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ANTIPROLIFERATIVE, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITIES OF CRUDE PLANT EXTRACTS OF *ASPHODELINE LUTEA* L. AND *PEGANUM HARMALA* L.

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ABSTRACT

In this study, we evaluated the biological activity of crude plant extracts of *Asphodeline lutea* and *Peganum harmala*. The in vitro antiproliferative activity, the effects on cell cycle phases, and the antioxidant and antibacterial activities of the crude extracts were investigated. The extracts revealed antiproliferative activity against three human cancer cell lines: MDA-MB-231 (breast), Hs-294T (melanoma), MV-4-11 (leukemia),and one non-tumorigenic human mammary gland epithelial cell line, MCF-10A. The crude extract of *Asphodeline lutea* appeared to be more active against all cell lines tested, while *P. harmala* extract revealed activity against only the MV-4-11human leukemia cell line. Both extracts differed in their activity toward cell cycle progression. After 72hours of treatment with *A. lutea* extract, we observed a shift of MV-4-11 cell percentage to the S-phase. On the other hand *P. harmala* tended to stop the cell cycle in G0/G1 phase. Nevertheless, the changes in cell cycle were not statistically significant when compared with the control group. Moreover, both crude plant extracts exhibited antioxidant activity. Antioxidant capacities of the extracts were expressed in terms of IC_{50} value of the extracts. Also, the minimum inhibitory concentration (MIC) studies indicated that the MIC of the crude plant extract of *P. harmala* was found to be 500 µg/ml for

all selected bacterial species, while for the crude plant extract of *A. lutea*, it was found to be 1500 µg/ml for *M. luteus*, *S. aureus*, and *B. subtilis*, and 2000 µg/ml for *E. Coli*.

KEYWORDS: Plant extracts, Antiproliferative activity, Antioxidant, Antibacterial activity.

INTRODUCTION

Natural products have long been used in alternative health care treatment and in the discovery of modern drugs (Kusuma et al., 2014). Plants have many polyphenols with various bioactivities, including antioxidant, antimicrobial and anticancer bioactivities (Dai and Mumper, 2010; Li et al., 2014).Due to the less toxic nature and adverse effects of polyphenols, the research on medicinal plants and their beneficial effects has increased (Pandey and Rizvi, 2009; Greenwell and Rahman, 2015).

Antioxidant activity of plants is attributed to the presence of polyphenols (Roleira et al., 2015). The phenols are considered to play an important role is in the protection of the organism from oxidative stress, which is a state of oxidant/antioxidant imbalance in the organism, where free radicals become stable by electron pairing with other molecules; often, these molecules are associated with oxidative stress, tissue damage and chronic diseases like cancer (Muniyandi et al, 2017).

Scientific interest in naturally derived compounds is growing because they are considered to have less toxic side effects compared to current treatments, such as chemotherapy (Greenwell and Rahman, 2015). Many plant species are already being used to treat or prevent cancer. Bioactive plant components like polyphenols, which are necessary for plant survival, are being investigated for their ability to inhibit growth and initiate apoptosis of cancerous cells (Street and Prinsloo, 2013). It has been reported that polyphenols may have apoptosis-inducing properties that can be useful in cancer treatment. The mechanism by which polyphenols are thought to carry out apoptosis initiation is through regulating the mobilization of copper ions, which are bound to chromatin-inducing DNA fragmentation (Greenwell and Rahman, 2015; Qin et al, 2012). In parallel, there is growing evidence for the potential of plant-derived compounds as inhibitors of various stages of tumorigenes and associated inflammatory processes, underlining the importance of these products in cancer prevention and therapy (Solowey et al, 2014).

Antimicrobial mode of action of plant extract might be related to the presence of phenolic compounds and different secondary metabolite on the active constituents (Murugan et al., 2013). Phenolic compounds are known to be synthesized by plants in response to microbial infection. It is therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms (Farzaei *et al.*, 2015). The biologically active constituents of plant extract are considered as antimicrobial agents, because of their ability to bind to bacterial adhesions and disturb the availability of receptors on the surface (Tiwari et al., 2015). However, the mechanism of active compounds via which they exert stronger antibacterial activity is attributed to their effect on cellular membranes (Padmini et al., 2010).

