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PHYTOCHEMICAL PROFILE, ANTIOXIDANT ACTIVITY AND HPTLC FINGERPRINTING OF *GERANIUM ROBERTIANUM* L. EXTRACTS

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ABSTRACT. The present study evaluated the phytochemical composition and antioxidant activity of some hydroalcoholic extracts of *Geranium robertianum*. Total polyphenol content (TPC) and total flavonoid content (TFC) were estimated using colorimetric methods, while antioxidant activity was evaluated by DPPH and ABTS assays. High performance thin layer chromatography (HPTLC) was performed to obtain the fingerprints of samples and for preliminary investigation of the presence of isoflavonoids in their composition. The leaf extract exhibited the highest TPC (9.47 ± 0.66 mg gallic acid equivalents/mL), TFC (736.66 ± 9.38 mg rutin equivalents/mL), and showed the strongest antioxidant activity in both DPPH (12.55 ± 1.01 mg vitamin C equivalents/mL) and ABTS (5.63 ± 0.34 µmol Trolox equivalents/mL) assays. Flowers extract demonstrated considerable levels of phenolic compounds and antioxidant activity. The chromatographic fingerprints are characteristic of each analyzed part of the plant. The extracts purchased from the market have fingerprints different from those prepared in the laboratory. Also, the obtained fingerprints highlight the possible presence of isoflavonoids in the composition of the analyzed extracts. These findings demonstrate that leaves and flowers of *Geranium robertianum* represent valuable source of natural antioxidants and provide new information regarding the phytochemical characteristics of commercial Romanian preparations.

Keywords: *polyphenolic compounds, flavonoid, antioxidant activity, HPTLC fingerprinting, isoflavonoid*

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INTRODUCTION

Plants are the main tool used in traditional medicine for the treatment of diseases [1], this being reported since antiquity, until the 19th century when they were replaced in medical treatments by the synthetic compounds [2]. Recently, plants regain attention due to their phytochemistry based on compounds with biological activity and their medical effects [3]; in 2010 the World Health Organization (WHO) estimated that approximately 80% of the world's population uses traditional herbal products in primary health care [4].

The *Geraniaceae* family comprises six genera of medicinal and ornamental plants, including *Geranium, Pelargonium, California, Monsonia, Hypseocharis,* and *Erodium*, which are cultivated or grow naturally in the wild flora [5]. Species belonging to the *Geraniaceae* are recognized for their biological activities such as antioxidant (*Geranium robertianum*) [2], antiviral, antiasthmatic (*Pelargonium sidoides, Geranium sanguineum*) [6], antiallergic *(Pelargonium graveolens*) [7], antitumor (*Geranium thunbergii*, *Geranium robertianum*) [5].

Geranium robertianum L. (*G. robertianum*), commonly known as herb-Robert or “năpraznic”, is a herbaceous species native to Europe, where it occurs spontaneously in forest ecosystems, forest edges, rocky slopes, and other shaded or semi-shaded habitats [8, 9]. Due to its wide ecological adaptability, the species is well distributed across temperate regions [2, 10].

From a botanical point of view, *G. robertianum* is a plant characterized by a fibrous rooting system. The stems branched in many directions from the base, being long, thin and fragile [8]. Their colour varies from all green to a mixture between red and green [2]. The leaves are usually greener with a triangular shape divided into three segments [2, 11]. At the time of flowering, the plant can have two to four flowers of five rounded petals arranged radially around the superior ovary and colored in various shades of pink and purple [11].

Traditionally, *G. robertianum* has been used in folk medicine for the treatment of inflammatory conditions, skin disorders, gastrointestinal complaints, and wound healing [2, 12]. These ethnopharmacological uses are supported by its rich phytochemical composition, which includes hydrolysable tannins (notably ellagitannins such as geraniin), flavonoids (rutin, quercetin and kaempferol derivatives) [13], and phenolic acids (ellagic acid, caffeic acid, gallic acid, and ferulic acid) [13, 14], considered to be responsible for its biological activities [2, 7].

Different products based on *G. robertianum* therapeutic effects could be purchased from market as food supplements [9]. The method of administration can be internal or external, depending on the form of the preparation and the purpose of use [10], but once administered, every kind of product can serve in a different way depending on the part of the plant used in preparation [2]. For example, *G. robertianum* leaves exert anti-inflammatory, antioxidant, antidiabetic and antirheumatic effects [9], the aerial parts are recommended in cancer, diabetes and for stopping nosebleeds [2]. It is worth mentioning that, most often, extracts from the leaves and aerial parts of *G. robertianum*, but not from the flowers, have been characterized in terms of their constituents and biological activity.

