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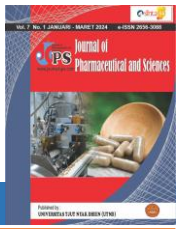

The Effect of Extraction Methods on Total Flavonoid Content and Antioxidant Activity of Kenop (*Gomphrena globosa*)

Pengaruh Metode Ekstraksi Terhadap Kadar Flavonoid Total dan Aktivitas Antioksidan Tanaman Kenop (*Gomphrena globosa*)

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Abstract

Gomphrena globosa is a medicinal plant with attractive pigments and therapeutic properties, including antihypertensive, anticancer, and antioxidant effects. These activities are mainly attributed to phenolic compounds such as flavonoids. Previous studies have shown that ethanolic extracts of *G. globosa* flowers and leaves obtained by maceration have potent antioxidant activity. However, no study has yet reported Soxhlet extraction of *G. globosa* leaves. Therefore, this study aimed to compare Soxhlet extraction and maceration extraction methods to evaluate their efficiency in extracting flavonoids and antioxidant activity. Flavonoid levels were determined using the aluminium chloride colourimetric method with quercetin as a standard, and antioxidant activity was evaluated using the DPPH radical scavenging assay. The results showed that maceration produced a higher flavonoid content (78.33 ± 2.14 mg QE/g extract) than Soxhlet extraction (57.43 ± 1.53 mg QE/g extract). Consistently, antioxidant activity was more potent in the macerated extract (IC_{50} = 53.93 ± 1.78 µg/mL) compared to the Soxhlet extraction (IC_{50} = 62.50 ± 0.39 µg/mL). Evaluation using the t-test showed that the antioxidant activities of these methods were significantly different ($p < 0.05$). These findings indicate that maceration is more effective in extracting flavonoids from *G. globosa* leaves, resulting in high antioxidant activity. Moreover, *G. globosa* leaves emerged as a promising natural antioxidant source for pharmaceutical and functional food applications.

***Keywords*: Antioxidant, Gomphrena globosa, Maceration, Soxhlet Extraction, Total Flavonoid Content**

Abstrak

Gomphrena globosa merupakan tanaman obat yang memiliki pigmen menarik dan khasiat terapeutik, termasuk antihipertensi, antikanker, dan antioksidan. Aktivitas ini terutama disebabkan oleh senyawa fenolik seperti flavonoid. Penelitian sebelumnya telah menunjukkan bahwa ekstrak etanol bunga dan daun *G. globosa* yang diperoleh melalui maserasi memiliki aktivitas antioksidan yang kuat. Namun, belum ada penelitian yang melaporkan penggunaan sokletasi untuk *G. globosa*. Oleh karena itu, penelitian ini bertujuan untuk membandingkan metode sokletasi dan maserasi guna mengevaluasi efisiensinya dalam mengekstraksi flavonoid dan aktivitas antioksidan. Kadar flavonoid ditentukan menggunakan metode kolorimetri aluminium klorida dengan kuersetin sebagai standar, sedangkan uji penangkapan radikal DPPH mengevaluasi aktivitas antioksidan. Hasil penelitian menunjukkan bahwa maserasi menghasilkan kandungan flavonoid yang lebih tinggi (78,33 ± 2,14 mg QE/g ekstrak) dibandingkan sokletasi (57,43 ± 1,53 mg QE/g ekstrak). Secara konsisten, aktivitas antioksidan lebih kuat pada ekstrak maserasi (IC_{50} = 53,93 ± 1,78 µg/mL) dibandingkan dengan ekstrak sokletasi (IC_{50} = 62,50 ± 0,39 µg/mL). Evaluasi menggunakan uji-t menunjukkan bahwa aktivitas antioksidan kedua metode ini berbeda secara signifikan ($p < 0,05$). Temuan ini menunjukkan bahwa maserasi lebih efektif dalam mengekstraksi flavonoid dari *G. globosa*, sehingga menghasilkan aktivitas

antioksidan yang tinggi. Selain itu, daun *G. globosa* muncul sebagai sumber antioksidan alami yang menjanjikan untuk aplikasi farmasi dan pangan fungsional.

