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IN VITRO ANTIOXIDANT ACTIVITY OF LEAF AND STEM EXTRACTS OF GREATER CELANDINE (*CHELIDONIUM MAJUS* L.) USING A HUMAN BLOOD MODEL

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Greater celandine (Chelidoniummajus L., CM) is an important plant in phytotherapy and traditional medicine.CM has been used extensively in folk medicine throughout Europe and in some Asian countries, particularly for the treatment of various ailments such as stomach cancer, stomach ulcers, liver and skin diseases. It also has potential anti-inflammatory, anticancer, antiviral, antimicrobial and antifungal properties.The present study was conducted to evaluate the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC)] in blood samples collected from healthy volunteers after in vitro incubation with extracts derived from stems and roots of greater celandine were used. The aim of this study was to evaluate the dose-dependent changes in biomarkers of oxidative stress in blood samples from healthy volunteers exposed in vitro to extracts of CM roots and stems collected from natural habitats in the area of the South Park in Słupsk (Pomeranian Province, northern part of Poland). Freshly washed plant samples were weighed, crushed and homogenised in 0.1 M phosphate buffer (pH 7.4) (1:19, w/w) at room temperature.The extracts were then filtered and used for analysis. Blood samples from healthy volunteers were preincubated with 4 mM phosphate buffer (pH 7.4) (control) and with CM stem and root extracts (at final concentrations of 5 and 2.5 mg/mL) at 37°C for 60 min.The results of our study showed a statistically significant decrease in TBARS levels in blood samples for root extracts at the final dose of CM extracts at both 5 and 2.5 mg/mL. We observed similar trends after in vitro incubation of blood samples with stem extracts of CM (at a final concentration of 2.5 mg/mL), where there was a statistically significant reduction in the concentration of TBARS compared to the untreated control samples. We also observed a statistically non-significant increase in TAC levels after in vitro incubation of blood samples with stem extracts of CM (at a final dose of 5 and 2.5 mg/mL) compared to control samples, while there was a statistically non-significant reduction in TAC levels after in vitro incubation of blood samples with root extracts of CM (at a final dose of 2.5 mg/mL) compared to untreated control samples. This study provides new insights into the understanding of the antioxidant properties of CM extracts.

Keywords: *Chelidoniummajus L., root and stem extracts, blood samples, lipid peroxidation, total antioxidant capacity*

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IN VITRO АНТИОКСИДАНТНА АКТИВНІСТЬ ЕКСТРАКТІВ ЛИСТЯ ТА СТЕБЛА ЧИСТОТІЛУ ВЕЛИКОГО (CHELIDONIUM MAJUS L.) НА МОДЕЛІ КРОВІ ЛЮДИНИ

Чистотіл звичайний (Chelidoniummajus L., CM) широко використовується в народній медицині по всій Європі та в деяких країнах Азії, зокрема для лікування різних захворювань,

таких як рак шлунка, виразка шлунка, захворювання печінки та шкіри. Він також має потенційні протизапальні, протипухлинні, противірусні, протимікробні та протигрибкові властивості. Це дослідження було проведено з метою оцінки рівня біомаркерів окисного стресу [реактивні речовини, які взаємодіють з 2-тіобарбітуровою кислотою (TBARS), загальна антиоксидантна активність (TAC)] в зразках крові, відібраних у здорових добровольців після інкубації in vitro з екстрактами, отриманими зі стебел і коренів чистотілу звичайного. Метою цього дослідження було оцінити дозозалежні зміни біомаркерів окиснювального стрессу в зразках крові здорових добровольців після інкубації in vitro з екстрактами коренів і стебел CM, зібраних у природному середовищі в районі Південного парку в Слупську (Поморське воєводство, північна частина Польщі). Свіжопромиті зразки рослин зважували, подрібнювали та гомогенізували в 0,1 М фосфатному буфері (pH 7,4) (1:19, мас./мас.) при кімнатній температурі. Зразки крові здорових добровольців попередньо інкубували з 4 мМ фосфатним буфером (pH 7,4) (контроль) та з екстрактами стебел та коренів CM (у кінцевих концентраціях 5 та 2,5 мг/мл) при 37°C протягом 60 хв. Результати нашого дослідження показали статистично істотне зниження рівня TBARS у зразках крові після інкубації з екстрактами коренів при кінцевій концентрації екстрактів CM 5і 2,5 мг/мл. Ми спостерігали подібні тенденції в рівнях TBARS після інкубації in vitro зразків крові з екстрактами стебел CM (у кінцевій концентрації 2,5 мг/мл), а зокрема статистично істотне зниження вмісту TBARS порівняно з необробленими контрольними зразками. Ми також спостерігали статистично неістотне підвищення рівня TAC після інкубації in vitro зразків крові з екстрактами стебел CM (у кінцевій концентрації5 і 2,5 мг/мл) порівняно з контрольними зразками, тоді як статистично істотне зниження рівня TAC спостерігали після інкубації in vitro зразків крові з екстрактами коренів CM (у кінцевій концентрації2,5 мг/мл) порівняно з необробленими контрольними зразками. Це дослідження дає нові дані щодо антиоксидантних властивостей екстрактів CM.

