

## Phytochemical Analysis and Anti-inflammatory Activity of Various Extracts Obtained from Floral Spikes of *PRUNELLA VULGARIS L.*

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### ABSTRACT

*Prunella vulgaris* L. also known as self-heal has been extensively used for treating various ailments in traditional system of medicine which indicates that the plant is an important source of various bioactive constituents. Current study was performed to carry out qualitative and quantitative phytochemical evaluation and anti-inflammatory activity of various extracts obtained from floral spikes of *P. vulgaris*. Different extracts were obtained from shade dried floral spikes of *P. vulgaris* using cold percolation method. The extracts were subjected to suitable phytochemical tests for the detection and estimation of various phytoconstituents. Aqueous and methanolic extracts were subjected to inflammatory activity in animal models using carrageenan paw edema method at a dose of 300 mg/kg of body weight. Diclofenac (10 mg/kg) was used as reference standard. Phytochemical analyses revealed the presence of flavonoids, tannins, saponins, carbohydrates, steroids, alkaloids and anthraquinone glycosides. The exact percentage of phytoconstituents obtained by quantitative estimation for alkaloids, saponins, tannins and carbohydrates was 0.255±0.001, 2.676±0.0141, 0.0988±0.005, 1.584±0.09 per 100 g of plant material, respectively. Both aqueous and methanolic extracts showed anti-inflammatory activity with maximum percentage inhibition of inflammation of 28.965 and 30.860, respectively while maximum percentage inhibition of inflammation shown by Diclofenac was 60.075. Phytochemical analyses revealed the presence various phytoconstituents which justify its role for the treatment of various ailments in folklore system of medicine. Besides it can be concluded that both aqueous and methanolic extracts show significant (P<0.05) anti-inflammatory activity comparable to reference standard diclofenac (P<0.01) when compared with control group.

**Keywords:** *Prunell vulgaris*, Phytoconstituents, Anti inflammatory, extracts.

### INTRODUCTION

Phytochemical analysis is an important parameter to identify plants for their medicinal properties<sup>1</sup>. Various plant constituents such as saponins, alkaloids, glycosides tannins etc have been documented to exhibit various biological activities including anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, antibacterial, antiviral, anticarcinogenic, antioxidant properties etc. <sup>2</sup>.

Inflammation is protective and defense mechanism of the body. It is thus, body's normal protective response to an

injury, irritation, surgery or even pathogenic attack<sup>3</sup>. Recruitment of inflammatory cells to sites of injury involves the concerted interactions of several types of soluble mediators which include the complement factor C5a, platelet-activating factor, and the eicosanoid LTB<sub>4</sub>. Besides several cytokines also play essential roles in orchestrating the inflammatory process, especially interleukin-1 (IL-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) <sup>4</sup>. Commonly employed drugs for the treatment of inflammation include NSAIDs, glucocorticoids etc. However, besides their high costs, severe adverse reactions and toxicity have been observed, which include gastrointestinal bleeding, ulceration, bleeding, renal damage & hypertension etc<sup>5</sup>. Thus screening of anti-

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inflammatory drugs is need of the hour and indigenous medicinal plants seem to play very important role in finding out better drugs.