Asphodeline lutea (L.) Rchb. (*A. lutea*), also known as King's Spear or Yellow Asphodel, is a perennial, landscaping plant of the family Asphodelaceae (Lazarova et al., 2016). It is a wild plant native to northern Africa, south-eastern Europe and Turkey and is used for both culinary and medicinal purposes (Todorova *et al.*, 2010). The fresh flowers of *A. lutea* are consumed in salads in different regions and its leaves have a good nutritional quality (Zengin et al., 2012). *A. lutea* is also in folk medicine as a diuretic and antispasmodic and for the treatment of anemia, tuberculosis, bronchial suffocation and hemorrhoids (Uysal et al., 2016).

The biological and medicinal properties of this species are not well studied. However, many studies have investigated the biological activities of *A. lutea in vitro* and *in vivo*. A previous published report concluded that *A. lutea* root extracts from Bulgarian and Turkish origins have anti-mutagenic, anti-microbial and anti-methicillin resistant *Staphylococcus aureus* (MRSA) activities (Uysal et al., 2016). In another study, *A. lutea* extract showed antioxidant effects against various induced oxidative stress *in vitro* (Lazarova et al., 2014). In addition, the chloroform and ethyl acetate extracts from *A.lutea* showed a strong antioxidant activity during autoxidation of triacylglycerols of lard and triacylglycerols of sunflower (Lazarova et al., 2009). *A. lutea* roots extract also demonstrated cytoprotective and antioxidant properties against carbon tetrachloride (CCl4)-induced liver injury *in vitro* and *in vivo* (Todorova *et al.*, 2010). Ivanova *et al.* (2010) showed that the methanol and chloroform extracts from *A. lutea* could result in a remarkable inhibition of multidrug resistance (MDR) in cancer cells. The principal bioactive components in *A. lutea* extract that have antioxidant properties are anthraquinones, naphthalenes, flavonoids and phenolic derivatives (Ivanova et al., 2008). These bioactive components have several biological activities *in vitro* and *in vivo*, including antioxidant (Zengin et al., 2016).

Peganum harmala L.(*P. harmala*), commonly known as Harmal or Syrian rue, is a perennial plant of the family Nitrariaceae and is widely cultivated in Middle East, central Asia, North Africa, India and Pakistan (Niroumand et al., 2015; Komeili, et al., 2016). It is a medicinally important herb that has been reported to have a broad spectrum of biological activities (Bourogaa et al., 2015). *P. harmala* is traditionally used for its antispasmodic, diuretic, sedative, analgesic and antirheumatic properties (Ullah et al., 2013). Moreover, *P. harmala* extracts have been reported to have a variety of potential health benefits, including antibacterial, antifungal, anti-inflammatory, antiviral, antioxidant and anticancer properties *in vitro* and *in vivo* (Mothana et al., 2011; Moloudizargari et al., 2013; Lamchouri, 2014).

The predominant active components of *P. harmala* that are the most relevant medically are alkaloids, with the β-carbolines being the most bioactive compounds (Aslam et al., 2014). The root and seeds contain several β-carbolines that are pharmacologically active, including harmine, harmaline, harmalol and Harman (Asgarpanah and Ramezanloo, 2012).These bioactive compounds have a wide range of potent therapeutic effects *in vitro* and *in vivo*, such as antioxidant, anticancer, anti-inflammatory, antifungal and antibacterial (Patel et al., 2012; Filali et al., 2015). A previous study demonstrated that *β*-carboline harmine was found to attenuate oxidative stress in rat prefrontal cortex and hippocampus through its antioxidant actions (Réus et al., 2010).

The aims of this study are to evaluate the antioxidant, antibacterial and antiproliferative activities of plant extracts of *Asphodeline lutea* and *Peganum harmala* L. collected from southern parts of Jordan (September 2016 to November 2016).

MATERIALS AND METHODS

The study was performed in the Biochemistry Lab. in the Biological Department, Mutah University and in the laboratory of Experimental Anticancer Therapy, Department of Experimental Oncology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences.

Plant materials

The fresh aerial parts of the plants *Asphodeline lutea* L. and *Peganum harmala* L. were collected from southern regions of Jordan during September to November, 2016. The common and scientific names are presented in Table (1).

Table (1).

Scientific Name	Common name	Family
Asphodeline lutea	King's Spear	Asphodelaceae
Peganum harmala	Harmal	Nitrariaceae

Cell lines

Three human cancer cell lines: MDA-MB-231 (breast), Hs-294T (melanoma), MV-4-11 (leukemia) and one non-tumorigenic human mammary gland epithelial cell line MCF-10A were chosen. A series of antiproliferative activity tests were performed (3- 4 test on each cell line in triplicate). Then, the cell cycle analysis were performed on the leukemia MV-4-11 cell line after treatment with tested extracts. The tested extracts were prepared freshly for each individual test.