Although *G. robertianum* is recommended to be used in hormonal deficiencies, in male and female sterility, frigidity, impotence, uterine bleeding of various etiologies, dysmenorrhea, etc. [15], no scientifically documented studies to support these actions have been published yet. Similar effects were also described for red clover (*Trifolium pratense*) and kudzu (*Pueraria lobada*) and they were associated with their isoflavonoid constituents which extert estrogenic activity [16].

This study aimed to provide a comparative phytochemical and antioxidant characterization of extracts obtained from different parts of plant of *G. robertianum* and also of some commercialy available extracts from Romanian market. In addition, HPTLC fingerprinting was performed to compare their chromatographic profiles and to preliminary investigate the presence of izoflavonoid constituents.

RESULTS AND DISCUSSION

Hydroalcoholic extracts prepared from flowers, leaves, stems and aerial parts of *G. robertianum* were comparatively evaluated in terms of total polyphenol (TPC) and flavonoid (TFC) content and antioxidant activity (AA), as well as chromatographic fingerprints, with extracts of aerial parts of the plants commercially available on the Romanian market, from four different manufacturers (A-D).

Therefore, TPC, TFC and AA of extracts were determined spectrophotometrically, and the results obtained are presented in **Table 1**. AA was evaluated *in vitro* using two free radicals, namely DPPH and $ABTS^{+\bullet}$.

Table 1. TPC, TFC and AA of *G. robertianum* extracts

Extract	TPC (mg GAE/mL)	TFC (µg RE/mL)	AA_{DPPH} (mg vit CE/mL)	AA_{ABTS} (µmol TroloxE/mL)
Flowers	8.91 ± 0.62	517.54 ± 8.15	10.97 ± 0.81	5.16 ± 0.25
Leaves	9.47 ± 0.66	736.66 ± 9.38	12.55 ± 1.01	5.63 ± 0.34
Stems	6.08 ± 0.34	358.02 ± 6.49	5.32 ± 0.30	2.39 ± 0.09
Aerial parts	9.33 ± 0.71	533.16 ± 8.74	11.24 ± 0.95	5.24 ± 0.33
A	8.76 ± 0.55	411.59 ± 7.21	10.57 ± 0.75	4.75 ± 0.29
B	7.14 ± 0.40	333.81 ± 6.75	8.65 ± 0.66	3.98 ± 0.15
C	6.22 ± 0.41	211.71 ± 5.34	7.94 ± 0.47	3.38 ± 0.11
D	5.31 ± 0.29	191.95 ± 5.11	6.78 ± 0.45	2.45 ± 0.08

The results are presented as mean ± standard deviations of three replicates
GAE-gallic acid equivalents, RE- rutin equivalents, vit CE- vitamin C equivalents

Total phenolic content

The TPC values of the analyzed samples ranged from 5.31 ± 0.29 to 9.47 ± 0.66 mg GAE/mL extract. Among the laboratory-prepared extracts, the leaf extract had the highest polyphenol content (9.47 ± 0.66 mg GAE/mL), followed by the aerial parts extract (9.33 ± 0.71 mg GAE/mL) The stem extract showed the lowest TPC value (6.08 ± 0.34 mg GAE/mL). Flowers also contain high amounts of phenolic compounds (8.91 ± 0.62 mg GAE/mL), contributing together with leaves to the high TPC level of extract of aerial parts of geranium. The commercial extracts exhibited lower and more variable values, ranging between 5.31 ± 0.29 and 8.76 ± 0.55 mg GAE/mL, which may be attributed to differences in raw material quality and extraction procedures, including solvent.

Total flavonoid content

A similar trend was observed for TFC, which varied between 191.95 ± 5.11 and 736.66 ± 9.38 mg RE/mL extract. The highest flavonoid content was determined in the leaf extract (736.66 ± 9.38 mg RE/mL), followed by the aerial parts extract (533.16 ± 8.74 mg RE/mL) and the flower extract (517.54 ± 8.15 mg RE/mL). The stem extract contained considerably lower flavonoid levels (358.02 ± 6.49 mg RE/mL), while commercial products showed the lowest and most variable values of TFC.

Antioxidant activity evaluation

The antioxidant activity measured by DPPH and ABTS assays followed the same general pattern as that observed for TPC and TFC. The leaf extract exhibited the highest antioxidant activity, reaching 12.55 ± 1.01 mg vit CE/mL (DPPH) and 5.63 ± 0.34 µmol TroloxE/mL (ABTS), respectively. Flower and aerial parts extracts also showed strong antioxidant activity, whereas stem extracts and several commercial products exhibited lower values. These results suggest that phenolic compounds, particularly flavonoids, are major contributors to the antioxidant capacity of *G. robertianum* extracts.

