# ANTISTRESS AND ANTIOXIDANT EFFECTS OF *PRUNELLA VULGARIS* LEAVES

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## Summary

The ethanolic extract of *Prunella vulgaris* (PV) (10, 25, 50 mg/kg, p.o) was tested for its adaptogenic activity by using forced swimming test and i*nvitro* antioxidant activity by using 1,1-Diphenyl, 2-Picryl – hydrazyl free radical, Nitric oxide scavenging activity and reducing power method. In antistress activity, pretreatment with PV extract significantly reduced the immobility time at 50 mg/kg was comparable to that of imipramine (20 mg/kg, i.p) treated group. Under *invitro* 1,1- Diphenyl, 2-Picryl – hydrazyl free radical and nitric oxide free radicals are considerably inhibited in a dose dependent manner and there is increase in absorbing power indicates increase in reducing power. The results suggest that it could be used for the treatment of oxidative stress induced disorder.

**Key words:** *Prunella vulgaris*, Adaptogenic, Antistress, Antioxidant

## Introduction

The word adaptogen is used by herbalists to refer to a natural herb product that increases the body's resistance to stresses such as trauma, anxiety and bodily fatigue. An adaptogen is nontoxic to the recipient. It produces a nonspecific response in the body - an increase in the power of resistance against multiple stressors including physical, chemical, or biological agents. It has a normalizing influence on physiology, irrespective of the direction of change from physiological norms caused by the stressor. Adaptogens have a normalizing effect on the body and are capable of either toning down the activity of hyper functioning systems or strengthening the activity of hypo functioning systems (1,2). Free radicals induced by per oxidation have gained much importance because of their use in several pathological conditions such as atherosclerosis, ischemia, liver disorder, neural disorder, metal toxicity and pesticide toxicity.

Together with other derivatives of oxygen, they are inevitable byproducts of biological redox reactions. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting the lipid per oxidation and by other mechanisms and thus prevent diseases (3,4). *Prunella vulgaris* (Family : Lamiaceae) also called as self heal or heart of the Earth grow along streams, around ponds and lakes, in road side ditches, wet prairies, as well as in drier habitats(5,6). It was reported that Phenolic acids are present in *Prunella vulgaris* (7) act as antilymphoma, exhibited immune stimulatory and anti inflammatory effect (8,9). Keeping in view if the specific pharmacological activities of various constituents of the herbs, the adaptogenic and antioxidant activity has been evaluated in the leaves extract of *Prunella vulgaris.*

## Methods

***Preparation of the extract*** The leaves of *Prunella vulgaris (PV)* were purchased from Munnalal Dawasaz (Mfg of ayurvedic medicine) and shade dried. The plant was authentified by an expert. The coarse powder was then macerated for 48 hrs and filtered using muslin cloth. The extracts were concentrated and evaporated to dryness under reduced pressure at 50$^{o}$c.

***Animals*** *A*lbino rats (160-180 g) of either sex were used. They were kept in standard plastic animal cages in groups of 6-8 animals, with 12 hr of light and dark cycle in the institutional animal house. The animals were fed with standard rodent diet and provided water *ad libitum*. After one week of acclimatization the animals were used for further experiments. Approval from the institutional animal ethical committee for the usage of animals in the experiments was obtained as per the Indian CPCSEA guidelines. Approval number of institutional Animal Ethical Committee for this study was IEAC/SUCP/06/2007.

***Acute toxicity test*** Acute toxicity of the preparation was determined using different groups of rats. The animals were fasted for 3 hrs prior to the experiment according to the recommended procedure (OECD guide no.425)**(**10). As per guidelines, the animals were observed for 48 hrs for any mortality was observed and the preparation was found to be safe up to dose of 5000 mg/kg b.wt

***Adaptogenic activity /Anti stress activity*** (11,12,13) The rats of either sex 120-150 gm were divided into 5 groups. Each group containing six animals.Group I – Control vehicle 10 ml/kg b.wt p.o, Group II – Imipramine 20 mg/kg b.wt i.p, Group III – PV 10 mg/kg b.wt.p.o, Group IV – PV 25 mg/kg b.wt .p.o and Group V – PV 50 mg/kg b.wt .p.o. The PV extract was solubilised in 1% CMC and administered to the Groups ( III, IV, and V) animals for 15 days.