*P. vulgaris* commonly known as self-heal, a low growing perennial herb, has been extensively used for treating various ailments like inflammation, eye pain, headache, and dizziness in traditional system of medicine. The plant has been evaluated for various biological activities. A number of studies carried on the *P. vulgaris* indicated its antiviral potential. It has been found to be active against HIV with the actions ranging from inhibition of viral replication, prevention syncytium formation and binding to CD4 receptors, inhibition of membrane fusion between the HIV and its target cells.<sup>6,7,8</sup> Different extracts from the plant have been found to be effective against HSV<sup>9,10</sup> and some lentivirus.<sup>11</sup> The Plant has shown immunomodulatory potential which was indicated by macrophage and T cell activation.<sup>12,13</sup> Extracts from the plant have been evaluated for their anti-inflammatory potential on various cell lines and were found to be affective in lowering the levels of pro-inflammatory mediators.<sup>14,15</sup> The plant has shown both in-vitro and in-vivo antioxidant potential this has been attributed to phenolic compounds such as caffeic acid, rosmarinic acid, rutin and quercetin.<sup>16,17</sup> Aqueous-ethanol extracts have shown to suppress blood glucose levels by enhancing insulin sensitivity.<sup>18</sup> Besides a compound Jiangtangsu isolated from *P. vulgaris* is claimed to have anti-diabetic potential and capability to restore pancreatic architecture.<sup>19</sup> Various extracts and isolated compounds have shown anticancer potential of the plant. A polysaccharide P32 isolated from the aqueous extract and oleanolic acid isolated from an ethanol extract have been found to induce apoptosis of lung adenocarcinoma cells.<sup>20,21</sup> Ethanol and aqueous extracts have shown significant cytotoxic effects on the various cancer cell lines besides having antioxidant potential.<sup>22</sup> Giri et al., (2011) and Park et al., (2010) through independent studies evaluated ethanol extract for nootropic activity and

claimed it to be affective for treatment of amnesic deficits and cognitive impairments by reducing acetylcholine esterase activity and exerting its effects via NMDA receptor signaling.<sup>23,24</sup> *P. vulgaris* as a component of herbal based dentifrice was found to be effective in reducing symptoms of gingivitis.<sup>25</sup>

In the current study extracts obtained from floral spikes of *P. vulgaris* were subjected to qualitative and quantitative phytochemical analysis and an effort has been made to find out the therapeutic effectiveness of *P. vulgaris* as anti-inflammatory agent.

## 2. MATERIALS AND METHODS

### 2.1. Collection and Identification of Plant Material

The whole plant of *Prunella vulgaris* was collected from the Gulmarg area of district Baramullah, Jammu & Kashmir, India and was authenticated by the curator Akhtar H Malik, Department of Taxonomy, University of Kashmir, under voucher specimen No. 1899-KASH Herbarium, University of Kashmir, 16/07/2013, Gulmargh, for further reference. The time of collection time was 11:00 am 14/07/2013.

### 2.2. Preparation of Extracts

After collection, the floral spikes were cut off from the plant and dried in shade for 20 days. The dried plant material was coarsely powdered and then extracted with water, ethanol, hydroalcohol (1:1) and methanol using fresh portion of plant powder each time adopting cold percolation method.

### 2.3. Qualitative phytochemical analysis

The powdered plant material/extracts viz. ethanolic, aqueous, hydroalcoholic and methanolic obtained from the above mentioned process of extraction were subjected to preliminary phytochemical screening for detection of various phytoconstituents.

#### 2.3.1. Test for Alkaloids

##### 2.3.1.1. Mayer's test

To detect the presence of alkaloids, few drops of Mayer's reagent were added to the extract cream colour

precipitate indicates the presence of alkaloids.<sup>26</sup>

#### 2.3.1.2. *Wagner's test*

Few ml of filtered extract plus few drops of Wagner's reagent along sides of tubes appearance of reddish brown precipitate indicates the presence of alkaloids.<sup>27</sup>

#### 2.3.1.3. *Dragendroff's Test*

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.<sup>28</sup>

#### 2.3.2. *Saponins*

##### 2.3.2.1. *Foam test*

To 0.5 ml of test solution added 0.5 ml distilled water, shook well, appearance of persistent foam indicates the presence of saponins.<sup>26</sup>

#### 2.3.3. *Tannins*

##### 2.3.3.1. *Ferric chloride (5%) test*

1ml of 5% FeCl<sub>3</sub> is added to the extract, presence of tannins is indicated by the formation of bluish black or greenish black precipitate.<sup>27</sup>

##### 2.3.3.2. *Lead acetate test*

2-3 ml of aqueous extract, few drops of lead acetate solution white precipitate indicates the presence of tannins.<sup>27</sup>

