Original article

Antihaemolytic activity of thirty herbal extracts in mouse red blood cells

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Reactive oxygen species (ROS) can lead to haemolysis and eventually to diseases such as thalassemia and sickle cell anaemia. Their action can be counteracted by the antihaemolytic activity of therapeutic agents. The aim of our study was to identify plants that most efficiently counteract ROS-caused haemolysis. From ten plants known for their antioxidant activity (*Orobanche orientalis* G. Beck, *Cucumis melo* L., *Albizzia julibrissin* Durazz, *Galium verum* L., *Scutellaria tournefortii* Benth, *Crocus caspius* Fischer & Meyer, *Sambucus ebulus* L., *Danae racemosa* L., *Rubus fruticsos* L., and *Artemisia absinthium* L.) we prepared 30 extracts using three extraction methods (percolation, Soxhlet, and ultrasound-assisted extraction) to see whether the extraction method affects antihaemolytic efficiency, and one extraction method (polyphenol extraction) to see how much of this action is phenol-related. Extract antihaemolytic activity was determined in mice red blood cells and compared to that of vitamin C as a known antioxidant. Nine of our extracts were more potent than vitamin C, of which *G. verum* (aerial parts/percolation) and *S. tournefortii* (aerial parts/polyphenol) extracts were the most potent, with an IC_{50} of 1.32 and 2.08 µg mL^{-1}, respectively. Haemolysis inhibition depended on extract concentration and the method of extraction. These plants could provide accessible sources of natural antioxidants to the pharmaceutical industry.

KEY WORDS: Galium verum*; hydrogen peroxide; percolation; phenols;* Scutellaria tournefortii*; Soxhlet; ultrasound-assisted extraction*

Oxidative damage and haemolysis caused by reactive oxygen species (ROS) have a major role in the development of diseases such as thalassemia, glucose-6-phosphate dehydrogenase deficiency, and sickle cell anaemia. Red blood cells (RBCs) are the primary targets of free radicals, owing to their high membrane concentrations of polyunsaturated fatty acids (linoleic and arachidonic acids in particular) and O_2 transport associated with redox active haemoglobin molecules, which are potent promoters of ROS. Oxidation depletes membrane protein content, deforms RBCs, and disturbs microcirculation (1-4). It is also implicated in haemolysis (5).

Haemolysis has long been used to measure free radical damage and counteraction by antioxidants. It is useful for screening for oxidising or antioxidising agents (6). Several herbal secondary metabolites such as flavonoids have been found to protect cells from oxidative damage. These compounds have been evidenced to stabilise RBC membrane by scavenging free radicals and reducing lipid peroxidation (3, 7).

Herbs are a rich source of flavonoids, phenolic acids, and alkaloids, some of which act as antioxidants (7-10). Nabavi et al. (9) studied the antioxidant and antihaemolytic activities of *Ferula foetida* in RBCs and a few other interesting studies have recently been reported, indicating the protective effects of plant

extracts against oxidative damage in intact RBC membranes (7, 9, 10).

The aim of this study was to investigate the antihaemolytic activity of herbal extracts from ten plants and identify promising alternatives to treating diseases associated with haemolysis. We also wanted to see which of the three extraction methods used - ultrasound-assisted extraction (UAE), Soxhlet extraction (SO), and percolation (PE) yielded more efficient extracts. Our third aim was to verify the protective effects (as claimed in literature, cf. 11, 12) of polyphenols extracted from two plants against oxidative stress. With these aims, we prepared 30 herbal extracts and evaluated their capacity to suppress RBC haemolysis. In addition, we also measured the haemolytic activity of the most potent extracts in the absence of H_2O_2 in order to determine their own oxidative toxicity to RBCs.

MATERIALS AND METHODS

Chemicals

Formic acid, 30 % hydrogen peroxide, and vitamin C, which was used as reference compound, were purchased from Merck (Darmstadt, Germany). All solvents were of analytical grade or purer. Distilled deionised water was prepared with the Ultrapure™ water purification system. Vitamin C was used in the same concentrations as the plant extracts.