Kata Kunci: **Antioksidan, *Gomphrena globosa*, Kadar Total Flavonoid, Maserasi, Sokletasi.**

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Introduction

Gomphrena globosa (known as kenop in Indonesia) is a medicinal plant widely recognised for its diverse therapeutic properties. This plant is often consumed as a herbal drink, not only for its health benefits but also because of its attractive colours, ranging from purple to pink and red. The pharmacological activities of *G. globosa* have been reported, such as antihypertensive, anticancer, antioxidant, anti-inflammatory, and detoxifying effects. [1]. Phytochemical investigations have revealed that *G. globosa* contains phenolic compounds, including flavonoids, flavonols, and phenolic acids [2]. This plant is rich in betacyanins, which belong to the flavonoid group [3]. Flavonoids are a group of phenolic compounds that have strong antioxidant properties. The role of flavonoids as antioxidants is to capture free radicals by donating one of their electrons [4].

Antioxidants are compounds that can neutralise the effects of free radicals [5]. Susilaningrum reported that the ethanolic flower extract of *G. globosa* obtained by maceration exhibited potent antioxidant activity with an IC_{50} value of 49.9 µg/mL. [6], while Ningrum reported that the leaf extract obtained by the same method showed an IC_{50} value of 100 ± 6.09 µg/mL [7], suggesting that both the ethanolic extract of the flowers and leaves of *G. globosa* possess significant antioxidant potential. Both studies used the maceration extraction method because it is highly suitable for materials such as flowers and leaves, which are susceptible to high temperatures and can damage the oil if exposed to excessive heat [8]. On the other hand, the Soxhlet extraction method is particularly advantageous for improving the efficiency of sample extraction. Despite the risk of causing thermal degradation, Soxhlet extraction was chosen to compare efficiency with the maceration method. *G. globosa* has potential as a natural antioxidant; however, no research has been conducted on the antioxidant activity of *G. globosa* using the Soxhlet extraction method. Therefore, this research was conducted to determine the effect of maceration and Soxhlet extraction methods on the total flavonoid content and antioxidant activity of the ethanol extract of *G. globosa*.

Experimental Section

Materials and Apparatus

The materials used in this research are an extract of *G. globosa*, 96% ethanol, 70% ethanol, concentrated HCl, Mg metal (Merck), 10% $AlCl_3$ (Merck), 1 M sodium acetate (Merck), quercetin (Merck), distilled water, methanol, vitamin C (Merck), and DPPH solution. The instruments used in the study are a UV-Vis spectrophotometer (Shimadzu), a maceration vessel, a Soxhlet vessel (M Tops), an oven (Memmert), a blender, a vortex, a rotary evaporator (Heidolph L-4000), a water bath (Memmert), and an analytical balance (Ohaus®).

Preparation and Extraction of Simplisia

Powdered samples of *G. globosa* were extracted using two methods. In Soxhlet extraction, 50 g of powder was refluxed with 500 mL of 96% ethanol at 90 °C for 5 hours, using eight cycles, until the solvent was

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removed. The mixture was then evaporated to yield a thick extract. Maceration involved soaking 50 g of powder in 500 mL of 96% ethanol for 3 days, stirring it intermittently, filtering, and then concentrating by evaporation [9].

Determination of Flavonoid Content

The presence of flavonoids in the extract was first identified using the Mg–HCl test. In this assay, 10 mg of the ethanolic extract of *G. globosa* was dissolved in 70% ethanol, then magnesium metal and concentrated HCl were added. The appearance of a purple-red colouration indicated the presence of flavonoid compounds. Quantitative determination of flavonoids was then carried out using the $AlCl_3$ colourimetric method with quercetin as the standard. A quercetin stock solution (100 ppm) was prepared and subsequently diluted to concentrations of 10–50 ppm. Each standard solution was mixed with methanol, 1 mL of 10% $AlCl_3$, 1 mL of 1 M sodium acetate, and distilled water to a final volume of 10 mL, and incubated for 40 minutes. The absorbance was then measured using a UV–Vis spectrophotometer at 433 nm to generate the calibration curve [10].

For the analysis, 15 mg of the extract was dissolved in 25 mL of methanol. Subsequently, 1 mL of this solution was mixed with methanol, 1 mL of 10% $AlCl_3$, 1 mL of 1 M sodium acetate, and distilled water to obtain a final volume of 10 mL. The mixture was incubated at room temperature for 40 minutes. The absorbance was then measured using a UV–Vis spectrophotometer at 433 nm. The total flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g) [10].