Ключові слова: *Chelidonium majus L., екстракти коренів та стебел, зразки крові, перекисне окиснення ліпідів, загальна антиоксидантна активність.*

INTRODUCTION

The disturbance of the natural balance between the rate of free radical oxidation and the activity of the body's antioxidant defences, which occurs under the influence of unfavourable external factors (environmental pollution, ultraviolet radiation, emotional stress, high content of easily digestible carbohydrates and fats in the diet with a simultaneous decrease in the content of bioantioxidants), plays an important role in the development of many diseases such as cardiovascular, oncological, gastrointestinal, etc. [9, 16, 26, 27]. In this regard, the search and study of promising non-toxic substances with antiradical and antioxidant activity is a very urgent task. It is known that medicinal plants are the main source of biologically active substances for living organisms, including humans [30, 37]. The medicinal effect of medicinal plants is related to the presence of pharmacologically active substances in them which, when they enter the body of animals and humans, exhibit physiologically active properties and have a healing effect. The main active constituents include flavonoids, polyphenols, phenolic acids, essential oils, tannins and vitamins [5, 12].

Greater celandine (*Chelidoniummajus* L., CM) has been used since ancient times to treat a variety of ailments. Celandine is a plant with bright yellow flowers. Greater celandine, also known as dovetail and considered a weed, is a member of

the poppy family (Papaveraceae). Native to Europe and western Asia, this perennial plant with small yellow flowers now grows all over the world [36]. When damaged, the stem secretes a visible yellow-orange sap known as 'devil's milk'. Its Latin name, *Chelidonium*, comes from the ancient Greek word chelidon and translates as 'swallow'. It is believed that the flowering of the celandine heralds the return of these birds from the south. From the time of Avicenna until the 18th century, celandine was used to treat eye diseases. Legend has it that swallows used its sap to save blind chicks [4, 36].

Celandine is most popular as a powerful external remedy for the treatment of skin defects: for the removal of warts, dry calluses, papillomas, pigment spots and some other benign skin formations [17].The aerial parts of greater celandine are most commonly used in medicine. The dried herb is used for solid and liquid extracts, and the juice is extracted from fresh stems and leaves. Herbalists in Eastern and Central Europe also use the roots. The plant is used to treat lung and liver diseases, to stimulate digestion and bile secretion, to fight infections and boost immunity, and to remove warts and skin ulcers. Chinese herbalists use greater celandine to improve circulation, combat cramps and menstrual pain, treat jaundice and more. Doctors confirm some of these effects. Research shows the benefits of celandine for the human body [35, 36].

The plant contains a group of alkaloids with a unique structure and various medicinal properties: chelidonine, coptisine, sanguinarine, chelerythrine, berberine [11, 25]. Chelidonine is the main alkaloid of celandine, named after the plant – *Chelidonium*. In standardised celandine products, the total alkaloid content is expressed as a percentage of chelidonine [2, 29]. Its concentration increases during the day, so the plant is usually harvested in the late afternoon. In the roots, the amount of alkaloids reaches 2-3%, and the aerial parts contain up to 1.5% of substances [29, 36].Celandine also contains: flavonoids (rutin, quercetin, luteolin), lectins, linoleic and oleic fatty acids, minerals (calcium, iron, potassium, magnesium, sulphur, phosphorus), chelidonic, caffeic and malic organic acids[2, 36].

Due to its immunostimulating properties, celandine may block the effects of immunosuppressive drugs such as corticosteroids [13, 21]. Celandine can damage the liver and may worsen the side effects of hepatotoxic drugs such as amiodarone (Cordarone), atorvastatin (Lipitor) and paracetamol [1, 28].Therefore, before using this plant material for medicinal purposes or as a source of antioxidants, it is necessary to study these properties of extracts or essential oils of a plant growing in a given geographical area.