Plants

Ten plant species were collected and their identity verified by Dr Bahman Eslami (Islamic Azad University of Qaemshahr, Iran). The voucher specimens were deposited in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Mazandaran University of Medical Sciences. Table 1 lists the plants, the parts, and the methods used for extraction. Plant materials were dried under dark conditions at room temperature for 2-3 weeks. The dry materials were milled, obtaining 2-3 mm particles.

Extraction methods

Extraction is the first important step in the recovery and purification of active ingredients from plants. The aim is to provide maximum yield and the highest quality of target compounds in the shortest time and at the lowest cost possible. Many techniques, such as conventional solvent extraction, microwave-assisted, and ultrasound-assisted extraction, have been developed to extract active ingredients (13). In this study, we opted for the three most common extraction methods: percolation, Soxhlet extraction, and ultrasound-assisted extraction.

For percolation, we used methanol to treat known amounts of each plant part at room temperature as described elsewhere (14, 15). The resulting extracts were concentrated over a rotary vacuum (Heidolph, Schwabach, Germany) at 35-40 °C until a crude solid extract was obtained, which was then freeze-dried (MPS-55 freeze-drier, Operon Co., Ltd., Gimpo, South Korea) for complete solvent removal.

In Soxhlet extraction, powdered samples were treated with methanol and extracted in an ISOLAB extractor (Wertheim, Germany) for 24 h. The extracts were then concentrated in a rotary evaporator (Heidolph, Germany) until the solvent was removed. The extracts were freeze-dried for complete solvent removal (14, 15).

In ultrasound-assisted extraction samples were treated with methanol and placed in an ultrasonic cleaning bath (Tecna3, Bologna, Italy) at a frequency of 100 kHz and temperature of 25±3 °C for 1 h to yield extracts, which were then separated from the residue by filtration and concentrated in a rotary evaporator until crude solid extracts were obtained. Followed freeze-drying for complete solvent removal (16).

Polyphenol extraction

Literature suggests that polyphenols act as antioxidants and protect RBCs against oxidative damage (11, 12, 17). These compounds were extracted from samples according to our recently published paper (16). The extraction was performed twice at 20 °C in a shaking incubator (115 W, Promax 1020, Heidolph, Germany). The extraction time was 30 min and the extracting solvent was 100 mL of methanol/acetone/water (3.5/3.5/3) containing 1 % formic acid. Extracts were combined and filtered through two layers of cheesecloth. The collected filtrate was centrifuged at 7000 g for 15 min. The supernatant was collected and evaporated under vacuum at 35-40 °C to remove methanol and acetone. Lipophilic pigments were then eliminated from the aqueous phase by two successive extractions in a separatory funnel with a twofold volume of petroleum ether. The aqueous phase was collected and further extracted by the equal volume of ethyl acetate three times in the separatory funnel. Three ethyl acetate phases were collected and

Table 1 *Plant names, extraction methods, part used, and Herbarium numbers*

Plant name	Extraction method	Part used	Herbarium number
Orobanche orientalis (broomrape)	Ultrasonic/Soxhlet	Aerial parts	MH1001
Cucumis melo (muskmelon)	Percolation/Soxhlet/Ultrasonic	Leaf/Fruit	MH1002
Albizia julibrissin (Persian silk tree)	Ultrasonic/Soxhlet/ Percolation	Leaf/Flower	MH1003
Galium verum (lady's bedstraw)	Percolation	Aerial parts	MH1004
Scutellaria tournefortii (skullcap)	Percolation/Soxhlet/Polyphenol	Aerial parts	MH1010
Crocus caspius (crocus)	Percolation/Polyphenol/Ultrasonic	Aerial parts/Bulb	MH1008
Sambucus ebulus (danewort)	Soxhlet/Percolation/Ultrasonic	Flower	MH1009
Danae racemosa (Alexandrian or poet's laurel)	Soxhlet	Aerial parts	MH1011
Rubus fruticsos (blackberry)	Percolation	Leaf	MH1015
Artemisia absinthium (wormwood)	Soxhlet/Percolation/ Ultrasonic	Aerial parts	MH1020

concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal.