Determination of Antioxidant Activity

Antioxidant activity was evaluated using the DPPH assay. A 35 µg/mL DPPH solution was prepared, protected from light, and its maximum absorbance wavelength was determined by UV-Vis spectrophotometry over 400–800 nm. Extracts obtained by maceration and Soxhlet extraction were dissolved in methanol to prepare concentrations of 5, 25, 50, 75, and 100 µg/mL. Each extract solution (1 mL) was mixed with 2 mL of DPPH in a 5 mL volumetric flask wrapped in aluminium foil, incubated in the dark for 40 min, and the absorbance was recorded. Vitamin C solutions 2, 4, 6, 8, and 10 µg/mL were prepared similarly and used as a positive control. Radical scavenging activity was expressed as percentage inhibition, and IC_{50} values were obtained from the concentration–response curves [9]. The magnitude of antioxidant power is calculated using the formula :

$$\% \text{ Inhibition } = \frac{\text{Abs control} - \text{abs sample}}{\text{abs control}} x\ 100\% \qquad (1)$$

Data Analysis

An independent t-test was performed to compare total flavonoid content and IC_{50} values of the ethanolic extracts of *G. globosa* obtained by maceration and Soxhlet extraction. Before conducting the t-test, data were assessed for normality and homogeneity of variances to ensure compliance with the assumptions of parametric analysis**.** Statistical significance was determined based on the p-value. Differences with $p > 0.05$ were considered not significant, indicating that variations between methods may have occurred by chance. In contrast, $p < 0.05$ was regarded as statistically significant, confirming that the extraction method had a measurable effect on total flavonoid content and IC_{50} values. [11].

Results and Discussion

This study evaluated the effect of different extraction methods on the yield, flavonoid content, and antioxidant activity of *G. globosa* extracts. Both maceration and Soxhlet extraction with ethanol were performed, and the resulting extracts were analysed qualitatively and quantitatively for flavonoid content and antioxidant capacity. Maceration for 3 days with 96% ethanol yielded 6.45 g of concentrated extract from 50 g of dried plant material, corresponding to a yield of 12.9% (w/w). In comparison, Soxhlet extraction of the same amount of material yielded 5.76 g of extract, corresponding to a 11.52% yield (w/w). These results indicate that maceration produced a slightly higher extract yield than Soxhlet extraction.

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The presence of flavonoids in both extracts was confirmed qualitatively using the Mg–HCl test. Quercetin is used as the positive control because it is a well-characterised natural flavonol widely distributed in plants. Upon reaction with Mg and HCl, quercetin produces a characteristic purple-red colouration, confirming the presence of flavonoid functional groups. These results indicate that both extraction methods successfully extracted flavonoids from *G. globosa*. Still, maceration yielded a higher flavonoid intensity than Soxhlet extraction, as evidenced by a more concentrated purple-red colour. The intensity of the purple-red colour formed, which correlates with the relative abundance of flavonoid compounds in the extract [12]. The lower response observed in Soxhlet extraction may be due to the degradation of thermolabile flavonoids during prolonged heating, whereas maceration at room temperature better preserved these compounds.

Total flavonoid content was determined using the $AlCl_3$ colourimetric method, which is based on the formation of stable complexes between $AlCl_3$ and the keto or hydroxyl groups of flavonoids, producing a measurable colour shift. Sodium acetate was added to enhance complex stability in the visible range. [13]. Quercetin was used as the reference standard because of its wide occurrence in plants and its ability to form complexes with $AlCl_3$, and was applied to generate a calibration curve [14]. Quercetin exhibited a maximum absorbance at 433 nm (0.715) and reached stability after 40 min. Sample extracts were analysed under the same conditions, and flavonoid content was calculated from the calibration curve and expressed as mg quercetin equivalents per gram of extract (mg QE/g). The results obtained from the determination of the flavonoid content can be seen in **Table 1**.