##### 2.3.3.3. *Bromine water test*

2-3 ml of aqueous extract, added few drops of bromine water solution, discoloration of bromine water indicated the presence of tannins.<sup>27</sup>

#### 2.3.4. *Flavonoids*

##### 2.3.4.1. *Shinoda test*

To dry extract, 5 ml of 95% ethanol, 3drops of HCl and 0.5 g of magnesium turnings were added. Pink colour formation indicates the presence of flavonoids.<sup>28</sup>

#### 2.3.5. *Glycosides*

##### 2.3.5.1. *Modified Borntrager's Test*

Extracts were 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 anthranquinone glycosides.<sup>28</sup>

##### 2.3.5.2. *Keller-Kiliani test*

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl<sub>3</sub>. The mixture was then poured into another test tube containing 2ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interphase indicates the presence of cardiac glycosides.<sup>27</sup>

##### 2.3.5.3. *Legal's Test*

Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.<sup>28</sup>

#### 2.3.6. *Terpenes*

2.3.6.1. To 2 ml of extract, 5 ml chloroform and 2ml conc. H<sub>2</sub>SO<sub>4</sub> was added. Reddish brown colouration of interface indicates the presence of terpenes.<sup>29</sup>

#### 2.3.7. *Carbohydrates*

##### 2.3.7.1. *Molish's test*

To 2.3ml extract, few drops of Molisch reagent was added shake well and conc. H<sub>2</sub>SO<sub>4</sub> was added from sides of the test tube, violet ring formation at the junction of two liquids indicates the presence of carbohydrates.<sup>30</sup>

##### 2.3.7.2. *Fehling's test*

1ml each of Fehling's A and B were mixed and boiled for 1 min. Equal volumes of test solution was added to the test tube and boiled for 5 min. Formation of brick red colour indicates the presence of reducing sugars.<sup>27</sup>

##### 2.3.7.3. *Benedict's test*

To a few drops of test solution added 2 ml of Benedict's reagent in test tube. Boiled for 2 min. on water bath and allowed it to stand. Red precipitate indicates the presence of reducing sugar.<sup>27</sup>

#### 2.3.8. *Proteins*

##### 2.3.8.1. *Biuret's test*

To 2ml of test solution added 2ml of 4% NaOH, to this added few drops of biuret reagent .Violet or pink color indicates the presence of proteins.<sup>27</sup>

##### 2.3.8.2. *Million's test*

To 3ml of test solution, 5ml of Million's reagent was

added. Appearance of white precipitate which turns brick red on warming indicates presence of proteins.<sup>27</sup>

#### 2.3.8.3. *Ninhydrin test*

To 3 ml of test solution added 3 drops of Ninhydrin solution, heated on boiling water bath for 10 min. Appearance of purple or blue colour indicates presence of proteins.<sup>27</sup>

#### 2.3.9. *Phenolics*

##### 2.3.9.1. *Ferric chloride (1%) test*

To 2ml of extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenolics.<sup>28</sup>

#### 2.3.10. *Amino acids*

##### 2.3.10.1. *Ninhydrin test*

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.<sup>27</sup>

#### 2.3.11. *Steroids*

##### 2.3.11.1. *Liebermann-Buchard test*

To 2 ml of test solution 2 ml of acetic anhydride and conc. Sulphuric acid was added. Blue green ring indicates the presence of terpenoids.<sup>28</sup>

##### 2.3.11.2. *Salkowski's Test*

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. H<sub>2</sub>SO<sub>4</sub>, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.<sup>28</sup>

#### 2.4. *Quantitative phytochemical analysis*

##### 2.4.1. *Determination of Alkaloids*

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered. The solution is allowed to stand for 4 h. The solution was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected. The precipitate was washed with dilute ammonium hydroxide and then filtered. Filtrate was

discarded and the residue is the alkaloid was dried and weighed.<sup>29</sup>

#### 2.4.2. *Determination of Saponins*

The coarsely ground samples each of 20 g were put into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The recovered layer heated in water bath until evaporation. After evaporation the samples were dried in the oven to a constant weight and the percentage of saponins was calculated according to following formulae.<sup>31,32</sup>

$$\% \text{Saonin} = \frac{\text{Weight of final filtrate}}{\text{Weight of samle}} \times 100$$