In the present study, biomarkers ofoxidative stress [2-thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC)] were measured in blood samples collected from healthy volunteers after *in vitro* treatment with extracts derived from stems and roots of greater celandine.The aim of this study was to evaluate the dose-dependent changes in biomarkers of oxidative stress in blood samples from healthy volunteers exposed *in vitro* to extracts of CM roots and stems

collected from natural habitats in the area of the South Park in Słupsk, in the Pomeranian province (northern part of Poland).

MATERIALS AND METHODS

***Collection of plant material and preparation of plant extracts*.**The plant material was collected from natural habitats on the territory of the South Park in Słupsk (54°28′08,5″N 17°02′56,0″E) in the Pomeranian Province (northern part of Poland). This area has been adapted for recreational purposes by creating a guarded swimming area, a permanent fireplace, benches and baskets, a place for camping and physical games, an access road and a car park. The collected roots and stems were taken to the laboratory for biochemical analysis. Freshly washed plant samples were weighed, crushed and homogenised in 0.1M phosphate buffer (pH 7.4) (1:19, w/w) at room temperature. The extracts were then filtered and used for analysis. The extract was stored at -25°C until use.

Collection of blood samples. Blood (10 mL) was collected from healthy volunteers by venipuncture. The study was approved by the Regional Research Ethics Committee of the Medical University of Gdansk, Poland (KB-31/18). All patients gave written informed consent before the start of the study procedures. Blood samples were drawn into commercially available tubes after overnight fasting. Venous blood samples (10 mL) were obtained from the antecubital vein of each participant using sterile disposable plastic syringes. Samples were collected at the same standardised time to minimise the effect of diurnal variation. Blood samples were stored at +4°C and used within 2 days for analysis of biomarkers of oxidative stress.

Blood samples were pre-incubated with 4 mM phosphate buffer (pH 7.4) (control) and with CM stem and root extracts (at final concentrations of 5 and 2.5 mg/mL) at 37°C for 60 min. This reaction mixture was gently shaken at fixed intervals during incubation at 37°C. A 4 mM phosphate buffer (pH 7.4) was used as a positive control.

The 2-Thiobarbituric acid reactive substances (TBARS) assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reactive substances (TBARS) using the method of Kamyshnikov (2004) for the determination of malonic dialdehyde (MDA) concentration [10]. This method is based on the reaction of the degradation product of lipid peroxidation, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to form a coloured adduct which is measured spectrophotometrically. The nmol of MDA per mL was calculated using an extinction coefficient of $1.56 \cdot 10^5$ $mM^{-1} \cdot cm^{-1}$.

Total antioxidant capacity (TAC) assay. Blood TAC levels were estimated by measuring TBARS levels after oxidation of Tween 80 according to Galaktionova and co-workers (1998) [3]. Plasma inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The absorbance of the solution obtained was measured at 532 nm. The absorbance of the blank was

defined as 100%. The content of TAC in the sample (%) was calculated from the absorbance of the blank.

Statistical analysis. Results are expressed as mean ± S.E.M. Statistical analysis was performed using the STATISTICA 13.3 package (TIBCO Software Inc., USA). All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors tests ($p > 0.05$). The significance of differences between levels of oxidative stress biomarkers (significance level, $p < 0.05$) was tested using the Mann-Whitney U test [34]. All statistical calculations were performed on separate data from each individual using STATISTICA 13.3 software (TIBCO Software Inc., USA).

RESULTS AND DISCUSSION

Figure 1 shows the values of TBARS levels obtained by incubating blood samples collected from healthy volunteers in the presence of aqueous extracts derived from roots and stems of CM. The final concentrations of the extracts in the blood samples were 5 mg/mL and 2.5 mg/mL.

Analysing the final dose of CM extracts at 5 mg/mL, we observed a statistically significant decrease in TBARS levels of 8.8% ($p < 0.05$) for root extracts (14.57 ± 0.44 nmol/mL) and 0.6% ($p > 0.05$) for stem extracts of CM (15.88 ± 0.46 nmol/mL) compared to the untreated control samples (15.97 ± 0.56 nmol/mL). We obtained similar results after *in vitro* incubation of the blood samples with root extracts of CM at a dose of 2.5 mg/mL, where we also recorded a statistically significant decrease in TBARS levels of 9.4% ($p < 0.05$) compared to the control samples (14.47 ± 0.49 nmol/mL*vs.* 15.97 ± 0.56 nmol/mL).We observed different trends after *in vitro* incubation of blood samples with stem extracts of CM (at a final concentration of 2.5 mg/mL), where there was a statistically significant reduction (by 11.4%, $p < 0.05$) in the concentration of TBARS compared to the control samples (14.15 ± 0.51 nmol/mL*vs.* 15.97 ± 0.56 nmol/mL) (Fig. 1).

