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ORIGINAL ARTICLE

Cytotoxic, anti-cancer, and anti-microbial effects of different extracts obtained from *Artemisia rupestris*

Cytotoxické, protirakovinné a antimikrobiální účinky extraktů z *Artemisia rupestris*

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Summary

Artemisia rupestris is a part of traditional Kazakh folk medicine. Extracts obtained from this plant are used to treat various diseases, including cancer. This study evaluates the anti-microbial, cytotoxic, and anti-cancer effects of different extracts of the plant. Different extraction techniques were used and the resultant activities were compared. Extracts of *A. rupestris* were prepared from the flowers plus the leaves and from the stems. The antimicrobial activity against *Candida albicans* and *Staphylococcus aureus* was quantified. Cell lines L1210 and THP-1 were used to evaluate the cytotoxic potential of these extracts *in vitro*. The anti-cancer effect was tested using L1210-induced tumorgenesis in mouse model. The aqueous extract of stems was the most active against *C. albicans*, whereas the methanolic extract of flowers plus leaves especially inhibited the growth of *S. aureus*. The aqueous extracts were found to be non-cytotoxic for both cell lines, whereas the lipophilic extracts showed cytotoxic effects. The extract obtained from flowers plus leaves was more cytotoxic than that from stems. The tested extracts showed no anti-cancer potential. The results obtained testify to the relatively safe consumption of aqueous extracts of *A. rupestris*, but lipophilic extracts showed toxic effects and their consumption should be considered more carefully.

Key words: L1210 cell line • THP-1 cell line • microwave-assisted extraction • ultrasonic-assisted extraction • *Candida albicans* • *Staphylococcus aureus*

Souhrn

Artemisia rupestris je součástí tradiční kazašské medicíny. Výtažky získané z této rostliny se používají k léčbě různých onemocnění včetně rakoviny. Tato studie hodnotí antimikrobiální, cytotoxické a protirakovinné účinky různých extraktů z této rostliny. Byly použity různé extrakční metody a výsledné biologické aktivity byly navzájem porovnány. Extrakty z *A. rupestris* byly připraveny z květů a listů, a ze stonků. Byla testována antimikrobiální aktivita proti *Candida albicans* a *Staphylococcus aureus*. Pro vyhodnocení cytotoxického potenciálu extraktů byly použity buněčné linie L1210 a THP-1. Vodný extrakt ze stonků byl nejúčinnější proti *C. albicans,* zatímco růst *S. aureus* inhiboval nejvíce methanolický extrakt z květů a listů. Vodné extrakty nevykazovaly cytotoxický účinek ani u jedné buněčné linie na rozdíl od lipofilních extraktů. Testované extrakty nevykazovaly účinek proti rakovině *in vivo*. Získané výsledky svědčí o relativně bezpečném použití vodných výluhů z *A. rupestris*, lipofilní extrakty vykazují toxické účinky, a proto je při jejich užití potřeba více opatrnosti.

Klíčová slova: L1210 buněčná linie • THP-1 buněčná linie • mikrovlnná extrakce • ultrazvuková extrakce • *Candida albicans* • *Staphylococcus aureus*

Introduction

Artemisia rupestris L. (Asteraceae) grows mainly in the Xinjiang Province of China, in Kazakhstan, Mongolia, Russia, and some European countries[1]. The plant is well-known in Chinese and Kazakh folk medicine and is used as an antiviral, antibacterial, anticancer, antianaphylactic, antidotal and anti-inflammatory agent[2–4]. The anti-inflammatory effect could be caused, at least partly,

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by the inhibition of NO production[5]. Insecticidal and repellent activities of constituents of the essential oil from *A. rupestris*[6] and anti-influenza properties[7], have also been identified in recent studies.

In Kazakh folk medicine, *A. rupestris* is prepared and drunk as a tea to treat such diseases as cancer, stomach pain, indigestion, jaundice, flu, the discomfort caused by fever, urticarial, and several types of hepatitis[4]. Evaporating the water from this tea forms a crude extract that is used to treat skin diseases, e.g., neurodermatitis, poisonous insect bites, and all kinds of skin injuries[8].

A. rupestris is known to be rich in essential oils[9], chlorogenic and rupestonic acid derivatives, flavonoids (aglycones, *O*-glycosides, *C*-glycosides, and *C,O*-glycosides), 2-phenoxychromones, and guaiane sesquiterpenoids[5, 10–14].

Chemical and biological studies of medicinal and edible plants have increasingly sought to find new types of naturally active compounds. Furthermore, consumers are becoming more interested in natural products that enhance their health. On the other hand, many plant extracts also have toxic activities and they should be prepared and consumed with care. In the present study, several kinds of extracts prepared from *A. rupestris* were evaluated for their anti-microbial and toxic effects. Until now, only a limited number of studies had evaluated these effects for compounds isolated from *A. rupestris*. None of them focused on testing the crude extracts that are used in traditional medicine, and not a single study using an *in vivo* model had been reported.

