Original Research Paper

Comparative evaluation of aqueous, alcoholic and hexane extracts of *Grammosciadium platycarpum* Boiss & Hausskn

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Abstract:

Grammosciadium Platycarpum Boiss is a rich source of phenolic and flavonoid compounds that have antioxidant activity. Antioxidants in the diet are important in terms of protecting the body against oxidative stress and maintaining health. This study aimed to investigate the antioxidant activity of mountain dill extract with different solvents. In this research, water, ethanol, methanol, and n-hexane solutions were used. The content of flavonoid, total phenol, and anthocyanin was investigated. The highest yield of flavonoid extraction with methanol solution was obtained (22.94±0.73 mg/g). The highest amount of anthocyanin measured was calculated related to extraction with ethanol solvent (3.40±0.12 mg/g). However, no significant difference was observed between different extracts obtained from different solutions in the amount of total phenol. The results indicated that extraction by alcohol solvent method has higher extraction efficiency than water and n-hexane solvent extracts. The type of solvent has a great effect on the anthocyanin and flavonoid content, which has a significant role in the antioxidant activity of the extract.

Keywords: *Grammosciadium platycarpum* Boiss & Hausskn, Ethanol, Methanol, n-Hexane, Aqueous extract.

1. Introduction

People's health is considered one of the most important pillars of society and one of the factors of society's growth and development [1]. Therefore, one of the main goals of the health sector of any country is to improve the health of the society. The health of society is also in the group of various factors, among which it can mention food supply and improvement of the hygiene level [2]. Apart from providing the health of society and their tremendous effects, chemical drugs have disadvantages such as high cost, lack of access in remote areas, increased microbial resistance, *etc*., which has highlighted the importance of medicinal plants [3]. By more precisely identifying the types of medicinal plants and their effective compounds, it is possible to reduce the burden of chemical drugs as an aid in the treatment of diseases. Some of these plants can be used not only as a seasoning in food preparation but also in traditional medicine [4].

Historically, plants have been crucial in the development of different societies, and the use of some medicinal plants has been traditional and is still widespread. Extensive research is being done to find the products and natural materials of these plants. Today, the implication of medicinal plants and recognizing their vital role in advancing national, regional, and global goals for achieving health, drug self-sufficiency, creating employment, and economic development is not hidden from anyone, and they are known as genetic reserves and treasures of the world [5].

The vast plateau of Iran consists of different climates and weather conditions, and for this reason, various plant species are found in it. Among the rich flora of Iran, which includes more than 8000 plant species, a significant number of them are plants that are called medicinal plants for some reason [6]. Since the second half of the 20th century, extensive pharmacodynamic research has been conducted on medicinal plants in many countries of the world, and important unknown compounds have been discovered in these plants and introduced to the pharmaceutical community, and subsequently, many herbal medicines have been prepared and marketed [7].

Currently, more than one-third of the medicines used in human societies are herbal medicines [8], due to the increasing acceptance of the medical community and consumers of these medicines, it seems that soon more than two-thirds of the medicines used for prevention and the treatment of various complications and diseases [9], as well as strengthening compounds, beauty, etc., should also be prepared and supplied from plant sources. In many European countries such as Sweden; Germany, Hungary, Austria, France, Italy, *etc*., between 40 and 60% of and in China, India, and Japan, about 80% of the needs of the pharmaceutical industry are supplied from plant sources, which shows their importance in ensuring the health of society and the global market [10, 11].

Grammosciadium platycarpum Boiss & Hausskn is a local and aromatic vegetable that grows mostly in the Zagros highlands and in the spring season with a lot of aroma and taste. Mountain dill has much more flavor than ordinary dill and is different in appearance. This plant retains much of its aroma and taste even after drying and does not lose its green color [12]. *G. platycarpum* with many properties plays a very substantial role in reducing blood pressure and sugar,

and it is also useful in the treatment of muscle problems such as back pain [13]. Mountain dill is a plant of the Apiacea of the Umbelliferae family with a height of 40-100 cm, its root is straight, conical, and white. The Apiacea family is rich in polyphenolic compounds and many of them such as rosemary, thyme, mint, and parsley are comprehended as an affluent source of antioxidants [13, 14].

