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ANTIOXIDANT ACTIVITIES OF PETROLEUM ETHER, ETHYL ACETATE, N-BUTANOL AND AQUEOUS EXTRACTS FROM *SPERANSKIA TUBERCULATA (BUNGE) BAILL* HERB

The article examines the antioxidant properties of various extracts of the herb Speranskia Tuberculata (Bunge) Baill. Crude extracts were obtained using petroleum ether, ethyl acetate, n-butanol and water. Subsequently, the dried extracts were dissolved in dimethylsulfoxide to prepare working solutions with 10 to 100 μg/mL concentrations. ***The work aims*** *to study the antioxidant effect of different concentrations of Speranskia Tuberculata (Bunge) Baill herb extracts obtained by dissolving in 4 solvents.* ***Methods****. The antioxidant activity of the extracts was evaluated, and total concentrations of phenolic compounds and flavonoids were determined.* ***Results****. N-butanol extracts demonstrated the lowest antioxidant activity, while the highest activity was characteristic of ethyl acetate and petroleum ether extracts. Using experimental data, IC_{50} was calculated for all solvents and both measurement methods. For the DPPH radical scavenging rate method, the IC_{50} ranged from 74 (n-butanol) to 35 μg/mL (petroleum ether). For the superoxide radical anion absorption rate method, the IC_{50} ranged from 41 (n-butanol) to 20 μg/mL (ethyl acetate). The determined concentrations of phenolic compounds varied from 280 (ethyl acetate) to 7 (petroleum ether) mg/GE/g. For flavonoids, the range was 167 (ethyl acetate) – 7 (petroleum ether) mg/RE/g. The obtained results made it possible to classify the studied extracts with high antioxidant activity, except for the n-butanol solution with an average activity level according to the DPPH radical absorption rate method results.* ***Conclusions****. Ethyl acetate extracts showed the highest total content of phenolic compounds and flavonoids, and ethyl acetate and petroleum ether extracts showed the highest antioxidant activity.*

Key words: *extracts of the herb Speranskia Tuberculata, methods of measuring the antioxidant activity of extracts, the total content of phenolic compounds and flavonoids.*

Цзеюань Сунь, Тетяна Деркач. АНТИОКСИДАНТНА АКТИВНІСТЬ ЕКСТРАКТІВ ПЕТРОЛЕЙНОГО ЕФІРУ, ЕТИЛАЦЕТАТУ, Н-БУТАНОЛУ ТА ВОДИ *SPERANSKIA TUBERCULATA (BUNGE) BAILL*

У статті досліджено антиоксидантні властивості різних екстрактів трави Speranskia Tuberculata (Bunge) Baill. Сирі екстракти отримані за допомогою петролейного ефіру, етил ацетату, н-бутанолу та води. У подальшому висушені екстракти були розчинені в диметилсульфоксиді для приготування робочих розчинів з концентраціями від 10 до 100 мкг/мл. ***Мета роботи*** *– дослідження антиоксидантної дії різних концентрацій екстрактів трави Speranskia Tuberculata (Bunge) Baill, отриманих розчиненням у чотирьох розчинниках.* ***Методи****. Оцінено антиоксидантну активність розчинів, визначені загальні концентрації фенольних сполук та флавоноїдів.* ***Результати.*** *Найменшу антиоксидантну активність продемонстрували екстракти, отримані за допомогою н-бутанолу, тоді як найвища активність була притаманна екстрактам з етил ацетату. Розраховано IC_{50} для всіх розчинників та обох методів вимірювання. Для методу швидкості поглинання радикалів DPPH, IC_{50} варіювалася в межах від 74 (н-бутанол) до 35 мкг/мл (петролейний ефір). Для методу швидкості поглинання аніон-радикалів супероксиду від 41 (н-бутанол) до 20 мкг/мл (етил ацетат). Визначені загальні концентрації фенольних сполук варіювалися від 280 (етил ацетат) до 7 (петролейний ефір) мг/GE/г. Для флавоноїдів інтервал склав 167 (етил ацетат) – 7 (петролейний ефір) мг/RE/г. Отримані результати дозволяють віднести досліджені екстракти до тих, що мають високу антиоксидантну активність за виключенням розчину н-бутанолу із середнім рівнем активності за результатами методу швидкості поглинання радикалів DPPH.* ***Висновки****. Найвищий вміст фенольних сполук та флавоноїдів показали екстракти, отримані за допомогою етил ацетату, а найвищу антиоксидантну активність –отримані за допомогою етил ацетату та петролейного ефіру.*

Ключові слова*: екстракти трави Speranskia Tuberculata, методи вимірювання антиоксидантної активності екстрактів, загальний вміст фенольних сполук та флавоноїдів.*

Introduction. The chemistry of free radicals has become very popular in research in recent decades, as it studies reactive forms of oxygen and nitrogen generated by our body under the influence of various external conditions or pathological conditions. Free radicals negatively alter lipids, proteins and DNA and cause many human diseases. At the same time, interest in antioxidants is growing, primarily because of their effect on free radicals [7]. Antioxidants clean the body's cells of free radicals and prevent or reduce damage caused by oxidation.