In this study, crude extracts prepared from *A. rupestris* were tested *in vitro* for their anti-microbial and cytotoxic effects on selected microbial strains and cancer cell lines. The effects of using different techniques to prepare the extracts the resultant on biological activities were also evaluated. The most active extracts were also tested *in vivo* for their antitumor effects on L1210-induced cancer in mice.

Experimental part

Chemicals and biochemicals

Fischer's medium (supplemented with 1.4 mM l-glutamine) and horse serum were purchased from Gibco (Loughborough, UK). RPMI 1640 medium and the penicillin-streptomycin mixture (100 U/mL of penicillin and 100 μg/mL of streptomycin) were obtained from Lonza (Verviers, Belgium). Phosphate-buffered saline (PBS), fetal bovine serum (FBS), 5-flucytosine, and ciprofloxacin were purchased from Sigma-Aldrich (Steinheim, Germany). Cell Proliferation Reagent WST-1 was obtained from Roche (Mannheim, Germany). Mueller Hinton Broth (MHB) was purchased from HiMEDIA Laboratories Pvt. Ltd. (Mumbai, India).

Plant material

The aerial parts (flowers, leaves and stems) of *A. rupestris* were collected from the Altai Mountain, East Kazakhstan region in August 2012. The plant was identified and authenticated by the herbalist (Dr. Salamat Ali) at the herbarium of the Food and Drug Inspection Center, Xinjiang, China, where a voucher specimen (No. 2013S0029) has been deposited.

Preparation of plant extracts

The plant material of *A. rupestris* was dried at room temperature and stored in dark, tight cotton bags to exclude humidity and light. In order to compare the cytotoxic activities of the different parts of the plant, the material was separated into two parts: the stems and the flowers plus the leaves. The stems were cut into pieces 1–5 mm long and were similar in diameter. Methanol 80% (v/v), ethanol 80% (v/v), chloroform 80% (v/v), and distilled water were used as extraction solvents. In order to determine the influence of extraction method on the activity of the plant material, simple boiling was chosen as the traditional method with microwave and ultrasonic extractions as modern methods.

For the extraction by the traditional method, ten grams of plant material were put into a 500 mL round bottom flask. One hundred milliliters of distilled water was poured into the flask, a reflux condenser was attached and the flask was heated to boiling for 2 h. Each extract was filtered, frozen, and lyophilized at reduced temperature and pressure (–40 °C, 35 mbar). The dry extracts were then used for the subsequent experiments.

One gram of plant material was mixed with 10 mL of the chosen solvent [methanol 80% (v/v), ethanol 80% (v/v), or chloroform 80% (v/v)], was put into the vessels of a microwave extraction system (START E, Milestone; Sorisole, Italy) where the microwave-assisted extraction was carried out. The extraction parameters were set up as follows: time: 15 min, temperature: 110 °C, power: 1000 W. After cooling, the samples were filtered, dried, and stored for subsequent analysis.

The ultrasonic-assisted extraction was similar to the microwave-assisted extraction. One gram of plant material in 10 mL of the chosen solvent was put into a water bath and sonicated for 15 min at a frequency of 20 kHz. After sonication, the samples were filtered, dried and stored for analysis.

The organic solvents (methanol, ethanol, and chloroform) were evaporated at room temperature under normal atmospheric pressure. The aqueous residues were freeze-dried under reduced pressure and to yield crude extracts.

The particular extracts were named as follows: AFMM – methanolic flowers plus leaves extract obtained by microwave extraction, AFMU – methanolic flowers plus leaves extract obtained by ultrasonic extraction, AFWB – aqueous flowers plus leaves extract obtained by boiling, AFWM – aqueous flowers plus leaves extract obtained by microwave extraction, ASMM – methanolic stems extract obtained by microwave extraction, ASMU – methanolic stems extract obtained by ultrasonic extraction, ASWB – aqueous stem extract obtained by boiling, ASWM – aqueous stems extract obtained by microwave extraction, AFEM – ethanolic flowers plus leaves extract obtained

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by microwave extraction, AFCM – chloroform flowers plus leaves extract obtained by microwave extraction.