The nontumorigenic human mammary gland epithelial cell line MCF-10A as well as all human cancer cell lines used in this study, namely, mammary gland cancer cell line MDA-MB-231, melanoma Hs-294T cell line, and leukemia cell line MV-4-11, were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, USA, LGC Standards, Poland). All cell lines are being maintained at the Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wroclaw, Poland).

Culture media

The MCF-10A cell line was cultured in Ham's F12 medium containing 5% horse serum (both from Gibco, Scotland, UK), 2 mM L-glutamine, 20 ng/ml epidermal growth factor, 0.5 mg/ml hydrocortisone, 100 ng/ml cholera toxin, and 10 µg/ml insulin (all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The MDA-MB-231 cell line was cultured in RPMI 1640 with GlutaMAX (Gibco, Scotland, UK/ Life Technologies Poland) medium adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 1.0 mM sodium pyruvate, and 10% fetal bovine serum (all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The MV-4-11 cell line was cultured in RPMI 1640 with GlutaMAX (Gibco, Scotland, UK/ Life Technologies Poland) medium containing 10% fetal bovine serum and 1.0 mM sodium pyruvate (both from Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The Hs-294T cell line was maintained in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Scotland, UK/ Life Technologies Poland) supplemented with 10% fetal bovine serum (HyClone, Life Technologies Poland) and 2 mM L-glutamine (from Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The solvent medium used for plant extracts was a mixture (1:1) of RPMI 1640 and Opti-MEM (Gibco, Scotland, UK/ Life Technologies Poland) media supplemented with 5% fetal bovine serum and 2 mM L-glutamine (both from Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

All culture media were supplemented with 100 U/mL penicillin (Polfa Tarchomin SA, Warsaw, Poland) and 100 µg/mL streptomycin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The cells were grown at 37°C in a humid atmosphere saturated with 5% CO_2.

Plant extracts

Two plant extracts were used in the antiproliferative assay study as well as in cell cycle analysis. The extracts were named: *Asphodeline lutea* (extract A), and *Peganum harmala (extract B)*. Cisplatin, the active cytostatic drug, was used as a control for the antiproliferative assay.

Samples of the extracts were prepared freshly, before adding to cells. Prior to usage, the extracts were weight and dissolved in absolute ethanol or water for injection to the concentration of 100 mg/mL and subsequently diluted in culture medium to reach the required concentrations (ranging from 1 to 1000 µg/mL).

The cytostatic compound, cisplatin (1 mg/mL, TEVA Pharmaceuticals Polska Sp. z o.o, Warsaw, Poland) was diluted in culture medium to reach the required concentrations (ranging from 0.01 to 10 µg/mL).

Methods

Plant processing and extraction

The fresh aerial parts from the selected plants were washed thoroughly with tap water at room temperature to remove dirt prior to the drying process. These washed aerial parts were dried in the shade at room temperature for 20 days. Then, they were ground by coffee blender into fine powder. The powder of each plant was stored in plastic bags until extraction. Thirty five grams of each plant powder were soaked in 350 mL (80%) methanol (MeOH) solution

(HAYMAN, England) and kept shaking at 150 rpm, in a dark place for three days at room temperature. The mixtures were then filtrated using buchner funnel under vacuum. The filtrate was centrifuged at 3000 rpm for 10 minutes. The extracts were concentrated to dryness in rotary evaporator under reduced pressure at 55°C. The extracts were left in open vials in fume hood for three days at room temperature. The resulting extracts were stored in a refrigerator at 4°C in a glass container until use (Cichewicz and Thorpe, 1996).

Determination of extraction yield

The percentage yield (%) (w/w) from all the dried extracts was calculated as: Yield (%) = (W1 * 100)/W2; where W1 is the weight of the extract after evaporation of solvent, and W2 is the weight of the plant powder (Dellavalle et al., 2011).

