Saudi Journal of Biological Sciences 29 (2022) 3213–3222

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Biological investigations on macro-morphological characteristics, polyphenolic acids, antioxidant activity of *Perilla frutescens* (L) Britt. grown under open field

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ARTICLE INFO

Article history:
Received 23 July 2021
Revised 28 January 2022
Accepted 30 January 2022
Available online 5 February 2022

Keywords:
Perilla
Phytochemistry
Functional food
Spice
Morphology

ABSTRACT

Perilla frutescens, perilla is a functional food, spice and medicinal herb and ornamental plant in the family of Lamiaceae. Thus, macro-morphological characteristics, phenolic acids, antioxidants of twelve accessions of *P. frutescens* grown under open field were studied. High polymorphism was found among the perilla accessions and macroscopic features of perilla genotypes showed variable results. Perilla can be classified into two clearly phenotypes green and purple, within these two other colours were appeared. A good level of biomass production was recorded for JTD3, 203P, PS2, 203P respectively. Principal component analysis was performed to cluster phenolic acids. GB phenotype exhibited the major content of polyphenols, followed by JTD3 then J1. Regarding antioxidant capacity, JTD3 showed the highest value followed by 203P and GB respectively. The HPLC analysis showed that the most abundant phenolic acids were ellagic acid which is accumulated in a higher percentage in NP606, 588P and JTD3 cultivars respectively, followed by salicylic acid and gallic acid. This is the first report of cultivation of various Perilla varieties under open field environmental conditions, not only to increase productivity but also to improve the quality. Therefore, the present study results confirm the importance of the Perilla species for human consumption, therapeutic and ornamental purposes.

1. Introduction

Perilla frutescens is a functional food, spice and medicinal herb as well as ornamental plant in the family of Lamiaceae. It is a significant therapeutic plant that is generally appropriated in China, India, Korea, Japan, Thailand, Taiwan and Southeast Asia (Ahmed, 2019, Ahmed and Zubaidy, 2020, Gaihre et al., 2021). It is generally called perilla or by different names (perilla mint, Chines basil, Korean perilla, beefsteak plant, purple mint, zisu in China, shiso in Japan, and tia to in Vietnam) and one of the important economic herbs that have been cultivated for more than 2000 years (Asif, 2011, Ahmed, 2019). The main types of perilla are red (purple), green and red/green perilla phenotypes with the important aerial parts used and huge variations in the morphology and taxonomy (Banno et al., 2004, Ahmed, 2019). Perilla also has been considered to be both a nectariferous and a polliniferous plant (Consonni et al., 2013). In China, *P. frutescens* leaves have been broadly utilized as a culinary spice and flavor and food specialist because of the fragrant taste. Its new leaves are usually utilized for preparing pickles or as an enhancement for crude fish dishes in Japan. It is a mainstream verdant vegetable in Korea, which is by and largely eaten as a pickle or wrapping with broiled meats. The seeds are powdered and added to soup for flavoring in Korea (Huang et al., 2011, Li et al., 2017). The herb has been traditionally used in folk medicine for the treatment of colds, cough, asthma, vomiting, stomach disorders, depression, intoxications with seafood and allergic reactions (Fujiwara et al., 2018, Ahmed and Sarosi, 2019). The bioactivity of perilla is widely studied and found that it has antioxidant,

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.01.059
1319-562X/

antimicrobial, antidepressive, anxiolytic, chemopreventive and antitumor, anti-inflammatory activities (Banno et al., 2004, Osakabe et al., 2004, Park et al., 2010), anti-allergic effects (Yang et al., 2021), keratinocyte ageing prevention (Lee and Park, 2021), hyperpigmentation diseases of skin and ageing (Mungmai et al., 2020). A recent study, for the first time, indicates that perilla leaf extracts (PLE) are able to limit the replication of SARS-CoV-2 by disabling the virion. These results could motivate further research into the therapeutic utility of PLE for COVID-19 prevention or treatment (Tang et al., 2021). Numerous studies reported the bioactive metabolites of perilla including phenolic acids, flavonoids, carotenoids, essential oils, fatty acids, triterpenes, phytosterols, tocopherols and policosanols (Meng et al., 2006, Zhou et al., 2014, Ahmed, 2019). The antioxidant content is increased by insect pollination, overall by honey bees, highlighting the important role of this pollination in the quality and nutraceutical value of this medicinal plant (Ferrazzi et al., 2017). Essential oils of perilla leaves are an important resource of natural medicinal products and recorded among commonly safe food flavorings for use in beverages, puddings, frozen dairy products, baked goods and processed vegetables and soups, and they may be mostly liable for the smell and taste of perilla (Chen et al., 2020). Rosmarinic acid is found in many Lamiaceae plant families including perilla, rosemary, sage, basil and mint and plays an anticarcinogenic role by two effects, anti-inflammatory and antioxidative activity (Osakabe et al., 2004).

Many medicinal plants were cultivated under different controlled environmental conditions not only to increase productivity but also to improve the quality. Therefore, in the present study, we examined the macroscopic characteristics, morphology, total polyphenols and phenolic acids, the antioxidant activity of twelve *P. frutescens* L. Britt accessions grown under open field.

2. Materials and methods

2.1. Reagents

Sodium sulphate (analytical grade), acetic acid (100%), ascorbic acid (C6H8O6), sodium acetate (0.05 M), sodium carbonate (99.5%), Folin-Ciocalteu phenol reagent, (≥95%), 2,4,6-tripyridyl-s-triazine (TPTZ) solution (≥98%), hydrochloric acid (37%), gallic acid (97.5–102.5%), iron-chloride (97%), methanol (99.5%), ethyl acetate (99.5%) were bought from Merck (Darmstadt, Germany). Mueller-Hinton Broth (MHB) and Mueller-Hinton Agar (MHA) were purchased from Difco, Becton Dickinson, Sparks, MD, USA.