Preparation of mice RBCs

A total of 30 male Swiss albino mice (20-25 g, Institute Pasteur of Iran) were used in this study. The animals were housed in standard cages with free access to food (standard laboratory rodent chow) and water. The animal house temperature was maintained at 23±3 °C with a 12-h light/12-h dark cycle (light on from 06:00 to 18:00 h). All of the experiments were conducted between 10:00 and 14:00 h. The experimental procedure was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (18).

The mice were killed under anaesthesia and their blood collected by heart puncture in heparinised tubes. RBCs were isolated and stored according to the method described by Ebrahimzadeh et al. (7). Briefly, blood samples were centrifuged (1500 *g*, 10 min), and RBCs separated from plasma and buffy coat and washed three times by centrifugation (1500 *g*, 5 min) in 10 volumes of 10 mmol L^{-1} phosphate buffered saline (pH 7.4; PBS). The supernatant and buffy coats of white cells were carefully removed with each wash. Washed RBCs were stored at 4 °C and used within 6 h (19).

Antihaemolytic activity assay

This assay is useful for screening for agents and their metabolites that have an oxidising or antioxidising activity (11). Peroxides such as H_2O_2 and cumene hydroperoxide (7), protein denaturation, and heat shock-induced damage have all been extensively studied in biological membranes (7, 20, 21).

The antihaemolytic activity of the extracts in our study was determined as described by Ebrahimzadeh et al. (7). Mice RBCs were diluted in PBS buffer to obtain a 4 % suspension. The plant extracts were prepared in PBS buffer at five concentrations: 0.25, 0.5, 1, 2, and 4 mg mL^{-1} (see our recently published paper under ref 22). To 2 mL of RBC suspension we added l mL of plant extract (in the above concentrations) and enough PBS to reach the final volume of 5 mL. After 5 min of incubation at room temperature, 0.5 mL of 0.3 % H_2O_2 was added to induce oxidative degradation of membrane lipids and the mixture was shaken at 37 °C for 240 min. The samples were then centrifuged at 1500 *g* for 10 min and the resulting supernatant was removed and used to evaluate their haemolytic activity using a spectrophotometer (UV–Visible EZ201, Perkin Elmer, Norwalk, CA, USA) at the absorbance wavelength of 540 nm. RBC lysis in the presence of H_2O_2 and absence of a plant extract was considered as 100 % haemolytic activity. Haemolysis in the presence extracts was calculated relative to this control haemolysis (22). Haemolysis inhibition was calculated as follows:

$$\% \text{ antihaemolysis} = [(A_o - A_1)/A_o] \times 100$$

where A_o was the absorbance of control (H_2O_2+RBC, without extract) and A_1 the absorbance in the presence

of the extract or vitamin C as the reference antioxidant (7, 9, 10) used in the same concentrations as the extracts (0.25-4 mg mL^{-1}). Each set of experiments was performed in triplicate and the inhibitory activity expressed as percentage. Based on the inhibition, the plant extracts were classified into four groups: (a) neutral, with haemolysis similar to control, about 100 %; (b) weak, with haemolysis >80 % of the control; (c) potent, whose haemolysis was <80 %; and (d) reverse, whose haemolytic activity was even higher than control (>100 %).

For potent extracts we calculated the half maximal inhibitory concentration (IC$_{50}$), which is the antioxidant concentration required to inhibit 50 % of haemolysis caused by H$_2$O$_2$. We also measured the haemolytic activity of potent extracts in the absence of H$_2$O$_2$ in order to see if and how much oxidative damage they alone caused to RBCs. For this experiment, we only used the highest concentration, 4 mg mL^{-1}.

