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EXTRACTION, PHYTOCHEMICAL SCREENING, AND QUANTIFICATION OF FLAVONOIDS, PHENOLS, AND ALKALOIDS IN *PRUNELLA VULGARIS*

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Abstract

This study aimed to evaluate the phytochemical composition of Prunella vulgaris extracts obtained using different solvents and quantify specific bioactive compounds present in each extract. Phytochemical screening revealed varying degrees of presence or absence of alkaloids, carbohydrates, glycosides, saponins, phenols, tannins, flavonoids, proteins, and diterpenes across chloroform, ethyl acetate, ethanolic, and aqueous extracts. Chloroform and ethyl acetate extracts showed predominantly negative results for alkaloids and other constituents, indicating a lower affinity for polar compounds like phenols, flavonoids, and alkaloids. In contrast, ethanolic and aqueous extracts exhibited a broader spectrum of phytochemicals. Ethanolic extract notably tested positive for flavonoids, phenols, proteins, and moderate alkaloid content, highlighting ethanol's effectiveness in extracting these compounds. Quantitative analysis confirmed that the ethanolic extract had the highest concentrations of total flavonoids (0.699 mg/100 mg), total phenols (0.565 mg/100 mg), and total alkaloids (0.245 mg/100 mg) among all tested extracts, suggesting ethanol as a suitable solvent for extracting diverse bioactive compounds from Prunella vulgaris. These findings underscore the potential medicinal uses of Prunella vulgaris extracts and suggest that solvent selection influences phytochemical composition and therapeutic properties.

Keywords: *Prunella vulgaris*, phytochemical screening, extraction solvents, ethanolic extract, flavonoids, phenols, alkaloids

Introduction

Prunella vulgaris, commonly known as self-heal or heal-all, is a perennial herbaceous plant that belongs to the Lamiaceae family (Chang et al., 2003). It is widely distributed across Europe, Asia, and North America, and has been traditionally used in herbal medicine for its purported therapeutic properties (Wojdyło et al., 2007). The plant is rich in bioactive compounds such as flavonoids, phenolic acids, alkaloids, and other phytochemicals, which contribute to its medicinal value (Gao et al., 2012).

Phytochemical screening of *Prunella vulgaris* extracts involves identifying and quantifying its bioactive components. Various solvent systems such as chloroform, ethyl acetate, ethanol, and water are used for extraction, each selectively extracting different classes of phytochemicals. The presence of alkaloids, flavonoids, phenolic acids, tannins, saponins, and other secondary metabolites is assessed using specific chemical tests. For instance, alkaloids can be detected using tests like Mayer's, Wagner's, and Dragendorff's tests, while flavonoids are identified by their reaction with lead acetate and alkaline reagents.

The study of phytoconstituents in Prunella vulgaris involves quantifying the content of major bioactive compounds such as flavonoids, phenolic acids, and alkaloids. Quantification is typically done using spectrophotometric methods based on specific calibration curves for standard compounds (e.g., quercetin for flavonoids, gallic acid for phenols, and atropine for alkaloids). These methods provide information into the concentration of these compounds in different parts of the plant and their potential health benefits.

Material and Methods

Selection of plant material

Selecting the right plant material is a foundational step in any horticultural endeavor, whether it is creating a vibrant garden, designing an eco-friendly landscape, or conducting scientific research. The process of choosing plants involves a thoughtful consideration of various factors, from environmental conditions to specific purposes and aesthetic preferences. The plants have been selected on the basis of its availability and folk use of the plant.

Collection of plant material

The flower of *Prunella vulgaris* were collected from Bhopal, Madhya Pradesh in the month of April, 2023. Drying of fresh plant parts was carried out in sun but under the shade. Dried flower of *Prunella vulgaris* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction procedure

The extraction of bioactive compounds from plant materials, such as the delicate flowers of *Prunella vulgaris,* represents a fundamental gateway to unlocking nature's pharmaceutical potential. *Prunella vulgaris,* commonly known as self-heal or heal-all, has long held a revered status in traditional herbal medicine for its purported therapeutic properties. The extraction process serves as a bridge between ancient herbal wisdom and modern scientific inquiry, offering a means to harness the plant's intricate chemical composition for medicinal, pharmaceutical, or research purposes.