2.2. Plant materials and growth conditions

The seeds of *Perilla frutescens* (L.) Britt varieties were obtained from the gene bank of the Department of Medicinal Plants, Budapest. Seeds were sown in greenhouse in 30/March/2017, using plant seedling tray then the seedlings with two–three leaves were transplanted directly to another tray on 10/May/2017, containing 30 seedlings for each accession named (PS1, PS2, PS3, 203P, 465P, 588P, J1, JTD3, NP-606, RauTiaTo, MP3 and GB) respectively. On 29/5/2017 we transferred the plantlets to the experimental research field plots in sorksor coordinates: 47.398820, 19.149270. The experimental design was a completely randomized block design with six replications for each treatment. The experimental plot area for each accession was 5 m^2 consisting of six rows and five lines. The distance between rows was 50 cm and each row contained five plants. The total number of plants in every plot was 30 plants and separated each other by a walking path of 0.5 m width. Plants were irrigated regularly using a sprinkler irrigation system and mechanical weed control was done periodically to maintain vigorous growth. No fertilizer neither organic nor non-organic were applied. Detailed characteristics of the soil are displayed in Table 1. The plant was cultivated in an organic manner no pesticides, fertilizers, genetically modified organisms, antibiotics, or growth hormones have been used.

2.3. Morphological and macroscopic characteristics

On 15/08/2017 we randomly selected five plants per accession in each plot and took measurements and the harvest processes were conducted by hand in the early morning. We measured plant height on the surface of the soil, a diameter of the plant in the third top of the plant. We noted the appearance of the plant and recorded their characteristics including colour of leaf surface, colour of the reverse side of leaf and colour of the stem (green, bright green, weak green, purple, dark purple, bright purple, weak purple), leaf shape (non-wrinkle, wrinkle, heavily wrinkle), leaf venation (pinnate, palmate, reticulate, dichotomous, parallel), leaf arrangement (alternate, opposite, subopposite, whorled), leaf margin (laciniate, serrate, crisped), degree of leaf pubescence and degree of stem pubescence (lightly pubescent, pubescent, heavily pubescent, more heavily pubescent). Fresh weight and stem weight have been recorded immediately after harvesting the plant before the flowering phase. Plants were dried in the shade at room temperature, and dry weight (drug) was recorded again by an analytical scale. Each measurement was performed in six replications. The voucher specimens of dried perilla herb (No. p-2) were deposited at the herbarium of the research centre. The air-dried plant material was kept in bags at room temperature until laboratory analysis.

2.4. Preparation of extracts

The dried leaves were separated from the stems and ground by means of an electric blender into a fine powder and sifted by using a stainless-steel sieve (500 μm hole size). 50 mL of boiling distilled water was added into 0.5 g of the powder from samples of each accession. The plant test solution was shaken and stored at room temperature for 24 h. The extracts were filtered using filter paper and stored in the refrigerator (4 °C) until the measurements were performed. The preparations were carried out in 6 replications.

2.5. Determination of total polyphenolic content

The total polyphenol content (TPC) of *Perilla frutescens* accessions was determined using the Folin-Ciocalteu method modified from Ahmed and Sarosi (2019). A stock solution of gallic acid was prepared by dissolving 5.1 mg in 10 mL of distilled water in a 100 mL volumetric flask. 7% Sodium carbonate (Na_2CO_3) solution was made up by dissolving 7.4 g of sodium carbonate anhydrous in 100 mL distilled water. A serial dilution of the gallic acid stock solution, containing 50 μl, 100 μl, 150 μl, 200 μl, 250 μl was prepared as a standard reference. A blank was also prepared. A portion of 0.5 mL from leaf water extracts was added to a test tube contain-

Table 1
Physico-chemical properties of open field soil.

Parameter	Value	Parameter	Value
Salt %	0.039	Ca%	0.489
pH	6.49	Mg mg kg–1	53
Humus%	1.17	Fe mg kg–1	109
NO3-N mg kg–1	1.24	Mn mg kg–1	37.8
P2O5 mg kg–1	291	Zn mg kg–1	1.73
K2O mg kg–1	36.7	Cu mg kg–1	3.47
CaCO3%	1		

ing 2.5 mL of Folin-Ciocalteu reagent (10% v/v) and shaken well. After 1 mint of incubation, 2 mL of sodium carbonate solution (0.7 M) was added and mixed again. Test tubes were placed into hot water (temperature 50 °C) for 5 min until the blue coloration appeared then the absorbance was recorded at 760 nm by using a Uv–visible spectrophotometer (Thermo Scientific™ Evolution 201/220). Quantification was done with respect to the standard curve of gallic acid and TPC content of samples was expressed as milligram gallic acid equivalent per gram of sample dry matter (mg GAE/g DM).

