Corrigendum: Green approach to obtain extracts of seven edible flowers (2021 IOP Conf. Ser.: Mater Sci Eng. 1031 012101)

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Description of corrigendum e.g.,

Page 5:
The Figure 1 section, the following graphic appears:

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Green approach to obtain extracts of seven edible flowers

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Abstract. Edible flowers are considered as a valuable source of bioactive compounds and are used as food and medicine with growing interest. Microwave extraction technique was employed as a green approach to obtain water extracts from seven edible flowers including Viola tricolor L., Cucurbita pepo L., Sambucus nigra L., Calendula officinalis L., Hibiscus rosa-sinensis L., Rosa damascena Mill. and Allium ursinum L. They were characterized in terms of total content of phenolics and flavonoids, as well as antioxidant potential. The total phenolic content in the edible flower’s extracts ranged between 12.08 ± 0.09 and 72.66 ± 0.60 mg gallic acid equivalent (GAE)/g dw and the total flavonoid content was established to vary between 2.36 ± 0.09 and 25.91 ± 0.09 mg quercetin equivalents (QE)/g dw. The highest values were reported in the extracts of Viola tricolor and Rosa damascena. Moreover, the same extracts displayed the highest antioxidant activity evaluated by six in vitro assays. The lowest activity was detected in the A. ursinum and C. pepo flowers extracts. In addition, the correlation between the conducted assays was studied. In conclusion, all of the studied edible flowers can be considered as promising sources of natural antioxidants in the food industry.

1. Introduction
Nowadays, the return to the natural and chemicals-free technologies is in vogue. In this regard, they should follow the main principles of green chemistry: 1) use of renewable plant resources; 2) use of alternative solvents (water or greases); 3) reduction of energy consumption (use of innovative technologies); 4) production of co-products instead of waste; 5) reduction of unit operations; 6) production of undenatured and biodegradable extract without contaminants [1]. This leads to the use of non-invasive techniques for the extraction of plant biologically active substances. The so-called green approaches have the main goal to shorten the length of extraction in an environmentally friendly way, at the same time, increasing the yield of extracted compounds. In this regard, the microwave-assisted method has proven to be significantly efficient and economically reasonable. Being among the modern extraction methods the microwave-assisted extraction (MAE) possesses several advantages. These are: the reduction of organic solvent consumption and in sample degradation, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement of extraction efficiency, selectivity, and kinetics of extraction. In addition, the usage of the contemporary methods for the extraction of plant materials is favoured [2]. Based on this, the microwave-assisted extraction is
preferred in the present study and water was introduced as an eco-friendliest solvent for a green extraction [3].

The diet of the modern people has undergone drastic changes in recent years in response to the stressful dynamic life. Food components are no longer considered only as nutrients, but also as conductors of health promoting properties. From ancient times plants and especially the edible flowers have found a place in the daily menu of people [4]. Nowadays, they are considered “modern” and are increasingly attracting the interest of the mass consumer. This is also because the edible flowers are a valuable source of biologically active substances.

A recent study focused on knowledge and consumption habits of edible flowers showed that the most common form of consumption of edible flowers is cooked (98.4%) and that the participants ate for the first-time edible flowers mostly in their own homes (76%) [5].

Edible flowers contain various secondary metabolites such as polyphenols, anthocyanins, flavonoids, carotenoids, and vitamin E [5-9]. These substances are considered as bioactive compounds and are thought to exhibit a variety of activities, including anti-allergic and anti-inflammatory actions, as well as anti-proliferative effects against cancer cells [7, 10-11]. Moreover, they can counteract the effects of reactive oxygen species (ROS) and free radicals quenching [6, 7, 12-15]. The importance of including the latter in the human diet has been studied in many epidemiological studies and those compounds are considered to prevent oxidative damage [16]. Plant polyphenols, which are natural antioxidants, are thought to have protective effects against diseases such as cardiovascular disease, cancer, and diabetes [17, 18].

Several methods for measuring antioxidant capacity have been proposed, classified as in vitro and in vivo methods. However, no analysis truly reflects the “total antioxidant capacity” of a particular sample. The total antioxidant capacity must take into account both lipophilic and hydrophilic capacity and, at least for physiological activity, must consider and differentiate both the transfer of hydrogen atoms (cooling of radicals) and the transfer of electrons (radical reduction). For this reason, it is recommended the use several in vitro methods for a comprehensive assessment of antioxidant potential [19].