Total antioxidant capacity (%) in blood samples obtained from healthy individuals after *in vitro* incubation with root and stem extracts derived from CM is shown in Figure 2.

We observed a statistically non-significant increase in TAC levels after *in vitro* incubation of blood samples with stem extracts of CM (at a final dose of 5 and 2.5 mg/mL) compared to control samples (70.12 ± 6.24% *vs.* 65.25 ± 5.17% after use of stem extracts of CM at a final concentration of 5 mg/mL; 67.35 ± 6.13% *vs.* 65.25 ± 5.17% after use of stem extracts of CM at a final concentration of 2.5 mg/mL). These increases were 7.46% ($p > 0.05$) and 3.22% ($p > 0.05$), respectively.After incubation of blood samples with root extracts of CM at a final dose of 5 mg/mL, we observed a statistically non-significant decrease in TAC levels of 5.3% ($p > 0.05$) compared to control samples (61.77 ± 6.85% *vs.* 65.25 ± 5.17%).After *in vitro* incubation of blood samples with root extracts of CM (at a final dose of 2.5 mg/mL), there was a statistically non-significant reduction (by 7.7%, $p > 0.05$) in

TAC levels compared to untreated control samples (60.23 ± 6.67% *vs.* 65.25 ± 5.17%) (Fig. 2).

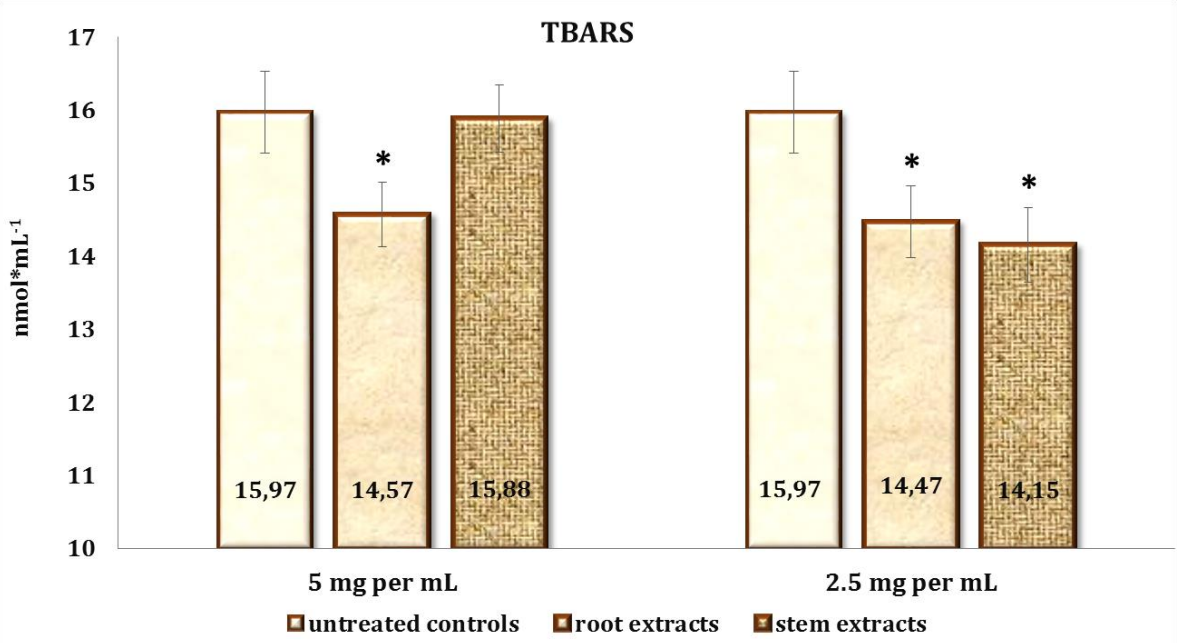

Fig. 1. TBARS content (nmol·mL^{-1}) as a biomarker of lipid peroxidation in blood samples obtained from healthy individuals after *in vitro* incubation with root and stem extracts derived from CM (M ± m, n = 8).
The final concentrations of the extracts in the blood samples were 5 mg/mL and 2.5 mg/mL.
*- Statistically significant differences ($p < 0.05$) compared with untreated control samples.