Determining the effective compounds is important for the more appropriate identification of medicinal plants. In this research, different solvents were used to evaluate the compounds of *G. platycarpum* extract.

2. Materials and Methods

2.1. Preparation of aqueous extract

The *G. platycarpum* was collected from around Darab in March 2022, washed, and dried in the shade after being approved by the botanist of the Darab Branch, Islamic Azad University. After grinding, 300 grams of dried *G. platycarpum* were added to two liters of boiled distilled water. The container was quickly removed from the heat and covered with aluminum foil. After 4 hours, the contents of the container were filtered using filter paper in the laboratory environment. The resulting container was placed on a bain-marie, after the evaporation of the solvent, a dry extract was obtained. After drying the extract, the opening of the container was closed and it was stored away from light and at a temperature of -21ºC [15].

2.2. Preparation of hydroalcoholic extract

To prepare methanolic and ethanolic extracts, the above method was used, but instead of water, 96% ethanol and 81% methanol were used.

2.3. Preparation of n-hexane extract

The plant was extracted using hexane solvent and maceration method. Five hundred grams of ground *G. platycarpum* were placed in a 2000 ml beaker, covered with hexane, and left for 72 hours. Then the hexane extract was removed and stored in a closed container. This operation was repeated two more times (3 times in total). The resulting extract was concentrated with a vacuum distillation device and hexane was separated from the extract. The temperature used was up to 40-47 ºC and the rotation speed was 60-70 rpm. The resulting extract was dehydrated by sodium sulfate.

2.4. Measurement of total phenol

The amount of total phenol in the extracts was analyzed using the Folin–Ciocalteu reagent. After preparing the extracts and performing the relevant calculations, standards (20, 40, 60, 80,100,120, and 140 ml) were prepared. First, up to 0.5 ml of each of the standards and extracts, 5 ml of Folin–Ciocalteu (10:1), and 4 ml of 7.5% sodium carbonate were added. After 15 minutes, absorbance was measured at 765 nm by the device (UV/vis 2800 spectrometer). The standard curve was drawn in terms of Gallic acid with different concentrations and the amount of plant phenolic compounds equivalent to Gallic acid was measured in mg per gram of dry plant powder. The equation of the regression line that shows the relationship between the concentration of Gallic acid and the absorption of solutions at a wavelength of 765 nm is as follows. In this relation, Y is the amount of absorption and X is the amount of phenolic compounds based on the equivalent of Gallic acid [16].

$$Y=0.0005X+0.016 \ (R^2= 0.998)$$

2.5. Measurement of total flavonoid

To calculate the flavonoid content of 0.5 ml of the extracts with 1.5 ml of solutions, 1 ml aluminum chloride 10% (10 grams of aluminum chloride in 100 ml of solutions), 0.1 ml of one molar potassium acetate (2.41 grams in 10 ml of distilled water) and 2.8 ml of distilled water were mixed. To prepare the control, the pure solution was replaced by extracts. The resulting solution was placed in the dark for 30 minutes and read at a wavelength of 415 nm by a spectrophotometer (UV/vis spectrometer 2800). To draw a standard curve of concentrations, different standard quercetin (10, 50, 100, 150, and 200 mg/ml) were used, and the curve was drawn with Excel. Then the equation of the y=bx+a obtained absorptions read from samples [17].

2.6. Anthocyanin measurement

0.1 g of plant tissue was completely ground in a Chinese mortar with 10 ml of acidic methanol (pure methanol and pure hydrochloric acid in a volume ratio of 1:99). The extract was poured into a screw-head test tube and kept in the dark for 24 hours at a temperature of 25 ºC. Then it was centrifuged for 10 minutes at a speed of 4000 revolutions per minute. The supernatant was absorbed at a wavelength of 550 nm. The extinction coefficient of 33000 cm/mole and the results were presented in micromoles per gram of dry weight.