A diet high in antioxidants can reduce the risk of many diseases (including heart disease and some cancers). For such applications, antioxidants of natural origin are preferred over synthetic ones, mainly because some known synthetic antioxidants, such as

butylated hydroxytoluene, butylated hydroxyanisole and others, pose a health hazard [7]. Some plants have great potential for use as functional ingredients or as additives in the food industry. Research on determining the antioxidant activity of natural food components continues rapidly, simultaneously emphasising the problem of finding new sources of plant raw materials.

The multi-thousand-year history of the development of traditional medicine, primarily in the countries of the East, remains partially unperceived and unknown to scientific pharmacy. The main reason is the lack of information about medicinal plants that can be obtained using modern analytical methods. The unknown combination of active pharmaceutical ingredients (APIs), the unexplored variation of the composition depending on the origin and other factors, and the unexplored reaction to the application of modern extraction and isolation methods restrain the spread of traditional treatment practices beyond the boundaries of traditional medicine.

A typical example of such a situation is the Chinese endemic herb *Speranskaya Tuberculata Bunge Bail.* On the one hand, this plant has been widely used in traditional Chinese medicine for centuries. On the other hand, the amount of information and publications devoted to this plant is minimal [10, 11], and the plant itself is still not included in the pharmacopoeias of the world's countries.

The work aimed to study the antioxidant effects of different concentrations of *Speranskia Tuberculata (Bunge) Baill* herb extracts produced by dissolution with four kinds of different solvents at the stage of crude extract production.

Materials and Methods. *Speranskia tuberculata (Bunge) Bail* herbs were purchased from Tongrentang Pharmacy Ltd. (Beijing, China). The cleaned of foreign impurities and dried herbs were powdered. The powder was sequentially extracted using a Soxhlet apparatus with petroleum ether (PE extract), ethyl acetate (EA), n-butanol (n-B) and deionised water (aqueous solution or AS extracts). The powder-solvent ratio was 1:10 w/v. Each extract was concentrated under reduced pressure, vacuum dried (-20°C), and then dissolved to necessary concentrations in dimethyl sulfoxide (DMSO). DMSO with 0.1% concentration was a blank control.

Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) is generally recognised as a rapid way to study antioxidant activity [2]. It is based on the study of free radical behaviour and includes spectrophotometric measurements of the capacity of antioxidants to scavenge DPPH radicals. This experiment consisted of a control group and 4 sample groups, conducted in 96-well plates for each group. Sample groups were prepared by dissolving 10 mg of different extracts into 180 μL of deionised water and adding 90 μL of 0.1 mM DPPH solution to the reaction system. The final concentrations of the extract samples were 5, 10, 20, 50 and 100 μg/mL. The control group used the same solvent volume to replace these 5 extracts, adding 90 μL 0.1 mM DPPH solution to each well. After mixing them thoroughly, it was placed in a shaker and reacted in the dark for 15 minutes. The absorbance of the sample (OD_A) and control (OD_{A0}) groups was measured at 520 nm using a SpectraMax M5 spectrophotometer; the s DPPH radical scavenging (DPPH) rate was calculated as

$$\text{DPPH rate} = \frac{OD_{A0} - OD_A}{OD_{A0}} 100\% \quad . \quad (1)$$

Another well-known method of studying antioxidant properties is the Pyrogallol autoxidation method [1]. Pyrogallol rapidly self-oxidises in the presence of molecular oxygen in an aqueous or alkaline environment.

The zero adjustment solution was prepared by placing 600 μL 0.1M Tris-HCl buffer (pH=8.2) and 600 μL distilled water in a centrifuge tube. Mix well and put them in a 25°C water bath for 20 minutes. Solution I: Take 600 μ L 0.1 M Tris-HCl buffer solution (pH=8.2) and 580 μL distilled water. Mix them and place the samples, set a control group, and the concentrations of the extracts to be tested in the sample groups are 5, 10, 20, 50 and 100 μg/mL. Solution II: 20 μL 5 mM solution of pyrogallol. Under water bath conditions, solution I and solution II were mixed at 37°C for 10 min, and the absorbance value of the solution was measured at 325 nm. During the measurement process, the first recording begins at 0 minutes, with an interval of 1 minute between each recording. The results of 5 consecutive measurements were recorded. The superoxide anion radical scavenging (SARS) rate was calculated as

$$\text{SARS rate} = \frac{\Delta A_0 - \Delta A_x}{\Delta A_0} 100\%, \quad (2)$$

where ΔA_x and ΔA_0 refer to the self-oxidation rate of the sample and control groups.