2.6. Antioxidant iron reduction assay (FRAP)

The ferric reducing antioxidant power (FRAP) assay was performed to determine the antioxidant capacity (AOC) of the perilla accessions according to the described method (Ahmed and Sarosi, 2019) with slight modifications. FRAP reagent solution was prepared freshly by mixing 50 mL acetate-puffer [(3.1) g sodium-acetate was dissolved in16 mL acetic acid, filled with 11 mL distilled water (DW) to make up acetate-puffer] + 5 mL TPTZ solution [2,4,6-tripiridil-S-triazine (TPTZ) solution was prepared by dissolving 0.03123 g TPTZ in 10 mL DW, then 33.6 Âμl HCl was added to the final solution] + 5 mL iron-chloride solution [0.054 g iron-chloride ($FeCl_3$) dissolved in 10 mL DW] in Florence flask. Serial dilutions of the ascorbic acid solution [prepared by dissolving 0.017613 mg in 10 mL DW, then 100 μl from this was diluted in 900 μl of DW], containing 10 μl, 20 μl, 30 μl, 40 μl, 50 μl was prepared as a standard reference for the calibration curve. Aliquots of 10 μl from test extracts of accessions were added to a test tube containing 1.5 mL FRAP solution plus 40 μl DW. A blank was also prepared with distilled water. Absorbance was read at 596 nm after one minute by using a Thermo Evolution 201 spectrophotometer. The final values were expressed in milligram ascorbic acid equivalent (AAE) per gram of sample dry matter (mg AAE/g DM). Measurements for each sample were carried out in six replications.

2.6.1. Extraction and HPLC analysis

The extraction method utilized for dried examples had as follows: 50 mL of%70 methyl alcohol (Methanol) was added to 4 g of dried sample and left at room temperature for 48 hrs. The filtration has been done using Ederol filter paper (medium pore filtering). The extract was concentrated to satisfactory volume to dispose of alcohol by utilizing an air conditioner. At that point as much as the volume of Petroleum Ether (50–60 boiling point) was added to the item, blended and shacked delicately, set in isolating channel, and left for a while to isolated unmistakably into two-layer. Thereby the major part of chlorophyll was dissolved in the petroleum ether. The upper aqueous was removed, and the bottom clear aqueous (extracts of phenolic compounds) were left then transferred to clean vials and injected into HPLC apparatus.

2.6.2. High performance liquid chromatography (HPLC) analysis

All standards were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in 62.5% aqueous methanol to yield concentrations ranging between 0.5 and 25 mg/L. The standards of phenolic compounds were Bisphenol, 2,4-Diaminophenol, Ellagic acid, Gallic acid, Salicylic acid, Tannic acid. Stock working solutions of the standards were stored in darkness at −18 °C. The analytical of high-performance liquid chromatography apparatus system was employed. The separation was achieved on the Analytical column: Eurospher 100, C18, 5 μm, 250 × 4.6 mm at ambient temperature. The mobile phase was acetonitrile + acetic acid 1% (40:60 v/v). The flow rate was 1 mL/min and the injection volume was 20 μl. The monitoring wavelength was 280 nm. The identification of each compound was based on a combination of retention time and spectral matching.

2.7. Statistical analysis

The data were analysed using the IBM SPSS Statistics 22 program. The results are reported as mean ± standard deviation (SD) of six replications and one-way analysis of variance (ANOVA) was employed for data comparison, following Tukey's honestly significant difference (HSD) test. Significant differences ($p < 0.05$) within rows were represented by different superscript letters. The correlation between the studied variables (antioxidant activity and total polyphenol content in the samples was also determined by a two-tailed Pearson correlation analysis. Principal component analysis (PCA) and the heatmap analysis were performed using Minitab Software V19 (Minitab LLC.) and https://biit.cs.ut.ee/clustvis/.

Caulis Perillae‟ and Perilla seed "Fructus Perillae‟.

3. Results

3.1. Macroscopic characteristics and drug yields

The characterization of medical herbs and consumed plants is significant to provide clear data concerning the quality and the intake, by consumers, of pharmaceutically active substances produced by specific cells of those plants. The plant has shown different variety after grown as appeared in Figs. 1 and 2. Perilla can be classified into two clearly phenotypes, green and purple, within these two other colours were appeared. Colour of leaf surface of these accessions displayed bright green such as PS2, 203P, 465P, J1 and NP606 and the only one genotype PS1 showed dark green. On the other hand, the rest taxa appeared to be weak, bright and dark purple. In addition to that, the reverse side of the mentioned leaf colour for the accessions displayed a slightly weak green (PS1, PS2, 203P, 465P, J1, NP606), the other species were bright, weak and dark purple. Regarding the leaf shape of the studied taxa, seven species were grown as non-wrinkle and four as wrinkle taxa. The type of leaf venation of all accessions was reticulate except PS1 and 465P which were pinnate. Leaves were arranged in opposite forms and most of the taxa leaf margin was serrate, two of them PS1 and PS2 were laciniate, while both J1 and NP606 were palmate, crisped respectively. With regard to the colour of stem appears to be purple for all species except two accessions with green stem colour 465P and PS1. Leaves and stem of all the accessions had trichomes but with varying degrees starting from lightly to heavily.

The morphological features of *Perilla frutescens* L. Britt genotypes showed variable results as it is shown in (Figs. 1, 2 and Table 2). JTD3 recorded the highest length of the plant (70.0 ± 4. 47 cm) followed by PS1 (68.20 ± 7.68 cm), and MP3 (67 ± 6.36 c m). Both PS2 and 588P accessions were kept the same level of plant height. On the other hand, NP606 (53.80 ± 7.14 cm) and GB (55 ± 3. 35 cm) reached the lowest length of the perilla herb. These accessions showed the widest diameter JTD3 (55.60 ± 4.45 cm), PS2 (52. 60 ± 10.05 cm), 203P (52.20 ± 9.70 cm), RauTiato (52.20 ± 4.49 cm), while 465P showed the lowest diameter (41.80 ± 6.05 cm). PS2, 203P and JTD3 produced the maximum fresh yield respectively (312.20 ± 134.68), (296.80 ± 108.34) and (262.80 ± 66.34) g/plant respectively, while others produced less fresh weight such as GB (133.40 g/plant), NP606 (174 g/plant) and PS3 (184.60 g/plant). Regarding the proportion of useful drug, we obtained the height dried yield drug for these phenotypes 203P (83 ± 29.11 g/plant), PS2 (77.60 ± 38.34 g/plant) and PS1 (69.80 ± 31.68 g/plant) whereas PS3 (43.80 ± 18.12 g/plant), GB (32 ± 7.77 g/plant) and

Fig. 1. Three important parts of Perilla; Perilla leaf "Folium Perillae", Perilla stalk.