Total phenolic and flavonoid content

Total phenolic content was determined using the Folin-Ciocalteu method (16). We first mixed sample extract (0.5 mL) with the Folin-Ciocalteu reagent (5 mL, diluted with distilled water at the 1:10 ratio) for 5 min and then added aqueous Na$_2$CO$_3$ (4 mL, 1 mol L^{-1}). After 2 h of incubation at room temperature, we measured the absorbance of reaction with a double-beam spectrophotometer (UV- Visible EZ201, Perkin Elmer, Norwalk, CA, USA) at 760 nm. The standard curve was prepared with 0, 50, 100, 150, 200, and 250 µg mL^{-1} solutions of gallic acid in methanol and water (50:50, v/v). Total phenol content is expressed as milligram of gallic acid equivalents (GAE) per one gram of extract.

Total flavonoid content was measured using the colourimetric aluminium chloride method (16). Briefly, 0.5 mL solutions of the extract in methanol were mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 mol L^{-1} potassium acetate, and 2.8 mL of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm. Total flavonoid content was calculated from a calibration curve which was prepared by quercetin solutions with methanol at concentrations ranging from 12.5 to 100 µg mL^{-1} and is expressed as milligram of quercetin equivalent (QE) per one gram of extract.
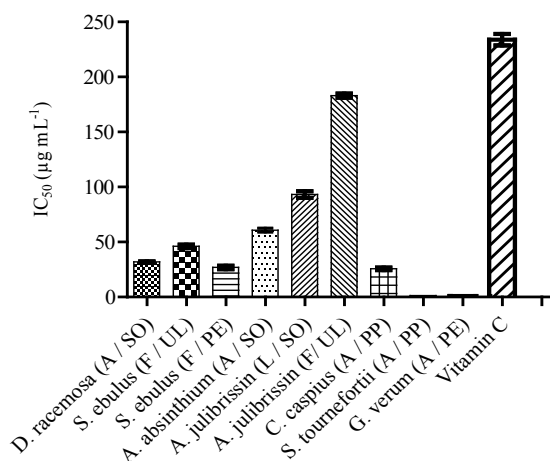

Figure 1 *IC$_{50}$ of the most potent extracts with antihaemolytic activity (less is better). Vitamin C served as reference compound.*
A: aerial part; F: fruit; L; leaf; SO: Soxhlet extraction; PE: percolation; UAE: ultrasound-assisted extraction; PP: polyphenol fraction

Statistical analysis

The results of the antihaemolysis assays are presented as means of three replicate measurements ± standard deviation (SD). The data were analysed using the analysis of variance (ANOVA) (p<0.05) and grouped using Duncan's multiple range test. The IC$_{50}$ values were calculated using linear regression analysis.

RESULTS AND DISCUSSION

Table 2 shows that the antihaemolytic activity of our extracts and extracted polyphenols varied a lot. The group with reverse effects (higher haemolysis than control) included *C. melo* (leaf/percolation, ultrasonic, and Soxhlet; fruit/Soxhlet and ultrasonic), *A. julibrissin* (leaf/ultrasonic; flower/Soxhlet), *S. tournefortii* (aerial parts/Soxhlet), *O. orientalis* (aerial parts/ultrasonic and Soxhlet), *C. caspius* (aerial parts/ percolation and ultrasonic; bulb/polyphenol), *A. julibrissin* (leaf/percolation; flower/percolation).

S. tournefortii (aerial parts/percolation) was the only neutral sample, showing no inhibitory effects on H$_2$O$_2$. Five samples, including *S. ebulus* (flower/ Soxhlet), *R. fruticsos* (leaf/percolation), *A. julibrissin* (flower/ultrasonic), *C. melo* (fruit/percolation), and *A. absinthium* (aerial parts/percolation) showed weak haemolysis inhibition.