Defatting of plant material

Flower of *Prunella vulgaris* were shade dried at room temperature. 67 gram shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration (Khandelwal, 2005). The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Dried powdered flower of *Prunella vulgaris* has been extracted with different solvents like chloroform, ethyl acetate, ethanol and water (non polar to polar solvents) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powder}} x100$$

Phytochemical screening

Phytochemical screening is a systematic approach used to identify the presence of various bioactive compounds within plant extracts. This screening process plays a crucial role in

assessing the medicinal or pharmacological potential of plant materials, including *Prunella vulgaris* flowers. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

1. Detection of alkaloids: Extract were dissolved individually in dilute hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Estimation of total phenolic content

Procedure

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.*, 2017). 10 mg Quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (Shamsa *et al.*, 2008).

Results and Discussion

The study focused on evaluating the phytochemical composition of *Prunella vulgaris* extracts obtained using different solvents and quantifying specific bioactive compounds present in each extract. Phytochemical screening revealed varying degrees of presence or absence of alkaloids, carbohydrates, glycosides, saponins, phenols, tannins, flavonoids, proteins, and diterpenes across the chloroform, ethyl acetate, ethanolic, and aqueous extracts.

Chloroform and ethyl acetate extracts showed negative results for alkaloids and mostly negative results for other constituents, indicating a lower affinity for extracting polar compounds such as phenols, flavonoids, and alkaloids. In contrast, ethanolic and aqueous extracts exhibited a broader spectrum of phytochemicals. Ethanolic extract notably tested positive for flavonoids, phenols, proteins, and moderate alkaloid content, indicating ethanol's effectiveness in extracting these compounds.

Quantitative analysis further substantiated these findings. The ethanolic extract displayed the highest concentrations of total flavonoids (0.699 mg/100 mg), total phenols (0.565 mg/100 mg), and total alkaloids (0.245 mg/100 mg) among all extracts tested. This underscores ethanol as a suitable solvent for extracting a diverse array of bioactive compounds from *Prunella vulgaris*.

These results have significant implications for potential medicinal uses of *Prunella vulgaris*, suggesting that different extraction solvents can selectively extract specific phytochemicals, influencing the plant's therapeutic properties. Future research could delve into the pharmacological activities of these extracts to understand their potential health benefits comprehensively.

Table 1: % Yield of *Prunella vulgaris*

S. No.	Extract	% Yield (w/w)
1.	Chloroform	0.85%
2.	Ethyl acetate	2.61%
3.	Ethanolic	5.47%
4.	Aqueous	8.02%

Table 2: Phytochemical screening of extract of *Prunella vulgaris*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	**Alkaloids**				
	Mayer's Test	-ve	-ve	-ve	-ve
	Wagner's Test	-ve	-ve	+ve	-ve
	Dragendroff's Test	-ve	-ve	-ve	-ve
	Hager's Test	+ve	-ve	+ve	
2.	**Carbohydrates**				
	Molisch's Test	-ve	-ve	+ve	-ve
	Benedict's Test	-ve	-ve	-ve	-ve
	Fehling's Test	+ve	-ve	+ve	+ve
3.	**Glycosides**				
	Modified	-ve	-ve	-ve	-ve
	Borntrager's Test	-ve	-ve	+ve	-ve
	Legal's test				
4.	**Saponins**				
	Froth Test	-ve	-ve	-ve	+ve
	Foam test	-ve	-ve	+ve	+ve
5.	**Phenol**				
	Ferric chloride test	-ve	-ve	+ve	+ve
6.	**Tannins**				
	Gelatin Test	-ve	-ve	+ve	+ve
7.	**Flavonoids**				
	Alkaline reagent	+ve	+ve	+ve	+ve
	test	+ve	-ve	+ve	+ve
	Lead acetate test				
8.	**Proteins**				
	Xanthoproteic test	+ve	+ve	+ve	+ve
9.	**Diterpenes**				
	Copper acetate	-ve	-ve	+ve	+ve
	Test				