Phytochemical analysis

The relative differences in content of five compounds were measured by an Agilent HP 1100 liquid chromatograph with diode array detector (DAD), which measures spectra in the 190 nm to 950 nm wavelength range. An XDB C18 column (50 mm × 2.1 mm, particle size 1.8 μm) was used for chromatographic separation. Gradient elution, starting with 10% methanol and 90% of 0.2% (v/v) of formic acid up to pure methanol for 36 minutes, was used. The temperature of the column compartment was set to 30 °C, the injection volume to 0.5 μL, the wavelength measured to 254 nm and the flow rate to 0.25 mL · min^{-1}. Quantitative comparison of areas of peaks gives the relative concentrations of these compounds.

An Agilent HP 1100 liquid chromatograph coupled with an MS ion trap detector was used for the confirmation of five secondary metabolites (5-caffeoylquinic acid, luteolin-7-hexose-pentose, apigenin-6,8-di-*C*-pentoside, 6-demethoxy-4'-*O*-methylcapillarisin, and rupestonic acid. An XDB C18 column (50 mm × 2.1 mm, particle size 1.8 μm) was used for chromatographic separation. Gradient elution, starting with 30% acetonitrile and 70% of 0.2% (v/v) of formic acid up to pure acetonitrile for 7 min, was used. The temperature of the column compartment was set to 30 °C, the injection volume to 0.5 μL and the flow rate to 0.25 mL · min^{-1}. MS parameters were set as follows: nebulizer pressure to 40 psi, drying gas to 10 L · min^{-1}, drying temperature to 350 °C, target mass to charge ratio to 1100, maximum ions in ion trap to 30000 and maximum ionization time to 300 ms.

Anti-microbial activity

Referential microbial strains of *Candida albicans* and *Staphylococcus aureus* ATCC 29213 were obtained from the laboratory of the Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno. The extracts to be tested were dissolved in dimethylsulfoxide (DMSO), serially diluted using 0.9% saline, and transferred to 96-well flat-bottom microplates in quadruplicates.

The fresh overnight culture of *C. albicans* was resuspended in RPMI 1640 medium to afford final a target inoculum of 2.5 × 10^5 CFU/mL in each well. The extracts to be tested were added to 100 μL of yeast suspension in the 96-well plate to obtain final concentrations of 256, 128, 64, and 32 μg/mL and growth was continual monitored for 48 h by measuring the absorbance at 600 nm every 30 min in a microplate reader (BMG Labtech Reader, Ortenberg, Germany) at 37 °C. The results were expressed as the area under the curve (AUC) for each of the growth curves. The plate was subsequently stored in a thermostat at 37 °C and further cultivation was carried out without a continual measurement. After 120 h, the total absorbance of culture was measured and the results were expressed graphically. Controls for both the growth (*C. albicans* in RPMI) and the blank (RPMI only) were included on each test plate. The fungistatic 5-flucytosine (1 μg/mL) was included as a positive control.

To test the antibacterial properties of the extracts, *S. aureus* was prepared in a way similar to that for *C. albicans*. The concentration of the inoculum was 6 × 10^5 CFU/mL in Mueller Hinton Broth and 100 μL of this suspension was placed into the wells of a 96-well plate. The bactericide ciprofloxacin (1 μg/mL) was included as a positive control. As it was for *C. albicans*, the growth was continually monitored for 24 h by measuring the absorbance at 600 nm every 30 min in a microplate reader at 37 °C. Then the plate was stored in a thermostat at 37 °C and the absorbance was measured after 120 h.

Quantitative comparison of the areas under the curves for 24 h for *S. aureus* and for 48 h for *C. albicans* shows the percentage of growth relative to that of the growth control for these periods. Quantitative comparison of the absorbances after 120 h gives the percentage of growth relative to that of the growth control after five days.

Cytotoxic activity in vitro

Murine lymphocytic leukemia cells L1210 and the human monocytic leukemia cell line THP-1 (ECACC, Salisbury, UK) were used to screen cytotoxic effects. Both of the cell lines were cultivated at 37 °C in a humidified atmosphere containing 5 % CO_2. The L1210 cells were placed in Fischer's medium supplemented with 10% (v/v) horse serum. The THP-1 cells were cultivated in RPMI 1640 medium supplemented with 2 mM of L-glutamine, 10% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Stabilized cells (3^{rd}–15^{th} passage) were used for all of the biological experiments.

Cells at a concentration of 500 000 cells/mL were seeded into 96-well plates in triplicate and incubated in 100 μL of serum-free medium at 37 °C. Measurements were taken 24 h after treatment with increasing concentrations of the test extracts dissolved in DMSO. The concentration of DMSO was 0.1% (v/v) in each well. The viability of the cells was measured by using the WST-1 test according to the manufacturer's manual. The amount of formazan created (which corresponds to the number of metabolically active cells in the culture) was calculated as a percentage of the control cells, which were treated only with DMSO and were designated as 100%.