NP606 (50 ± 16.59 g/plant) obtained the minimum amount of the herbal drug.

3.2. Polyphenolic content and antioxidant capacity

The antioxidant capacity is a common method used to evaluate many medicinal and aromatic plants, foods, crops, fruits, edible plants, vegetables to show their scavenging activities against radical sources concerning phenolic acids to benefit human health. Because of these, we studied the antioxidant capacity of twelve accessions of perilla leaves as well as total polyphenolic content which are considered as drugs and nutraceuticals among many Asian and other communities. The quantitative analytical results are summarized in Table 3. GB (Green/Purple colour) phenotype exhibited the major content of polyphenols with significant difference ($p < 0.05$) according to Tukey HSD (248.93 ± 13.25 mg GAE/g dry mass), followed by JTD3 (Purple colour) variety which accumulated the second highest content of polyphenols (236.22 ± 3.28 mg GAE/g dry mass) then J1 (Green colour) (232.83 ± 20.55 mg GAE/g dry mass). There is no difference for both (Green colour) accessions 203P and 465P in terms of secondary metabolite accumulation including phenolic compounds and the results of both analyses showed a similar pattern. In contrast, PS1 (Green colour) and 588P (Purple/Green colour) RauTiaTo (Purple colour) were produced of phytochemicals (phenolics) at low content compared to other species.

The antioxidant capacity of perilla accessions exhibited significant differences ranged from (137.61 to 206.83 mg AAE/g DM). JTD3 (Purple colour) showed the highest value (206.83 ± 2 mg AAE/g DM) and statistically significant than all of them ($P < 0.05$), following 203P (Green colour) and GB (Green/Purple colour) (188.36 ± 17.61 and 186.63 ± 14.46 mg AAE/g DM) respectively. Both taxa J1 (Green colour) and PS3 (Purple) showed almost the same amount (181.72 ± 12.49 and 180.24 ± 2.94 mg AAE/g DM). On the other hand, the mixed phenotype (Green/Purple colour) NP606 showed the lowest antioxidant capacity among all the studied genotypes (137.61 ± 7.18 mg AAE/g DM) following the (Green colour) accession PS1 and (Purple colour) accession RauTiaTo (143.50 ± 5.68 and 149.98 ± 6.78 mg AAE/g DM) individually. Fig. 3 shows the relationship between antioxidant capacity and total polyphenol content of perilla phenotypes.

3.3. Determination of polyphenolic compounds by HPLC

In this investigation, the concentration of individual components in various perilla accessions was quantified. HPLC chromatogram of different *P. frutescens* species was performed and found polyphenolic compounds such as (bisphenol, 2,4-Diaminophenol, ellagic acid, gallic acid, salicylic acid, tannic acid) in different percentages as shown in (Table 4). The results showed that the most abundant phenolic acids were ellagic acid which is accumulated in a higher percentage (4.31, 3.8, 3.7 %) in NP606, 588P and JTD3 species respectively. The second most higher phenolic substance was salicylic acid which is accumulated in the perilla cultivars GB, PS3 and NP606 ranged from (3.17, 3.12, 2.97 %) respectively. Two perilla cultivars NP606 and GB seem to had the higher percentage of gallic acid content. In addition, 203P and GB accumulated nearly equal content of tannic acid (1.02%). On the other hand, the level of biphenol and 2,4-Diaminophenol content in all studied perilla taxa was not reached one percentage.

Phenolic acids of perilla genotypes were subjected to principal component analysis (PCA). According to the loading plot obtained by principal component analysis (PCA), a total of 67.7% variance was revealed among original data (Fig. 4a). The first (PCA1) exhibited 44.6% and the second (PCA2) indicated 23.1% variance across perilla phenolic acids. In (Fig. 4a) score plot, phenolic acids are seen significantly distributed with three prominent groupings which are gallic acid and ellagic acid, salicylic acid, and other components. In this (Fig. 4b) loading plot perilla genotypes have large positive loadings on PC1. According to the heatmap analysis (Fig. 4c), the

Fig. 2. Field view of different genotypes of *Perilla frutescens* grown under open field condition photo (Hiwa M. Ahmed).

perilla genotypes were clustered into three main groups. Group one (RauTiaTo, 588P, NP606, GB and JTD3) cultivars contained a higher percentage of gallic acid and ellagic acid. Group two (PS2, PS3, 203P and 465p) were rich in salicylic acid and group three contained other phenolic components.