Pearson's correlation coefficients (r) were also calculated to evaluate relationships between TPC, TFC and AA determined by both methods. Statistical significance was set at $p < 0.05$. Pearson correlation analysis revealed strong positive relationships between TPC and AA_{DPPH} ($r = 0.942$, $p < 0.001$) and AA_{ABTS} ($r = 0.974$, $p < 0.001$), while TFC was also significantly correlated with AA_{DPPH} ($r = 0.814$, $p = 0.014$) and AA_{ABTS} ($r = 0.841$, $p = 0.009$). In addition, a strong correlation was observed between TPC and TFC ($r = 0.884$, $p = 0.004$), confirming the contribution of flavonoids to the total phenolic content and to the antioxidant activity of extracts.

The present findings are consistent with previous studies reporting *G. robertianum* as a rich source of phenolic compounds with strong antioxidant properties [2, 11, 17]. Literature data indicate that leaves generally contain higher levels of phenolic compounds than stems, which agrees with the trends observed in the present study [13, 17, 18]. The higher accumulation of bioactive compounds in leaves can be explained by their metabolic role and exposure to environmental stress factors such as UV radiation and oxidative stress. Also, the lower values of TPC, TFC and antioxidant activity of stem extracts agree with previous observations, showing reduced accumulation of phenolic compounds in supporting tissues [19].

In addition, extraction solvent and methodology are known to significantly influence phenolic yield and antioxidant activity. This may explain the variability observed among commercial products, which likely reflects differences in extraction procedures, solvent composition, plant material quality, and storage conditions [2].

An important contribution of this study is the evaluation of the flower extract of *G. robertianum*. To our knowledge, no previous study has reported the TPC, TFC and AA of extracts prepared exclusively from the flowers of this species, although the literature has mentioned that the infusion of flowers or leaves obtained from *G. robertianum* has been used for infertility problems [20]. In this regard, the label of each analyzed extract mentions that the product is also recommended for use as an adjuvant in sterility and sexual dynamics disorders in both sexes, in ovarian cyst cancer, polycystic ovaries, etc. These recommendations, as well as the high content of polyphenols (8.91 ± 0.62 mg GAE/mL) and flavonoids (517.54 ± 8.15 µg RE/mL) and the antioxidant activity determined in the analyzed extract, demonstrate that *G. robertianum* flowers could be a valuable source of bioactive compounds.

Also, this study provides the first comparative characterization of some commercially available *G. robertianum* hydroalcoholic extracts marketed in Romania. The considerable variability observed among products highlights the need for standardization and importance of quality control in herbal preparations.

HPTLC fingerprinting

High-performance thin layer chromatography (HPTLC) was used to obtain the phytochemical fingerprints of the investigated extracts of *G. robertianum*. Plants of the genus *Geranium*, including *G. robertianum*, have been frequently studied for the identification and determination of their bioactive constituents, but to our knowledge, the HPTLC fingerprinting of flowers, leaves, stems and hydroalcoholic extracts available on the Romanian market has not been reported. However, a study in which the chromatographic fingerprinting of polyphenols from alcoholic extracts obtained from aerial parts of four wild-grown *Geranium* species (*G. dissectum*, *G. lucidum*, *G. pusillum*, and *G. robertianum*) from Romania was reported [12]. Therefore, hydroalcoholic extracts from flowers, leaves, stems and aerial parts of the plant, as well as four extracts from aerial parts of the plant obtained from four different producers in Romania, were fingerprinted by HPTLC.

The proposed method considered not only the fingerprinting of *G. robertianum* extracts in terms of separation of polyphenols, but also the attempt to identify some of the most common compounds from the isoflavone class (genistin, daidzin, genistein and daidzein), as they are known to act as phytoestrogens. The idea that *G. robertianum* might contain polyphenols from the phytoestrogen class came from the recommendations for the use of plant extracts, mentioned by manufacturers on the products analyzed (Table 3), but not yet scientifically supported.

Seven mobile phase systems (Table 2) were tested to obtain a good chromatographic separation of the compounds of interest. The best separation was obtained with mobile phase 4, chloroform-ethyl acetate-methanol-water, 4:2:2.2:1 (*v/v*). This was then optimized to ensure the good separation of the constituents from the *G. robertianum* extracts, so that the chromatographic separations were further performed with the solvent mixture consisting of chloroform-ethyl acetate-methanol-water, 1:2:1.2:0.4 (*v/v*).