Anti-tumor activity in vivo

The testing of *in vivo* anti-tumor activity was carried out using male DBA/2 SPF mice (AnLab, Prague, Czech Republic) with an average weight of 20 ± 5 g. The experiment was approved by the Animal Welfare and Protection Committee of the University of Veterinary and Pharmaceutical Sciences Brno. The animals were kept in a Sealsafe NEXT – IVC Blue Line Housing System (Tecniplast, Varese, Italy) to ensure the best experimental conditions and eliminate the risk of intergroup cross-contamination. The temperature in the room ranged from 20 to 24 °C. Sterile, commercially supplied feed and sterile drinking water were provided as needed.

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After a week of acclimatization, the experimental animals were divided into 5 groups of 10 animals each. Group I was used as a negative intact control, group II as a positive control (i.e., with induced tumorigenesis and without any treatment), group served III as a reference cisplatin group, group IV for AFMM, and group V for ASMM. With the exception of Group I (intact control), each of the groups was administered a suspension of mouse leukemia cells (L1210) at a dose of 1×10^6 intraperitoneally.

The animals were then weighed daily and clinical conditions, including any signs of tumor growth, changes in behavior, or sudden death were monitored during the next 10 days. After these 10 days, group III was administered cisplatin at a dose of 2 mg/ kg i.p. and groups IV and V received the test extracts, dissolved in 2% DMSO, at a dose of 10 mg/kg i.p. daily for a period of 5 days. On each of the following six days, the animals were weighed, clinical conditions were observed, and any mortality was recorded. Animals were sacrificed if they lost more than 50% of their starting weight or if severe toxicological problems were apparent. The remaining animals were sacrificed 22 days after the application of L1210 cells. During the consequent necropsy, organs were visually examined and tissue samples were taken for morphological and histopathological examination.

The experimental data describing the survival of the animals were expressed as a Kaplan-Meier survival curve.

Statistical analysis

Analysis of the Kaplan-Meier curve followed by a Mantel-Cox test was used to statistically evaluate the in vivo toxicity. Values of $p < 0.05$ were considered to be statistically significant. GraphPad Prism 5.01 (GraphPad Software Inc., La Jolla, CA, USA) was used for the analysis.

Results and discussion

Different extraction techniques were used and the quantity of identified compounds were compared (Fig.1 and Fig. 2). Stem extracts were rich in content compounds and five compounds were identified there – 5-caffeoylquinic acid (**1**), luteolin-7-hexose-pentose (**2**), apigenin-6,8-di-*C*-pentoside (**3**), 6-demethoxy-4'-*O*-methylcapillarisin (**4**), and rupestonic acid (**5**). On the other hand, flower plus leave extracts possessed only rupestronic acid (**5**), but its concentration was ~10-timer higher than in stem extracts (Fig.1 and Fig. 2). Obtained data also indicates that the tradition extract preparation (boiling in water) allows to gain greater amount of compounds, which were identified in this study. The microwave extraction was more effective than ultrasonic in the isolation of above mentioned compounds.

The highest concentrations (256 µg/mL) of the extracts were used to evaluate their anti-microbial activity against *C. albicans* and *S. aureus* (Fig. 3). Lower concentrations showed no effects (data not shown).

Extracts of flowers plus a leaves most inhibited the growth of *S. aureus* (Fig. 3A). Neither the application of different extraction techniques (microwave- or ultrasonic-assisted) nor the solvents used for extraction (methanol, water) significantly affected the ability of extracts to