4. Discussion

4.1. Macroscopic characteristics and drug yields

There is not much research addressed to compare the current phenotypes and genotypes of perilla thus in our study, we can obviously classify those accessions into two phenotypes green and purple (red) colour. This was also noted by our previous study carried out on cultivated some varieties under controlled condition Phyto-chamber (Ahmed and Sarosi, 2019). To classify perilla, morphological features are often used as an indicator including e.g. size, shape, and color of the leaves and seeds; the degree of leaf serration; flower color (Hu et al., 2010). There is no doubt, geographic conditions play a major role in determining some of these diverse features (Lee and Ohnishi, 2001). The leaf morphology of perilla seems to be the best taxonomic characteristic to differentiate among cultivars, the morphology of the perilla leaves offered distinct characteristics for each cultivar. In the current results, five taxa were found to be green colour after cultivation in the open field which are (PS1, PS2, 203P, 465P and J1), and three other varieties appeared to be purple colour including (PS3, JTD3 and RauTiaTo), while the rest population appeared to be mixed colour (green/purple or purple/green) which are (PS1, 465P, 588P, NP606, MP3 and GB). Genetic and phenotypic variability, misidentification, instability of extracts, toxic components and contaminants are intrinsic problems associated with medicinal and aromatic herbs (Yi and WetzStein, 2010). Liu et al. (2013) demonstrated different colour obtained from various 12 batches of perilla collected from 8 different regions of China, a similar trend was also observed in the current study. The same author found most of the leaves were small, fragmented, rolled-up, while in the current study no rolled-up leaves were detected. The plant height was not comparatively constant among the studied taxa, only ′JTD3‘reached higher values (70.0 ± 4.47 cm) than others. Rouphael et al. (2019) showed that green perilla genotypes resulted in higher yield and biomass production and higher content of dry matter than red perilla in response to salinity applied as chemical eustressor. Ghimire et al. (2017) showed that the average in Chinese accessions was higher for plant height, leaf length, leaf width than the Japanese accessions. Lin et al. (2020a, 2020b) studied red and green perilla phenotypes under temperature and water-stressed conditions and they found a variation in morphological traits, polyphenols and antioxidants with a lower value for all parameters compared to the current study. The morphological and anatomical features are important parts of pharmacobotanical control as the first steps towards the correct identification of medicinal herbs. Even though the phytotherapeutic medicine of the herb depends on the plant material to extract the active component not all the parts of the

Table 2
Macroscopic characteristics of perilla accessions after grown in open field condition.

No	Accessions	Colour of leaf surface {green, bright green, weak green, purple, dark purple, bright purple, weak purple}	Colour of reverse side of leaf {green, bright green, weak green, purple, dark purple, bright purple, weak purple}	Leaf shape (non-wrinkle, wrinkle, heavily wrinkle)	Leaf venation (pinnate, palmate, reticulate, dichotomous, parallel)	Leaf arrangement (alternate, opposite, Subopposite, whorled)	Leaf margin	Colour of stem {green, bright green, weak green, purple, dark purple, bright purple, weak purple}	Degree of leaf pubescence (lightly pubescent, pubescent, heavily pubescent, more heavily pubescent)	Degree of stem pubescence, (lightly pubescent, pubescent, heavily pubescent, more heavily pubescent
1	PS1	Dark green	Weak green	Non-wrinkle	Pinnate	Opposite	Laciniate	Weak green	More heavily pubescent	Heavily pubescent
2	PS2	Bright green	Weak green	Wrinkle	Reticulate	Opposite	Laciniate	Purple	Pubescent	Heavily pubescent
3	PS3	Dark purple	Bright purple	Non-wrinkle	Reticulate	Opposite	Serrate	Dark purple	Lightly pubescent	Pubescent
4	203P	Bright green	Weak green	Wrinkle	Reticulate	Opposite	Serrate	Purple	Pubescent	More heavily pubescent
5	465P	Bright green	Slight green	Non-wrinkle	Pinnate	Opposite	Serrate	Bright green	Heavily pubescent	More heavily pubescent
6	588P	Weak purple	Bright purple	Non-wrinkle	Reticulate	Opposite	Serrate	Purple	Pubescent	Pubescent
7	J1	Bright green	Weak green	Wrinkle	Reticulate	Opposite	Palmate	Weak Purple	Pubescent	More heavily pubescent
8	JTD3	Dark purple	Weak purple	Heavily wrinkle	Reticulate	Opposite	Serrate	Dark purple	Pubescent	More heavily pubescent
9	NP606	Bright green	Weak green	Non-wrinkle	Reticulate	Opposite	Crisped	Dark purple	Pubescent	Lightly pubescent
10	RauTiaTo	Bright purple	Bright purple	Non-wrinkle	Reticulate	Opposite	Serrate	Dark purple	Lightly pubescent	More heavily pubescent
11	MP3	Bright purple	Weak purple	Non-wrinkle	Reticulate	Opposite	Serrate	Dark purple	Lightly pubescent	Heavily pubescent
12	GB	Dark purple	Dark purple	Wrinkle	Reticulate	Opposite	Serrate	Bright purple	Lightly pubescent	Lightly pubescent

plant, therefore the morphological descriptions of the herbal drug are crucial to avoid misidentification as it is often seen in the literature and sometimes contradictory and incomplete (de Oliveira et al., 2018). Glandular trichomes might indicate the presence of lipophilic substances on the plant parts. As Zhou et al. (2021) suggest that the active components are produced predominantly in peltate-glandular perilla trichomes. Transcriptomes were used to study genes associated with bioactive component production that served to unravel the biosynthesis of secondary components in this folk medicinal herb (Zhou et al., 2021).