#### 2.4.3. *Determination of Tannins*

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 1ml of 0.016M K<sub>3</sub>Fe(CN)<sub>6</sub>, followed by 1ml of 0.02M FeCl<sub>3</sub> in 0.10M 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<sub>3</sub>PO<sub>4</sub> 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.<sup>33</sup>

#### 2.4.4. *Determination of Carbohydrates*

0.5g of plant material was extracted with 80% ethanol. Extract was dissolved in 10 ml of water. Different aliquots were prepared and final volume was made to 1ml by water. 5

ml of 96% of concentrated H<sub>2</sub>SO<sub>4</sub> 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 was plotted using different concentrations at 10 mg% glucose.<sup>30</sup>

2.5. Biological activity

2.5.1. Experimental animals

Male Albino Wistar rats weighing 150–200 gm were employed for assessing the anti-inflammatory activity. They were fed with a standard pellet diet and water ad libitum. The animals were maintained at 25oC to 28oC with 40-70% RH and 12 hour light/dark cycles and were fasted for 12 hours prior to the experiment. Studies were performed in accordance with CPCSEA guidelines after obtaining due approval (Approval No. F-IAEC (Pharm.Sc.)APPROVAL/2013/23).

2.5.2. Acute oral toxicity

Dose selection was done according to OECD 425 guidelines (2008) following limit test using wistar albino rats. On the basis of acute toxicity study 1/10th of the dose was selected for further experimental evaluation.

2.5.3. Drugs and Chemicals

The analytical grade drugs used for the test were normal saline, Carageenan (Sigma Aldrich), Diclofenac Sodium (Voveran, Novartis India Ltd.)

2.5.4. Anti-inflammatory activity

Eighteen rats were used in this study and they were divided into three groups of six rats per group. Each group received one of the following treatments: plant extract, (300 mg/kg body weight, p.o), Diclofenac (10mg/kg body weight) or control normal saline (0.9%, 0.1ml) administered orally. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline in the right paw of rats (Winter et al). The paw volume was measured at 0, 1, 2, 3, 4 and 5hr. after carrageenan injection using a Plethysmometer. Anti-inflammatory activity was measured as the percentage reduction in edema level when drug was present, relative to control.<sup>34,35</sup> The percentage inhibition of the inflammation was calculated from the formula:

$$\text{Percentage inhibition} = \left[ \frac{V_c - V_t}{V_c} \times 100 \right]$$

$V_c$   
= Mean increase in paw volume of control group

$V_t$   
= Mean increase in paw volume of treated group /standard group

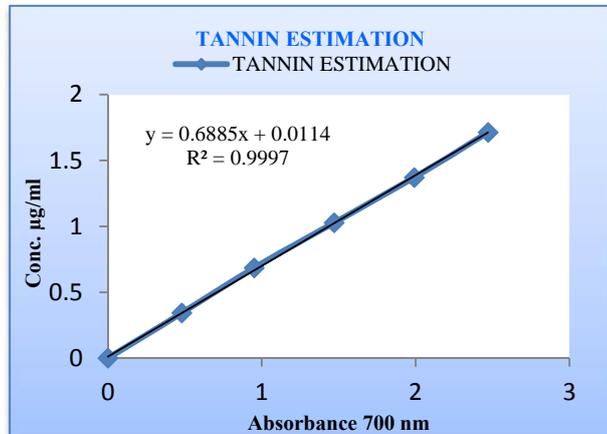
3.RESULTS AND DISCUSSION

Table 1. Result of Phytochemical screening in various extracts obtained from floral spikes of *Prunella vulgaris*.