Antibacterial activity

Antibacterial activities of the different extracts were investigated by the disc diffusion method (Alzoreky and Nakahara, 2003). The crude plant extracts were dissolved in 10% DMSO (M. TEDIA.Company.INC. USA) in addition to MeOH. Four to five well-isolated colonies of the same morphological type were selected and inoculated into tubes containing 5mL Muller-Hinton broth (Biolab .CE. Hungary) and incubated at 37°C with shaking at 150 rpm until the turbidity of the bacterial growth achieved. The bacterial cells in the broth culture were counted using hemocytometer. A volume ranged from 0.5 to 3 mL of each broth culture was added to the prepared sterile nutrient agar cooled to about 45-50^{o}C to obtain 2 X 10^{6} cell/mL inoculum concentration of the organism, then poured into sterile Petri dishes and allowed to solidify for bout 45-60min (Zwadyk, 1972).

For each bacterial strain, three sterile 6.0 mm antimicrobial susceptibility discs were used, the first and the second discs were loaded with 1000 and 2000 µg of plant extract, respectively, and the third was loaded with 10% DMSO as a negative control. The discs were left to dry, then placed on the surface of the agar plate under aseptic conditions. Nalidixic acid, Tetracycline, Oxacillin and Streptomycin discs were used as positive controls. The plates were left on the bench for about 2hrs to allow the extract diffuse properly into the agar. All plates were incubated for 18-24hr at 37^{o}C except *M. luteus* at 27^{o}C (optimal growth temperature). The studies were done in triplicates to ascertain the results obtained. Growth inhibitory activity was calculated by measuring the diameter of clear zone around the disc using a ruler (Romero et al., 2005).

Determination of MIC

The minimum inhibitory concentration (MIC) of plant extracts against the bacterial growth was determined. $2x10^6$ cell/ml was inculcated into tubes of 5ml Muller-Hinton broth containing a serial dilution of plant extract (0 – 2 mg/ml). Cultures were incubated at growth temperature for 24h. Bacterial growths were measured by UV/VIS spectrophotometer (PG instruments Ltd) at 600nm (Garcia et al., 2002). The MIC was determined by inhibition of visible growth on lowest concentration of plant extract containing media as compared with visible growth on extract free media (Patel et al., 2011).

Determination of total phenolic content

The amount of total phenolic compounds (TPCs) in extracts were determined according to the folin-ciocalteu procedure (Singleton et al., 1965). Samples (0.2mL) were introduced into test tubes, 1.0mL of folin-ciocalteu's reagent (s. d. fine- CHEM Ltd) and 0.8mL of sodium carbonate (BDH .GPR. England) (7.5%) were added (the final concentration of the plant extract in the solution was 500µg /mL). The tubes were mixed and allowed to stand for 30min. Absorption at 760nm was measured. The concentration of total phenolic compound was calculated by comparison with the absorbance of standard gallic acid (GA) (s. d. fine-CHEM Ltd) curve at different concentrations, and the total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram plant extract.

Antioxidant Activities

2,2-dipheny l-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical assay was carried out spectrophotometrically as described by reference (Tepe et al., 2005). Aliquots (50µL) of various concentrations (the final concentration of the plant extract in the solution was from 0 to 2000µg/mL) of the extracts were added to 5mL of 0.004% methanol solution of DPPH (Sigma- Aldrich. USA). After incubating the samples for 30min at room temperature, the absorbance was read against a methanol at 517nm. All determinations were done in triplicate. Inhibition of free radical scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = 100 \times (\text{Abs control} - \text{Abs sample})/\text{Abs control}$$

Where the control (containing all reagents except the tested compound) and Abs sample is the absorbance of the tested compound. Extract concentration providing 50% inhibition (IC_{50}) was determined from a graph plotting percentage inhibition against extract concentration. Trolox® (Sigma- Aldrich. USA) (final concentration 0 to 1.5µg/mL) was used as a standard

for the construction of the calibration curve, and the DPPH radical-scavenging activities were expressed as mg Trolox® equivalents per gram of plant extract (Chung et al., 2002).