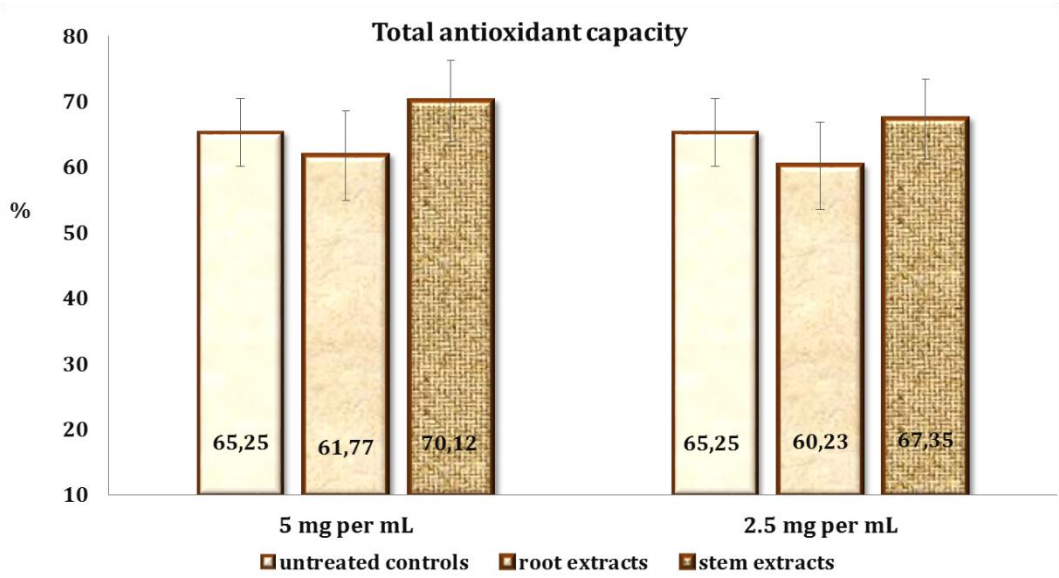

Fig. 2. Total antioxidant capacity (%)in blood samples obtained from healthy individuals after *in vitro* incubation with root and stem extracts derived from CM (M ± m, n = 8).
The final concentrations of the extracts in the blood samples were 5 mg/mL and 2.5 mg/mL

The results of recent years have provided new insights into the preliminary steps of developing a high-value product such as CM for phytomedicine applications through promising metabolic variations with antioxidant and anticancer potentials.Metabolic variations, antioxidant potential and cytotoxic effects were

investigated by Nile and co-workers (2021) in the different plant parts like leaf, stem, flower, pod and root of CM using spectroscopic and chromatographic methods [20]. Total phenolics and flavonoids were analysed in the different parts of CM. The leaf showed a higher flavonoid content (137.43 mg/g), while the pod showed the highest phenolic content (23.67 mg/g) when compared to the stem, flower and root. In the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) antioxidant assay, the flower extract showed 57.94% activity, while the leaf, pod and root extracts showed 39.10%, 36.08% and 28.88% activity respectively. The pod and leaf extracts demonstrated the potential effect, showing 45.46 and 41.61% activity respectively in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Similar to the phosphomolybdenum assay, the flower showed higher antioxidant activity (46.82%) than the other plant parts. The *in vitro*sulphorhodamine (SRB) assay facilitated the evaluation of the cytotoxic effect against HeLa and CaSki human cervical cancer cells. The extract showed a dose-dependent inhibitory effect on both cell lines. The highest cytotoxic effect was observed in the pod and flower extracts after 48 h exposure at 1000 μg/mL [20].