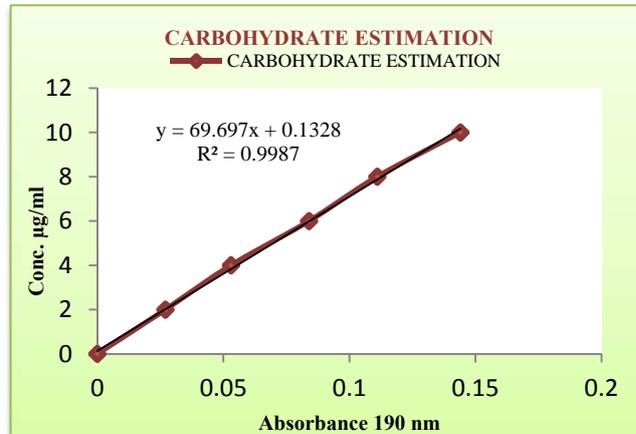
S. No	Phytochemical Test	Plant extracts			
		Aqueous	Ethanollic	Hydroalcoholic	Methanolic
1.	<b>Test For Alkaloids</b>				
	Mayer's Test	+	++	+	+
	Wagner's Test	-	+	+	+
	Dragendroff's Test	-	+	+	+
2.	<b>Test For carbohydrates</b>				
	Molish Test	+++	++	+++	++
	Fehling test (reducing sugars)	+++	++	++	+
	Benedict test (Mono Saccharides)	++	+	++	+
	Selwinoff test (Fructose)	-	-	-	-
3.	<b>Test For Proteins</b>				
	Ninhydrin Test	-	-	-	-

S. No	Phytochemical Test	Plant extracts			
		Aqueous	Ethanollic	Hydroalcoholic	Methanolic
4.	<b>Test for Saponins</b>				
	Foam Test	+++	++	++	+
5.	<b>Test for Tannins</b>				
	Lead Acetate	+++	++	+++	++
	5% FeCl <sub>3</sub>	+++	++	+++	++
	Bromine Water	+++	++	+++	++
6.	<b>Test for Cardiac glycosides</b>				
	Keller –Killian Test	-	-	-	-
	Baljet Test	-	-	-	-
	Legal Test	-	-	-	-
7.	<b>Test for Anthraquinone glycosides</b>				
	Borntranger test	++	+	++	++
8.	<b>Test for Steroids</b>				
	Salkowski Test	+++	+++	++	+++
9.	<b>Test for Flavonoids</b>				
	Shinoda Test	++	+++	++	+++
10.	<b>Test for Terpenes</b>	+	+++	+	++
11.	<b>Test for amino acids</b>	-	-	-	-
12.	<b>Test for Phenolics</b>	++	+++	++	+++

+ = slightly present. - = Absent. ++ = substantially present, +++ strongly present.



Graph 1: Standard curve of 10-3M Gallic acid for tannin estimation



Graph 2: Standard curve of 10% glucose for carbohydrate estimation

**Table 2. Result of quantitative estimation of phytochemical constituents in floral spikes of *Prunella vulgaris*.**

S.No.	Bioactive agent	Quantity in gram percentage
1.	Alkaloids (dry powder)	0.255±0.001
2.	Saponins (dry powder)	2.676±0.010
5.	Tannins (dry powder)	0.099±0.005
6.	Carbohydrates (dry powder)	1.584±0.090

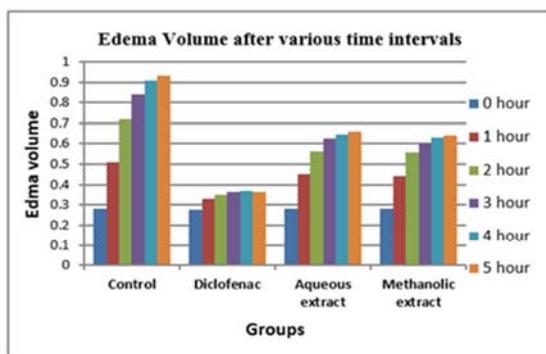
**Table 3. Shows Volume of mercury displaced by rats treated with standard aqueous and methanolic extracts in ml at various time intervals in hours**

