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Original Research Article

In vitro anti-oxidant and anti-inflammatory activities of hydroalcoholic extract of leaves of *Valeriana Jatamansi*

Manjinder Kour[1], Harsimran Singh[1], Jagdeep Kaur[1]*

[1]Department of Pharmacology, Sri Sai College of Pharmacy, Punjab, India

*Corresponding author's email: jagdeepkaur10@gmail.com

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Abstract

The study aims to evaluate in vitro anti-oxidant and anti-inflammatory activities of hydroalcoholic extract of leaves of *Valeriana jatamansi*. In addition, the phytochemical screening of the extract had been carried out. The anti-oxidant activity was compared with the ascorbic acid standard whereas anti-inflammatory activity was compared with diclofenac sodium. Phytochemical screening evaluated the presence of flavonoids, alkaloids, phenols, tannins and terpenoids. With the increase in the concentration of extract, anti-oxidant and anti-inflammatory property increased. IC50 value of plant extract revealed that the extract has more potent antioxidant activity as compared to Vitamin C which may be attributed to the presence of flavonoids, tannins and phenols in the extract.

Key words

Valeriana jatamansi, Anti-oxidant, Anti-inflammatory, Phytochemical screening.

Introduction

The drug is intended to use in various purposes like diagnosis, prevention, treatment of the disease and maintenance of health. There are four sources of drugs: natural source, semi-synthetic source, synthetic source and biosynthetic source. Natural sources are the most abundant source of drugs [1]. The natural sources are divided into four types: plant source, animal source, mineral source and micro-organisms source [2]. Herbal drugs are use of plants for medicinal purposes. About 80% of the world population are depending upon herbal drugs [3]. Herbal drugs are great demand in the developed world for primary health care

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because of their efficacy, safety and lesser side effects [4]. India has richest medical plants traditional in the world [5]. According to WHO (World Health Organization), the use of herbal treatment throughout the world is two to three times much better than that conventional drugs [6]. The most of effective drugs are herbal plants base such as digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) [7].

Various advantages of herbal drugs are: herbal drugs are very cheap, herbal drugs do not have any side effects, as they are free from chemicals, easy available, easy biodegradable, easy to handle and herbal drugs can be brought without prescription and they are available in all most all health stores [3, 4]. Lack of studies on the isolation and purification of the synthetic compounds, the herbal drugs are most commonly used in the various diseases. There are number of herbal drugs like *Emblica officinalis, Ocimum sanctum, Zingiber officinalis, Curcuma longa, Azadirachta indica, Cinnamomum zeylanicum* act as anti-oxidant, anti-inflammatory, anti-diabetic, anti-cancer and cardioprotective agents [7].

Valeriana jatamansi is a perennial herb and tetraploid species belonging to family Valerianaceae. *Valeriana jatamansi* is commonly named as Tagar, Sugandhawal and Jatamansi. It is widely distributed in Western Himalayan, Kashmir, Garhwal, Khasi hills and Bhutan at the heights of 2500-3000 meters [8, 9]. The herb attains the height up to 40-50 cm with a thick horizontal root stick. The leaves are 2.5-7.5 cm long. The flowers are unisexual, pinkish white in color and 2-7 cm long. Leaves are persistent, long petioled, deeply cordate-ovate and toothed or sinuate. The fruits are oblong, compressed and hairy. The rhizomes are irregular in shape and quite characteristic and bitter in taste [9]. The seeds are planted in the nursery in February-March. Harvesting of *Valeriana jatamansi* is done during September to November. The whole plant is pulled out and only rhizomes are collected [8]. The major chemical constituents of *Valeriana jatamansi* are valerenic acid (sesquiterpenoids), valepotriates (iridiod esters), alkaloids, baldrinal, homobaldrinal, amino acid, phenolic acid, flavonoids, valerosidatum, chlorogenic acid, caffeic acid and fatty acid [8, 10]. The reported activities of *Valeriana jatamansi* are anti-oxidant [11], anti-inflammatory [12, 13], anti-diarrhoeal [14] and anti-microbial [11]. Further, the reported activities of *Valeriana jatamansi* are anti-oxidant due to the presence of phenolic compounds [11], anti-inflammatory due to the presence of volatile oils [13], anxiolytic activity due to the presence of valtrate [15, 16, 17], anti-diarrhoeal due to the presence of flavonoids [14] and anti-microbial due to the presence of volatile oils [13]. The traditional uses of *Valeriana jatamansi* are liver protection, sleep improvement, skin disease, obesity, wound healing, anti-spasmodic and snake poisoning [18].

