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PHYTOCHEMI CAL SCREENING OF *PRUNELLA VULGARIS* L. – AN IMPORTANT MEDICINAL PLANT OF KASHMIR

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ABSTRACT

Kashmiri medicinal plant (*Prunella vulgaris*) was analyzed for its chemical composition and amount of bioactive constituents. The results showed that the herb contains on an average alkaloid (1120 mg %), saponins (350 mg %), phenolics (55.785 mg %) and tannins (52.25 mg %). The medicinal plant contained carbohydrates (375 mg %), proteins (441.6 mg %) and lipids (2403.8 mg %). Role of these bioactive principles are discussed according to their folkloric use in Kashmir valley. Besides the herb is of great importance as far as its other clinical application are concerned. This quantitative estimation can be used for comparative evaluation of bioactive constituents with other populations of *Prunella vulgaris* present in different parts of the world and can be used for selection of superior quality of this herb to use in pharmaceutical industries.

Keywords: Phytochemical Screening, Kashmir, folkloric remedies, *Prunella vulgaris.*

INTRODUCTION

Prunella vulgaris L. has 1 to 2 feet long stem with leaves notched on the edge. The flowers are purple in color which sprouts at top of the stem. Flowers are in full bloom mostly from June to August. *P. vulgaris* L. (labiatae), is also known as self heal. It was popular in European and Chinese medicine for curing sore throat, fever and enhance wound healing. The plant is perennial herb which is wild in Kashmir valley and is widely used. The herb has tremendous medicinal importance and not a single traditional composite unani medicine of sore throat, common cold and head ache is without this herb. In Kashmir, the herb is used in unani medicine as brain tonic in chilly winters, as it is boiled and inhaled in the form of steam which clears phlegm and reduces head ache. In Kashmir the herb is among one of the ingredients of composite traditional medicine which is used for bathing pregnant women after she delivers her baby. The aqueous extract of this herb is recently used in clinical treatment of herpetic keratitis (Xu *et al*., 1999).The herb has great medicinal value and is used as hypotensive, antibiotic, antiseptic, antirheumatic, antipyretic, antibacterial, antioxidant, diuretic, vermifuge (Duke, 1985). It is used in treatment of wounds, ulcers and sores (Chiej, 1984).

Aqueous extract of *P. vulgaris* contain an anti-HIV active compound named as Prunellin which is chemically a polysaccharide (Tabba *et al*., 1989).When screening test of different commonly used herbs were done it was found that *P. vulgaris* exerts remarkable anti-HIV activity (John *et al*., 1994). Its antiviral action was also reported against the herpes simplex virus (Zheng, 1990). The aqueous extract of this herb inhibits anaphylactic shock and allergic reactions (Shin *et al*., 2001). It protects rat RBC against haemolysis and kidney, brain homogenates against lipid peroxidation (Liu and Ng, 2000). Immune modulatory effects of *P. vulgaris* was carried on monocytes (Xuya, fang *et al*., 2005). It contains a high content of rosamarinic acid which makes plant more usable as far as its therapeutic applications are concerned (Markova *et al*., 1997).

MATERIAL AND METHODS

Collection of plant Material

The whole plant of *P. vulgaris* was identified and collected from Naranag area (District Ganderbal) of Kashmir. The plant was shade dried for some days and ground into powder with the help of an grinder and latter it was stored in air tight bottles for further use.

Qualitative screening

Tannins: To 2 ml of aqueous extract 2 ml of 5% $FeCl_3$ was added. Formation of yellow brown precipitate indicates that tannins are present (Jigna *et al*., 2007).

Alkaloids: To the 2 ml methanolic filtrate, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayors reagents/Wagner's reagent/ Dragendroff reagent was added. Formation of Orange precipitate indicates the presence of alkaloids (Oguyemi, 1979).

Saponins: Aqueous extract of 2 g powder was made and subjected to frothing test. Frothing persistence indicated presence of saponins. Latter the froth was mixed with few drops of olive oil. Formation of emulsion indicates presence of saponins (Sofowora, 1993).