Treatment	Dose	Volume of mercury displaced in ml at various time intervals in hours					
		0	1	2	3	4	5
Control (Negative)	0.1 ml (normal saline)	0.280±0.003	0.510±0.003	0.720±0.005	0.842±0.002	0.908±0.003	0.930±0.003
Diclofenac	10 mg/kg bdw	0.280±0.002	0.328**±0.002	0.350**±0.003	0.362**±0.002	0.368**±0.002	0.362**±0.002
Aqueous extract	300 mg/kg of bdw	0.280±0.003	0.453*±0.003	0.565*±0.003	0.628*±0.003	0.645*±0.002	0.662*±0.003
Methanolic extract	300 mg/kg of bdw	0.280±0.004	0.442*±0.003	0.558*±0.003	0.600*±0.003	0.633*±0.002	0.643*±0.002

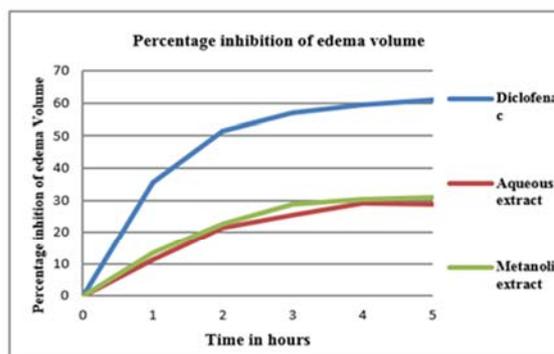
All values are expressed as mean ± SEM (n=6), Significance of difference in control and treatment groups were determined by one way analysis of variance (ANOVA) followed by Dunnet comparison tests using GraphPad instat software . \*\*\* P<0.001 Extremely significant, \*\*P<0.01 Very significant, \* P<0.05 are significant

**Table 4. Percentage inhibition of inflammation at various time intervals of control group and rats treated with standard drug, aqueous extract and methanolic extract**

Treatment	Dose	Percentage inhibition of inflammation various time intervals in hours					
		0	1	2	3	4	5
Control	0.1 ml (Normal saline)	00	00	00	00	00	00
Diclofenac	10 mg/kg bdw	00	35.686	51.389	57.007	59.471	61.075
Aqueous extract	300 mg/kg bdw	00	11.176	21.528	25.416	28.965	28.817
Methanolic extract	300 mg/kg bdw	00	13.333	22.500	28.741	30.286	30.860



Graph 3: Shows paw edema volume of Rats treated with standard drug methanolic and aqueous extracts after various time intervals



Graph 4: Shows Percentage inhibition of edema volume in Rats treated with standard drug aqueous and methanolic extracts.

Phytochemical screening of the extracts revealed the presence of flavonoids, tannins, saponins, carbohydrates, steroids in high concentration. Methanolic and ethanolic extracts showed higher concentration of flavonoids, phenols, terpenoids, and steroids while aqueous extracts showed higher concentration of saponins and tannins. Besides alkaloids and anthraquinone glycosides were found in smaller concentration as evident from colour intensity (Table 1). The exact percentage of phytoconstituents obtained by quantitative estimation for alkaloids and saponins were found out to be  $0.255 \pm 0.001$ ,  $2.676 \pm 0.0141$  grams per 100 g of plant material, respectively. Tannin estimation was done using method adopted by Graham, 1992 using standard curve of 10-3 M gallic acid ( $y=0.6885x+0.0114$ ,  $R^2=0.9997$ ) (Graph 1) was found out to be  $0.099 \pm 0.005$  grams per 100 g of plant material. Similarly carbohydrate estimation was done using method adopted by Krishnaveni, 1989 using standard curve of 10% glucose ( $y=69.697x+0.1328$ ,  $R^2=0.9987$ ) (Graph 2) and was found out to be  $1.584 \pm 0.090$  g/100 g of plant material. The results of phytochemical analysis provided detailed information (both qualitative and quantitative) of various phytoconstituents of *P. vulgaris*. Rasool et al. performed phytochemical analysis of *P. vulgaris* which showed difference from our results both qualitatively and quantitatively.<sup>36</sup> Our results showed the presence of terpenes, flavonoids, steroids and absence of proteins and amino acids unlike that of Rasool et al. This is most likely due to fact that our study was performed on floral spikes of *P. vulgaris* compared to whole plant used by Rasool et al. The presence of above mentioned phytoconstituents justify the use of *P. vulgaris* in the folklore system of medicine. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2.<sup>37</sup> This property may explain the mechanisms of anti-oxidative action of *P. vulgaris*. Flavonoids serve as health promoting compound