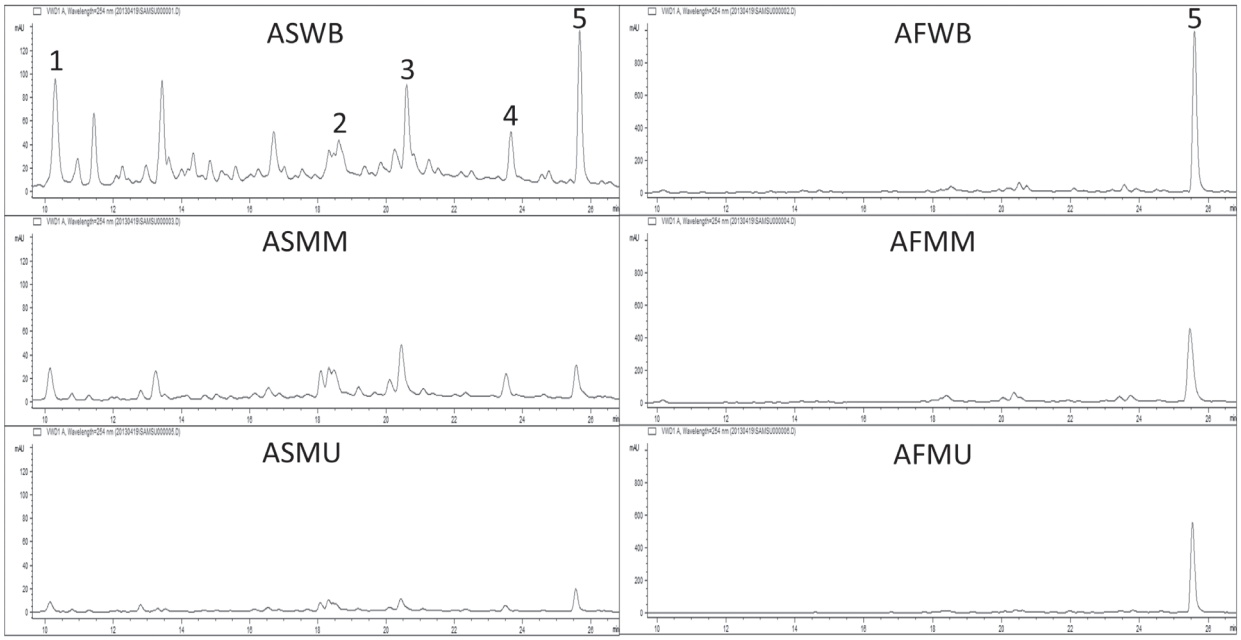


Fig. 1. Compound determined by HPLC-DAD (software ChemStation for LC 3D Systems, Agilent Technologies) and HPLC-MS (LC/MSD Trap Software 5.3, Bruker Daltonik 6 mbH) in different extracts. Scale of Y axis for stem extracts is 0–140 mAU and for flower plus leave extracts 0–1000 mAU

AFMM – methanolic flowers plus leaves extract obtained by microwave extraction, AFMU – methanolic flowers plus leaves extract obtained by ultrasonic extraction, AFWB – aqueous flowers plus extract obtained by boiling, AFWM – aqueous flower and leaves extract obtained by microwave extraction, ASMM – methanolic stems extract obtained by microwave extraction, ASMU – methanolic stems extract obtained by ultrasonic extraction, ASWB – aqueous stems extract obtained by boiling

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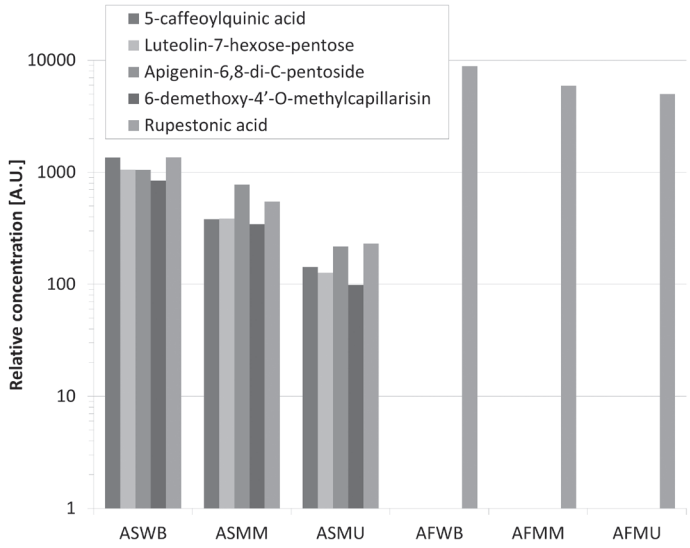


Fig. 2. Content of identified compounds in different extracts
AFMM – methanolic flowers plus leaves extract obtained by microwave extraction, AFMU – methanolic flowers plus leaves extract obtained by ultrasonic extraction, AFWB – aqueous flowers plus extract obtained by boiling, AFWM – aqueous flower and leaves extract obtained by microwave extraction, ASMM – methanolic stems extract obtained by microwave extraction, ASMU – methanolic stems extract obtained by ultrasonic extraction, ASWB – aqueous stems extract obtained by boiling

inhibit the growth of *S. aureus*. Bacterial growth was inhibited by between 40% and 60% after 24 h. After 120 h of cultivation, the percentage inhibition of growth was ~80% for methanolic extracts of flowers plus leaves (AFMM and AFMU) and ~70% for the aqueous flowers plus leaves extract (AFWB). The aqueous extract of stems (ASWB) inhibited growth the least. After 120 h cultivation, the inhibition of bacterial growth was 40%. After 24 h of cultivation in the presence of ASWB, the amount of *S. aureus* was 140% as compared to the untreated culture. The methanolic stems extracts (ASMM and ASMU) were more efficient. The percentage of inhibition after 24 h of cultivation was about 40% and after 120 h it was from 50% to 70%.