2.7. Statistical Analysis

The method of analysis of variance (ANOVA) was applied. Statistical analysis was done using SPSS 18 software, and graphs were prepared with Excel. All experiments were performed with three repetitions.

3. Results

As demonstrated in Table 1 and Figure 1, the highest amount of flavonoid was related to the methanolic extract (22.94±0.73 mg/g) followed by the ethanolic extract (13.57±0.16 mg/g). The lowest amount of this substance was recorded from the aqueous extract (6.89±0.33 mg/g). The results of phytochemical evaluation revealed that high amounts of secondary compounds in inhibiting free radicals, in addition to confirming the health benefits of this plant as a natural emollient and anti-inflammatory, can be used in the production of effective medicinal products, and since in plants, phenolic compounds and flavonoids always act as scavengers of free radicals and hydrogen donors, performing as effective antioxidants.

Table 1. Comparison of some phytochemicals of *G. platycarpum* extracts (mg g^{-1})

Extracts	Flavonoids	Anthocyanin	Phenol
EE	13.57 ± 0.16^{b}	3.40 ± 0.12^{a}	4.72 ± 0.49
ME	22.94 ± 0.73^{a}	1.64 ± 0.23^{bc}	4.45 ± 0.07
AE	6.89 ± 0.33^{d}	1.99 ± 0.12^{b}	4.34 ± 0.27

HE	12.04±0.35^{c}	1.57±0.10^{c}	3.21±0.17
P-value	0.000	0.000	0.350

EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract, HE: n-Hexane extract

The use of different solvents for extracting had no significant effect on the amount of total phenol, but numerically, the lowest amount of phenol (3.21±0.17 mg/g) was related to n-Hexane extract (Table 1 and Figure 1).

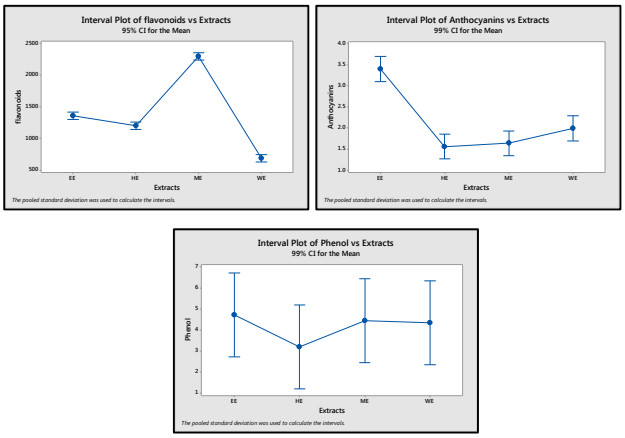

Figure 1. Some phytochemicals interval plot of *G. platycarpum* extracts

The highest amount of Anthocyanin (3.40±0.12 mg/g) was in the ethanolic extract and the lowest amount was related to the n-Hexane extract (1.57±0.10 mg/g).

Table 2 illustrates the correlation between phytochemical compounds. The highest positive correlation was obtained between anthocyanin and phenol (0.408), but this value was not statistically significant. The non-significance of correlation is probably due to the small number of data.

Table 2. Pearson correlation of some phytochemicals of *G. platycarpum* extracts

	Flavonoids	**Anthocyanin**
Anthocyanin	-0.158 (0.623)	
Phenol	0.141 (0.663)	0.408 (0.188)

Number in parentheses is P-value.

4. Discussion

Recently, due to serious concerns about the carcinogenic potential of synthetic antioxidants that have been widely used, there is a great interest in finding new and safe antioxidants from natural sources. Plants have received much attention as sources of biologically active substances, including antioxidants and anticancer agents. Scientific information about the antioxidant properties of different plants, especially those that are less used in food and medicine, is still lacking. Therefore, evaluating such properties is an interesting and useful task, especially for finding new sources of natural antioxidants, functional foods, and food ingredients [18].

