sciendo

DOI: 10.2478/fv-2018-0025

FOLIA VETERINARIA, 62, 3: 36—40, 2018

IN VITRO EVALUATION OF BIOLOGICAL EFFECTS OF DANDELION (*TARAXACUM OFFICINALE*) EXTRACTS

Marcinčáková, D.[1], Červeňáková, N.[1], Miłek, M.[2]

[1]Department of Pharmacology and Toxicology, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
Slovakia
[2]Department of Chemistry and Food Toxicology, Faculty of Biology and Agriculture
University of Rzeszów, Ćwiklińskiej 1a, 35-601 Rzeszów
Poland

dana.marcincakova@uvlf.sk

ABSTRACT

Dandelion (*Taraxacum officinale*) of the *Asteraceae* family is known for its pharmacological effects and has been used in therapy for centuries. Currently extracts of all parts of this plant are used — root, leaves and flowers. The extracts are prepared using various extraction agents that may significantly affect the effectiveness and therapeutic spectrum of the extracts. The aim of this study was to use three different solvents for the preparation of the extracts from dandelion (*Taraxacum officinale*) leaves and flowers, namely triton X-100 (2%), nonidet P-40 (2%) and acetone (30%). After extraction, the extractants were evaporated and the dried extracts were dissolved in water to obtain a series of solutions of the concentrations: 125, 250, 500 and 1000 µg.ml^{-1}. The biological effects of the extracts were investigated by means of the MTT test of cell viability. Rabbit kidney epithelial cells (RK13) exposed to the extracts for 24 and 48 hours were used as a model cell line. We observed that the acetone extract of dandelion leaves and flowers at lower concentrations caused an increase in the viability of the treated cells in comparison with the control cells which were not exposed to the extracts ($P < 0.05$). At the same time, we observed a significant effect of the solvent used for the preparation of the dry extracts on the viability of the cells. The residues of the extractants caused a decrease in the cell viability almost to zero, which in fact means the death of the cells. The selection of the correct extractant for the preparation of the extracts is essential regarding the use of extracts in the pharmaceutical or cosmetic industries.

Key words: cell culture; MTT; solvent; viability

INTRODUCTION

The pharmaceutical industry constantly maintains an interest in the herbaceous plants owing to the fact that phytotherapy is perceived as a part of modern medicine. Officinal plants play an important role in the treatment and prevention of various diseases and disorders. Phytotherapy is based on the use of pharmacologically effective ingredients

found in plants in order to achieve the required therapeutic effect on target molecules, enzymes and receptors [3, 12].

Dandelion (*Taraxacum officinale*) of the *Asteraceae* family belongs among plant species commonly grown and widely-spread throughout Slovakia, containing many effective ingredients. Owing to their broad spectrum of pharmacological profile and safety of applications, they are widely used in the therapy and prevention of various diseases and organism disorders, such as: digestive and urinary tract disorders, inflammatory processes, pro-thrombotic states and oxidation stress. The whole plant — leaves, flowers, stems and roots – have curative use. Many pharmaceutical products are prepared on the plant basis and a great number of herbs are used for the preparation of various tea blends. Medicinal preparations of the current modern phytotherapy include not only infusions, decoctions or tinctures, but also dry standardized extracts or single individual fractions of such extracts. Extracts are the raw material of the pharmaceutical industry that suit to the current requirements of processing, analysis and control as well as the synthetic medicines [8, 12]. The preparation of these dry extracts involves the use of various extraction reagents. Solvents are widely used in the chemical industry in many applications, for example: in the processing (synthesis, separation, purification of active ingredient), medicinal formulations and during cleaning and washing, and exhibit different properties [4]. It is assumed that drying involves evaporation of the solvents. The effect of extractants on the live organism is frequently unknown and can even be harmful.

The aim of our study was to validate the effect of extracts of the herbaceous plant dandelion (*Taraxacum officinale*), prepared by means of three different extractants, acetone, nonidet P-40 and triton X-100, on a live cell line — model line of rabbit kidney epithelial cells RK13 — and by means of the observation of the viability of these cells to compare the influence of the solvents used in the preparation of dry extracts.