The anti-proliferative assay

The antiproliferative tests were performed on all four cell lines: MCF10A, MDA-MB-231, MV-4-11 and Hs-294T (Wietrzyk et al., 2007). Briefly, 24 hr prior to the addition of the tested compounds, MCF10A, MDA-MB-231, MV-4-11 and Hs-294T cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1.0×10^5/mL. To determine the *in vitro* cytotoxicity of test compounds, the assays were performed after 72-h exposure of the cultured cells to the varying concentrations of tested compounds (total plate incubation time: 96 h) using sulforhodamine B (SRB) assay or MTT assay for MV-4-11 cell line (non - adherent). All cell lines were exposed to each tested compound at four different concentrations in the range of 1–1000μg/mL. The activity of the tested agents was compared to the activity of cisplatin (TEVA Pharmaceuticals Poland, Warsaw, Poland). In addition, control wells loaded with ethanol based on the solubility of the tested agents were maintained. Absorbance of each solution was read using Synergy H4 (BioTek Instruments USA) at a wavelength of 540 nm. Entire washing procedure was performed on Biotek EL-406 washing station (for plates undergoing SRB procedure; MCF10A, MDA-MB-231, Hs-294T). The results were calculated as IC_{50} value (inhibitory concentration 50%)—the dose (μg/mL) of tested agent which inhibits proliferation of 50% of the cancer cell population. IC_{50} values were calculated in Prolab-3 system based on Cheburator 0.4, Dmitry Nevozhay software for each experiment (Nevozhay, 2014). Each sample (concentration) of extract was tested in triplicate in a single experiment, which was repeated at least three times.

Cell cycle analysis

In this study the flow cytometer Fortessa FACS BD from Becton Dickinson was used.

For the cell cycle analysis, the human leukemia MV-4-11 cell line was used. The cells were seeded in a 6-well plate at a density of 3×10^5 cells/2 mL. Next, after 24 h, the tested extract A and extract B were added in a volume of 2 mL to the cells. For all extracts, dilutions the MV-4-11 dedicated medium was used. The final concentration of the tested extracts was 12.5 μg/mL and 1.25 μg/mL for cisplatin. The cells were exposed to the tested extracts for 72 hrs. Next, the cells growing in suspension were collected, counted with trypan blue solution, centrifuged (+4°C, 5', 324 x g), re-suspended in 70% cold ethanol and frozen at −20 °C for at

least 24h. After that, cells were transferred to 5mL propylene tubes (dedicated for flow cytometry analysis), washed in PBS and centrifuged (+4°C, 10', 324 x g). Then, the RNase solution (in PBS, 8µg/mL) was added (250µL for 2.5-5× 10^5 cells) and cells were incubated for 60min at the 37°C. After that, the cells were placed on ice, propidium iodide PBS solution (50µg/mL) was added to the cells at the amount of 25 µL and the cells were incubated for 30 min. Next, the flow cytometry analysis of cell cycle was performed using Cell Quest software (BD). The analysis of the obtained results was performed using Diva software (BD). For each sample the percentage of cells in each cell cycle phase was determined. The fluorescence intensity is proportional to the DNA amount in the cells.

RESULTS AND DISCUSSION

The methanolic extracts yield (%) of *P. harmala* and A. *lutea* were found to be 19.9 and 13.57 %, respectively (Table 2).The amount of total phenolic compounds (TPCs) in extracts was 0.89 and 0.61 GAE mg /g methanolic extract of *P. harmala* and A. *lutea*, respectively (Figure 1). These results indicated that extract B has higher TPCs (0.89 mg/g) than extract A (0.61 mg/g).

Table 2: Weight and percentage yield (%) of crude plant extracts of A. lutea and P. harmala.

Plant extracts	Weight of MeOH plant extract (g)	Yield of MeOH plant extract (%)
A. lutea	4.75	13.57
P. harmala	6.98	19.9

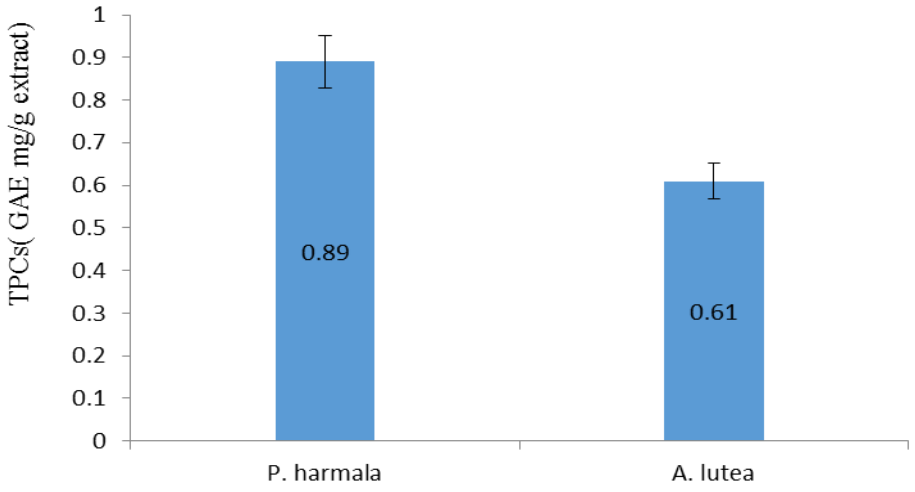

Figure 1: Total phenolics compounds of methanolic extract of *P. harmala* and A. *lutea* expressed as gallic acid equivalent (GAE) milligrams per gram (g) of the extract. Mean ± SD.