Table 1. Results of Determination of Total Flavonoid Content from Kenop

Extraction Method	Total Flavonoid Content ($\bar{x}$ ± SD)
Soxhlet Extraction	57.43 ± 1.53
Maceration	78.33 ± 2.14

Antioxidant activity was assessed using the DPPH assay, a widely employed method due to its simplicity, sensitivity, and low sample requirement [15]. The assay is based on the reduction in absorbance associated with the colour change that occurs when DPPH radicals are scavenged by hydrogen-donating compounds, forming a more stable product [16]. The maximum wavelength was determined by UV-Vis spectrophotometry to ensure accurate absorbance measurement [17]. Vitamin C, a natural water-soluble antioxidant and potent reducing agent, was used as a positive control [18].

The ethanolic extracts of *G. globosa* obtained by Soxhlet extraction and maceration methods exhibited vigorous antioxidant activity. The IC_{50} values were 62.50 ± 0.39 µg/mL for Soxhlet extraction and 53.93 ± 1.78 µg/mL for maceration. Both extraction methods showed 50% inhibition of the DPPH radical across concentrations of 50-100 µg/mL. In comparison, vitamin C showed extreme antioxidant activity, with an IC_{50} value of 5.61 ± 0.26 µg/mL, corresponding to 50% inhibition at concentrations of 4-6 µg/mL. The results obtained from the determination of the antioxidant activity can be seen in **Figure 1**.

The total flavonoid content of *G. globosa* extracts obtained by maceration was 78.33 ± 2.14 mg QE/g extract, while Soxhlet extraction yielded 57.43 ± 1.53 mg QE/g extract. An independent t-test showed a p-value < 0.05, indicating a significant difference between the two methods. The higher flavonoid content observed in the maceration method is attributed to extraction at room temperature, which minimises thermal degradation of flavonoids. In contrast, continuous heating during Soxhlet extraction may promote degradation of heat-sensitive compounds. These findings are consistent with [8], who reported that ethanol extraction at room temperature is more suitable for preserving flavonoid compounds.

Antioxidant activity measured by the DPPH assay showed IC_{50} values of 62.50 ± 0.39 µg/mL and 53.93 ± 1.78 µg/mL for Soxhlet extraction and maceration, respectively. In the ethanolic extracts of *G. globosa*, antioxidant constituents such as flavonoids and phenolics are intermixed with various inactive components, resulting in a lower effective concentration of active compounds. In contrast, vitamin C is a pure, highly reactive, and water-soluble molecule that can efficiently and directly interact with free radicals, thereby exhibiting a lower IC_{50} value [19]. The t-test again demonstrated a significant difference ($p < 0.05$), with the lower IC_{50} value for the macerated extract indicating more potent antioxidant activity than that of the Soxhlet-extracted extract. The heating process in Soxhlet extraction may contribute to this finding. Although heating in Soxhlet extraction can improve extraction efficiency, it may also decrease antioxidant stability due to thermal oxidation [9]. On the other hand, the ethanolic extract of *G. globosa* contains flavonoid-related

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pigments known as betacyanins, primarily represented by gompherin I, II, and III. These compounds are decarboxylated derivatives of betacyanins that can form during thermal processing, due to the betalain pigments' intrinsic heat sensitivity and structural instability at elevated temperatures [20]. Overall, these findings demonstrate that the extraction method significantly influences both flavonoid content and antioxidant properties, with maceration yielding higher flavonoid levels and more potent antioxidant activity than Soxhlet extraction.