Anthocyanins belong to the broad group of phenolic compounds that are collectively called flavonoids. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylylium salts. Anthocyanin as well as other phenolic compounds can act as antioxidants by donating hydrogen to highly reactive radicals and preventing the production of more oxidation products. The consumption of anthocyanins has been calculated to be 9 times more than other food flavonoids in the diet [19, 20]. Today, consumers are very concerned about the use of chemical preservatives in foods. Therefore, they tend to use safe and natural food products.

In this study, solvents such as water, ethanol, methanol, and hexane were used. The results showed that the choice of solvent significantly affected the extraction performance. The results showed that the water/methanol mixture had the highest extraction performance. However, this solvent is expensive, not safe, and requires more caution to be used as a solvent. Malviya et al. [21] reported the highest performance with a water/ethanol mixture (50:50) and the lowest performance with water. For this reason, some authors recommend that despite its safety and low price, water is not suitable for extracting phenolic compounds [22]. Recently, researchers estimated that the mixture of ethanol: acidic water with acetic acid increases the extraction yield. The use of solvents together increases the extraction performance of phenols and antioxidant molecules [23].

5. Conclusion

Considering the importance of anthocyanin and flavonoid compounds in terms of food color and antioxidant activity, as well as for the optimal use of food industry waste, in this research, the extraction of anthocyanin and flavonoid compounds from mountain dill was done with the help of different solvents. The results showed that the extraction by alcohol solvent method has higher extraction efficiency and anthocyanin content than water and n-hexane solvent extracts. While the content of phenolic compounds in different solvents did not show any significant difference. The type of solvent has a great effect on the anthocyanin and flavonoid content, which has a significant role in the color stability and antioxidant activity of the extract.

Conflicts of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper's content.

References

1. Rosen MA. Issues, concepts and applications for sustainability. Glocalism: Journal of Culture, Politics and Innovation. 2018;3:1-21.
2. American Diabetes Association Professional Practice Committee. 1. Improving care and promoting health in populations: standards of medical care in diabetes—2022. Diabetes Care. 2022 Jan 1;45(Supplement_1):S8-16. https://doi.org/10.2337/dc22-S001
3. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial

therapeutics: potential avenues of biocompatible drug discovery. Metabolites. 2019 Nov 1;9(11):258. https://doi.org/10.3390/metabo9110258