The strongest inhibitory effect on the growth of *C. albicans* was found for the aqueous stems extract obtained by boiling (ASWB) as shown in Figure 3B. The method of extraction and the solvent used for the extraction affected the anti-microbial effect. The aqueous stems extract (ASWB) was the most effective, inhibiting

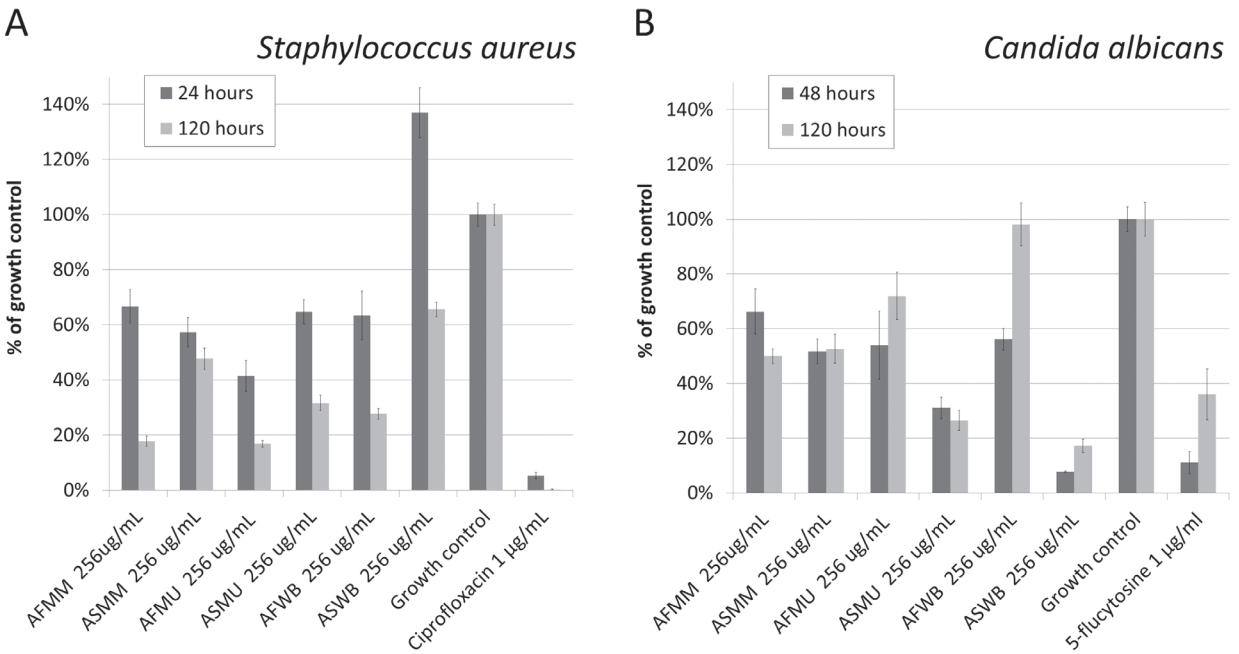


Fig. 3. Anti-microbial effects of the test extracts on Staphylococcus aureus (A) and Candida albicans (B). The cells were treated with extracts at a concentration of 256 µg/mL and the viability was monitored by measuring the absorbance at 600 nm in a microplate reader at 37 °C. The results are expressed as the mean ± SE for four independent experiments
AFMM – methanolic flowers plus leaves extract obtained by microwave extraction, AFMU – methanolic flowers plus leaves extract obtained by ultrasonic extraction, AFWB – aqueous flowers plus extract obtained by boiling, AFWM – aqueous flower and leaves extract obtained by microwave extraction, ASMM – methanolic stems extract obtained by microwave extraction, ASMU – methanolic stems extract obtained by ultrasonic extraction, ASWB – aqueous stems extract obtained by boiling