Antibacterial activity

As shown in table 3 and figure 2, the crude plant extract of *P. harmala* (at both concentrations) has antibacterial activity against all four bacterial species. The inhibition zone for all species was higher at 2000 µg/disc concentration than 1000 µg/disc concentration. The results showed that *M. luteus* was found to be the most sensitive, followed by *E. coli*, *S. aureus* and *B. subtilis*. The inhibition zone for all bacteria species ranged from 12 to 17 mm at 2000 µg/disc concentration.

In addition, table 4 and figure 2 show that the four bacteria species were insensitive to the crude plant extract of *A. lutea* at 1000 µg/disc concentration. However, *M. luteus*, *S. aureus* and *B. subtilis*, but not *E. coli*, were sensitive to the extract of *A. lutea* at 2000 µg/disc concentration. The results illustrated that the inhibition zone was 8 mm for *M. luteus*, *S.aureus* and *B. subtilis* at 2000 µg/disc concentration (Figure.2).

In the present study, the extracts were used from 500 to 2000 µg/mL for MIC studies. The MIC of the crude plant extract of *P. harmala* was found to be 500 µg/ml for all bacterial species. While for the crude plant extract of *A. lutea*, it was found to be 1500 µg/ml for *M. luteus*, *S. aureus* and *B. subtilis*, while 2000 µg/ml for *E. coli* (Figure 2).

Table 3: Antibacterial activity of methanolic crude extracts of P. harmala at different concentrations on the bacterial growth.

Bacteria	Zone of inhibition (mm)	
	1000 µg/disc	2000 µg/disc
S. aureus	8.67 ± 0.58	12.33 ± 0.95
E. coli	8.0 ± 0.75	13.0± 0.98
B. subtilis	8.5 ± 0.5	12.0 ± 1
M. luteus	13.0 ± 1	17.0 ± 1

Data are expressed as mean ± SD, where n = 3.

Table 4: Antibacterial activity of methanolic crude plant extracts of A. lutea at different concentrations on the bacterial growth.

Bacteria	Zone of inhibition (mm)	
	1000 µg/disc	2000µg/disc
S. aureus	-ve	8.0
E. coli	-ve	-ve
B. subtilis	-ve	8.0
M. luteus	-ve	8.0

Data are expressed as mean ± SD, where n = 3.

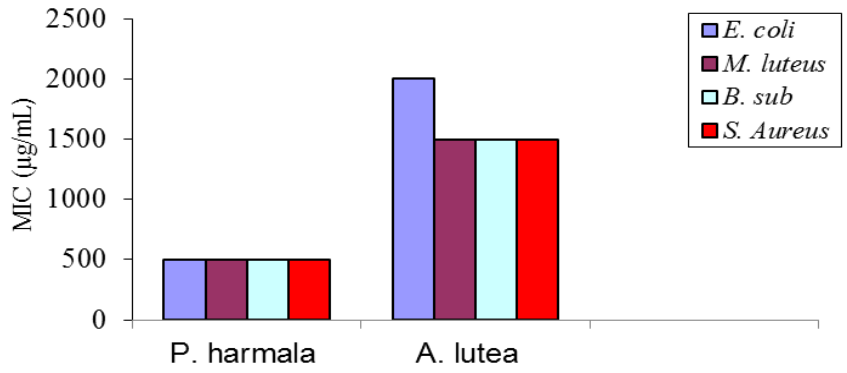

Figure 2: Minimum inhibitory concentration of methanolic crude plant extracts of *A. lutea and P. harmala* for all species.

These results illustrate that the higher capacity of extract B as an antibacterial activity (MIC 500 µg/ml) against the selected bacterial species may be due to the high content of TPCs (0.89 mg/g).

Antioxidant activity

The antioxidant activities of the plant extracts was evaluated by DPPH radical scavenging assay. Both crude plant extracts of *A. lutea and P. harmala* exhibited antioxidant activity (Figure 3). The antioxidant capacities of the extracts were expressed in terms of IC_{50} value of the extracts (Figure 3).