CM extracts show very high cytotoxic activity and can be recommended for further experiments to confirm their potential use in the treatment of various human cancers.The cytotoxic effect of *Chelidoniummajus* L., *Mahoniaaquifolium* (Pursh) Nutt. and *Sanguinariacanadensis*L. extracts, obtained from different parts of these plants at different stages of vegetation, on FaDu, SCC-25, MCF-7 and MDA-MB-231 cancer cells was investigated by Tuzimski and co-workers (2023). Almost all the extracts tested showed higher cytotoxicity against these cancer cells than the anticancer drug etoposide. The highest cytotoxicity against the FaDu, SCC-25, MCF-7 and MDA-MB-231 cancer cell lines was obtained for the *S.candensis* extract collected before flowering. The cytotoxicity of extracts from different parts of CM, collected at different vegetative stages, was also evaluated on melanoma cells (A375, G361 and SK-MEL-3). The highest cytotoxic activity against melanoma A375 cells was observed for the CM root extract with an IC_{50} of 12.65 μg/mL. The same extract was the most cytotoxic against SK-MEL-3 cells (IC_{50} = 1.93 μg/mL), while the highest cytotoxic activity against G361 cells was observed after exposure to the extract obtained from the herb of the plant. The cytotoxic activity of CM extracts against melanoma cells was compared with the cytotoxicity of the following anticancer drugs: etoposide, cisplatin and hydroxyurea. In most cases, the IC_{50} values obtained for the anticancer drugs were higher than those obtained for the CM extracts [31].

Petruczynik and co-workers (2019) investigated the cytotoxic activity of extracts obtained from CM,*Berberis* sp.,*Thalictrumfoetidum*L. containing different alkaloids on selected cancer cell lines [24]. The cytotoxic effect of the tested plant extracts and the respective alkaloid standards was investigated using human pharyngeal squamous carcinoma cells (FaDu), human tongue squamous carcinoma cells (SCC-25), human breast adenocarcinoma cell line (MCF-7) and human triple negative breast adenocarcinoma cell line (MDA-MB-231). All of the plant extracts

studied exhibited cytotoxic activity against cancer cell lines: FaDu, SCC-25, MCF-7 and MDA-MB-231. The highest cytotoxic activity against FaDu and MDA-MB-231 cells was observed for CM root extract, while the highest cytotoxic activity against SCC-25 and MCF-7 cells was estimated for *Thalictrumfoetidum* root extract. There were significant differences in the cytotoxic activity of extracts obtained from the roots and herbs of CM and *Thalictrumfoetidum* [24].

The substantial cytotoxicity and proapoptotic activity of sanguinarine, berberine, and CM extract indicate the utility of these compounds in anticancer therapy [22]. Och and co-workers (2019) evaluated the cytotoxicity and proapoptotic activity of sanguinarine, berberine, and extracts of CM and *Berberisthunbergii* DC. IC_{10}, IC_{50} and IC_{90} doses were determined against haematopoietic cancer cell lines using trypan blue staining. Changes in the expression of 18 apoptosis-related genes in cells exposed to IC_{10}, IC_{50} and IC_{90} were evaluated by real-time PCR. Sanguinarine and CM extract exhibited significant cytotoxicity against all cell lines tested. A lower cytotoxic activity was observed for berberine. *Berberisthunbergii* extract had no effect on cell viability. Berberine, sanguinarine and CM extract altered the expression of apoptosis-related genes in all cell lines tested, indicating induction of apoptosis [22].

Secondary metabolites present in the crude extract and latex of CM (alkaloids such as berberine, coptisine, chelidonine, chelerythrine, sanguinarine and protopine) have been shown to be biologically active compounds with a wide range of pharmacological functions. Berberine, an isoquinoline alkaloid extracted from plants, has a wide range of biological activities, including inhibiting the growth of a variety of cancer cell lines [33].In addition, the components of the natural plant protoberberine fraction (BBR-F) extracted from CM may represent promising novel photosensitive agents and can be used as natural photosensitizers in cancer photodynamic therapy. Warowicka and co-workers (2019) investigated the potential anticancer effect of a protoberberine alkaloid fraction (BBR-F) isolated from CM on HeLa and C33A cervical cancer cells after light irradiation (PDT treatment). An effective reduction in HeLa and C33A cell viability was observed after PDT treatment of BBR-F treated cells. Furthermore, microscopic analysis revealed several morphological changes in the cells that occurred during apoptosis. Apoptosis of HeLa and C33A cells was also characterised by biochemical changes in cell membrane composition, activation of intracellular caspases, disruption of mitochondrial membrane potential ($\Delta\psi m$) and generation of reactive oxygen species (ROS) [33].