4.2. Polyphenolic content and antioxidant capacity

Numerous studies have shown the health benefits of metabolites, especially polyphenols. Perilla contains considerably high contents of plant-derived bioactive agents which are important for the supplement company with curative and preventive properties for public health, including antioxidant, antidiabetic, anticancer properties (Ahmed, 2019, Igarashi and Miyazaki, 2013). In the current study, there is little higher content was found for JTD3 (Purple colour) phenotype which was cultivated under open field (236.22 ± 3.28 mg GAE/g dry mass), compared to our previous study (234.2 ± 2.723 mg GAE/g DM), where the plant was cultivated in a growth chamber under controlled conditions (Ahmed and Sarosi, 2019). In the current study, the same accession had a paramount antioxidant capacity, this means that AOC is well correlated to TPC as previous studies exhibited a high correlation between TPC and antioxidant activity (Jun et al., 2014, Ahmed and Sarosi, 2019). Of all fractions, the ethyl acetate fraction of purple *Perilla frutescens* had the highest antioxidant activity (Jun et al., 2014) while in this research water extract showed a similar pattern for the purple phenotype. The antioxidant properties of green perilla leaves exhibited potent effects based on (DPPH, 86%; ABTS, 90%) at a concentration of 100 μg/mL (Lee et al., 2017). In this study, mixed colour phenotypes showed a higher content of phenolic acids than other perilla phenotypes, as Meng (2009) demonstrated that the antioxidant activity and phenolic compounds of perilla varieties may partly be correlated with the foliage colour. These results propose that the production of phenolic acids in herbal medicines may be affected by a number of factors including environmental stress (light, temperature, location, and moisture) and agronomic conditions (cultivars, years, sowing periods and genetics) (Ahmed and Sarosi, 2019, Getahun et al., 2019). The plant is cultivated in an organic manner no pesticides, phytohormones, antibiotics, fertilizers, genetically modified organisms have been used, and consequently, the phytochemicals are fully natural compounds without any produced side effects. Rouphael et al. (2019) exhibited that red perilla genotype other than green perilla accumulated higher content of total polyphenols when the plant grown in response to salinity applied as chemical eustressor. The same pattern has been discovered in the present study where mixed and purple perilla genotypes cultivated in open field accumulated much more secondary metabolite polyphenols. Lin et al. (2020a, 2020b) studied red and green perilla phenotypes under temperature and water-stressed conditions and they found a variation in polyphenols and antioxidants with a lower value for both phenotypes compared to the current study which we obtained a much higher result.

4.3. Determination of polyphenolic compounds by HPLC

Other studies also found different phenolic acids in perilla leaves. For instance, ethyl acetate fraction of *P. frutescens* leaves presented high active ingredient content of gallic acid (Wang et al., 2021), while in our study much higher content was detected. Ellagic acid is found in many therapeutic plants and vegetables. It

Table 3
The phytochemical analysis and morphological features of perilla genotypes grown under open field condition.

No.	Accessions name	Phenotypes colour	Statistics	Antioxidant capacity (mg AAE/g DM)	Total polyphenol content (mg GAE/g DM).	Height (Cm)	Diameter (Cm)	Stem weight (g/plant)	Leaf weight (g/plant)	Fresh Weight (g/plant)	Dry Weight (g/plant)
1	PS1	Green	Mean	143.50^{b}	176.54^{a}	68.20^{b}	47.20^{b}	**29.60^{b}**	**40.20^{b}**	**240.60^{b}**	**69.80^{b}**
			Std. Deviation	5.68	9.69	7.68	5.49	**14.40**	**17.35**	**104.99**	**31.68**
2	PS2	Green	Mean	167.52^{d}	201.74^{c}	65.40^{b}	52.60^{b}	**36.60^{b}**	**41.00^{b}**	**313.20^{b}**	**77.60^{b}**
			Std. Deviation	6.93	7.59	10.89	10.05	**19.18**	**19.22**	**134.68**	**38.34**
3	PS3	Purple	Mean	180.24^{e}	214.07^{c}	59.80^{b}	47.80^{b}	**20.80^{b}**	**23.00^{b}**	**184.60^{b}**	**43.80^{b}**
			Std. Deviation	2.94	5.38	7.60	4.92	**10.68**	**7.72**	**85.25**	**18.12**
4	203P	Green	Mean	188.36^{e}	220.56^{c}	63.00^{b}	52.20^{b}	**38.20^{b}**	**44.80^{b}**	**296.80^{b}**	**83.00^{b}**
			Std. Deviation	17.61	21.97	8.56	9.70	**14.30**	**14.89**	**108.34**	**29.11**
5	465P	Green	Mean	159.66^{c}	220.82^{c}	58.20^{b}	41.80^{a}	**24.40^{b}**	**29.80^{b}**	**236.40^{b}**	**54.20^{b}**
			Std. Deviation	3.38	6.28	9.74	6.05	**16.85**	**18.88**	**146.24**	**35.54**
6	588P	Purple/ Green	Mean	164.79^{d}	176.13^{a}	65.40^{b}	49.80^{b}	**25.80^{b}**	**30.20^{b}**	**210.00^{b}**	**56.00^{b}**
			Std. Deviation	4.14	2.16	7.71	5.53	**8.59**	**7.93**	**62.65**	**14.41**
7	J1	Green	Mean	181.72^{e}	232.83^{e}	55.20^{a}	45.60^{b}	**27.40^{b}**	**35.00^{b}**	**229.40^{b}**	**62.40^{b}**
			Std. Deviation	12.49	20.55	4.17	3.32	**13.44**	**10.37**	**96.72**	**23.52**
8	JTD3	Purple	Mean	206.83^{f}	236.22^{e}	70.00^{b}	55.60^{b}	**33.00^{b}**	**34.20^{b}**	**262.80^{b}**	**67.20^{b}**
			Std. Deviation	2.00	3.28	4.47	4.45	**9.76**	**8.23**	**66.34**	**17.96**
9	NP606	Green/ Purple	Mean	137.61^{a}	201.88^{c}	53.80^{a}	45.20^{b}	**20.80^{b}**	**29.20^{b}**	**174.00^{b}**	**50.00^{b}**
			Std. Deviation	7.18	17.01	7.14	4.17	**7.00**	**9.93**	**54.45**	**16.59**
10	RauTiaTo	Purple	Mean	149.98^{c}	197.72^{b}	59.40^{b}	52.20^{b}	**24.00^{b}**	**30.60^{b}**	**194.40^{b}**	**54.60^{b}**
			Std. Deviation	6.78	12.55	6.62	4.49	**12.43**	**12.45**	**82.83**	**24.77**
11	MP3	Green/ Purple	Mean	156.09^{c}	222.25^{c}	67.00^{b}	50.20^{b}	**20.60^{b}**	**30.00^{b}**	**188.00^{b}**	**50.60^{b}**
			Std. Deviation	9.07	7.34	6.36	6.18	**5.99**	**7.67**	**43.02**	**12.35**
12	GB	Green/ Purple	Mean	186.63^{e}	248.93^{e}	55.00^{a}	47.40^{b}	**13.20^{a}**	**18.80^{a}**	**133.40^{a}**	**32.00^{a}**
			Std. Deviation	14.46	13.25	3.35	5.08	**3.66**	**5.34**	**32.52**	**7.77**