4. Tiwari P. Recent trends in therapeutic approaches for diabetes management: a comprehensive update. Journal of diabetes research. 2015 Aug;1-11. https://doi.org/10.1155/2015/340838
5. Asaah EK, Tchoundjeu Z, Leakey RR, Takousting B, Njong J, Edang I. Trees, agroforestry and multifunctional agriculture in Cameroon. International journal of agricultural sustainability. 2011 Feb 1;9(1):110-9. https://doi.org/10.3763/ijas.2010.0553
6. Heshmati GA. Vegetation characteristics of four ecological zones of Iran. International Journal of plant production. 2007 Mar;1(2):25-24.
7. Husain GM, Khan MA, Urooj M, Kazmi MH. Pharmacodynamic Evaluation: Herbal Medicine. Drug Discovery and Evaluation: Methods in Clinical Pharmacology. 2020:483-97. https://doi.org/10.1007/978-3-319-68864-0_52
8. Patridge E, Gareiss P, Kinch MS, Hoyer D. An analysis of FDA-approved drugs: natural products and their derivatives. Drug discovery today. 2016 Feb 1;21(2):204-7. https://doi.org/10.1016/j.drudis.2015.01.009
9. Wemrell M, Merlo J, Mulinari S, Hornborg AC. Two-thirds of survey respondents in southern Sweden used complementary or alternative medicine in 2015. Complementary medicine research. 2017 May 23;24(5):302-9. https://doi.org/10.1159/000464442
10. Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. Journal of traditional and complementary medicine. 2017 Apr 1;7(2):234-44. https://doi.org/10.1016/j.jtcme.2016.05.006
11. Talebi, E., Haghighat Jahromi, M., Khosravi Nezhad, M., Rowghani Haghighi Fard, E. Herbal plants as an appropriate stimulus with prophylactic potential in livestock: A review. Safe Future and Agricultural Research Journal (SFARJ), 2022; 1(1): 11-19. https://doi.org/10.22034/sfar.2022.158473
12. Babashpour-Asl M, Piryaei M. Analysis of *Grammosciadium platycarpum* Boiss. & Hausskn. Essential Oil Using of Three Dimensional Nanocomposite Based on MgAl Layered Double Hydroxide as a Fiber Coating of SPME. Journal of Medicinal plants and By-product. 2021 Sep 1;10(2):161-7. https://doi.org/10.22092/jmpb.2020.351310.1237
13. Nickavar B, Kamalinejad M, Mohandesi S. Comparison of the components of the essential oils from leaves and fruits of *Grammosciadium platycarpum*. Chemistry of natural compounds. 2006 Nov;42:686-8. https://doi.org/10.1007/s10600-006-0252-x
14. Yüce E, Paksoy MY, Bagci E. Essential oil composition of two Grammosciadium DC Species, *G. platycarpum* (Boiss et Hausskn) Schischk and *G. macrodon* Boiss (Apiaceae), from Turkey. Tropical Journal of Pharmaceutical Research. 2016 Mar 4;15(2):411-4. https://doi.org/10.4314/tjpr.v15i2.26
15. Poursafavi Z, Tabaei SJ, Kheirandish F, Mohebali M, Kamalinezhad M, Vajdian R. Effect of olive leaf aqueous and hydroalcoholic extract on Leishmania tropica Glucantime resistance and sensitivity *in vitro*. Pejouhesh dar Pezeshki (Research in Medicine). 2018 Mar 10;42(1):46-52.
16. Talebi, E., Rowghani Haghighi Fard, E., Navabi, M., Eatemadi, M. Evaluating the Effect of Two Types of Thyme Essential Oils (*Zataria Multiflora* & *Ziziphora Clinopodioides* Lam) on Some Productive Traits and Blood Parameters in Broilers. Poultry Science Journal, 2021; 9(1): 107-119. https://doi.org/10.22069/psj.2021.18831.1668
17. Nemati Z, Barzegar R, Khosravinezhad M, Talebi E, Safaei HR. Chemical composition and antioxidant activity of Shirazi *Thymus vulgaris* essential oil. Future Natural Products. 2018 Apr 1;4(2):26-32.
18. Arabshahi-Delouee S, Urooj A. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. Food chemistry. 2007 Jan 1;102(4):1233-40. https://doi.org/10.1016/j.foodchem.2006.07.013
19. Assous MT, Abdel-Hady MM, Medany GM. Evaluation of red pigment extracted from purple carrots and its utilization

as antioxidant and natural food colorants. Annals of Agricultural Sciences. 2014 Jun 1;59(1):1-7. https://doi.org/10.1016/j.aoas.2014.06.001

20. Wallace TC. Anthocyanins in cardiovascular disease. Advances in nutrition. 2011 Jan 1;2(1):1-7. https://doi.org/10.3945/an.110.000042
21. Malviya S, Arvind, Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. Journal of food science and technology. 2014 Dec;51:4132-7. https://doi.org/10.1007/s13197-013-0956-4
22. Masci A, Coccia A, Lendaro E, Mosca L, Paolicelli P, Cesa S. Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction. Food chemistry. 2016 Jul 1;202:59-69. https://doi.org/10.1016/j.foodchem.2016.01.106
23. Rezaei-Saraji M, Ghorbani M, Mahoonak AS, Tabarestani HS, Ghasemnezhad A. Extraction of phenolic and flavonoid compounds of *Grammosciadium platycarpum* Boiss. & Hausskn extract and their microencapsulation in alginate hydrogel. Food Processing and Preservation Journal, 2022 14 4: 91-109.