Table 2 *The haemolytic activity of plants at different concentrations.*

Plant names (part used)	Method	Concentration (mg mL^{-1})				
		4	2	1	0.5	0.25
S. tournefortii (aerial parts)	Percolation	100.0±0.81	100.0±0.94	100.0±0.95	100.0±0.17	100.0±0.26
S. tournefortii (aerial parts)	Polyphenol	35.8±0.76	46.5±0.37	48.5±0.85	49.3±0.93	97.6±0.69
S. tournefortii (aerial parts)	Soxhlet	114.2±0.62	112.3±0.75	111.2±0.81	107.4±90	97.2±0.63
S. ebulus (flower)	Soxhlet	85.3±0.93	93.8±0.68	92.5±0.82	93.1±0.57	94.2±0.58
R. fruticsos (leaf)	Percolation	87.5±0.72	97±0.68	98.1±0.59	99.3±0.39	100.2±0.75
C. melo (fruit)	Percolation	96.4±0.94	98.2±0.83	99.2±0.07	99.5±0.09	99.6±0.59
C. melo (fruit)	Soxhlet	108.0±0.51	107.8±1.01	104.0±0.68	100.1±0.71	97.2±0.91
C. melo (fruit)	Ultrasonic	121.4±0.55	120.5±1.12	119.3±0.95	107.5±0.22	87.5±0.61
C. melo (leaf)	Percolation	121.1±0.80	119.3±0.29	114.1±0.45	112.4±0.98	111.9±0.37
C. melo (leaf)	Soxhlet	119.9±0.12	117.4±0.65	115.8±0.26	111.6±0.71	101.4±0.31
C. melo (leaf)	Ultrasonic	106.9±0.47	102.7±1.01	97.4±0.04	91.5±0.57	88.8±0.06
A. absinthium (aerial parts)	Percolation	87.7±0.47	83.2±0.48	88.1±0.19	91.8±0.69	91.2±0.19
D. racemosa (aerial parts)	Soxhlet	65.5±0.39	80±0.58	80.5±0.96	83.3±0.83	94.4±1.00
S. ebulus (flower)	Ultrasonic	79.3±0.41	84.1±0.29	86.6±0.59	91.6±0.18	98.4±0.09
S. ebulus (flower)	Percolation	72.3±0.16	78.5±0.68	86.6±0.71	98.6±0.56	99.8±0.26
A. absinthium (aerial parts)	Ultrasonic	75.9±1.08	88.6±0.91	90±0.99	90.7±0.08	95.2±0.83
A. absinthium (aerial parts)	Soxhlet	74.9±0.13	85±0.76	91.4±034	95.1±0.98	98.9±0.80
A. julibrissin (leaf)	Soxhlet	62.2±0.38	83.1±0.85	86.1±0.82	92.9±0.94	100.1±0.09
A. julibrissin (flower)	Ultrasonic	82.1±0.09	89.2±0.17	92.6±0.57	94.4±0.67	98.8±0.61
A. julibrissin (flower)	Soxhlet	129.7±0.43	127.4±0.09	121.0±0.81	120.1±0.18	110.6±0.72
A. julibrissin (leaf)	Ultrasonic	123.8±0.91	118.8±0.85	116.8±0.47	103.2±0.70	95.4±0.58
A. julibrissin (leaf)	Percolation	126.2±0.63	124.4±0.45	117.0±0.94	98.7±0.51	90.6±0.09
A. julibrissin (flower)	Percolation	92.3±0.33	90.1±0.83	88.0±0.66	86.8±0.41	83.4±0.91
C. caspius (aerial parts)	Polyphenol	67.7±0.41	75.5±0.96	85.5±1.40	88.8±0.74	96.1±0.90
C. caspius (bulb)	Polyphenol	97.7±0.59	89.1±0.19	75.9±0.07	75.3±0.94	67.9±0.79
C. caspius (aerial parts)	Ultrasonic	114.0±0.41	113.9±0.73	110.8±0.84	109.4±0.69	104.8±0.05
C. caspius (aerial parts)	Percolation	130.6±0.38	112.1±0.69	109.4±0.19	104.9±0.81	100.3±0.77
G. verum (aerial parts)	Percolation	34.5±0.27	45.5±0.06	48.8±0.54	52.0±0.83	65.4±0.91
O. orientalis (aerial parts)	Soxhlet	94.0±0.41	91.4±0.49	91.4±0.83	85.2±0.91	80.4±0.29
O. orientalis (aerial parts)	Ultrasonic	128.2±1.30	117.2±1.01	112.4±0.91	110.9±0.66	86.5±0.82
Vitamin C		83.3±0.11	90.6±0.10	98.7±0.26	99.3±0.38	99.8±0.51