MATERIALS AND METHODS

Preparation of extracts

In the experiment we investigated the action of the extracts of dandelion (*Taraxacum officinale*, *Asteraceae*) leaves and flowers. The plants originated from the Subcarpathian region (Rzeszow, Poland). About 10—15 dandelion plants were collected from one location (a meadow outside of the town) in May, 2017, and were air dried. One gram of dried, pulverized dandelion leaves or flowers was extracted with 20 ml of proper solvent in an ultrasonic bath (Sonorex RK 30, Bandelin, Germany) for 30 min. The following solvents were used: aqueous acetone 30 % v/v; aqueous solution of nonidet P-40, 2 % v/v; aqueous solution of triton X-100, 2 % v/v. Nonidet and triton are non-ionic surface active substances — viscous liquids easily soluble in water (Sigma Aldrich, Germany).

The extracts were then centrifuged (10 min, 6500 × g) and the supernatants were collected. After removal of acetone by vacuum evaporation, all supernatants were lyophilised (24 h, lyophylisator Alpha, Christ GmBH, Osterode am Harz, Germany) to obtain dry extracts. Immediately before the experiments, we prepared a series of fresh water solutions from the dried extracts of concentrations: 125, 250, 500 and 1000 $\mu g.ml^{-1}$.

Cell cultivation

Rabbit kidney epithelial cells RK13 (ATCC® CCL-37™) were selected as a model cell line for evaluation of the biological action of the prepared extracts. The cells were grown in a complete culture medium EMEM (Earl's Minimal Essential Medium; Lonza, Belgium) with 10 % foetal bovine serum, 1 % L-glutamine, 0.1 % gentamycin, 0.5 % Fungizone (amphotericin B) and 1 % antibiotic (combination of penicillin and streptomycin) at 37 °C in an atmosphere containing 5 % CO_2 until they reached approximately a 90 % confluent monolayer. During cultivation, the cells were regularly checked for the absence of mycoplasma contamination [15].

MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay

The cell suspension (100 µl) of density 22,000 cells per well area was cultivated in 96-flat bottom well plates (Greiner Bio One, Greiner, Germany). After 24-hour cultivation, the cells in the growth phase were exposed to extracts of dandelion leaves and flowers for 48 hours. Wells with the cells not exposed to the action of extracts served as a control. After 24 and 48 hours of exposure, the medium was removed from wells and the adhered cells were washed with PBS (100 µl). PBS was then removed and 90 µl of cultivation medium and 10 µl (concentration 5 $mg.ml^{-1}$) of yellow tetrazolium stain MTT 3-(4,5-dimethylthiazol-

2-yl)-2,5-diphenyltetrazolium bromide) was added to each well. In metabolically active, i.e. live cells, this stain turns to a purple formazan. After 4-hour incubation in a CO_2 cell incubator, the MTT medium was replaced by concentrated DMSO (100 µl) solution to dissolve formazan crystals. Immediately after addition of Sorensen glycine buffer (12.5 µl per well), the absorbance at 570 nm was read (spectrophotometer Synergy HT, Biotek, Winooski, VT, USA).

Statistical processing of results

The spectrophotometrically determined absorbance values were converted to % of cell viability by the following formula:

% = absorbance of sample × 100/absorbance of control

The cells not exposed to extracts served as a control (viability 100 %). The results were presented as means (n = 3) with standard deviation (± sd). The results were evaluated statistically using the software Graf Pad Prism 3.00 (GraphPad Software, San Diego, CA, USA) and Dunnett's comparison test. The significance level was set to $P < 0{,}05$.

RESULTS

Changes in the viability of cells following their exposure to the extracts of dandelion leaves are summarised in Fig. 1. An increase in the metabolic activity above 100 % was observed after 24-h exposure to acetone extracts of concentrations 125 and 250 $\mu g.ml^{-1}$ and after 48-h exposure to extract of concentration 500 $\mu g.ml^{-1}$.

In the case of extracts prepared with nonidet and triton, almost all values were low, close to zero ($P < 0.05$) which indicated the death of cells. Viability of all cells exposed to extracts differed significantly ($P < 0.05$) from the viability of untreated cells with the exception of acetone extracts of concentrations 250 and 500 $\mu g.ml^{-1}$ after 24-h exposure ($P > 0.05$).