The purpose of this study was to evaluate Viola tricolor L., Cucurbita pepo L. flowers, Sambucus nigra L., Calendula officinalis L., Hibiscus rosa-sinensis L., Rosa damascena Mill. and Allium ursinum L. as a good source of biological compounds with antioxidant effects. In this regards, microwave-assisted extraction of the selected edible flowers was performed and their antioxidant potential was assessed by six reliable in vitro methods. Correlation analysis between the methods was also performed.

2. Materials and methods

All reagents used in this study were of analytical grade and purchased from Merck Chemicals (Germany) and Sigma-Aldrich (Germany).

2.1. Sample preparation

Seven edible flowers from Bulgaria were subjected to extraction and analyses - Viola tricolor L. (VT), Cucurbita pepo L. flowers (CP), Sambucus nigra L. (SN), Calendula officinalis L. (CO), Hibiscus rosa-sinensis L. (HR), Rosa damascena Mill. (RD) and Allium ursinum L. (AU) (Table 1). The plant materials were obtained either from a local shop or purchased from local pharmacies in fragmented and dry condition. The flowers were further dried to constant weight by drying in a vacuum-oven at 60 °C. They were subsequently ground using an electric grinder to a fine powder and stored at ambient temperature in air-tight containers before extraction. Microwave-assisted extracts were prepared as follows: 1 g of plant material was mixed with 20 ml of water and put in a microwave oven (LG MB4047C) for 30 s, at 800 W output power. All extracts were then filtered and stored at 4 °C without adding any preservatives until analyses.
Table 1. Edible flowers studied.

| №  | Plant species        | Family            | Common name         | Local name            |
|----|----------------------|-------------------|---------------------|-----------------------|
| 1  | Viola tricolor       | Violaceae         | Wild pansy          | Tritzvetna temenuga    |
| 2  | Cucurbita pepo       | Cucurbitaceae     | Zucchini blossom    | Tzvjat ot tikvichka   |
| 3  | Sambucus nigra       | Adoxaceae         | Elder               | Tzvjat ot buz         |
| 4  | Calendula officinalis| Asteraceae        | common marigold     | Neven                 |
| 5  | Hibiscus rosa-sinensis| Malvaceae       | rose mallow         | Hibiskus              |
| 6  | Rosa damascena       | Rosaceae          | Damask rose         | Maslodajna roza       |
| 7  | Allium ursinum       | Amaryllidaceae    | ramsons, wild garlic| Tzvjat ot div chesyn  |

2.2. Total polyphenol content analysis (TPC)
The total polyphenol content was analyzed using the Folin-Ciocalteu method of Kujala et al. [20] with some modifications. Each sample extract (1 ml) was mixed with 5 ml of Folin-Ciocalteu’s phenol reagent and 4 ml of 7.5% Na₂CO₃. The mixture was vortexed well and left for 5 min at 50°C. After incubation, the absorbance was measured at 765 nm. The TPC in the extracts was expressed as mg gallic acid equivalent (GAE) per g dry weight.

2.3. Total flavonoid content (TFC)
The total flavonoid content was evaluated according to the method described by Kivrak et al. [21]. An aliquot of 0.5 ml of the sample was added to 0.1 ml of 10% Al(NO₃)₃, 0.1 ml of 1 M CH₃COOK and 3.8 ml of ethanol. After incubation at room temperature for 40 min, the absorbance was measured at 415 nm. Quercetin was used as a standard and the results were expressed as mg QE/g dw.

2.4. Antioxidant activity (AOA)

2.4.1. DPPH radical scavenging activity. The ability of the extracts to donate an electron and scavenge DPPH radical was determined by the slightly modified method of Brand-Williams et al. [22]. Freshly prepared 4x10⁻³ M methanolic solution of DPPH was mixed with the samples in a ratio of 2:0.5 (v/v). The light absorption was measured at 517 nm at room temperature after 30 min incubation. The DPPH radical scavenging activity was presented as a function of the concentration of Trolox. Trolox equivalent antioxidant capacity (TEAC) and was defined as the concentration of Trolox having equivalent AOA expressed as the μM Trolox per g dw.

2.4.2 ABTS radical cation decolorization assay. The radicals scavenging activity of the extracts against radical cation (ABTS⁺) was estimated according to a previously reported procedure with some modifications [23] The results were expressed as TEAC value (μM TE/g dw).

2.4.3. Ferric reducing antioxidant power assay (FRAP). The FRAP assay was carried out according to the procedure of Benzie and Strain [24]. The FRAP reagent was prepared fresh daily and was warmed to 37°C prior to use. The absorbance of the reaction mixture was recorded at 593 nm after incubation at 37°C for 4 min. The results were expressed as μM TE/g dw.