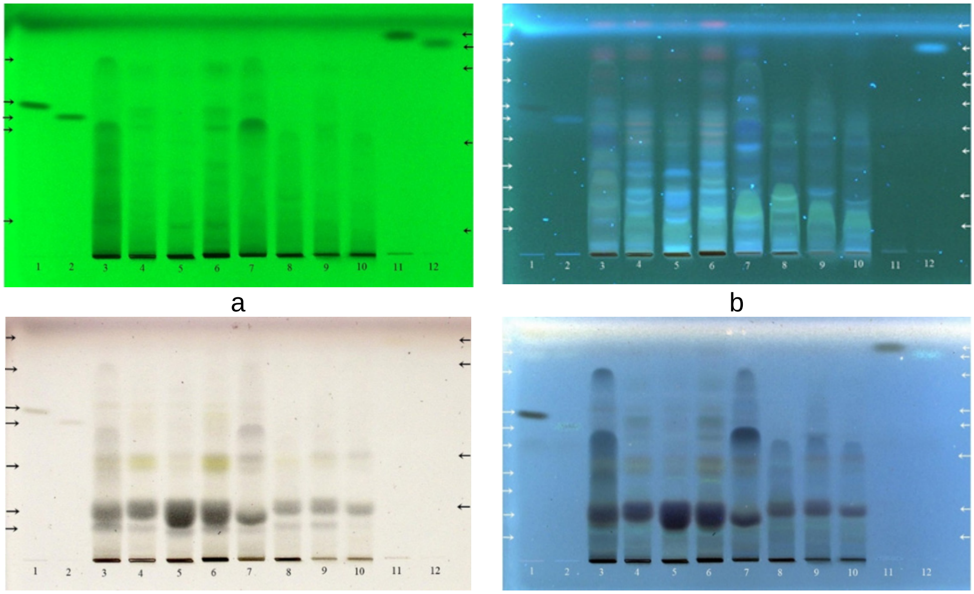
Table 2. The composition of the tested mobile phases

No.	Mobile phase composition	Ref.
1	Chloroform-methanol, 19:1 (*v/v*)	[21]
2	Chloroform-methanol-water, 9:1:0.1 (*v/v*)	[22]
3	Chloroform-methanol-water-acetic acid, 60:30:10:0.5 (*v/v*)	[22]
4	Chloroform-ethyl acetate-methanol-water, 4:2:2.2:1 (*v/v*)	[23]
5	Ethyl acetate-butanone-formic acid-water, 5:3:1:1 (*v/v*)	[24]
6	Ethyl acetate-methanol-acetic acid-water, 60:30:3:0.5 (*v/v*)	[25]
7	Benzene-ethyl acetate-formic acid, 10:3:1 (*v/v*)	[26]

The image of the plate under UV light at 245 nm (Figure 1a) shows that the extracts prepared in the laboratory from the leaves and aerial parts of the plant have similar chromatographic fingerprints, the intensity of the bands characteristic of the separated compounds being higher in the aerial parts extract. Moreover, the fingerprint of the aerial parts extract highlights two compounds contained only in the flower and stem extracts, separated in the lower half of the plate. The chromatographic fingerprint of the flower extract differs greatly from that of the leaf and stem extracts, but seems to be very similar to that of the extract from producer A, highlighting in both samples the presence of a compound in high concentration that appears separately in the middle area of the plate and which could be contained in lower concentration in the extracts from leaves and aerial parts, as well as in the extract from producer C. Also, the fingerprint of extract of the aerial parts prepared in the laboratory is very different from those of the samples from producers A, B and D.

Seven mobile phase systems (Table 2) were tested to obtain a good chromatographic separation of the compounds of interest. The best separation was obtained with mobile phase 4, chloroform-ethyl acetate-methanol-water, 4:2:2.2:1 (*v/v*). This was then optimized to ensure the good separation of the constituents from the *G. robertianum* extracts, so that the chromatographic separations were further performed with the solvent mixture consisting of chloroform-ethyl acetate-methanol-water, 1:2:1.2:0.4 (*v/v*).