The values of IC_{50}, which characterise the amount of inhibitor needed to inhibit a biological process by 50%, were calculated using graphs plotting the scavenging percentage against sample concentrations.

At a final study point, the contents of flavonoids and phenolic compounds were measured. Flavonoids and other phenolic compounds prevent lipid peroxidation and act as scavengers of radicals, such as superoxides, lipid peroxides, and hydroxylated compounds. All these lead to the inactivation of single oxygen molecules and the prevention of the activity of lipoxygenases [4, 9]. Thus, determining the content of flavonoids and phenolic compounds adds completeness to the study results of antioxidant properties under the influence of extracts.

When determining the total content of flavonoids, accurately pipette 0, 0.4, 0.8, 1.2, 1.6, and 2.0 mL of rutin standard solution into a 10 mL volumetric flask, and add 2.0, 1.6, 1.2, 0.8, 0.4, and 0 mL of 60% ethanol solution, respectively. Add 0.5ml of 5% sodium nitrite solution and shake well, then make it stand for 6 minutes. Add 0.5 mL of 10% aluminium nitrate solution and make it stand for 6 minutes. Add 4.0 mL of 4% sodium hydroxide solution, and add 60 % ethanol to the volume, shake well, and make a stand for 15 minutes. Measure the value of absorbance at 510 nm using a SpectraMax M5 spectrophotometer. Then, the content of flavonoids of 4 kinds of extracts was evaluated according to the absorbance value. The total flavonoid content was expressed as mg of rutin acid equivalents (RE) per g of extract.

To determine the total content of phenolic compounds, accurately weigh 25 mg of gallic acid standard solution, dissolve it in water and bring it to volume in a 250 mL volumetric flask to obtain a 0.1 mg/mL standard stock solution. Take 0.0, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mL of standard stock solutions of gallic acid and place them in 25 mL stoppered test tubes. Add 1 mL of folinol reagent, shake well, and add 2 mL of 12% Na_2CO_3 solution. Bring to 25 mL with water and shake well. Measure the absorbance value at 765 nm using a SpectraMax M5 spectrophotometer after reacting in a dark environment at room temperature for 2 hours. Then, the total phenolic compound content was evaluated using the absorbance values and finally expressed as mg of gallic acid equivalents (GE) per g of extract.

Results. The results of determining the DPPH and SARS rates depending on the concentration of solutions and type of extracts are shown in Fig. 1.

For both methods, n-butanol extracts show the lowest antioxidant activity, and EA and PE extracts show the highest. For other solutions, the two methods used give mixed results. The results of determining the total content of phenolic compounds and flavonoids are demonstrated in Fig. 2. The obtained results for the two substances correlate well. It is unequivocally indicated by the high Pearson correlation coefficient, which equals 0.9945 at the level of two-sided significance p = 0.005. Extracts based on ethyl acetate (EA extracts) show the highest content of extracted phenolic compounds and flavonoids, while petroleum ether extracts (PE extracts) have the lowest content.

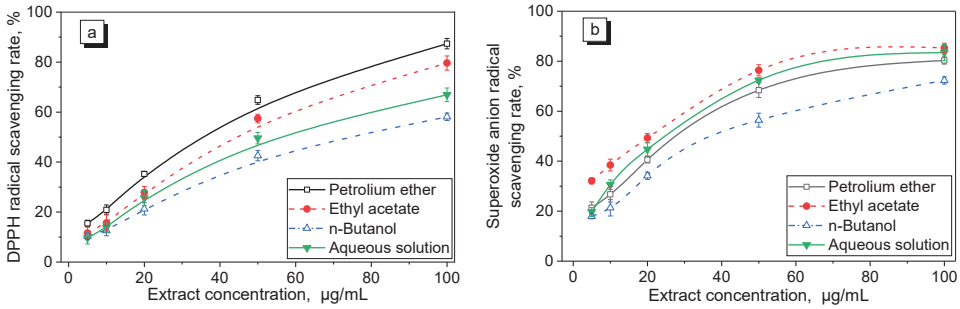

Fig. 1. Relative values (%) of DPPH (a) and SARS rates (b) as a function of solute concentrations extracted with 4 different solvents