The investigation of the metabolic activity of the model cell line treated with extracts of dandelion flowers showed a marked increase in the activity of cells already after 24-h exposure (Fig. 2). Similar to the action of dandelion leaf extracts, the acetone extracts affected positively the viability of cells. In the case of concentrations 125 and 250 $\mu g.ml^{-1}$, an increase in viability of the treated cells above 100 % was observed after 24-h of the action of extracts ($P < 0.05$). After exposure to concentrations 500 and 1,000 $\mu g.ml^{-1}$, the metabolic activity of the cells decreased to 80 %. On the contrary, the viability of the cells exposed to the extracts of dandelion flowers prepared with nonidet and triton, was very low, almost zero, resembling the action of leaf extracts. All differences in the activity in comparison with the control were significant ($P < 0.05$).

DISCUSSION

Dandelion (*Taraxacum officinale*) exhibits a broad pharmacological profile and with regard to the occurrence of undesirable effects, it is classified as safe. A number of studies have confirmed its pharmacological effects — diuretic, choleretic, anti-inflammatory, antioxidant, anticancerigenic, antihyperglycaemic, anticoagulant and prebiotic [14].

In our investigations of the biological activity of dandelions we observed the effects of different extracts of dandelion leaves and flowers on the viability of a model cell line RK13. We detected a significant influence of the solvent used for the preparation of dry extracts. The common methods of selection of the most suitable solvent uses the available powerful tools that help to reduce the effort by focusing on a pre-selected set of solvents suitable for the relevant processing step [4]. Acetone dissolves many hydrophilic and lipophilic plant components, is water-miscible, volatile and its toxicity for the biological test is low. Although water is used as a general solvent, plant extracts prepared with organic solvents exhibit higher antimicrobial activity in comparison to water extracts. [11]. Our study showed an increased metabolic activity of cells exposed to acetone extracts from dandelion leaves and flowers. One can assume that this was related to the influence of some specific components as dandelions contain a large amounts of polyphenols and flavonoids (19 %) [7]. Triton X-100 and nonidet P-40 belong to the most used non-ionic surface active substances utilized for lysis of cells at extraction of proteins and other cell organelles and also as a reagents that induce the permeabilisation of membranes [2]. Surfactants are also used as modifiers of aqueous extraction in the so-called micelle mediated extraction (MME). Formation of surfactant micelles in a solution is a factor improving solubilization of various analytes. The MME method is widely used in sample preparation processes, also for extraction of biologically active plant metabolites, e. g. terpenes [10, 13], alkaloids [9] or polyphenols [1, 5, 6]. The removal of

surfactant from the extract is problematic, therefore even after lyophilisation some residues of this compound remain in the sample. Despite complete evaporation of the solvent during preparation of dried powdered extracts, comparative studies proved the influence of solvent residues on metabolic activity or the viability of the cells. Their residues most likely caused a decrease in the metabolic activity of the treated cells down to the zero level, i. e. to cell death. The cytotoxic effects of surfactants has been confirmed when various substances of this type were tested on cells. Triton X-100, used in our research, is classified as one of the most cytotoxic surfactants [1]. Nonidet P-40 is structurally similar, so it can be concluded, that its effects may be comparable. Our study showed that the selection of suitable solvent for obtaining plant extracts is very important as it can have adverse effects on live organisms. Thus, despite the positive effects of the relevant plant, the final impact on health can be negative.