The fluorescence detection (366 nm) before derivatization (Figure 1b) provided additional important information about some constituents useful for evaluation and authentication of closely related samples. Thus, the images obtained at 366 nm confirm that the chromatographic fingerprints of the leaf and aerial part extracts are very similar, also highlighting in the middle of the plate a succession of three light orange and orange-pink zones characteristic of compounds found in both samples, but also in the stem extract in a much lower concentration. The flower extract has a different fingerprint from all the other samples, highlighting separate characteristic compounds in the lower half of the plate. It can also be clearly seen that in the middle part of the plate there is a blue zone characteristic of a compound contained in high concentration in both the flower extract and the extract from producer A. This is not found in the leaf extract nor in the aerial parts extract prepared in the laboratory. The print of the extract from the stems is different from that of the leaves and stems, highlighting the presence of characteristic light blue bands separated in the lower third of the plate. Extracts from producers B, C and D have very similar fingerprints, showing few separated constituents, the difference between them being mainly given by the intensity of the separated bands. This justifies the lower TPC, TFC and AA values of these samples compared to those prepared in the laboratory and that from producer A.



a b

c d

Figure 1. HPTLC fingerprints of samples on silica gel $60F_{254}$ with chloroform-ethyl acetate-methanol-water,1:2:1.2:0.4(*v/v*) as mobile phase. a-UV 254nm, b-UV 366nm, c-Derivatization with 10% H_2SO_4 in ethanol, white light, d-Derivatization with 10% H_2SO_4 in ethanol UV 366nm. Tracks: 1-genistin, 2-daidzin, 3-flowers, 4-leaves, 5-stem, 6-aerial parts, 7-A, 8-B, 9-C, 10-D, 11-genistein, 12-daidzein

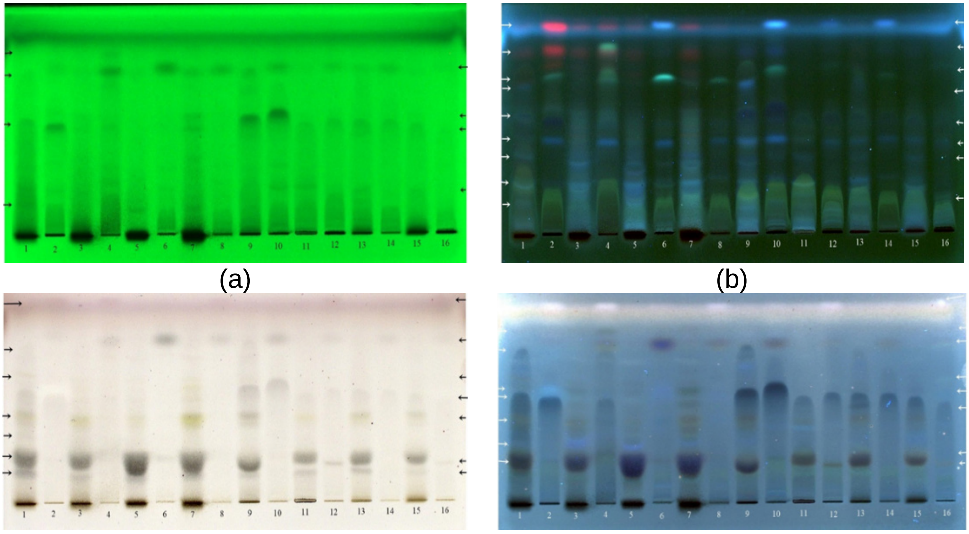
The images of the plate in white light and at 366 nm after derivatization support the information provided in the other detection modes. It can also be seen that there are two zones, one at the bottom of the plate (gray in white light and light green at 366 nm) and another in the middle (colored in gray-yellow in white light and light brown at 366 nm) that are found in the chromatographic fingerprints of all the analyzed samples.

Another aim of HPTLC fingerprinting was to check if the analyzed samples contain isoflavone. Therefore, some standards of isoflavone were analyzed together with *G. roberianum* extracts. The image of the plate under UV light at 254 nm before derivatization (Figure 1a), highlights the separation of the isoflavonoid standards at values of $R_{f\ daidzin}$ = 0.54, $R_{f\ genistin}$= 0.58, $R_{f\ daidzein}$ = 0.82 $R_{f\ genistein}$ = 0.86. Comparing the chromatographic fingerprints of the analyzed samples, it can be seen that the leaf extract and the aerial part extract show a compound separated at R_f = 0.54 which could be daidzin, the intensity of its characteristic band being higher in the aerial part extract. Also, in the fingerprint of extract A, a band at R_f = 0.58 can be observed that could be attributed to genistin. Moreover, the compound separated at R_f = 0.54 appears as light blue zones in leaf and aerial extracts when the plates were documented at 366 nm, similar to the characteristic band of the daidzin (Figure 1b). Derivatization with 10% H_2SO_4 solution in ethanol allows genistin to be visualized in white light (Figure 1c) as a yellow-brown colored zone, the characteristic shade of isoflavones with a -OH group in position 5. Daidzin appears as a gray-yellow zone, less intensely colored than genistin, due to the absence of the -OH group in position 5 from its structure. Thus, the fingerprints of the extracts from flowers, leaves, stems and aerial parts of the plant, as well as the extract from producer A, obtained in white light after derivatization, show the presence of zones at R_f =0.58, like that characteristic of genistin. Also, the images of the plates at 366 nm after derivatization (Figure 1d), confirm the presence of this compound in all the mentioned samples, but also in the extract from producer C. The image of the plate under white light after derivatization shows the presence of gray-yellow zones in the extracts from leaves and aerial parts, at R_f = 0.54, similar to daidzin. On the other hand, the image of the plate under UV light at 366 nm, after derivatization, does not clearly highlight the presence of this compound.