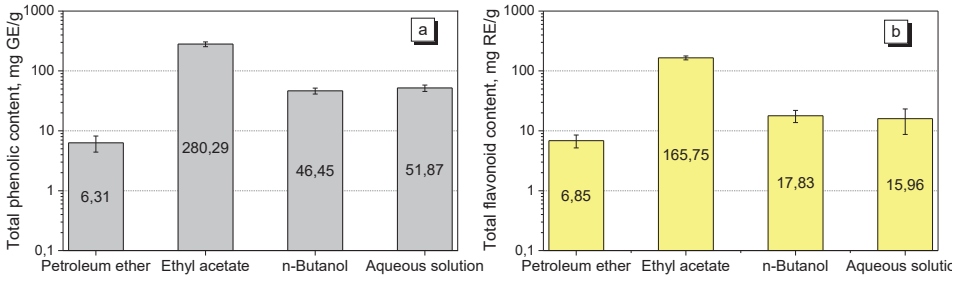

Fig. 2. The total contents of phenolic compounds (a – in mg/GE/g) and flavonoids (b – in mg/RE/g) extracted with 4 different solvents

This observation may have a straightforward explanation. Flavonoids are natural phenolic compounds with a 15-carbon skeleton consisting of two aromatic rings and a cyclohexane ring fused to one of these aromatic rings. According to their structure, they can be classified as anthocyanins, catechins, flavones, flavonols, flavanols, flavanones, and isoflavonoids [5]. The potential closeness of the extracted compounds' chemical structures may underlie close interaction mechanisms with different solvents.

Discussion. The recalculation of the experimental values of the rates of DPPN and SARS into IC_{50} values allowed one to estimate the degree of antioxidant activity of individual extracts (Fig. 3a). Although there is no agreed criterion, it is often assumed that substances with IC_{50} less than 50 μg/mL have high antioxidant activity, with IC_{50} in the range of 50-100 μg/mL – medium activity [3]. Except for the DPPN test result for n-butanol, all other results indicate a high level of activity of the extracts (Fig. 3a). At the same time, the correlation between the two activity tests does not look ideal (Pearson's coefficient 0.795 at the level of two-sided significance p=0.2025).

It is also well known that phenolic compounds, especially flavonoids, have high antioxidant properties and play an essential role in fighting free radicals [4, 7]. The antioxidant mechanism of flavonoid action involves transferring a hydrogen atom to free radicals – the faster and easier the hydrogen transfer, the greater the antioxidant power. However, if we compare the determined contents of phenols and flavonoids (Fig. 2) and the corresponding IC_{50} values (Fig. 3a), the lack of correlation between them is striking. Evidently, the mechanisms of the formation of antioxidant activity of phenols and flavonoids are more complicated than a simple linear correlation.

The solvent polarity has a complex effect on the extraction of bioactive compounds. Thus, the extraction of flavonoids and phenolic compounds may decrease with increasing polarity of the solvent while antioxidant activity and free radical scavenging activity increase [6]. Figure 3b illustrates that IC_{50} first increases (the activity decreases) with increasing polarity. In strongly polar solutions, the activity changes the trend and begins to grow. The inhomogeneous dependence of the activity and extractions in different solvents on polarity may be the reason for the complex correlation between the content of phenols and flavonoids and the antioxidant activity of the solutions.

Conclusions. Extracts based on ethyl acetate (IC_{50} according to the results of two methods were 20-42 μg/mL) and petroleum ether (29-35 μg/mL) showed the highest, and those based on n-butanol the lowest antioxidant activity (41-74 μg/mL). The total content of phenolic compounds and flavonoids ranged from 7 mg/GE/g and 7 mg/RE/g (petroleum ether) to 280 mg/GE/g and 166 mg/RE/g (ethyl acetate).

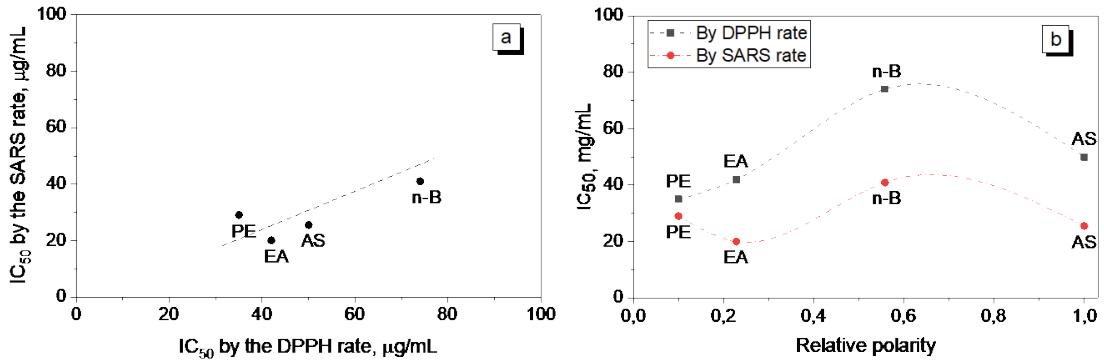

Fig. 3. IC_{50} measured by DPPH and SARS rates (a) and shown as a function of the relative polarity of solvents (b). The relative polarities were taken from [8]

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