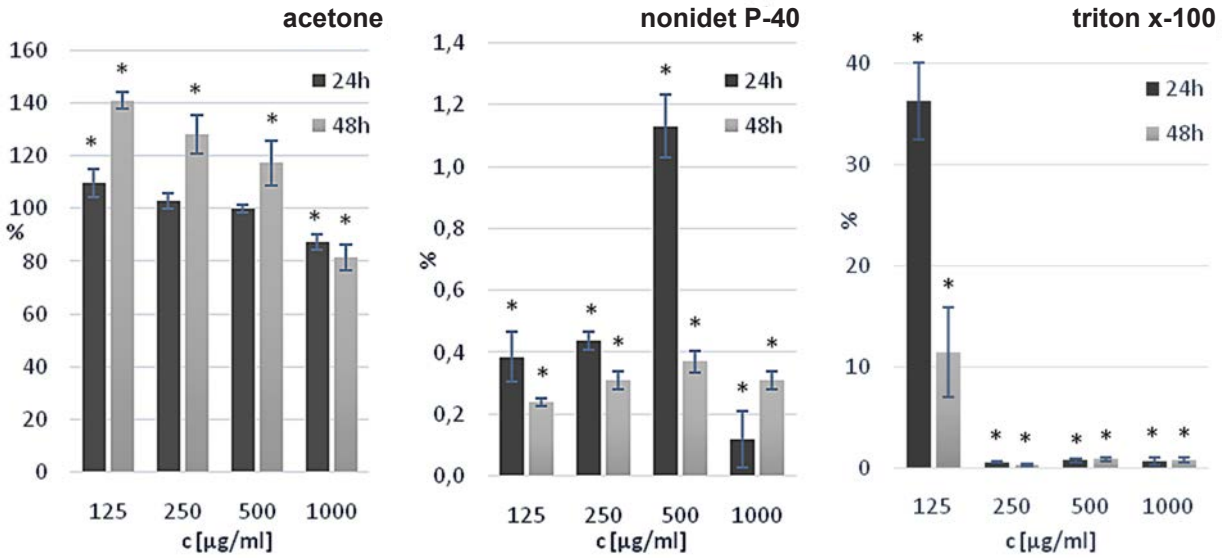

Fig. 1. Changes in the viability of cells (%) in comparison with the control (100%)after exposure to extracts prepared with acetone, nonidet and triton from dandelion leaves in relation to extract concentrations (µg.ml^{-1})

*the differences in comparison with the control were significant at the level of P < 0.05

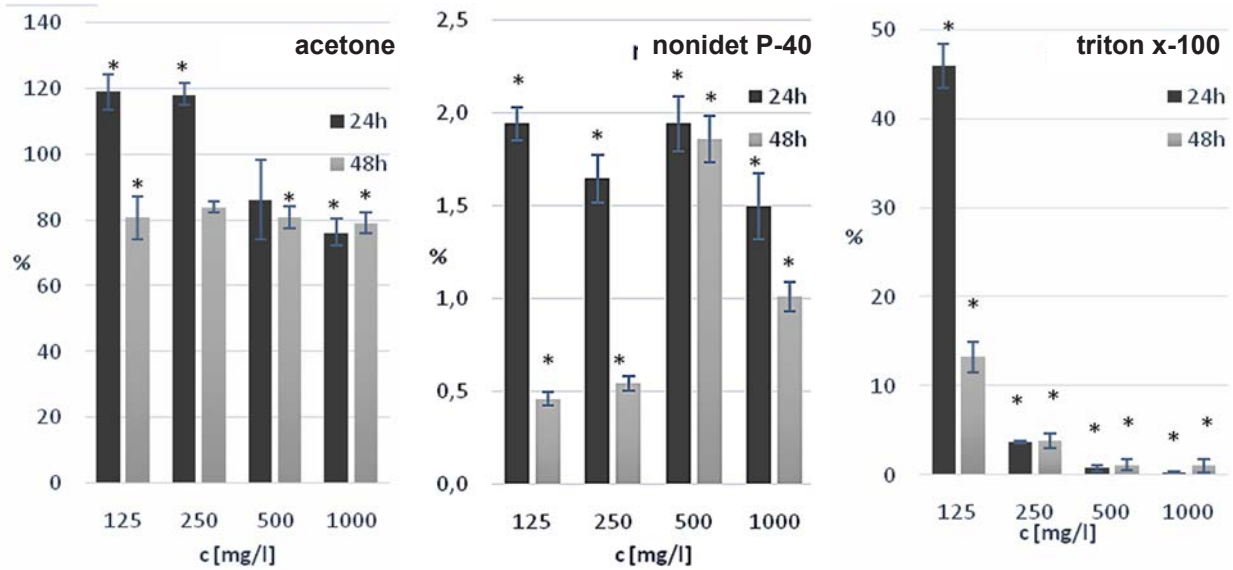

Fig. 2. Metabolic activity of cells (%) in comparison with the control (100 %) after exposure to extracts prepared with acetone, nonidet P-40 and triton x-100 from dandelion flowers in relation to extract concentrations (µg.ml^{-1})

*the differences in comparison with the control were significant at the level of P < 0.05

CONCLUSIONS

Extracts of medicinal plants are frequently used in phytotherapy. The selection of solvents used for their preparation is very important as it affects their biological action. Acetone appears a suitable solvent for preparation of extracts from dandelions with regard to the complex of components present in this plant. Exposure of model cells to acetone extracts caused a significant increase in their metabolic activity above 100 % which can be considered a supportive effect. On the contrary, the use of surface active substances, ionic detergents, affected negatively metabolic activity and viability of cells, in fact it caused their death. The residues of extractants can markedly affect the action of dry plant extracts and eventually have a direct negative effect on live organisms. This stresses the importance of the selection of the correct extractants in order to ensure the optimum use of beneficial components of medicinal plants and also the way of application of the extract in cosmetic or pharmaceutical industries.