Regardless of how the chromatographic plate was documented, daidzein and genistein could not be identified in any of the analyzed samples. This may be because most isoflavones are present as glycosides in plants and also in hydroalcoholic extracts. Therefore, for the separation and identification of aglycones, the literature has described the need to perform an acidic hydrolysis step, followed by extraction in ethyl acetate [23].

The plate image at 254 nm (Figure 2a) obtained after the chromatographic separation of hydrolyzed extracts shows that in the fingerprints of hydrolyzed extracts of leaves and aerial parts of the plant the presence of a band at

R_f = 0.86, of weak intensity in the case of the aerial parts extract, which could be attributed to genistein. This observation is supported by the fact that in the fingerprints of the hydrolyzed extracts of flowers, leaves and aerial parts, after derivatization with 10% ethanolic solution of H_2SO_4, characteristic zones are observed at the same R_f, both in white light and at 366 nm. Also, in the chromatographic fingerprint of the hydrolyzed flower extract, at 366 nm after derivatization, the presence of a light blue zone at R_f = 0.54 which appears to be similar with daidzin can be observed. Furthermore, in the hydrolyzed extract from producer D, the presence of a light blue band at R_f = 0.82, similar to the characteristic band of daidzein, can be highlighted.



(a) (b)

(c) (d)

Figure 2. HPTLC fingerprints of samples on silica gel 60F254 with chloroform-ethyl acetate-methanol-water,1:2:1.2:0.4(v/v) as mobile phase. a-UV 254nm, b-UV 366nm, c-Derivatization with 10% H_2SO_4 in ethanol, white light, d-Derivatization with 10% H_2SO_4 in ethanol UV 366nm. Tracks: 1-flowers, 2-hydrolyzed flowers, 3-leaves, 4- hydrolyzed leaves, 5-stem, 6-hydrolyzed stem, 7-aerial parts, 8- hydrolyzed aerial parts, 9-A, 10- hydrolyzed A, 11-B, 12- hydrolyzed B, 13-C, 14- hydrolyzed C, 15-D, 16-hydrolyzed D.

The results obtained suggest the possible presence of the investigated isoflavones in the analyzed *G. robertianum* extracts. However, further analysis using more selective techniques would be required for clear identification and quantification of these compounds.

CONCLUSIONS

G. robertianum extracts showed high levels of phenolic compounds and significant antioxidant activity, particularly in leaves and flowers. Strong positive correlations between phenolic content and antioxidant activity confirmed the important contribution of polyphenols and flavonoids to the antioxidant potential of the investigated extracts. Considerable differences were observed among the commercial hydroalcoholic preparations, highlighting the influence of extraction and processing conditions on phytochemical composition and emphasizing the need for quality control and standardization.

HPTLC fingerprinting of extracts revealed distinct phytochemical profiles and suggested the possible occurrence of isoflavonoid compounds. However, further studies using more selective analytical techniques are required for definitive identification. The results provide new information regarding flower extracts and commercial Romanian preparations, contributing to a better understanding of the phytochemical variability of *G. robertianum* and supporting its potential use as a natural source of bioactive compounds.

EXPERIMENTAL SECTION

Chemicals

All reagents used in the study were of analytical grade. Ethanol 96%, 1,1-diphenyl-2-picrylhydrazyl radical, (DPPH•), potassium persulfate ($K_2S_2O_8$), 2,2 ′-azino-bis(3-ethylbenzothiazoline-6- sulfonic acid) ($ABTS^+$), 6-hydroxy2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ascorbic acid (vitamin C), gallic acid (GA), rutin, aluminum chloride ($AlCl_3$), Folin-Ciocalteau reagent 2N, sodium acetate (CH_3COONa), sodium carbonate (Na_2CO_3), sulfuric acid 95-98%, hydrochloric acid were purchased from Merck (Darmstadt, Germany). The solvents used for the chromatographic analysis were ethyl acetate, acetic acid, formic acid, butanone, benzene and methanol, and were purchased from Millipore (Bedford, USA).