***Forced Swimming test*** (14) The rats are individually forced to swim inside a vertical plexiglas's cylinder (height: 40cm; diameter: 18cm, containing 15cm of water maintained at 25$^{o}$C). Rats are placed in the cylinders for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5-6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water the rats are removed and allowed to dry in a heated enclosure 32$^{o}$C before being returned to the cages. They are again placed in the cylinder 24 h later and the total duration of immobility is measured during a 5 min test.

Floating behaviour during this 5min period has been found to be reproducible in different groups of rats. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. Standard drug Imipramine was administered one hour prior to testing. The results are shown in **Table 1** and depicted in **Figure 1.**Statistical Analysis Statistical analysis was performed using One way ANOVA followed by Dunnets test. P values <0.05 were considered significant.

***InVitro Antioxidant Activity***

***DPPH Radical Scavenging Activity*** (15,16,17) DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diagnostic molecule. The reduction capacity of DPPH radical was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. 0.1Mm solution of DPPH in ethanol was prepared and 0.1 ml of this solution was added to 3.0 ml of PV extract solution in water at different concentrations (10-100 ug/ml). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Experimental was done in triplicate. The difference in the absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as percent scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Scavenging effect (%) = [ 1 – Abs. of sample / Abs. of control] x 100

The results are shown in **Table 2** and depicted in **figure 2**

***Nitric oxide scavenging*** (15,16,17) The nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH is inhibited by antioxidants which compete with oxygen to react with nitric oxide. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine (NED) was read at 546 nm. Nitric oxide was generated from sodium nitroprusside and measured by the Griess reagent. Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4) saline was mixed with different concentrations (10, 20, 40, 50, 60, 80, 100 ug/ml) of PV extract and incubated at 25$^{o}$C for 150min. To 1 ml of the incubated solution, 1 ml of Griess reagent (1% sulphanilamide, 2% O – Phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride) was added. Absorbance was measured at 546 nm. % inhibition of OD was calculated by using the formula. Experiment was done in triplicate. Capability to scavenge the nitric oxide radical was calculated by using the equation: % inhibition = [ 1 – Abs. of sample / Abs. of control] x 100

The results are shown in **Table 3** and depicted in **figure 3**

***Reducing power***(18) Substances which have reduction potential react with potassium ferricyanide ($Fe^{3+}$) to form potassium ferrocyanide ($Fe^{2+}$) which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm. Different doses of Ethanolic extract of PV were mixed in 1 ml of distilled water so as to get 20, 40, 60, 80, 100 ug concentration. This was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50$^{o}$C for 20 min. A portion (2.5 ml) of tricholoro acetic acid (10%) was added to the mixture, which was then centrifugated at 3000 rpm for 10 min.

The upper layer of the solution (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction indicate increase in reducing power.The % reducing power was calculated by using the formula:

% increase in the absorbance = Control OD – Test ODx 100

Control OD

The results are shown in **Table 4** and depicted in **figure 4**

## Results

**EFFECT OF PV EXTRACT ON IMMOBILITY TIME IN FST IN RATS Table – 1**

| S.No | Group | Immobility Time In Seconds |
|---|---|---|
| 1 | Control 1% CMC | 180 ± 3.2 |
| 2 | Standard Imipramine (20mg/kg) | 105 ± 2.19 |
| 3 | PV Extract 10mg/kg | 160 ± 6.4* |
| 4 | PV Extract 25mg/kg | 135 ± 3.3** |
| 5 | PV Extract 50mg/kg | 120 ± 4.0** |