as a results of their anion radicals.<sup>38</sup> The presence of flavonoids support the usefulness of *P. vulgaris* in folklore remedies in the treatment of stress related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores.<sup>39</sup> The phenolic compounds contribute to the anti-oxidative properties of this plant and its usefulness in herbal medicament. Tannins were found in this plant and they are reported to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable antioxidant, antiviral, antitumoractivities.<sup>40</sup> The plant was found to contain saponins, known to exhibit their anti-inflammatory, anti-mutagenic, antiviral, antibacterial, antifungal and anti-tumour activities.<sup>41</sup> The plant showed positive test for steroids which are very important constituents for the synthesis of sex hormone.<sup>42</sup> The plant showed positive results for terpenoids as well, which have also been reported to possess anti-hepatotoxic activity.<sup>43</sup> Phytochemical analyses thus revealed the presence various phytoconstituents which justify its role for the treatment of various ailments in folklore system of medicine.

The present study also revealed the anti-inflammatory effect of aqueous and methanolic extracts of *P. vulgaris* in animal models. Carrageenan induced hind paw edema is a standard experimental model of acute inflammation sensitive to cyclooxygenase to evaluate the effect of non-steroidal anti-inflammatory agents. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasation, increased tissue water and plasma protein exudation, along with neutrophil extravasation, due to the metabolism of arachidonic acid.<sup>3</sup> Both aqueous and methanolic extracts from *P. vulgaris* showed significant anti-inflammatory activity ( $P < 0.05$ ) at dose of 300mg/kg of body weight in rats. Table 3 indicates the change in paw edema volume which was observed at various time intervals for rats of control group and those treated with aqueous, methanolic extracts and standard drug diclofenac. This is reflected graphically which shows there is less increase in edema

volume in rats administered with aqueous, methanolic extracts and standard drug diclofenac (Graph 3). Further it can be observed from the Graph 3 that edema volume at each time interval is maximum for control group and minimum for rats treated with standard drug diclofenac. However edema volume is rats treated with aqueous and methanolic extract is also less than rats of control group and comparable to standard group. Table 4 gives percentage anti-inflammatory effect shown by aqueous and methanolic extract and standard drug diclofenac. The maximum percentage inhibition of paw edema by aqueous (28.965%) and methanolic extract (30.860%) was at 4th and 5th hour respectively while as for diclofenac showed maximum percentage inhibition (61.075) of paw edema at 5th hour. These results are reflected graphically (Graph 4) where it can be observed that percentage inhibition of inflammation increases gradually with time. Further it can be observed from the graph that anti-inflammatory effect of methanolic extract is slightly better than aqueous extract. The anti-inflammatory effect of both the extracts is however comparable to standard drug diclofenac. Hwang et al. (2013) and Kim (2012) performed independent anti-inflammatory studies on *P. vulgaris* adopting in-vitro models.<sup>14,15</sup> Results of both the studies indicated anti-inflammatory potential of the plant which is in agreement with our results where we had adopted in-vivo model. However it is pertinent to mention that Hwang et al used hexane fraction unlike our study