Materials and sample preparation

The plant material used in this investigation was collected in June 2021, from Strâmtura (47.778890N, 24.144720E), Maramureş County, Romania. The aerial parts of the plants were collected during the flowering period, and after drying in a dark, well-ventilated space at room temperature, samples of entire aerial parts, flowers, leaves and stems were separated and grounded.

Also, four *G. robertianum* food supplements available on the market in Romania, produced by Faunus Plant (A), Farmanat Poieni (B), Aroma plant Bonchiş (C) and Dorel Plant (D) were purchased. These are hydroalcoholic extracts from aerial parts of plant, and their characteristics, according to the manufacturers' labels, are presented in Table 3.

Although the Romanian Pharmacopoeia recommends the extraction of polyphenolic compounds in ethanol-water 7:3 (v/v), in this study an extraction system consisting of ethanol-water, 1:2.5 (v/v), similar to those used by the producers of extracts A-D (Table 3) was chosen. The extraction of bioactive compounds was done by maceration, using 1 g of dried grounded plant material and 10 mL of ethanol-water, 1:2.5 (*v/v*). After maceration at room temperature, in the dark for 10 days, the extracts were decanted and centrifuged for 15 min at 6000 rpm using a C2006 centrifuge (Centurion Scientific Limited, Bosham, UK). The resulting supernatants were collected and stored in dark bottles, at 4◦C until further analysis.

Table 3. Characteristics of analyzed *Geranium robertianum* food supplements

Extract	Ingredients	Administration	Recommendation
A	aerial parts of plant 20%, ethanol min. 30% *v/v*	internal use: 5 mL in 50ml water or tea, 3-4 times/day, on an empty stomach	male and female sterility, frigidity, impotence, uterine bleeding of various etiologies, dysmenorrhea, benign tumors of various locations, cancer (uterine, lung, intestinal, breast or other locations), diarrhea, intestinal inflammation, irradiation, sequelae after irradiation
B	whole plant, hydroalcoholic solution 34°	5 mL as it is or diluted in water or tea, 3-5 times/day	male and female sterility, frigidity, impotence, low immunity, menstrual cycle disorders, cysts and uterine bleeding of various etiologies, dysmenorrhea, benign tumors of various locations, cancer (uterine, lung, intestinal, breast or other locations), diarrhea, intestinal inflammation, chronic fatigue
C	aerial part plant 26%, water 50%, grain alcohol (96%)- 24%.	5 mL, 3 times/day, 30 min before main meals, on an empty stomach	male and female sterility, frigidity impotence, uterine bleeding of various causes, benign tumors of various locations, dysmenorrhea intestinal inflammation, diarrhea, uterine fibroids, ovarian cysts, supporting the body after irradiation
D	aerial parts of plant – 20%; hydroalcoholic solution (96° alcohol: water 1:2)– 80%	5 mL diluted in tea, 3 times/day, 30 min before main meals	breast, lung, ovarian, intestinal, gastric cancer, ovarian cysts, polycystic ovaries, hematuria, irradiation, sterility and sexual dynamics disorders in both sexes, low spermatogenesis and sperm motility, hemorrhagic, leukorrhea, chronic diarrhea, gastroenteritis, hemorrhagic enteritis, cystitis

Spectrophotometric Measurements

Spectrophotometric measurements were performed at room temperature, in triplicate using a T80+ UV-Vis spectrophotometer (PG Instruments, Lutterworth, UK).

Total Phenolic Content (TPC)

TPC was determined according to the Folin–Ciocalteu assay with slight modification [27]. Thus, 0.3 mL of each extract properly diluted were mixed with 1.5 mL Folin–Ciocalteu's reagent (0.2 N). After 5 min, 1.2 mL sodium carbonate (0.7 M) were added, and then all the mixtures were incubated for 2 h, at room temperature in dark conditions. The absorbance was measured at 760 nm and the TPC was calculated from the calibration curve ($y = 0.009x + 0.1247$, $R^2 = 0.9996$) obtained in the same conditions, using solutions of gallic acid with concentrations between 0 and 100 µg/mL. The results were expressed as mg gallic acid equivalents/mL of extract (mg GAE/mL).