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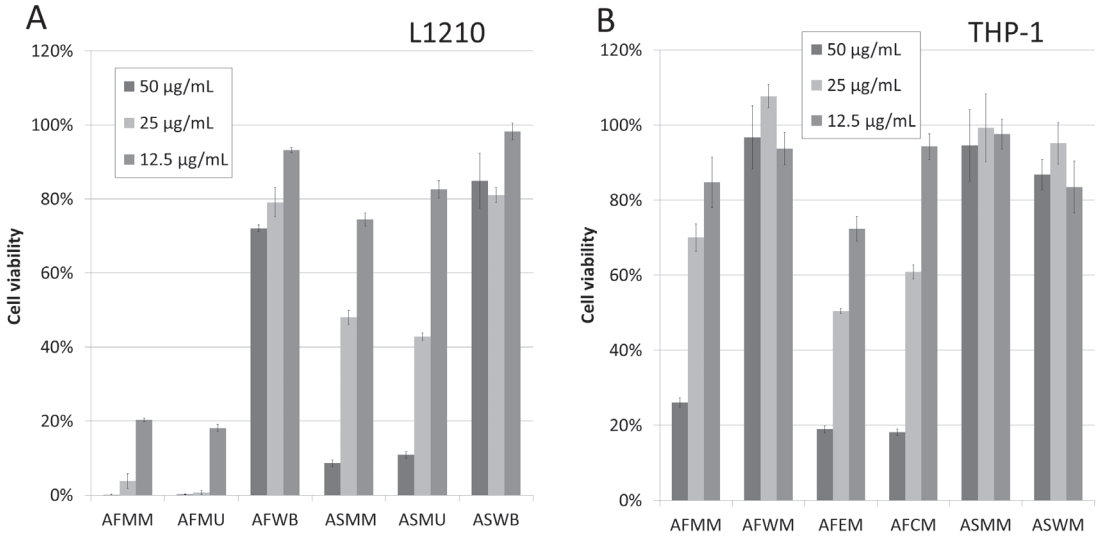


Fig. 4. Cytotoxic effects of the test extracts on L1210 (A) and THP-1 (B) cell lines. Cells were treated with the extracts at concentrations of 50, 25, and 12.5 µg/mL and the cell viability was measured 24 h later using WST-1 reagent. The results are expressed as the mean ± SE for three independent experiments
AFMM – methanolic flowers plus leaves extract obtained by microwave extraction, AFMU – methanolic flowers plus leaves extract obtained by ultrasonic extraction, AFWB – aqueous flowers plus leaves extract obtained by boiling, AFWM – aqueous flowers plus leaves extract obtained by microwave extraction, ASMM – methanolic stems extract obtained by microwave extraction, ASMU – methanolic stems extract obtained by ultrasonic extraction, ASWB – aqueous stems extract obtained by boiling, ASWM – aqueous stems extract obtained by microwave extraction, AFEM – ethanolic flowers plus leaves extract obtained by microwave extraction, AFCM – chloroform flowers plus leaves extract obtained by microwave extraction

growth of the yeast by about 90% after 48 h. The least effective of the stems extracts was the methanolic stems extract obtained by the microwave extraction (ASMM), which inhibited growth of the yeast by around 50%. The methanolic stems extract obtained by ultrasonic extraction (ASMU) was more efficient, inhibiting growth by around 70%. It seems, therefore, that the compounds most active against of *C. albicans* may be hydrophilic substances such as derivatives of chlorogenic acid, which have been found in *A. rupestris*[12]. Chlorogenic acid derivatives have high antifungal activities[15]. These substances are thermolabile and could, therefore, be degraded by the heat of microwave extraction.

Extracts obtained from flowers plus leaves were less effective against *C. albicans*. The aqueous flowers plus leaves extract (AFWB) was the least effective, inhibiting the growth of yeast around 40% after 48 h; the percentage of growth after 120 h was identical to that of the growth control. Methanolic flowers plus leaves extracts (AFMM and AFMU) likewise showed growth inhibited by about 40% after 48 h, but after 120 h the inhibition was only about 30 %.

Obtained results showed that the aqueous stems extract obtained by boiling (ASWB) was the most active against *C. albicans*, whereas *S. aureus* was especially inhibited by methanolic flowers plus leaves extracts (AFMM and AFMU). Extracts obtained by microwave extraction showed slightly lower anti-microbial effect in comparison with ultrasonic extraction.

Results from this study agree with previous studies. The anti-microbial activities of ethanolic, methanolic, and hexane extracts obtained from *Artemisia absinthium*, *A. annua*, and *A. vulgaris* have been studied. Extracts obtained from these plants were tested against five Gram-positive and two Gram-negative bacteria. The results showed the alcoholic extracts to be more effective against the microorganisms tested[16]. The polyphenol contents and anti-microbial activities of extracts obtained from the dried aerial parts of two *Artemisia* species (*A. vulgaris* and *A. campestris*) by using classical, ultrasonic, and Soxhlet extractions have been compared. Ultrasonic treatment had a positive effect on the yield of the extracted substance and the kinetics of its extraction, but the extract obtained by classical extraction had greater total contents of phenolic compounds and flavonoids than those obtained by the two modern extraction techniques. The anti-microbial activity of *A. campestris* extracts was greater than that of *A. vulgaris*. The extracts obtained from both of these species were found to affect yeast more than bacteria[17].