Oxidative stress is defined as the balance between anti-oxidants and reactive oxygen species (ROS) is disturbed due to depletion of anti-oxidants or accumulation of ROS [19, 20, 21]. Oxidative stress involved in various pathological conditions such as cancer [22, 23], atherosclerosis [24], hypertension [25], ischemia [26, 27], diabetes [28], acute respiratory distress syndrome [29], idiopathic pulmonary fibrosis [30], chronic obstructive pulmonary disease [31, 32] and asthma [33, 34].

Inflammation is defined as the local tissue response to cellular injury which caused by various agents such as physical agents, chemical agents, infective agents or immunological agents [35]. Inflammation is a process in which body's immune system protects us from harmful stimuli

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such as pathogens to prevent infections and to restore the body cells to normal state [36]. It is characterized by redness and swelling with heat and pain [35]. Inflammation can be classified either as acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leucocytes from the blood into the injured tissues [37, 38]. Chronic inflammation is prolonged inflammation and characterized by simultaneous destruction and healing of tissues from the inflammatory process [39].

The effect of free radical scavenging property and anti-inflammatory action had yet not been elucidated in the leave part of *Valeriana jatamansi*. Therefore, the main objective of this investigation was to carry out phytochemical screening, examine in-vitro anti-oxidant and anti-inflammatory effect of hydroalcoholic extract of leaves of *Valeriana jatamansi.*

Material and methods

Plant material

Leaves of *Valeriana jatamansi* was collected and authenticated from Dr. Y. S. Parmar University, Solan Himachal Pradesh.

Method of preparation of extract

Preparation of hydroalcoholic extract of *Valeriana jatamansi*

The leaves of the plant were washed with running tap water and then shade dried. Leaves were cut into small pieces and grinded into coarse powder using a blender. Then 50 g of coarse powder were defatted by using 140 ml of petroleum ether and further the marc was extracted by using 50% ethanol in soxhlet apparatus. The extract was concentrated on water bath and this extract was stored in airtight container in a cool place.

Phytochemical screening

The hydroalcoholic extract of the plant was screened for the presence of various phytoconstituents such as carbohydrates, saponin, glycosides, protein, amino acids, alkaloids, terpenoids, tannins, flavonoids, phenolic compounds, and carbohydrates [6, 7].

Principle of DPPH assay

The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-b-picrylhydrazyl; DPPH) is a stable free radical. It has delocalised spare electron over the molecule as a whole. Due to this reason the molecule does not dimerize. The delocalization of electron provides deep violet color to it, which has characteristic band absorption in ethanol solution at about 517 nm. When a solution of DPPH is mixed with that of a substrate which can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. The anti-oxidant potential of an agent is determined through its free radical scavenging in terms of change in optical density of DPPH [40].

Procedure for anti-oxidant activity

The working solutions (10, 20, 40, 60, 80, 100 µg/ml) of the extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml. 1 ml of DPPH solution (0.1 mM in methanol) was mixed with 3 ml of sample extracts and standard solutions separately. The mixture was shaken and kept for 30 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of extracts was determined at 517 nm using UV spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Vitamin C was used as reference standard. DPPH (3 ml of 0.1 mM) and methanol (1 ml) was used as blank.

The DPPH radical scavenging activity was calculated using the following formula:

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DPPH radical scavenging activity (% inhibition) = [(A0–A1/A0) × 100]

Where A0 is the absorbance of the blank, and A1 is the absorbance of extract mixed with DPPH.

IC50 value (inhibitory concentration at which DPPH radicals where scavenged by 50%) was obtained by interpolation from linear regression analysis [11].

Procedure for anti-inflammatory activity

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the test extract so that final concentrations become 10, 20, 40, 60, 80, 100 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2 ˚C in a BOD incubator for 15 minutes and then heated at 70 ˚C for 5 minutes. After cooling, their absorbance was measured at 660 nm (UV spectrophotometer) by using vehicle as blank. Diclofenac sodium at the final concentration of (10, 20, 40, 60, 80, 100 µ/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 × [Vt / Vc – 1]

Where, Vt = absorbance of test sample, Vc = absorbance of control [41].