+ve= positive, -ve= negative

Table 3: Estimation of total flavonoids, phenol and alkaloids content of *Prunella vulgaris*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)	Total alkaloids content (mg/ 100 mg of dried extract)
1.	Chloroform	0.128	-	0.075
2.	Ethyl acetate	0.321	-	-
3.	Ethanolic	0.699	0.565	0.245
4.	Aqueous	0.387	0.352	-

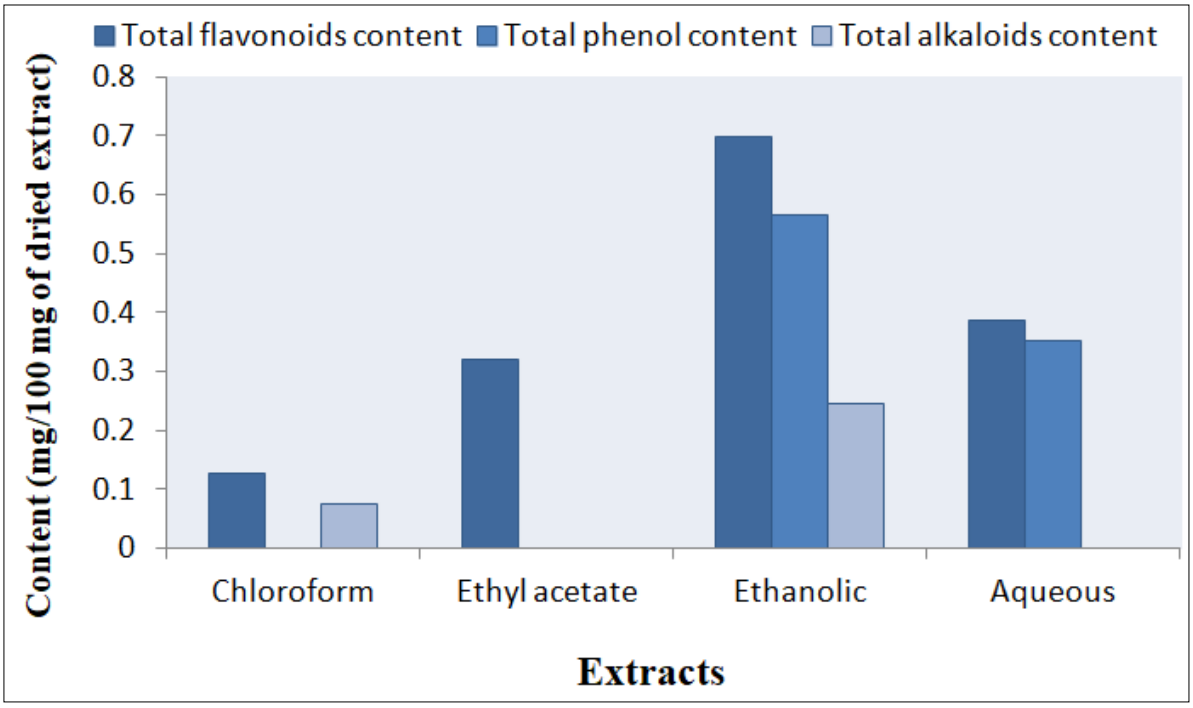

Figure 1: Graph of Estimation of total flavonoids, phenol and alkaloids content of *Prunella vulgaris*

Conclusion

In conclusion, this study comprehensively explored the phytochemical composition of *Prunella vulgaris* extracts using various solvents and quantified specific bioactive compounds present in each extract. Phytochemical screening revealed distinct profiles

across chloroform, ethyl acetate, ethanolic, and aqueous extracts, highlighting their varied affinity for extracting alkaloids, flavonoids, phenols, and other phytoconstituents. The ethanolic extract emerged as particularly rich in flavonoids, phenols, proteins, and alkaloids, indicating ethanol's efficacy in extracting these beneficial compounds from Prunella vulgaris. Quantitative analysis confirmed the highest concentrations of total flavonoids, phenols, and alkaloids in the ethanolic extract, underscoring its potential for therapeutic applications.

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