2.4.4. Cupric ion reducing antioxidant capacity assay (CUPRAC). CUPRAC assay was performed according to the method of Ak and Gülçin [25]. To a test tube were added 1 ml of CuCl₂ solution (1.0x10⁻²M), 1 ml of neocuproine methanolic solution (7.5x10⁻³M), and 1 ml NH₄Ac buffer solution (pH 7.0), and mixed; 0.1 ml of the sample followed by 1 ml of water were added (total volume of 4.1 ml), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of herbal extracts was measured as μM TE/g dw.
2.4.5. Oxygen radical absorbance capacity assay (ORAC). The method developed by Ou et al. [26] was used with some modifications. This method measures the ability of an antioxidant to neutralize peroxide radicals and is based on the inhibition of the decline of fluorescence of fluorescein during its oxidation in the presence of an antioxidant. The thermal decomposition of 2,2’-azobis(2-amidinopropane) dihydrochloride (AAPH) is used as a peroxide radical generator. The results are expressed in μM Trolox equivalents per gram of extract. The excitation wavelength of 485 nm and emission wavelength of 520 nm were used.

2.4.6. Hydroxyl radical averting capacity assay (HORAC). The method was developed by Ou et al. [27], and measures the ability of an antioxidant to form complexes in conditions of Fenton reaction, caused by the interaction between Co (II) and H₂O₂. The results are expressed in μM gallic acid equivalents per gram of extract. The excitation wavelength of 485 nm and emission wavelength of 520 nm were used.

2.5. Statistical analysis
Analytical determinations were performed in triplicate and the results were expressed as mean±SD (MS Excel 2003 software). Statistical analysis of the data was performed by one-way ANOVA and Tukey-Kramer post-hoc test (α=0.05), as described by Assaad et al. [28]. Correlation coefficient (r) was calculated from the coefficient of linear regression (R²) of the plot TPC/TF versus each AOA method using Microsoft Excel 2017.

3. Results and discussion
The present study focuses on seven edible flower species belonging to different botanical families that are of potential interest to consumers. They were characterized in terms of total phenolic content, total flavonoid content and antioxidant potential. The relationship between these analyses is presented as a correlation coefficient.

Microwave extraction was chosen as an approach due to the need to simulate the normal use of herbs in everyday life. On Figure 1 were represented the total phenolic content in the edible flower’s extracts ranged between 12.08 ± 0.09 and 72.66 ± 0.60 mg gallic acid equivalent (GAE)/g dw and the total flavonoid content was established to vary between 2.36 ± 0.09 and 25.91 ± 0.09 mg quercetin equivalents (QE)/g dw. In respect of TPC, the highest values were reported in the extracts of Rosa damascena and Viola tricolor followed by Sambucus nigra (30.28±0.35 mg GAE/g dw) and Hibiscus rosa-sinensis (28.20±0.23 mgGAE/g dw).

As regard the total flavonoid content the highest values were established in Viola tricolor and Sambucus nigra extracts followed by the Rosa damascena one (Figure 1). There were significant differences between the different species tested, with the exception of S. nigra and R. damascena extracts, where the values appeared to be quite similar. Based on the results V. tricolor seems to be a good source of phenolic compounds mostly flavonoid compounds. In this regard, Koike et al. [29] reported Viola tricolor as a new functional food consisting of various flavonoids. Moreover, these beautiful flowers have wide application as food – both medicinal and functional [29]. Interestingly, the highest in total phenolic compounds extract (R. damascena one) was established to consists of less total flavonoid substances. This could be attributed to the presence of non-flavonoid phenolic substances in the plants [30, 31]. The total phenolic content varied in the different varieties of plants and each plant extract contained a lower total flavonoid content than the total phenolic content, due to the presence of non-flavonoid phenolic substances in plants [30, 31].

Similar results were established for infusions of edible flowers - from 11.94 ± 0.20 to 70.29 ± 0.62 mg GAE/g dw, whereas for decoction higher values for TPC were reported – from 56.66 ± 0.48 to 135.82 ± 1.50 mg GAE/g dw, resp. [32]. The same tendency was observed for the TFC, as well. For infusions the reported values were from 1.11 ± 0.03 to 15.89 ± 0.23 mg QE/g dw [32], which are relatively similar to those established for the microwave extracts. In recent study, Ak et al. [33] investigated the TPC of various extracts of Calendula officinalis flowers establishing values in range from 30.96 ± 0.17 to 34.27 ± 0.40 mg GAE/g extract, as the highest values were reported for the
microwave one confirming the scientific interest to the technique and the potential efficacy of the extraction. Moreover, the microwave extraction is faster and eco-friendly (substantial savings of energy and a reduced environmental burden - less CO$_2$ rejected in the atmosphere) compared to decoction and infusion techniques [34]. In addition, Zheng et al. [35] established similar results in respect of the TPC for C. officinalis acetone/water/acetic acid extract.