The Results are expressed as mean of six animals, n=6

* $P < 0.05$ when compared to control ** $P < 0.001$ when compared to control

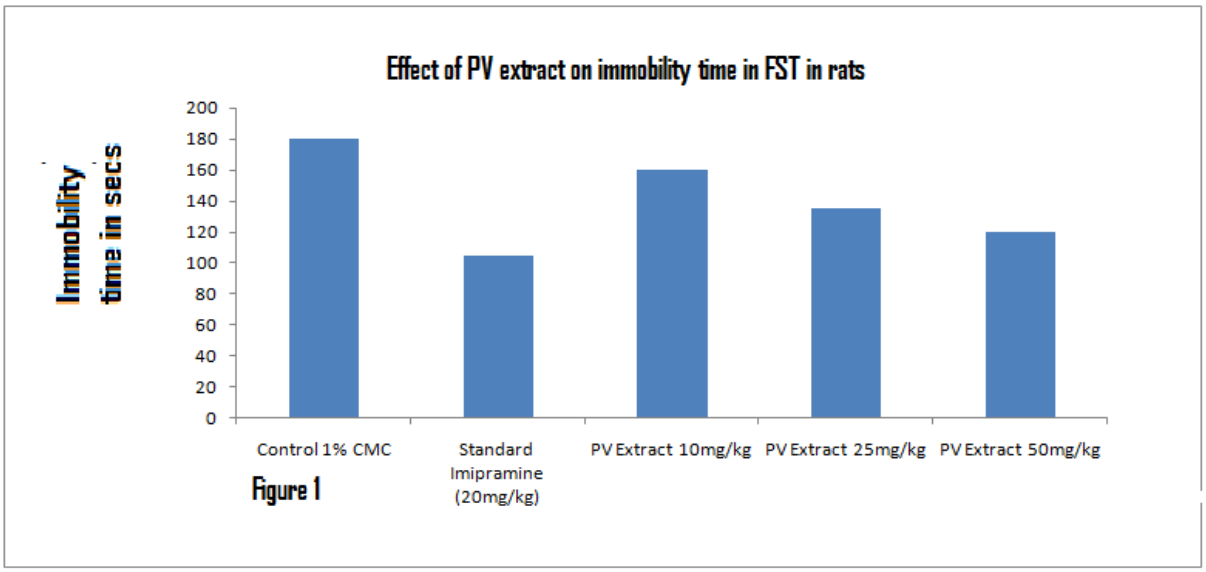
Effect of PV extract on immobility time in FST in rats
Immobility time in secs
200
180
160
140
120
100
80
60
40
20
0
Control 1% CMC
Standard Imipramine (20mg/kg)
PV Extract 10mg/kg
PV Extract 25mg/kg
PV Extract 50mg/kg

Figure 1

**DPPH radical scavenging activity**

**Table – 2**

| Conc. of extract (ug/ml) | Absorbance at 517 nm | % Inhibition |
|---|---|---|
| 0 (Control) | 0.884 | 0 |
| 10 | 0.628 ± 0.004* | 28.95 |
| 20 | 0.317 ± 0.002* | 64.14 |
| 40 | 0.192 ± 0.002* | 78.25 |
| 60 | 0.151 ± 0.003* | 82.91 |
| 80 | 0.093 ± 0.002* | 89.47 |
| 100 | 0.078 ± 0.004* | 91.17 |

Values are mean of three replicates

*P < 0.001 when compare to control.

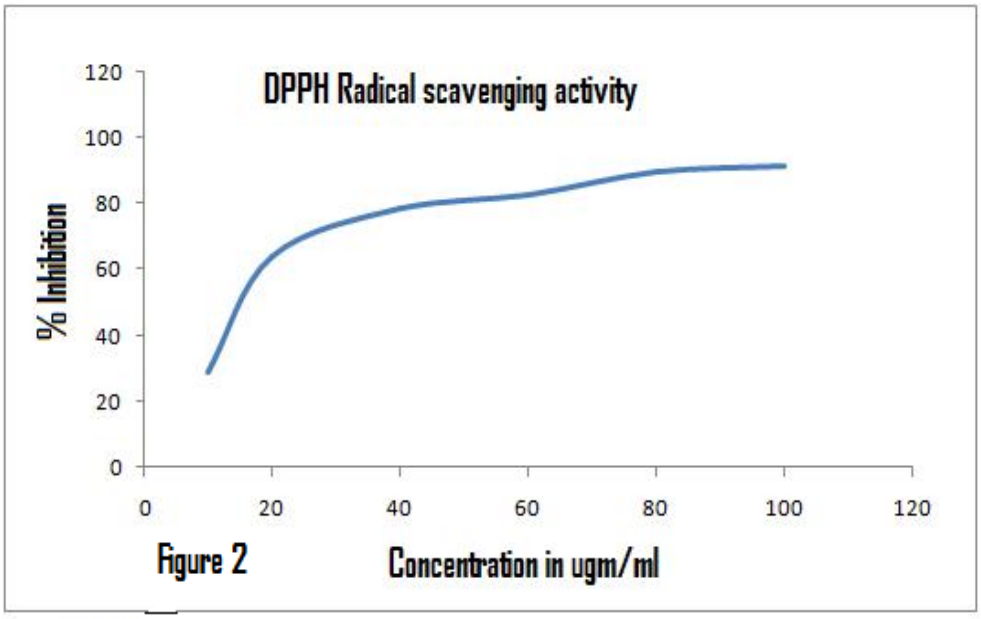


Figure 2

**Nitric oxide scavenging method**

**Table – 3**

| Conc of extract ( ug/ml) | Absorbance at 546 nm | % Inhibition |
|---|---|---|
| 0 (Control) | 0.869 | 0 |
| 20 | 0.546 ± 0.005* | 37.16 |
| 40 | 0.336 ± 0.002* | 61.33 |
| 60 | 0.252 ± 0.004* | 71.00 |
| 80 | 0.189 ± 0.003* | 78.25 |
| 100 | 0.147 ± 0.002* | 83.08 |

Values are mean of three replicates *P < 0.001 when compare to control.