Results

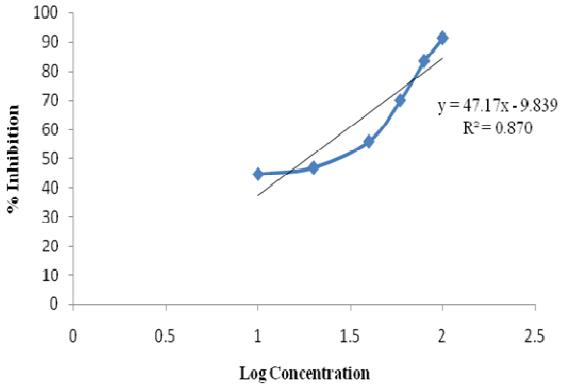
Phytochemical screening

The phytochemical screening of hydroalcoholic extract of *Valeriana jatamansi* showed the presence of flavanoids, alkaloids, phenols, tannins and terpenoids.

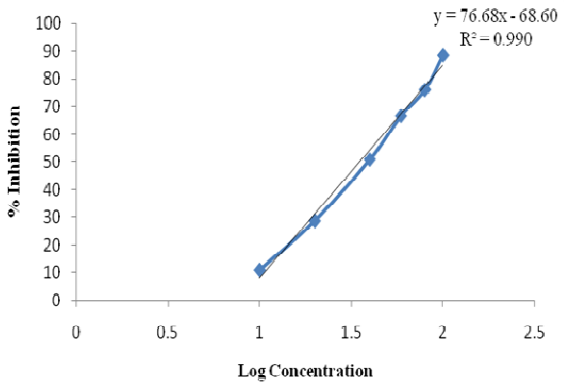
Anti-oxidant activity using DPPH free radical scavenging method

In the DPPH free radical scavenging activity, *Valeriana jatamansi* was evaluated for their free radical scavenging activity with ascorbic acid as standard compound and the entire test were preformed in triplicate series. The IC50 was calculated for test compounds as well as ascorbic acid as standard by linear regression analysis of dose response curve plotting between % inhibition and log concentration as per **Table - 1** and graphically represented as per **Graph - 1(a)** and **Graph - 1(b)**.

Graph - 1(a): Log concentration vs. % inhibition for *Valeriana jatamansi* by DPPH free radical scavenging assay method.



Graph - 1(b): Log concentration vs. % inhibitions for Vitamin C by DPPH free radical scavenging assay method.



The scavenging effect increased with the increasing concentrations of test compounds. The IC50 value for *Valeriana jatamansi* was 0.7, which were comparatively lower than the IC50

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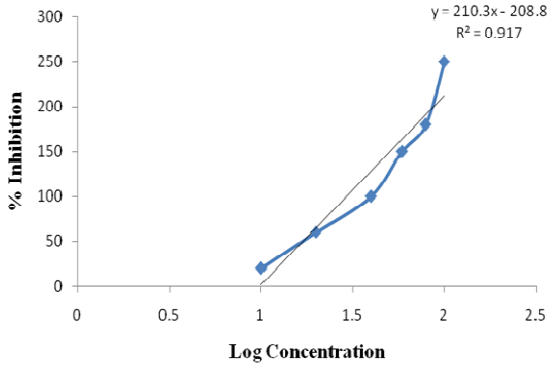
(1.4) of ascorbic acid. IC50 value of *Valeriana jatamnsi* showed high scavenging activity than ascorbic acid.

Anti-inflammatory activity

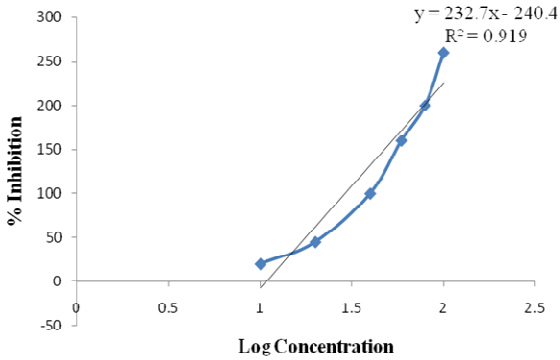
The anti-inflammatory effect of herbal extract (*Valeriana jatamansi*) and diclofenac sodium against denaturation of egg albumin was given in **Table – 2**. The IC50 was calculated for test compounds as well as diclofenac sodium as standard and the entire test were preformed in triplicate. The IC50 was calculated for test as well as diclofenac sodium as standard by linear regression analysis of dose response curve plotting between % inhibition and log concentration as per **Table - 2** and graphically represented in **Graph - 2(a)** and **Graph - 2(b)**.

