii* extracts measured using MTT assay and the determined IC50

Extract	% Viability ± SEM (IC_{50} µg/ml ± SEM)	
	MCF 7	A549
Methanol	23.41 ± 1.61 (29.58 ± 1.42)	129.58 ± 7.50 (ND)
Chloroform	13.54 ± 0.34 (14.96 ± 0.54)	11.92 ± 0.51 (19.78 ± 0.59)
***n*-Hexane**	59.96 ± 6.09 (132.97 ± 1.89)	140.61 ± 5.14 (ND)

ND: not determined. The results are the mean and SEM of three replicates.

DISCUSSION

In this study, phytochemical screening of *A. Pavarii* indicated the presence of simple and poly phenolic active compounds. These compounds are documented as disease preventive with antimicrobial activity and to reduce the risk of cancer [16, 17]. The methanolic and chloroform extracts showed a good antioxidant activity but n-hexan showed negative results. These results may be due to the polarity of the solvent. n-hexane is a non-polar solvent and therefore it may be did not extract the active constituent in contrast to methanol and chloroform. The results support the previously reported finding for this solvent [8]. The antimicrobial activity for methanolic extract was also high against the three tested strains of bacteria compared to chloroform and n-hexan extracts, results indicated the polarity of the solvent plays an important role in the extraction of the active ingredient and consequently on its microbial activity. According to the American National Cancer Institute, the IC50 limit to consider a crude extract promising for further purification should be lower than 30µg/ml [18]. The anti-proliferative activities recorded in this study are in accordance with this limit. IC_{50} values of the methanolic extract for MCF7 cell line and chloroform extract for both MCF7 and A549 are well within this limit. The results in this study suggest that *A. Pavarii* plant has a strong and consistent anti-proliferative effect on MCF7 and A549 cell lines.

CONCLUSION

In conclusion, *Arbutus pavarii* might be considered as a potential source of metabolites which could be developed as precursors for antimicrobial and anticancer drugs.

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