Chelidonine was able to suppress the LPS-induced inflammatory response both *in vitro* and *in vivo*, which was related to the TLR4/NF-κBsignalling pathway, which was perturbed by chelidonine. Liao and co-workers (2018) used RAW264.7 macrophages and mice to investigate the anti-inflammatory effects of chelidonine. They found that chelidonine significantly suppressed LPS-induced NO and PGE2 production, as well as iNOS and COX-2 mRNA and protein expression. In addition, LPS-induced pro-inflammatory cytokines such as TNFα and IL-6 were also

attenuated by chelidonine. What's more, LPS-induced activation and degradation of IκBα followed by translocation of p65 from the cytoplasm to the nucleus were attenuated by chelidonine. Furthermore, chelidonine even significantly inhibited LPS-induced TLR4 expression. These researchers demonstrated that chelidonine significantly reduced serum levels of TNFα, IL-6 and PGE2 in LPS-stimulated mice [15].

Studies by others have shown that CM cell cultures are rich in polyphenolic compounds and isoquinoline alkaloids, which have been shown to have antimicrobial, antioxidant and anti-inflammatory properties. Chelerythrine, a natural benzo-phenanthridine alkaloid of CM, inhibited inflammatory and pain responses in several *in vivo* and cell models used by Lanfeld and co-workers (1981). *In vivo*, i.p. administration of the alkaloid (1-5 mg/kg) alleviated oedema of the mouse ear, paw oedema of the rat and abdominal contraction (pain response). The isolated peritoneal macrophages also showed a dose-dependent reduction in the expression of prostaglandin E_2 and cyclooxygenase-2 after treatment with 0.0001-1 μg/mLchelerythrine. The alkaloid fraction and sanguinarine were effective against carrageenan-induced rat paw oedema, but chelerythrine showed less activity [14]. However, the later study by Mikołajczak and co-workers (2015) showed that different water extract fractions at relatively high doses of 200 mg/kg body weight failed to alleviate inflammation in a similar model. The crude water extract treatment actually exacerbated paw inflammation. Conversely, the extracts containing mainly coptisin and chelidonine were effective in the hot plate test for antinociceptive properties, suggesting a supramedullary mode of action [19].

Chelidonine has central nervous system effects similar to those of morphine, but weaker, and spasmolytic effects on smooth muscle similar to those of papaverine, but also weaker. It is also a spindle inhibitor. Chelerythrine is a strong skin and mucous membrane irritant, a central nervous system depressant and a local anaesthetic [6, 23]. Sanguinarine is a vasorelaxant and an inhibitor of smooth muscle contraction. It is also an inhibitor of acetylcholinesterase and 5-lipoxygenase and has antimicrobial activity [7, 18]. Coptisin was found to have neuroprotective, cytotoxic, monoamine oxidase inhibitory and cardioprotective bioactivities [32].

In a study by Jang and co-workers (2021), chelidonine was found to inhibit the proliferation of BxPC-3 and MIA PaCa-2 human pancreatic cancer cells in a dose- and time-dependent manner, confirming its apoptotic potential. In addition, flow cytometry analysis revealed that over 50% of BxPC-3 and MIA PaCa-2 cells exhibited early and late phase apoptosis after exposure to chelidonine (1 μM) for 24 h. These changes in expression levels following chelidonine treatment were further confirmed by analysis of transcription factor activity in both pancreatic cancer cell lines [8].

CONCLUSIONS

The present study was conducted to evaluate the biomarkers of oxidative stress in blood samples collected from healthy volunteers after *in vitro* incubation with

extracts derived from stems and roots of greater celandine were used. The aim of this study was to evaluate the dose-dependent changes in biomarkers of oxidative stress in blood samples from healthy volunteers exposed *in vitro* to extracts of CM roots and stems collected from natural habitats in the area of the South Park in Słupsk, in the Pomeranian province (northern part of Poland).The results of our study showed a statistically significant decrease in TBARS levels in blood samples for root extracts at the final dose of CM extracts at both 5 and 2.5 mg/mL. We observed similar trends after *in vitro* incubation of blood samples with stem extracts of CM (at a final dose of 2.5 mg/mL), where there was a statistically significant reduction in the concentration of TBARS compared to the untreated control samples. We also observed a statistically non-significant increase in TAC levels after *in vitro* incubation of blood samples with stem extracts of CM (at a final dose of 5 and 2.5 mg/mL) compared to control samples, while there was a statistically non-significant reduction in TAC levels after *in vitro* incubation of blood samples with root extracts of CM (at a final dose of 2.5 mg/mL) compared to untreated control samples. This study provides new insights into understanding the antioxidant properties of CM extracts.

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