The relative haemolysis of control was 100 %. Data are presented as %. Each experiment was performed in triplicate. Lower values indicate higher antihaemolytic activity

The remaining nine samples, namely *D. racemosa* (aerial parts/Soxhlet), *S. ebulus* (flower/ultrasonic and percolation), *A. absinthium* (aerial parts/ultrasonic and Soxhlet), *A. julibrissin* (leaf/Soxhlet), *C. caspius* (aerial parts/polyphenol), *S. tournefortii* (aerial parts/ polyphenol), and *G. verum* (aerial parts/percolation) showed strong antihaemolytic activity. These nine were also more potent than the reference vitamin C (IC_{50}=235±5.26 μg mL^{-1}). Their IC_{50} is shown in Figure 1. The most potent were *G. verum* (aerial parts/ percolation) and *S. tournefortii* (aerial parts/ polyphenol), whose respective IC_{50} of 1.32 and 2.08 μg mL^{-1} was significantly lower than that of vitamin C (p<0.001). Their antihaemolytic activity grew with concentration.

The haemolytic activity of the nine potent compounds in the absence of H_2O_2 was below 7 % (data not shown), which suggests that they are not toxic. Unlike *A. julibrissin* and *C. melo*, whose activity in most cases intensified H_2O_2-induced haemolysis.

C. caspius showed contradictory findings, as its polyphenol fraction extracted from the aerial parts strongly inhibited H_2O_2-induced haemolysis, but its extracts obtained by percolation and ultrasound intensified it.

Although the Soxhlet extract of *S. ebulus* showed weak antihaemolytic activity, extracts obtained with other methods were potent against haemolysis. Similarly, percolation rendered *A. absinthium* weak while ultrasound and Soxhlet yielded potent antihaemolytic extracts. Clearly, extraction methods deserve a more comprehensive research in this respect.

Various studies have recently investigated the potential of phenols against oxidative damage in RBCs, suggesting a possible interaction between flavonoids and RBC membrane lipids and proteins that are generally targeted by lipid peroxidation (12, 17, 23). Divya et al. reported (24) considerable antihaemolytic activity of the methanolic extracts of *Bombax ceiba pentandra* fruit and spike (14.57 and 19.14 % haemolysis *vs*. 100 % for negative control, respectively) owing perhaps to their high phenol and flavonoid content and good antioxidant activity. Deepinderjeet et al. (25) also related the antihaemolytic effects of *Calendula officinalis* (IC_{50}=120.5±0.4), *Juglans regia* (IC_{50}=148.8±1.4), and *Ficus bengalensis* (IC_{50}=214.1±1.5 μg mL^{-1}) to high flavonoid content and good antioxidant activity. A report by Yang et al. (26) showed high haemolysis inhibition by fermented soybean meal extract at concentrations of 2-10 mg mL^{-1} (IC_{50} value 4.599 mg mL^{-1}). The extract also had potent *in vivo* antioxidant activity. Our phenol and flavonoid findings are shown in Table 3. Antihaemolytic activity strongly correlated with total phenolic content (r^2=0.693) but not with flavonoid content (r^2=0.029). Our findings are in agreement with studies showing that polyphenols protect RBCs from oxidative stress or increase their resistance to oxidative damage (12, 27). They also confirm the correlation between total phenolic content and antioxidant activity from studies on different foodstuffs such as fruit and vegetables (28-30).