A. rupestris is commonly used in traditional Asian folk medicine, but no cytotoxic study had been evaluated until now. Extracts from flowers plus leaves and from stems were prepared by using different extraction methods (microwave extraction, ultrasonic extraction, and boiling water) and used for the initial cytotoxic experiments on the murine L1210 cell line (Fig. 4A). When different extraction techniques were used with

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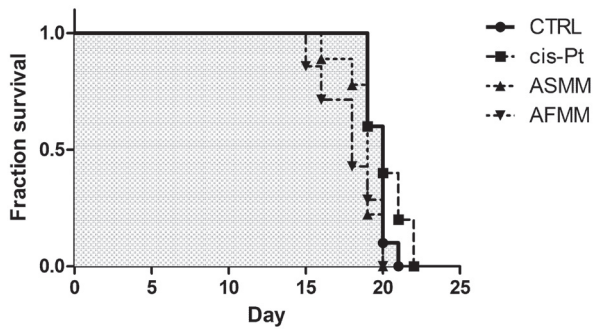


Fig. 5. Kaplan-Meier survival curve. Mice with L1210-induced cancerogenesis were treated with extracts (ASMM and AFMM) 10 mg/kg and with cisplatin (cis-Pt) 2 mg/kg, and the percentage of surviving mice was evaluated on every day of the 22 day experiment. The grey area indicates the survival of the untreated (CTRL) group
AFMM – methanolic flowers plus leaves extract obtained by microwave extraction, ASMM – methanolic stems extract obtained by microwave extraction

the same solvent (methanol), the cytotoxic effects were comparable (compare AFMM with AFMU and ASMM with. ASMU). This indicates that the extraction method did not influence the cytotoxic potential significantly. On the other hand, replacing the less polar methanol solvent with polar aqueous solvent dramatically reduced the cytotoxicity (compare AFMM with AFWB and ASMM with ASWB). Hydrophilic compounds, which were extracted by boiling in water, are generally less toxic than hydrophobic compounds, due, at least in part, to their lesser ability to penetrate through biomembranes. The greatest difference observed was between the parts of the plant used. The flowers plus leaves extracts were more toxic than the extracts taken from stems. It may be that the flowers are more toxic because they contain alkaloids that are not found in the stems[10].

After the initial tests on the L1210 cell line, the microwave technique was chosen for the preparation of all of the extracts to be evaluated for their cytotoxic effects on human cell line THP-1 (Fig. 4B). In general, the human monocyte cell line THP-1 was less sensitive to the extracts used than the murine lymphocytic cell line L1210. Neither of the two aqueous extracts tested (AFWM and ASWM) showed any cytotoxic effect, indicating that the traditional folk ways of using *A. rupestris*, i.e., preparing it as a tea, are safe. However, when less polar solvents, e.g., methanol, ethanol or chloroform, are used, the toxic action is significant. Interestingly, the methanolic stems extract (ASMM) showed no cytotoxic effect on the THP-1 cell line in contrast to its effect on L1210 cells, perhaps because of a significantly different sensitivity to one or more specific compounds present in the extract. This agrees with previous studies that showed the L1210 cell line to be more sensitive to different xenobiotics than the THP-1 cells[18, 19].

The anti-cancer potential of *A. rupestris* was evaluated using the L1210-induced cancerogenesis on a murine model. Two extracts active *in vitro* were selected for evaluation *in vivo* – methanolic extract flowers plus leaves and methanolic stems extract (AFMM and ASMM, respectively). Histological examination of cancer sections showed no positive effect of the test extracts on the growth of the cancer. Moreover, mice treated with *A. rupestris* extracts had significantly shorter life spans than the untreated group ($p = 0.041$ for AFMM and $p = 0.048$ for ASMM) (Fig. 5). The greater toxicity found for the flowers plus leaves extract (AFMM) *in vivo* agrees with the *in vitro* observation, where this extract also showed greater activity than the extract obtained from stems (ASMM).

Conclusions

A. rupestris has long been used in traditional folk medicine, but only a limited number of studies describing its biological activities have been reported. This study is the first to evaluate the toxic effects of crude extracts of *A. rupestris in vitro* and *in vivo*. The results indicate that the application of aqueous extracts is safe. Lipophilic extracts, on the other hand, had significant cytotoxic effects. No anti-cancer potential of the selected extracts was demonstrated, perhaps because the dosage of the material the administered was too large or they may be effective against cancers other than lymphomas those induced by the *i.p.* injection of L1210 cells. The application of different extracts or lower dosages might show different effects that support folk wisdom. Considerably more evaluation of the bioactivities of *A. rupestris* is warranted. Results from this study indicate that the aqueous stems extracts are active against *Candida albicans*, whereas *Staphylococcus aureus* is especially inhibited by methanolic flowers plus leaves extracts. Extracts prepared by microwave extraction possess the least anti-microbial activity, perhaps because of heat degradation of one or more constituent compounds.

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Conflict of interest: none.

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