$^{a-f}$ Values with different superscript letters in the same column are significantly different ($P < 0.05$) according to Tukey HSD.

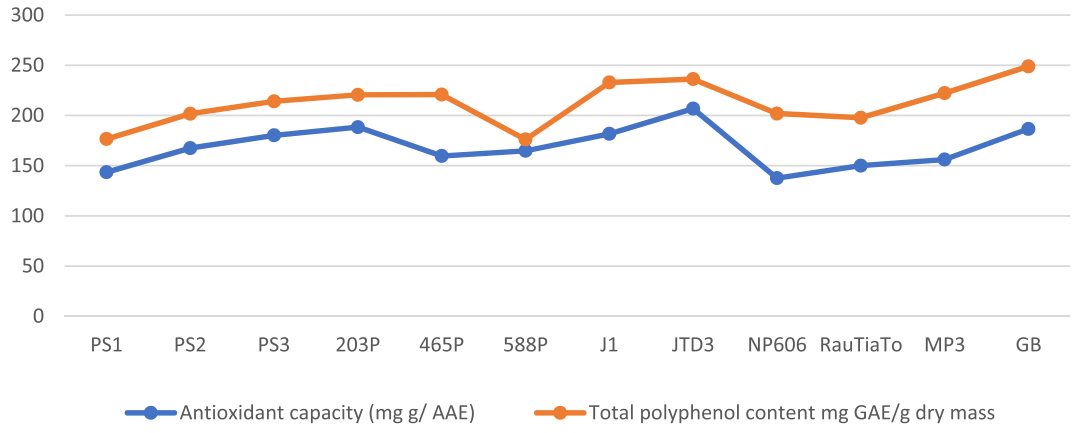

Fig. 3. The relationship between antioxidant capacity and total polyphenol content of perilla phenotypes.

may be found in free form or as complex compounds (ellagitannins), which can be converted to ellagic acid and its metabolites, such as urolithins (Rios et al., 2018). Ellagic Acid has been shown to have antioxidants (Zeb and Akbar, 2018, Tošović and Bren, 2020), osteoarthritis (Lin et al., 2020a, 2020b), diabetes (Ahmad, 2022), anti-inflammatory (Cornélio et al., 2013), antiplatelet activity (Chang, 2013), Neuroprotective (He at al., 2020). Kang and Lee (2011), found three different phenolic acids from purple perilla which were caffeic acid, rosmarinic acid, and rosmarinic acid methylester. Naringin, hesperidin, myricetin, benzoic acid and quercetin were reported to be the major phenolic components in all the Japonica accessions studied by Ghimire et al. (2019). Rosmarinic acid and scutellarin were the most abundant polyphenolic compounds accumulated in higher amounts in the top leaves of the perilla plant than the lower leaves (Gaihre et al., 2021). Rosmarinic acid has been shown to have anti-inflammatory, antiallergic, antioxidant, hepatoprotective, antibacterial, antiviral, and antinociceptive activities (Mikami-Konishide et al., 2013, Gaihre

Table 4
The percentage composition of phenolic acids extracted from *Perilla* species grown under open field condition using HPLC.

NO	Samples	Bisphenol	2,4-Diaminophenol	Ellagic acid	Gallic acid	Salicylic acid	Tannic acid
	R/t	8.73	8.59	3.7	1.8	3.55	4.7
1	PS2	0.4	0.4	0.4	0.88	2.17	0.76
2	PS3	0.2	0.2	0.9	0.88	3.12	0.77
3	203P	0.15	0.18	1.07	0.34	2.91	1.02
4	465P	0.4	0.42	1.23	0	4.3	0.21
5	588P	0.3	0.28	3.8	2.6	2.61	0.21
6	JTD3	0.17	0.14	3.7	0.54	2.9	0.52
7	NP606	0.12	0.15	4.31	2.18	2.97	0.77
8	RauTiaTo	0.16	0.19	3.4	2.37	2.11	0.83
9	MP3	0.09	0.102	1.22	0.66	2.13	0.41
10	GB	0.3	0.14	3.6	2.12	3.17	1.04