Graph - 2(a): Log concentration vs. % inhibition for *Valeriana jatamansi* by anti-inflammatory activity.



The anti-inflammatory activity was increased with increased in concentration of test compound. The IC50 value for *Valeriana jatamansi* was 1.49 which was comparatively higher than the IC50 (1.533) of diclofenac sodium. The IC50 of extract showed equivalent anti-inflammatory activity as diclofenac sodium.

Graph - 2(b): Log concentration vs % inhibitions for Diclofenac sodium by anti-inflammatory activity.



Discussion

In our study hydroalcoholic extract of leaves of *Valeriana jatamansi* at a dose of 10, 20, 40, 60, 80, 100 µg/ml, showed anti-oxidant and anti-inflammatory activities.

Oxidative stress is the balance between anti-oxidants and reactive oxygen species (ROS), which is disturbed due to the generation of ROS [20]. The oxidative stress involved in various pathological conditions such as atherosclerosis, hypertension, ischemia, diabetes, etc [24-26, 28]. ROS generates oxidative stress which leads to molecular damage and causes cellular effects [42]. The preliminary phytochemical screening of *Valeriana jatamansi* leaves revealed that hydroalcoholic extract showed the presence of carbohydrates, flavonoids, alkaloids, terpenoids and phenols. The hydroalcoholic extract of *Valeriana jatamansi* shows anti-oxidant activity at different concentration i.e., 10, 20, 40, 60, 80 and 100 µg/ml. In our study, the screening of the anti-oxidant activity of plants *Valeriana jatamansi* had revealed its capacity to scavenge the free radicals by using DPPH method at high concentration may be due to the presence of alkaloids and polyphenol content i.e., thymol and carvacrol. IC50 value of *Valeriana jatamansi* were 0.7 which were comparatively lower than

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the IC50 (1.4) of ascorbic acid as standard. *Valeriana jatamansi* showed significant anti-oxidant activity when compared with standard.

Inflammation is a local tissue response to cellular injury which caused by various agents such as physical agents, chemical agents, etc [35]. The inflammation involved in various pathological conditions such as rheumatoid arthritis, asthma, chronic hepatitis, etc [43]. The hydroalcoholic extract of *Valeriana jatamansi* in in-vitro anti-inflammatory activity exhibits increased anti-inflammatory effect with increasing concentration of test compounds. The IC50 value for *Valeriana jatamansi* were 1.49 which were comparatively lower than the IC50 (1.52) of diclofenac sodium as standard. Our study revealed that *Valeriana jatamansi* had anti-inflammatory activity at high concentration may be because it inhibits mediator of inflammation due to the presence of flavonoids, tannins and polyphenols components.

Conclusion

On the basis of results obtained in the present study, the following salient findings can be summarized.

- The DPPH free radical scavenging effect increased with the increasing concentrations of test compounds and this can be attributed to free radical scavenging activity of many of the active ingredients present in the extract.
- The anti-inflammatory activity increased with the increasing concentrations of test compounds due to the presence of flavonoids that inhibits the production of pro-inflammatory cytokines and chemo-tactic agents.

Therefore, it had been concluded from the above mentioned findings that the *Valeriana jatamansi* exhibits anti-oxidant and anti-inflammatory activities. Thus, further studies are warranted to elucidate the mechanisms responsible for the anti-oxidant and anti-inflammatory activities at the molecular levels.

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Table – 1: DPPH free radical scavenging activity of Test compound and Standard.

Conc. (µg/ml)	Log Conc. (µg/ml)	% Inhibition of *Valeriana jatamansi* (VJ)	% Inhibition of Ascorbic acid (AA)	IC50 value of VJ	IC50 value of AA
10	1	44.8	11.11	0.7	1.39
20	1.3	47.05	28.84		
40	1.6	55.96	50.94		
60	1.77	70.01	66.66		
80	1.9	83.37	76.05		
100	2	91.25	88.6		

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Table – 2: Anti-inflammatory activity of Test compound and Standard.

Conc. (µg/ml)	Log Conc. (µg/ml)	% Inhibition of *Valeriana jatamansi* (VJ)	% Inhibition of diclofenac sodium (DS)	IC50 value of VJ	IC50 value of DS
10	1	20	20	1.49	1.533
20	1.3	60	45		
40	1.6	100	100		
60	1.77	150	160		
80	1.9	180	200		
100	2	250	260		