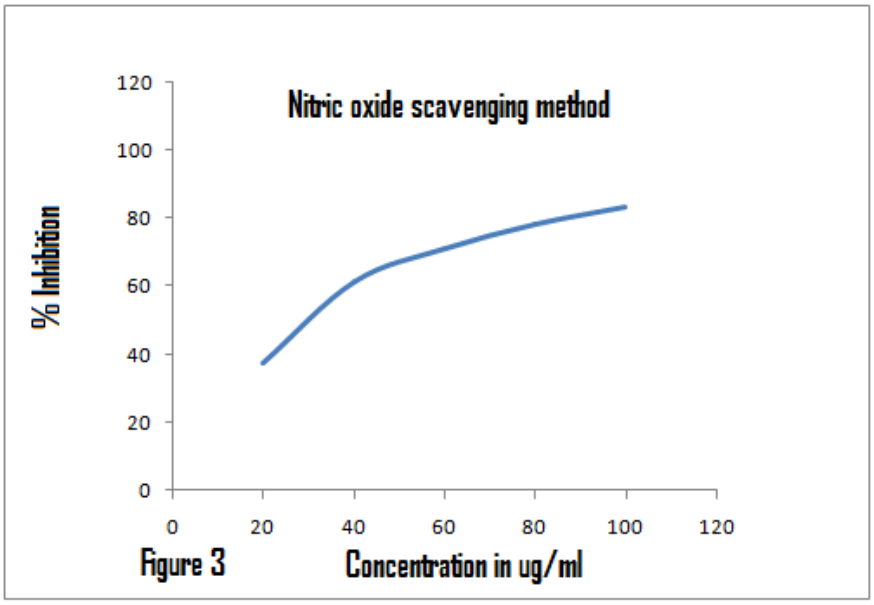


Figure 3

**Reducing Power method**

**Table 4**

| Conc of extract ( ug/ml ) | Absorbance at 700 nm | % Inhibition |
|---|---|---|
| 0 (Control) | 0.852 | 0 |
| 10 | 0.852 ± 0.006* | 114.2 |
| 20 | 0.062 ± 0.004* | 110.1 |
| 40 | 0.138 ± 0.003* | 101.2 |
| 60 | 0.231 ± 0.002* | 90.25 |
| 80 | 0.406 ± 0.03* | 69.7 |
| 100 | 0.638 ± 0.002* | 42.4 |