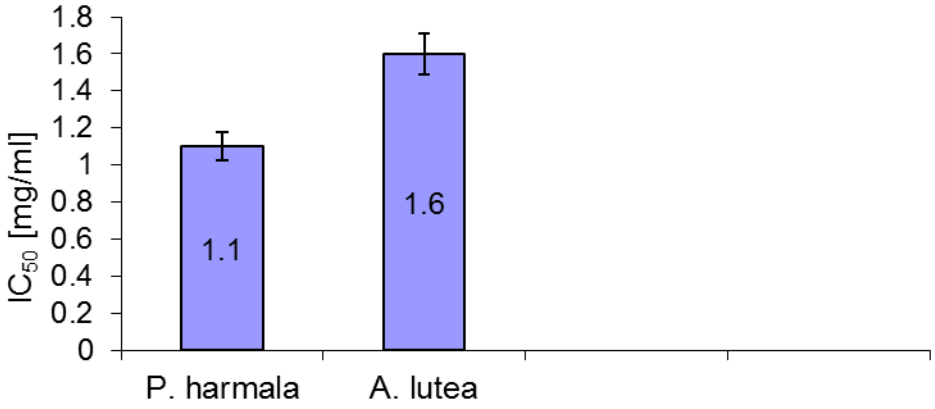

Figure 3: The concentration of crude plant extracts of *A. lutea and P. harmala* required to inhibit 50% (IC_{50}) of the initial DPPH free radical. Mean ± SD, where n = 3.

It was found that The IC_{50} value inversly proportional to the high antioxidant capacity. Results of Methanolic extracts of *P. harmala* showed higher antioxidant capacity than extract of *A. lutea* as evidenced by the low IC_{50} value of extracts of *P. harmala*.

It was reported that the antioxidant and antimicrobial activities of plant extracts varies from one plant to another. This may be due to many factors such as the effect of climate, soil composition, age and vegetation cycle stage on the quality, quantity and composition of active molecules (phenolic compounds) in the extract (Masotti et al., 2003; Angioni et al., 2006). Moreover, different studies found that the type of solvent has an important role in the process of extraction (Al-Zubaydi et al., 2009).

The antiproliferative assay

The obtained results from the antiproliferative assay are presented in Table 5 and Figure 4.

Table 5: Antiproliferative activity of plant extracts of *A. lutea* and *P. harmala*. Mean ± SD, where n = 3.

Extract	MCF-10A	MDA-MB-231	Hs-294T	MV-4-11
	IC50 (mean ± SD) [µg/mL]			
Extract A	43.2±12.6	23.3±4.5	21.7±7.4	21.9±4.1
Extract B	700.3±1,2	n.a	n.a	298.4±91.9
Ethanol	n.a.	n.a.	n.a.	n.a.
Cisplatin	4.7±1.5	6.7±0.8	0.8±0.2	1.49±0.9

n.a. - not active

Figure 4 presents the percent of proliferation inhibition after treatment with extracts A and B on all four cell lines used. The data are presented as mean percent of proliferation inhibition with standard deviation for each cell line separately at the concentrations used.

The results obtained in this study show that the most active compound against all cell lines tested is the extract A. Which exhibited the lowest IC_{50} value against all cell lines tested. The extract B showed activity against only human leukemia cell line MV-4-11, but ten times lower than that of extract A. Interestingly, both extracts revealed about 2 times lower antiproliferative activity against normal epithelial cell line MCF-10A, compared to the cancer cell lines tested, which may indicate selectivity towards neoplastic cells, these observations agreed with Ramesh results (Ramesh et al., 2009). However, the antiproliferative activity of tested compounds did not corresponds to the antioxidant properties, and it was found to be inversely proportional to the TPCs. Therefore, we decided to evaluate the influence of the tested extracts on the cell cycle using MV-4-11 leukemia cell line.