![Graph A](image1)

**Figure 1.** Total phenolic content, mg GAE/g dw (A) and total flavonoid content, mg QE/g dw (B) of edible flower microwave extracts. Values are means ± SEM, n = 3 per treatment group. Means in a column without a common superscript letter differ (P<0.05) as analysed by one-way ANOVA and the TUKEY test.

The established total phenolic and flavonoid compounds are considered important antioxidant components. They are responsible for deactivation of free radicals based on their ability to donate hydrogen atoms to free radicals based on their structural characteristics [36].

Different in vitro methods are used in order to assess AOA. They tend to simulate the processes that occur in human body. However, none of them is 100 % reliable. That is why it is recommended to use as many as possible when the AOA of a sample is characterised. Several investigations have reported the antioxidant potential of edible flowers [13, 15, 32, 37]. The results for the antioxidant potential of the studied microwave extracts are presented in Table 2. The highest antioxidant activity according to the six in vitro assays was established in V. tricolor, R. damascena, S. nigra and H. rosa-sinensis. On the other hand, the lowest activity was detected in the A. ursinum and C. pepo flower extracts, which is in correspondence with previous study of edible flowers [32]. The antioxidant potential of the microwave extract of R. damascena was evaluated the highest in all the assays (excl. of HORAC assay). The same tendency was reported in recent study on edible flowers [32]. Such results are not surprising since the R. damascena is claimed with significant antioxidant and radical scavenging capacity [38]. Interestingly, the wide known with its activity A. ursinum plant [39] was evaluated with the lowest antioxidant potential which could be contributed to the flower part of the plant. Most of the research papers were focused on leaves of A. ursinum [39, 40]. However, Zheng et. al. [35] reported similar antioxidant activity to our results in respect of DPPH and ABTS assays for C. officinalis acetone/water/acetic acid extract (38.21±0.63 and 74.06 ± 0.63 µM TE/g, resp.). However, some edible flowers are more explored then others. Rosa damascena and C. officinalis extracts have been the subject of various research papers [9, 32]. Pires et al. [9] reported that C. officinalis and R. damascena water extracts exhibited Trolox EC50 of 1.37±0.08 and 0.18±0.02 µg /ml toward DPPH assay, resp.

To assess the inhibitory capacity of phenolic compounds against ROS, data on the correlation of antioxidant activity and phenolics concentration are often reported. In this regard, the contribution of TPC and TFC to AOA was studied by plotting all available data from the samples about these methods in a graph (Figure 2). Linear regression is then sought. In this way, more detailed information is generated. Based on these plots, the corresponding correlation coefficients were calculated and the results are presented in Table 3.
Table 2. *In vitro* antioxidant activity of seven edible flower microwave extracts according to DPPH, ABTS, FRAP and CUPRAC (µM TE/g dw), ORAC (µM TE/g dw) and HORAC (µM GAE/g dw) assays.

| Sample/Assay | TEAC<sub>DPPH</sub> | TEAC<sub>ABTS</sub> | TEAC<sub>FRAP</sub> | TEAC<sub>CUPRAC</sub> | HORAC | ORAC |
|--------------|----------------------|----------------------|----------------------|-----------------------|-------|-------|
| VT           | 132 ± 7.03<sup>b</sup> | 215 ± 2.35<sup>b</sup> | 369.32 ± 3.68<sup>b</sup> | 510 ± 5.89<sup>b</sup> | 20500 ± 2210<sup>a</sup> | 44300 ± 1050<sup>c</sup> |
| CP           | 40.1 ± 1.22<sup>d</sup> | 62.6 ± 0.4<sup>c</sup> | 79.68 ± 1.02<sup>c</sup> | 90 ± 1.22<sup>c</sup> | 6940 ± 59.5<sup>b</sup> | 12700 ± 554<sup>e</sup> |
| SN           | 101 ± 2.64<sup>e</sup> | 134 ± 0.922<sup>d</sup> | 218.01 ± 3.54<sup>d</sup> | 326 ± 2.33<sup>d</sup> | 9050 ± 468<sup>b</sup> | 75900 ± 697<sup>b</sup> |
| CO           | 48.5 ± 0.196<sup>e</sup> | 65.2 ± 0.168<sup>e</sup> | 88.44 ± 0.93<sup>e</sup> | 143 ± 2.86<sup>d</sup> | 6150 ± 112<sup>b</sup> | 16000 ± 519<sup>e</sup> |
| HR           | 116 ± 1.3<sup>c</sup> | 149 ± 4.23<sup>c</sup> | 307.53 ± 4.96<sup>c</sup> | 314 ± 0.643<sup>c</sup> | 7543 ± 222<sup>b</sup> | 25400 ± 344<sup>d</sup> |
| RD           | 406 ± 2.83<sup>a</sup> | 609 ± 2.05<sup>a</sup> | 960.25 ± 12.40<sup>a</sup> | 872 ± 8.88<sup>a</sup> | 16700 ± 576<sup>a</sup> | 101000 ± 2770<sup>a</sup> |
| AU           | 5.64 ± 0.0578<sup>c</sup> | 24 ± 0.304<sup>c</sup> | 39.33 ± 1.95<sup>f</sup> | 23.9 ± 0.228<sup>c</sup> | 5950 ± 177<sup>b</sup> | 42100 ± 111<sup>c</sup> |