ACKNOWLEDGEMENT

This study was supported by the National Reference Laboratory for Pesticides of the University of Veterinary Medicine and Pharmacy in Košice and the project VEGA No. 1/0408/17.

REFERENCES

1. **Arechabala, B., Coiffard, C., Rivalland, P., Coiffard, L. J., De Roeck-Holtzhauer, Y., 1999:** Comparison of cytotoxicity of various surfactants tested on normal human fibroblast cultures using the neutral red test, MTT assay and LDH release. *J. Appl. Toxicol.*, 19, 163—165.
2. **Bi, W., Tian, M., Row, K. H., 2011:** Extraction and concentration of tanshinones in *Salvia miltiorrhiza* Bunge by task-specific non-ionic surfactant assistance. *Food Chemistry*, 126, 1985—1990.
3. **Csupor, D., 2015:** *Phytotherapy, a Textbook for Pharmacy Students* [online]. University of Szeged, Szeged, Department of Pharmacognosy, 229 pp.
4. **Gani, R., Jimenez-Gonzalez, C., Kate, A. T., Crafts, P. A., Jones, M., Powell, L., 2006:** A modern approach to solvent selection: although chemists' and engineers' intuition is still important, powerful tools are becoming available to reduce the effort needed to select the right solvent. *Chemical Engineering*, 113, 30—43.
5. **Hosseinzadeh, R., Khorsandi, K., Hemmaty, S., 2013:** Study of the effect of surfactants on extraction and determination of polyphenolic compounds and antioxidant capacity of fruit extracts. *PloS one*, 8, e57353.
6. **Jiang, L., Zhou, G., Li, Y., 2011:** Micelle-mediated extraction for the analysis of chlorogenic acid, rutin and quercetin in honeysuckle by HPLC-UV. *J. Liq. Chromatogr. Relat. Technol.*, 34, 1473—1487.
7. **Koley, D., Bard, A. J., 2010:** Triton X-100 concentration effects on membrane permeability of a single HeLa cell by scanning electrochemical microscopy (SECM). *Proceedings of the National Academy of Sciences*, 107, 16783—16787.
8. **Martinez, M., Poirrier, P., Chamy, R., Prüfer, D., Schulze-Gronover, C., Jorquera, L. et al., 2015:** *Taraxacum officinale* and related species — An ethnopharmacological review and its potential as a commercial medicinal plant. *J. Ethnopharmacol.*, 169, 244—262.
9. **Memon, A. A., Memon, N., Bhanger, M. I., 2010:** Micelle-mediated extraction of chlorogenic acid from Morus laevigata leaves. *Sep. Purif. Technol.*, 76, 179—183.
10. **Shi, Z., He, J., Chang, W., 2004:** Micelle-mediated extraction of tanshinones from *Salvia miltiorrhiza* Bunge with analysis by high-performance liquid chromatography. *Talanta*, 64, 401—407.
11. **Schütz, K., Carle, R., Schieber, A., 2006:** Taraxacum — A review on its phytochemical and pharmacological profile. *J. Ethnopharmacol.*, 107, 313—323.
12. **Ştefania, G., Vâtcă, S., Vâtcă, A., 2016:** The use of medicinal plants in the human civilisations. *Agriculture—Science and Practice*, 3—4, 46—50.
13. **Sun, C., Lin, H., 2008:** Application of non-ionic surfactant in the microwave-assisted extraction of alkaloids from *Rhizoma Coptidis*. *Anal. Chim. Acta*, 612, 160—164.
14. **Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, K., 2011:** Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, 1, 98—106.
15. **Young, L., Sung, J., Stacey, G., Masters, J. R., 2010:** Detection of mycoplasma in cell cultures. *Nature Protocols*, 5, 929—934.

Received June 22, 2018
Accepted August 22, 2018