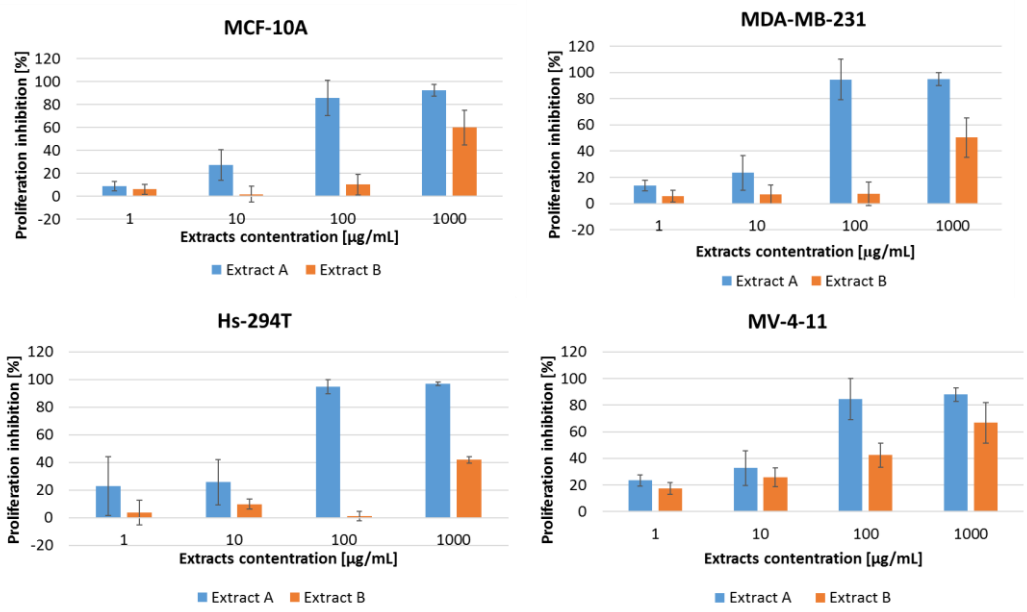

Figure 4: Proliferation inhibition (%) of human cell lines: MCF-10A, MDA-MB-231, Hs-294T and MV-4-11. Mean ± SD, where n = 3.

Cell cycle analysis

It was known that some plant polyphenolic compounds may arrest the cell cycle at G0/G1 phase, while others stop cell cycle at G2M stage (Shin et al., 2013; Suh et al., 1995). Our results presented in Figure 5 showed that extract A has tendency to increase the percentage of MV-4-11 cells in S stage, similarly to cisplatin. In parallel, the decrease of MV-4-11 cells in G_2M cell cycle phase was observed. After treatment with extract B, results showed an increase in the percetage of cells in G_0/G_1 (Fig. 5).Nevertheless, the changes in cell cycle achieved by extract A and B were not statistically significant when compared to the control group.The amount of dead cells (the sub G_1 population) did not achieved more than 5% fluctuating between control group and tested extracts.Based on the above studies, we can suppose, that the mechanism of antiproliferative activity of tested plants extracts is not directly related to their antioxidative properties and rather is not related to cell cycle arrest. Shin reported (Shin et al., 2013), that several moieties in the structure of polyphenols are necessary for G1 cell cycle arrest. Therefore further studies are needed to define the exact chemical composition of tested extracts and then to elucidate exact mechanism of action.

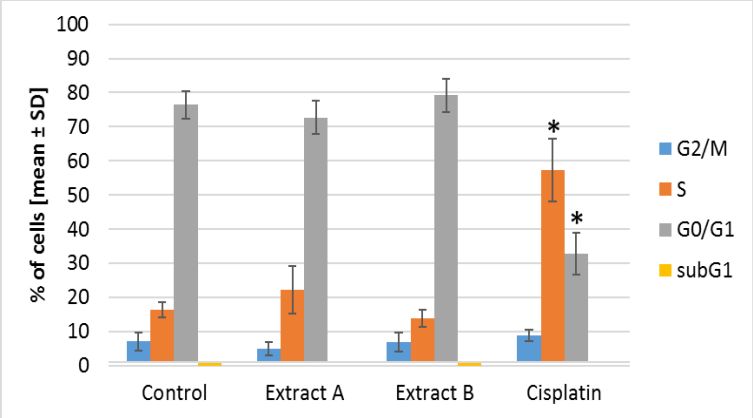

Figure 5: Cell cycle phases of MV-4-11 human leukemia cells after 72hrs treatment with the tested extracts. Mean ± SD, where n = 3. * $p<0.05$ as compared to control, Sidak's multiple comparisons test.

CONCLUSION

Methanol extracts of *A. lutea and P. harmala* have different levels of antioxidant and antibacterial activity, also possess high effects on the antiproliferative activities and on cell cycle phases. These effects may be due to the phenolic compound composition and concentration in the crude extracts. It was found a strong relationship between total phenolic content of plants extracts and antioxidant and antibacterial activity.

Further studies are needed to purify the active components in the respective plant extracts, which are responsible for the antioxidant, antimicrobial, and antiproliferative activities.

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