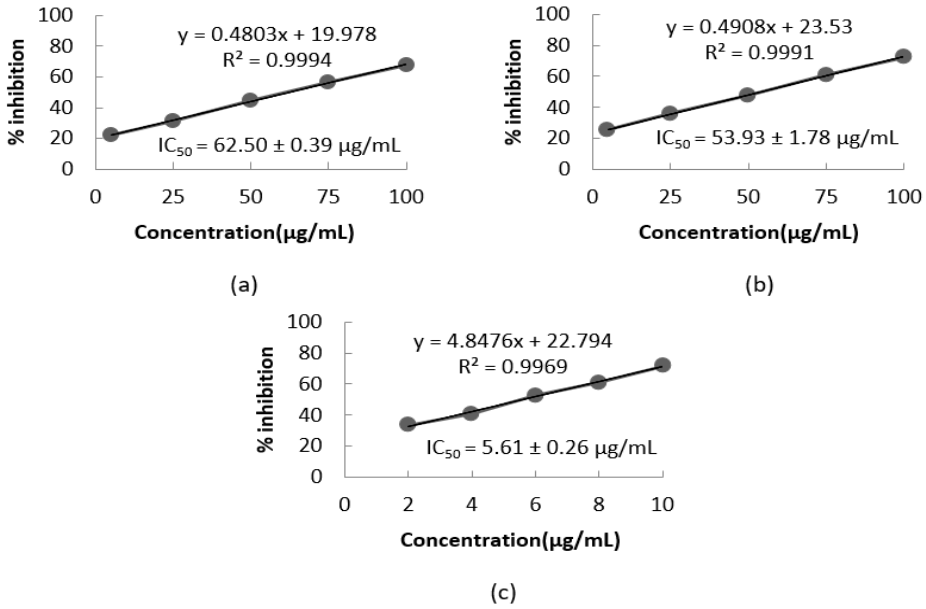

Figure 1. Results of Antioxidant Activity Test of *G. globosa* Extract (a) Soxhlet Extraction, (b) Maceration, (c) Vitamin C

Conclusions

The extraction method significantly altered the total flavonoid content and antioxidant activity of *G. globosa* ethanolic extracts. Maceration yielded a higher flavonoid content, 78.33 ± 2.14 mg QE/g extract, compared to Soxhlet extraction, 57.43 ± 1.53 mg QE/g extract. Consistently, DPPH assay results showed more vigorous radical scavenging activity in the macerated extract IC_{50} = 53.93 ± 1.78 µg/mL than in the Soxhlet extraction IC_{50} = 62.50 ± 0.39 µg/mL. Although both methods produced extracts with vigorous antioxidant activity, maceration proved superior, likely due to reduced thermal degradation of flavonoid compounds. Overall, maceration is recommended as a more suitable extraction method for obtaining high-quality flavonoid- and antioxidant-rich ethanolic extracts from G. globosa, as it yields better extract quality despite requiring a longer processing time**.** These findings highlight the potential of *G. globosa* as a natural antioxidant source and underscore the importance of optimising extraction conditions to maximise bioactive compound yield. Further investigations, including in vivo studies and formulation development, are warranted to explore its applications in the pharmaceutical and functional food industries.

References

[1] Agustina L, Amaranggana AP, Yuliati N, Damayanti N. Formulasi Sediaan Nutrasetikal Teh Celup Kombinasi Bunga Rosella (Hibiscus sabdariffa) dan Bunga Kenop (Globe amaranth) dengan Variasi Konsentrasi Stevia sebagai Perasa. Heal Med Sci 2024;1:10. https://doi.org/10.47134/phms.v1i3.220.

[2] Rohmah MK, Rahman E, Dan Y, Ambari Y. Uji Kesetaraan Aktivitas Antioksidan Seduhan Bunga Kenop (Gomphrena globosa L.) dengan Vitamin C Menggunakan Metode DPPH Equivalence Test of Antioxidant Activity of Steeping Knob Flower (Gomphrena globosa L.) with Vitamin C Using DPPH Method. vol.. 24. n.d.

[3] Bujak T, Zagórska-Dziok M, Ziemlewska A, Nizioł-łukaszewska Z, Lal K, Wasilewski T, et al. Flower Extracts as Multifunctional Dyes in the Cosmetics Industry. Molecules 2022;27.

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

https://doi.org/10.3390/molecules27030922.

[4] Husna PAU, Kairupan CF, Lintong PM. Tinjauan Mengenai Manfaat Flavonoid pada Tumbuhan Obat Sebagai Antioksidan dan Antiinflamasi. EBiomedik 2022;10:76–83.

[5] Rahmi H, Studi Agroteknologi Fakultas Pertanian P, Singaperbangsa Karawang Jl Ronggowaluyo UH, Jambe Timur T, Karawang K. Review : Aktivitas Antioksidan dari Berbagai Sumber Buah-buahan di Indonesia. J Agrotek Indones 2017;2:34–8.

[6] Susilaningrum DF, Wijayanti N. Antioxidant potential and cytotoxic assay of ethanol extract of gomphrena globosa L. Flower. AIP Conf. Proc., vol. 2260, American Institute of Physics Inc.; 2020. https://doi.org/10.1063/5.0015685.