Total flavonoids content (TFC)

TFC of was determined by a colorimetric method described in the Romanian Pharmacopoeia [28], using $AlCl_3$. Aliquots of 0.5 mL of each extract optimally diluted were mixed with 0.4 mL of 25 g/L $AlCl_3$ solution, 0.5 mL of 100 g/L CH_3COONa solution and 4 mL distilled water. After 15 min, the absorbance was measured at 430 nm and TFC was determined from the calibration curve of rutin ($y = 0.0063x +0.0084$, $R^2 = 0.9995$) obtained in a range of concentration of 0-120 µg/mL. TFC of extracts were expressed as µg rutin equivalents/mL (µg RE /mL).

Antioxidant activity (AA)

Antioxidant activity of geranium extracts was determined by DPPH assay [29] and ABTS assay [30], with some modifications.

DPPH assay

Aliquots of 0.25 mL of each extract properly diluted were added to 3.0 mL of 0.09 mg/mL solution of DPPH prepared in ethanol. After 20 min, the absorbance was measured at 517 nm. Also, for each analyzed sample, the absorbance of a mixture obtained from 0.25 mL of sample solvent and 3.0 mL of DPPH solution was measured in parallel, in the same conditions.

Both measured absorbance values were considered in the calculation of the antioxidant activity. The antioxidant activities of extracts were determined based on the calibration curve ($y = 1.4714x$, $R^2 = 0.9996$) obtained in the same conditions, using solutions of vitamin C with different concentrations between 0.150 and 0.275 mg/mL. The antioxidant activities (AA_{DPPH}) of samples were expressed as mg vitamin C equivalents/mL (mg vit CE/mL).

ABTS assay

First, the cationic radical $ABTS^{\bullet+}$ was generated from the reaction of 7 mmol/L ABTS diammonium salt solution with 2.45 mmol/L $K_2S_2O_8$. These solutions were mixed in a ratio of 1:1 (*v/v*) and incubated for 24 h at room temperature, in the dark. Then, $ABTS^{\bullet+}$ solution was freshly diluted so that their absorbance measured at 734 nm to be between 0.800 and 0.900. Mixtures obtained from 0.5 mL of each extract properly diluted and 3.0 mL $ABTS^{\bullet+}$ solution were incubated at room temperature in dark for 15 min and then the absorbance was measured at 734 nm. Also, for each analyzed sample, the absorbance of a mixture obtained from 0.3 mL of sample solvent and 3.0 mL of $ABTS^{\bullet+}$ solution was measured in parallel, in the same conditions. In the calculation of the antioxidant activity, both measured absorbance values were considered. The antioxidant activities were using the calibration curve ($y = 3.8759x + 0.1191$, $R^2 = 0.9998$) obtained with Trolox solutions in a range of 1.10-1.35 µmol/mL. The results (AA_{ABTS}) were expressed as Trolox equivalents (µmol TroloxE/mL).

Hydrolysis of extracts

Acidic hydrolysis of extracts was performed according to Chen et al. [23], with some modifications. Thus, aliquots of 1.0 mL of 0.1 M hydrochloric acid were added to 1.0 mL of each extract and the obtained mixtures were incubated for 2h at 96°C, using a water bath. The hydrolyzed extracts were then extracted twice with 1.0 mL portions ethyl acetate. The resulting ethyl acetate fractions were pooled and made up to 2.0 mL in a volumetric flask.

HPTLC analysis

Aliquots of 16 µL of each extract and 5 µL of each standard 0.1 mg/mL solutions of genistin, daidzin, genistein and daidzein were applied as 8 mm bands at 15 mm from the low edge of the plate with a flow rate of 40 nL/s using a semi-automatic application device Linomat 5 (CAMAG, Basel, Switzerland). The chromatographic analysis was performed on silica gel 60F_{254} HPTLC plates 20 cm × 10 cm, Merck (Darmstadt, Germany), using a mixture of chloroform-ethyl acetate-methanol-water, 2:4:2.4:0.8 (v/v) as the mobile phase. The plates were developed to a distance of 80 mm at room temperature in normal chromatographic twin trough chamber (CAMAG, Basel, Switzerland), pre-saturated for 30 min with the mobile phase. After development, the dried plates were sprayed with 10% sulphuric acid ethanolic solution, using an electro-pneumatic device TLC Sprayer (CAMAG, Basel, Switzerland). The plates were then heated at 105°C for 10 min using the TLC Plate Heater 3 (CAMAG, Basel, Switzerland). The detection was done in UV light at 254 and 366 nm before and after derivatization and in white light after derivatization. The documentation of the plates was performed using a TLC visualizer device Digistore 2 (CAMAG, Basel, Switzerland). All CAMAG devices were controlled by winCATs 1.4.3 software.

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