Values are means ± SEM, n = 3 per treatment group. Means in a column without a common superscript letter differ (*P*<0.05) as analyzed by one-way ANOVA and the TUKEY test.

**Figure.** 2. Linear regression analysis of antioxidant activity versus total phenolic content (A) and total flavonoid content (B). The obtained coefficients of determination are as follow: for graph A - CUPRAC - 0.8993, FRAP - 0.8645, ABTS- 0.8283, DPPH - 0.8993, ORAC - 0.7135 and HORAC - 0.7172; for graph B - CUPRAC - 0.2742, FRAP - 0.1133, ABTS- 0.1018, DPPH - 0.0811, ORAC - 0.0989 and HORAC - 0.7966. The antioxidant activities were expressed in µMTE/g (CUPRAC, FRAP, ABTS, DPPH), in mMTE/g (ORAC) and in µM GAE/g (HORAC).
Based on the results, the AOA is contributed mainly to the phenolic compounds as the correlation factor ranges between 0.8447 and 0.9483 for all the methods. The variation in the correlation coefficients could be contributed to the different principle on which the assays are based. As regards the correlation between TFC and antioxidant capacities, a low relationship was observed. The correlation coefficients of 0.3146, 0.8925, 0.5236, 0.3365, 0.2849 and 0.3190 for ORAC, HORAC, CUPRAC, FRAP, DPPH, and ABTS respectively, were calculated (Table 3). This is in consonance with the other author [35]. Meanwhile, correlation coefficient of 0.5745 between the TPC and TFC was established, which indicate the presence of the flavonoid compounds among the phenolics but they seem to contribute less to the established antioxidant potential. The results confirmed that the phenolic compounds are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups and thus the phenolic content of plants may contribute directly to their antioxidant action [41]. Several investigations of the antioxidant activity of plant extracts have confirmed a correlation between total phenolic content and antioxidant activity [42, 43]. In contrast to this, a recent study demonstrated a negative relation between antioxidant activity and TPC of strawberry [44].

|          | ORAC  | HORAC | CUPRAC | FRAP  | DPPH  | ABTS  | TFC  |
|----------|-------|-------|--------|-------|-------|-------|------|
| TPC      | 0.8447| 0.8469| 0.9483 | 0.9298| 0.9101| 0.9313| 0.5745|
| TFC      | 0.3146| 0.8925| 0.5236 | 0.3365| 0.2849| 0.3190|      |

4. Conclusions
Microwave-assisted extraction was successfully applied for the green extraction of biologically active substances of seven edible flowers. Particularly promising are aqueous based methods, since water is a cheap, safe and abundant solvent and the limited use of toxic organic solvents is a consumer’s, ecological and processing demand.

In all of the studied aqueous extracts was established the presence of polyphenols and flavonoids. The analysis of the antioxidant activity outlined the *R. damascena* and *V. tricolor* extracts as the most powerful ones. Nevertheless, all of the studied edible flowers can be considered as sources of natural antioxidants in the food industry and can be used as potential functional foods to counterbalance the effect of reactive oxygen species (ROS) and oxidative stress. The high correlations between TPC values and the antioxidative values indicated that phenolic content contributed towards the antioxidant capacities of these flowers. However, a lower correlation was found between TFC values and the antioxidant assays. Hence, MWE comes up as an appropriate method for the extraction of antioxidants from the investigated edible flowers - *Viola tricolor*, *Cucurbita pepo* flowers, *Sambucus nigra*, *Calendula officinalis*, *Hibiscus rosa-sinensis*, *Rosa damascena* and *Allium ursinum*.

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