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Cardiac glycosides: To 2 ml alcoholic filtrate, 1 ml glacial acetic acid and 1-2 drops of $FeCl_3$ was added followed by 1 ml of concentrated H_2SO_4. Appearance of brown ring at the interface indicates presence of cardiac glycosides. A violet ring may also appear below the brown ring (Trease and Evans, 1989).

Terpenes; To 2 ml of aqueous extract, 5 ml chloroform, 2 ml acetic anhydride and concentrated H_2SO_4 was added carefully to form layer. Reddish brown coloration of interface indicates terpenes (Harborne, 1973).

Flavonoids: 2 g plant material was extracted in 10 ml alcohol or water. To 2 ml filtrate few drops of concentrated HCl followed by 0.5 g of zinc or magnesium turnings was added. After 3 minutes magenta red or pink color indicated the presence of flavonoids (Jigna *et al*., 2007).

Phenolics: To 2 ml of alcoholic or aqueous extract, 1 ml of 1% ferric chloride solution was added. Blue or green color indicates phenols (Martinez, 2003).

Quantitative screening

Alkaloid determination: 2.5 g of the powder was extracted using 100 ml of 20% acetic acid in ethanol. The solution was covered for almost 4 hours. Filtrate was concentrated to 25 ml. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed (Edeoga *et al*., 2005 and Okwu and Josiah, 2006).

Saponin determination: 10 g of sample was mixed with 100 ml of 20% aqueous ethanol. The mixture was kept for 4 hours on water bath shaker at 55˚ C. Filtrate was again extracted in same manner. The combined extract were concentrated to 40 ml over water bath at 90˚C. Concentrate obtained was transferred into a separating funnel and 10 ml of diethyl ether was added to it. After shaking vigorously aqueous layer was recovered and ether layer was discarded. The process was repeated. To the aqueous layer n-butanol was added. The whole mixture was washed in separating funnel twice with 10 ml 5% of aqueous NaCl. Upper part was retained and heated in water bath until evaporation. Latter it was dried in oven to a constant weight (Obadoni and Ochuko, 2001; Edeoga *et al*., 2005).

Phenolics determination: 0.5-1 g of sample powder was extracted with 10 ml of 80% ethanol. Supernatant was evaporated to dryness and extract was redissolved in 5ml water. Different aliquots 0.1-1 ml were pipetted into test tubes and final volume was made to 3ml by water. 0.5 ml of folin's reagent followed by 20% Na_2CO_3 equal to 2ml was added respectively. Tubes were vortexed and kept in boiling water for one minute. After cooling absorbance was read at 650 nm against blank. A standard curve was prepared using different concentrations of 100 mg% catechol (Malick and Singh, 1980).

Tannins determination: 2g of plant powder was extracted thrice in 70% acetone. After centrifuging the sample supernatant was removed. Different aliquots were taken and final volume to 3 ml was adjusted by distilled water. The solution after vortexing were mixed with 1 ml of 0.016M $K_3Fe(CN)_6$, followed by 1 ml of 0.02M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. 5 ml of stabilizer (3:1:1 ratio of water, H_3PO_4 and 1% gum arabic) was added followed by revortexing. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001M gallic acid (Graham, 1992).

Carbohydrate determination: 0.5 g of plant material was extracted with 80% ethanol. Extract was dissolved in 10 ml water. Different aliquots were prepared and final volume was made to 1 ml by water. 5 ml of 96% of concentrated H_2SO_4 was added followed by shaking and incubation for 40 min at room temperature. 1 ml of 5% phenol was added to each tube and absorbance was taken at 490nm. Standard curve using different concentrations of 25 mg% glucose (Krishnaveni *et al*., 1984).

Proteins determination: 1g plant material was extracted using 10 ml water added with few drops of triton X- 100. Supernatant was extracted in acetone and the pellet obtained was dissolved in 0.1 M NaOH. Aliquots were prepared and final volume was made to 1 ml by distilled water. 5 ml of copper reagent was added to tubes, mixed well and incubated for 10 minutes. 1 ml of folin's reagent was mixed. Tubes were incubated for 30 min at room temperature and absorbance was taken at 700 nm. Standard curve was prepared using 50 mg % BSA (Lowry *et al*., 1951).