[7] Ningrum NA, Wijayanti N. Antioxidant properties of Gomphrena globosa leaves Extract. AIP Conf. Proc., vol. 2260, American Institute of Physics Inc.; 2020. https://doi.org/10.1063/5.0015689.

[8] Kurniawati A. Pengaruh Jenis Pelarut Pada Proses Ekstraksi Bunga Mawar Dengan Metode Maserasi Sebagai Aroma Parfum. J Creat Student 2017;2:74–83. https://doi.org/10.15294/jcs.v2i2.14587.

[9] Dewi I, Maisaroh S. Perbandingan Metode Sokletasi dengan Maserasi terhadap Daya Aktivitas Antioksidan Bunga Tasbih (Canna hybrida Hort.). J Farm Higea 2020;12.

[10] Suharyanto S, Hayati TN. Penetapan Kadar Flavonoid Total Ekstrak Buah Gambas (Luffa acutangula(L.) Roxb.) dengan Metode Spektrofotometri UV-Vis. Pharmacon J Farm Indones 2021;18:82–8. https://doi.org/10.23917/pharmacon.v18i01.10916.

[11] Amelia D, Simatupang N, Sinuraya BJ, Ekonomi F, Prima U. Pengaruh Harga, Citra Merek Dan Kualitas Pelayanan Terhadap Kepuasan Pelanggan Pt. Jne Cabang Medan. J Manaj 2020;7:11–24.

[12] Singh P, Arif Y, Bajguz A, Hayat S. The role of quercetin in plants. Plant Physiol Biochem 2021;166:10–9. https://doi.org/10.1016/j.plaphy.2021.05.023.

[13] Tanamal MT, Papilaya PM, Smith A. Kandungan Senyawa Flavoid Pada Daun Melinjo (Gnetum gnemon L.) Berdasarkan Perbedaan Tempat Tumbuh. BIOPENDIX J Biol Pendidik Dan Terap 2017;3:142–7. https://doi.org/10.30598/biopendixvol3issue2page142-147.

[14] Rissa Laila Vifta K, Alviyan Shutiawan M, Maulidya A, Yuswantina R. Skrining Flavonoid Ekstrak Buah Parajito (Medinilla speciosa Blume) Asal Kabupaten Kududs dan Semarang Dengan Pembanding Kuersetin dan Rutin. Media Inf Penelit Kabupaten Semarang 2021;4:2021–4.

[15] Julizan N. Validasi Penentuan Aktifitas Antioksidan Dengan Metode Dpph. Kandaga– Media Publ Ilm Jab Fungsional Tenaga Kependidikan 2019;1. https://doi.org/10.24198/kandaga.v1i1.21473.

[16] Mutmainna Tamrin, Achmad Kadri Ansyori, Hayatus Sa'adah. Uji Aktivitas Antioksidan Ekstrak Etanol Biji Buah Nyirih (Xylocarpus granatum) Dengan Metode DPPH Secara Spektofotometri UV-VIS. J Ris Kefarmasian Indones 2024;6:233–48. https://doi.org/10.33759/jrki.v6i2.527.

[17] Sukmawati, Sudewi S, Pontoh J. Optimasi dan Validasi Metode Analisis Dalam Penentuan Kandungan Total Flavonoid Pada Ekstrak Daun Gedi Hijau (*Abelmoschus manihot* L.) Yang Diukur Dengan Spektrofotometer UV-VIS. Pharmacon 2018;7:32–41.

[18] Rozi F, Majiding CM, Nuzul M, Ash A, Aryatika K. Analisis Kapasitas Antioxidant Minuman Sumber Vitamin C Antioxidant Capacity Analysis of Vitamin C Source Drink 2025;1:1–7.

[19] Mehmood A, Javid S, Khan MF, Ahmad KS, Mustafa A. In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC Chem 2022;16:1–10. https://doi.org/10.1186/s13065-022-00858-2.

[20] Drobnicka N, Sutor K, Kumorkiewicz-Jamro A, Spórna-Kucab A, Antonik M, Dziedzic E, et al. Phytochemical molecules from the decarboxylation of gomphrenins in violet gomphrena globosa L.—floral infusions from functional food. Int J Mol Sci 2020;21:1–19. https://doi.org/10.3390/ijms21228834.

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