**Values are mean of three replicates** ***P < 0.001 when compare to control.**

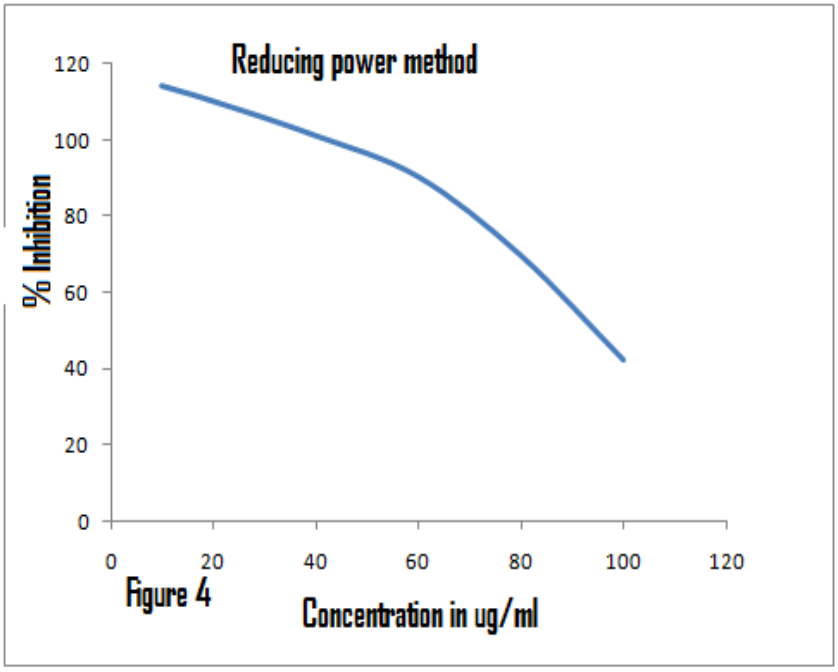


Figure 4

Animals were observed for mortality by acute toxicity test in mice for 48 hrs. No mortality was observed and the PV extract was found to be safe up to a dose of 5000 mg/kg. PV extract result decrease in immobility time in concentration dependent manner, the extract at 50 mg/kg shows significant decrease in immobility ($p<0.001$) as compared to control. The results suggest that the PV extract posses anti-stress activity. It may be due to the presence of phenolic acids in *Prunella vulgaris* (7) posses immune stimulatory, anti inflammatory effects, antilymphoma activity (8,9). In the DPPH radical scavenging assay the drug at various concentrations of ethanolic solution of the drug produced inhibition of DPPH in dose dependent manner. The obtained results of absorbance and % inhibition showed decrease in the concentration of DPPH radical due to scavenging ability of extracts and at 100 µg/ml of ethanolic extract exhibits 91.1% inhibition $IC_{50}$ values were found to be 30 µg/ml. The nitric oxide generated from sodium nitro prusside when react with oxygen from nitrite, which inhibited by antioxidants by competing with oxygen to react with nitric oxide. The % inhibition and $IC_{50}$ values found to be 83.08% and 45 µg/ml. The PV extract exhibits significant antioxidant activity with low $IC_{50}$ values in these two methods. As there is increase in absorbance indicated significant increase in reducing power.

## Discussion

There is extensive evidence that single-dose administration of adaptogens activates corticosteroid formation, and that repeated dosage with adaptogens normalizes the levels of stress hormones, such as adrenocorticotropic hormone (ACTH). It is known that the blood level of corticosteroids increases as a result of long-term training or adaptation, and that a trained organism responds to stress stimuli with only mildly increased activity of the hypothalamic-pituitary axis (HPA), as opposed to a very pronounced increase in activity seen in untrained states**.** In other words, adaptogens apparently increase the ability of the stress system to respond to stress stimuli in a manner that tends to preserve homeostasis, particularly by modulating the biosynthesis of eicosanoids - including prostaglandins E2 and F2, 5-hydroxyeicosatet racenoic acid (5-HETE), 12-HETE, and leukotriene B4. Moreover, adaptogens also appear to regulate the basal level of the arachidonic acid and also to do this under various stressful conditions, such as immobilization, heavy physical exercise, and radiation injury. Although there is a difference in the mode of action and pharmacologic activity of different adaptogens. The primary site of action of adaptogens appears to be the HPA, and their secondary sites of action the liver and components of the immune and cardiovascular systems. The excessive activity of the stress system is associated with increased arousal or anxiety, increased blood pressure, gastrointestinal dysfunction, and suppression of the immune response. Adaptogens are also thought to function primarily due to their antioxidant and free radical scavenging effects (1,2). Free radicals are chemical entities that can exist separately with one or more unpaired electrons. Antioxidants may offer resistance oxidative stress by scavenging free radicals. Free radical or reactive oxygen species (ROS) are produced during biochemical redox reactions as part of normal physiological cell metabolism (protection from infectious organism) and as a response to environmental factors such as UV light, cigarette smoke, environmental pollutants and gamma radiations. Once formed, ROS attack cellular components causing damage to lipids, proteins and DNA, which can initiate numerous diseases, including cancer, atherosclerosis, rheumatoid arthritis, diabetes, liver damage and central system disorders. Living organisms have a large number of antioxidants, including macro and micro molecules and enzymes which represent total antioxidant activity of the system play a central role in preventing oxidative stress (3,4).

The results suggest that the PV extract posses anti-stress activity. It may be due to the presence of phenolic acids *Prunella vulgaris* (7) posses immune stimulatory, anti inflammatory effects, anti lymphoma activity (8,9). The ethanolic extract of PV produced significant % inhibition in the levels of DPPH and NO and significant increase in reducing power. In conclusion, the present study shows that PV extract posses adaptogenic activity and antioxidant activity. Further studies are in progress to identify the mechanisms underlying the pharmacological activity observed.

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