To conclude, the nine extracts that showed high antihaemolytic activity in our study, *G. verum* (percolation) and *S. tournefortii* (polyphenol fraction) could serve as easily accessible sources of natural antioxidants for the pharmaceutical industry.

Table 3 *Phenol and flavonoid content of the nine most potent extracts*

Plant name	Extraction method	Part used	Phenol content[a] / mg g^{-1} of extract	Flavonoid content[b] / mg g^{-1} of extract
A. julibrissin	Ultrasonic	Flower	340	136
A. julibrissin	Soxhlet	Leaf	688	121
G. verum	Percolation	Aerial parts	329	56
S. tournefortii	Polyphenol	Aerial parts	735	151
C. caspius	Polyphenol	Aerial parts	191	82
S. ebulus	Percolation	Flower	56	14.5
D. racemosa	Soxhlet	Aerial parts	256	131
S. ebulus	Ultrasonic	Flower	81	52
A. absinthium	Soxhlet	Aerial parts	267	136

[a] gallic acid equivalents of extract; [b] quercetin equivalent; mg g^{-1} of extract

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Sažetak

Antihemolitička aktivnost trideset biljnih ekstrakata u mišjim eritrocitima

Reaktivni kisikovi spojevi (ROS) mogu dovesti do hemolize te u konačnici do bolesti poput talasemije i anemije srpastih stanica. Takvo se djelovanje može ublažiti ili spriječiti terapijskim djelovanjem antihemolitika. Cilj je ovog istraživanja bio izdvojiti biljke koje najuspješnije sprječavaju hemolizu uzrokovanu reaktivnim kisikovim spojevima. U tu smo svrhu pripremili 30 ekstrakata biljaka poznatih po svojem antioksidacijskom djelovanju: *Orobanche orientalis* G. Beck, *Cucumis melo* L., *Albizzia julibrissin* Durazz, *Galium verum* L., *Scutellaria tournefortii* Benth, *Crocus caspius* Fischer & Meyer, *Sambucus ebulus* L, *Danae racemosa* L., *Rubus fruticsos* L. te *Artemisia absinthium* L. Rabili smo tri uobičajene ekstrakcijske metode (perkolacija, Soxhlet i ultrazvučna ekstrakcija) kako bismo utvrdili utječe li metoda na anihemolitičku aktivnost ekstrakta te smo u nekoliko uzoraka ekstrahirali polifenole kako bi se vidjelo koliko je ta aktivnost povezana s njihovom razinom. Antihemolitičku smo aktivnost mjerili u mišjim eritrocitima i usporedili je s onom vitamina C, koji je poznati antioksidans. Izdvojeno je devet ekstrakata sa snažnijom aktivnosti od vitamina C, od kojih su ekstrakti *G. verum* (zračni dijelovi/perkolacija) odnosno *S. tournefortii* (nadzemni dijelovi/polifenoli) bili najsnažniji, s inhibicijskom koncentracijom (IC_{50}) od 1,32 odnosno 2.08 µg mL^{-1}. Inhibicija hemolize ovisila je o koncentraciji ekstrakta te o metodi ekstrakcije. Ove bi biljke mogle poslužiti farmaceutskoj industriji kao lako dostupni izvori prirodnih antioksidansa.

KLJUČNE RIJEČI: *fenoli*; Galium verum; *perkolacija*; Scutellaria tournefortii; *Soxhlet*; *ultrazvučna ekstrakcija*; *vodikov peroksid*

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