Lipids determination: 1g plant sample was dissolved in ether and stirred for a hour. Mixture was centrifuged, supernatant dried and dissolved in ethanol. 0.1 ml of alcohol was taken as blank, olive oil as standard and test sample as unknown respectively. 2 ml of concentrated H_2SO_4 and 5 ml of phosphovanillin reagent was added and mixed well, incubated for 30 min. Absorbance was read at 540nm (Ganai *et al*., 2005).

RESULTS AND DISCUSSION

Screening of the plant material revealed the presence of the phenols, saponins, alkaloids, tannins and terpenes. Quantitative estimations of bioactive constituents are summarized in table. Besides estimations of carbohydrates, lipids and protein was being carried out.

Table: Phytochemical Screening of *Prunella vulgaris* L.

Bioactive Agents	Presence(+) / Absence(-)	Quantity/100g of plant material* (i.e. g %)
Alkaloid	+	1.53 ± 0.02
Phenols	+	0.056±0.005
Tannins	+	0.05±0.001
Cardiac Glycosides	–	–
Flavonoids	–	–
Saponins	+	0.35±0.0025
Carbohydrates	+	0.375±0.0012
Lipids	+	2.44±0.002
Proteins	+	0.44±0.0025

*Results are mean ± SD of triplicate determination on the basis of dry weight.

Prunella vulgaris has been tremendously used these years for its good taste and nutritional value as it has good content of carbohydrate, protein and fat (Launert, 1981).Our results also confirm high values of carbohydrates, lipids and proteins. Presence of carbohydrates is also in accordance with Tabba *et al*., 1989; Xu *et al*., 1999 who confirms presence of anti-HIV polysaccharide prunellin in it. Our report suggest that it contains good phenolics content. In addition, presence of phenolic compounds indicates that the plants are anti-microbial agent (Okwu and Okwu, 2004). This is agreed with the findings of Yeung, 1985 who reported that this herb is effective in the treatment of typhoid fever and other bacterial infections, particularly infections caused by *Escherichia coli*, *Bacillus typhi*, *Pseudomonas* and *Mycobacterium tuberculli.* Presence of phenolic component rosamarinic acid is also confirmed by Lamaison *et al*., 1991.This might be the reason that traditional healers in Kashmir prescribe steam inhalation of this herb to clear phlegm from chest and brain cavity, hence reduces chest infections and head ache. It is general trend in Kashmir to use it as decoction for bathing pregnant women after a month of delivering her baby. Traditional healers consider it to be as brain tonic which can give stamina to head, acts as disinfectant and remove general weakness of body of mother.

The herb also contains saponins which is used to stop bleeding and in treating wounds and ulcers as it helps in red blood cell coagulation (Okwu and Josiah, 2006). This is in conformity with Chiej, 1984; Grieve, 1984; Foster, 1990. So among folkloric remedies of Kashmir it has also been used by nursing mothers for sore cracked nipples. These findings are also reported by Anthony, 2003. Apart from saponins, other secondary metabolite constituent detected in the herb are alkaloids. Alkaloids have numerous functions and among them foremost are their analgesic, antispasmodic and bactericidal effects (Okwu and Josiah, 2006). The results are in relation with Grieve, 1984; Launert, 1981; Yeung, 1985; Duke and Ayensu, 1985. Herb is also rich in tannins and contribute property of astringency i.e., fasten the healing of wounds and inflamed mucous membranes (Okwu and Josiah, 2006). This perhaps explain why traditional medicine healers in Kashmir often use it in treating wounds and burns.

This study will prove useful in the comparative studies of the amount of bioactive principles present in this herb with its other species and populations belonging to different regions with different climatic conditions. This data can also help us to choose the superior race of this valuable herb with greater quantity of medically and therapeutically important phytochemicals.

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