where polar fractions i.e. methanolic and aqueous extracts were used for the evaluation of anti-inflammatory activity. Thus, it can be explained that phyto-constituents responsible for the anti-inflammatory activity in the two cases must be different i.e., polar phyto-constituents in our case and non-polar in case of study performed by Hwang et al. Based on HPLC analysis of ethanolic extract Kim suggested that anti-inflammatory activity could be due to rosmarinic acid. Further it is pertinent to mention that we used extracts from floral spikes of *P. vulgaris* unlike whole plant extracts used in other studies. Inflammation is a biphasic event. The first phase, which lasts for zero to 1 hour, involves release of pro-inflammatory mediators like histamine, serotonin, and bradykinin. The second phase lasts for 1 to 6 hours and involves release of TNF- $\alpha$ , IL-1 $\beta$ , COX-2, and prostaglandins increasing the severity of swelling in carrageenan induced paw edema.<sup>44,45</sup> Scavenging of these mediators may be responsible for anti-inflammatory effect of *P. vulgaris*.

## CONCLUSION

It can be concluded that *P. vulgaris* is a therapeutically potential herb which can be attributed to its phytoconstituents and justifies its use in folklore system of medicine. Besides, aqueous and methanolic extracts also showed anti-inflammatory activity which was quite comparable to the one observed for diclofenac at 10mg/kg and the results were found statistically significant.

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## التحليل الفيزيائي والنشاط المضاد للالتهابات النافعة من المستخلصات المختلفة التي تم الحصول عليها من

## زهرة لـ PRUNELLA VULGARIS L

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## ملخص

تم استخدام *Prunella vulgaris* L. المعروف أيضاً باسم الشفاء الذاتي على نطاق واسع لعلاج الأمراض المختلفة في النظام التقليدي للطب مما يشير إلى أن النبات هو مصدر مهم لمختلف المكونات النشطة بيولوجياً. أجريت الدراسة الحالية لإجراء تقييم كيميائي نباتي نوعي وكمي ونشاط مضاد للالتهابات من مقتطفات مختلفة تم الحصول عليها من طفرات *P. vulgaris*. تم الحصول على مقتطفات مختلفة من المسامير الزهرية المجففة للظلال *P. vulgaris* باستخدام طريقة الترشيح البارد. تعرضت المستخلصات لاختبارات كيميائية نباتية مناسبة لاكتشاف وتقدير مختلف المكونات النباتية. تعرضت المستخلصات المائية والميثانولية للنشاط الالتهابي في النماذج الحيوانية باستخدام طريقة نمة مخبل الكاراجينان بجرعة 300 ملغم / كغم من وزن الجسم. تم استخدام ديكلوفيناك (10 ملغ / كلغ) كمعيار مرجعي. كشفت التحليلات الكيميائية النباتية عن وجود مركبات الفلافونويد والعفص والسابونين والكربوهيدرات والمنشطات والقلويات وجليكوسيدات الأنتراكينون. كانت النسبة المئوية الدقيقة لمكونات النبات التي تم الحصول عليها عن طريق التقدير الكمي للقلويات ، السابونين ، العفص والكربوهيدرات  $0.001 \pm 0.255$  ،  $0.0141 \pm 2.676$  ،  $0.005 \pm 0.0988$  ،  $0.09 \pm 1.584$  لكل 100 غرام من المواد النباتية ، على التوالي. أظهرت كل من المستخلصات المائية والميثانولية نشاطاً مضاداً للالتهابات مع الحد الأقصى لنسبة تثبيط الالتهاب من 28.965 و 30.860 على التوالي بينما كان الحد الأقصى لنسبة تثبيط الالتهاب الذي أظهره ديكلوفيناك 60.075. كشفت التحليلات الكيميائية النباتية وجود العديد من المكونات النباتية التي تبرر دورها لعلاج الأمراض المختلفة في نظام الطب الشعبي. بالإضافة إلى أنه يمكن الاستنتاج أن كلا من المستخلصات المائية والميثانولية تظهر نشاطاً مضاداً للالتهابات كبيراً ( $P > 0.05$ ) مشابهاً لمرجع ديكلوفيناك المرجعي القياسي ( $P > 0.01$ ) بالمقارنة مع مجموعة التحكم.

الكلمات الدالة: *Prunella vulgaris* ، Phytoconstituents ، مقتطفات مضادة للالتهابات.

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