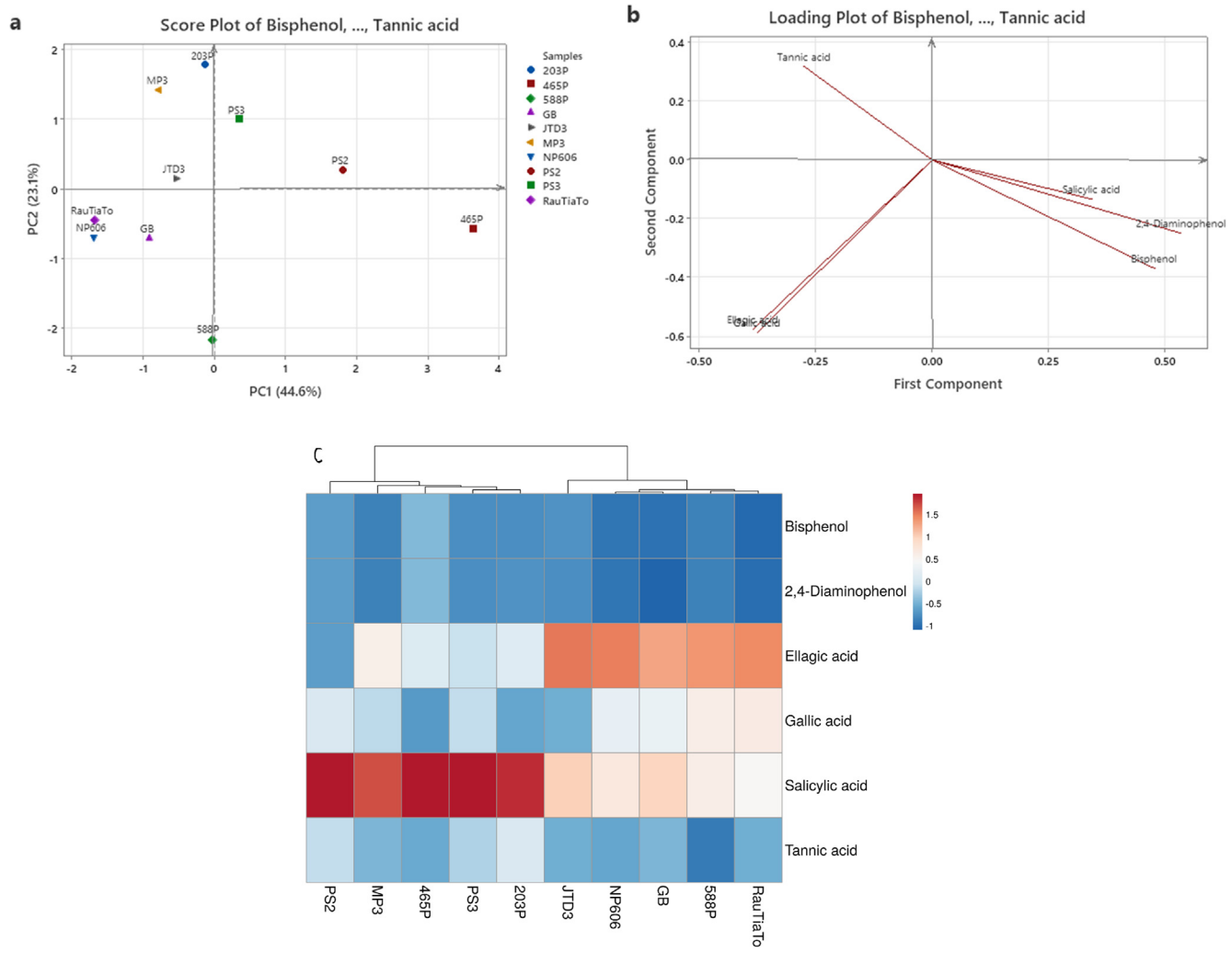

Fig. 4. Principal component analysis (PCA) Score plot (PC1 × PC2) (a), Loading plot (b) and Heatmap (c) for visualization of ten perilla genotypes (PS2, PS3, 203P, 465P, 588P, JTD3, NP606, RauTiaTo, MP3, GB) based on phenolic compositions (Bisphenol, 2,4-Diaminophenol, Ellagic acid, Gallic acid, Salicylic acid, Tannic acid) by HPLC.

et al., 2021). This demonstrates that various cultivars have different phenolics as components. Besides, it has been seen that the phenolic substance of plants can be impacted by different factors, including reaping time, plantation practice, climatic condition, variety, natural conditions, stockpiling time, and temperature (Ahmed and Sarosi, 2019, Getahun et al., 2019), and genetic background of perilla when grown under the same conditions and periods (Deguchi and Ito 2020).

5. Conclusion

High polymorphism was found among the perilla accessions and macroscopic features of perilla genotypes showed variable results among studied cultivars. The perilla herb has shown different varieties after grown and can be classified into two clearly phenotypes green and purple, within these two other colours are appeared. Regarding the proportion of useful drugs, we obtained

the highest drug yield for these green phenotypes 203P, PS2 and PS1 than that of purple phenotypes. GB (Green/Purple colour) phenotype exhibited the major content of polyphenols with significant difference ($p < 0.05$) according to Tukey HSD (248.93 ± 13.25 mg GAE/g dry mass), followed by JTD3 (Purple colour) variety which accumulated the second highest content of polyphenols then J1 (Green colour). Concerning antioxidant capacity, JTD3 (Purple colour) showed the highest value (206.83 ± 2.0 mg AAE/g DM) and statistically significant than all of them ($P < 0.05$), followed by 203P (Green colour) and GB (Green/Purple colour) respectively. The HPLC analysis showed that the most abundant phenolic acids were ellagic acid which is accumulated in a higher percentage in NP606, 588P and JTD3 cultivars respectively, followed by salicylic acid and gallic acid. Further study is necessary in future to detect a new molecular mechanism responsible for the development of morphology and biosynthesis of the major chemicals in *P. frutescens*. These results confirm the importance of perilla cultivars for the purpose of medicinal and aromatic, functional foods and ornamental plants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Stipendium Scholarship and Newcasle Center for Natural Therapy for fellowships and financial support. The author also thanks Dr. Szilvia Sarosi, Dr Peter Radacsi and Dr. Eva Zambori for helping during the fieldwork and analysis.

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