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Research Article

EVALUATION OF ANTIOXIDANT PROPERTIES OF *Valeriana wallichi* TO SCAVENGE FREE RADICALS.

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ABSTRACT

Antioxidants have imperative anticipatory roles, not merely on detrimental changes in the aroma and dietetic eminence of food, although on tissue damage in an assortment of human diseases. Cellular smash up or else oxidative injury arising from free radicals or reactive oxygen species (ROS) nowadays appears the elementary mechanism underlying a numeral of human being neurodegenerative disorders, viral infections, diabetes, inflammation, autoimmune pathologies along with digestive system disorders. Free radicals are generated all the way through customary metabolism of drugs, ecological chemicals and erstwhile xenobiotics as well as endogenous chemicals, particularly stress hormones (adrenalin and noradrenalin). Accumulated substantiation suggests that ROS can be scavenged during chemoprevention utilizing innate antioxidant compounds present in foods and medicinal plants. The Methanolic extracts of *Valeriana wallichi* was used in present study and also screened for the presence of phyto-chemicals viz. alkaloids, flavonoids, tannins, saponins, glycosides etc and their effect on 2,2-Diphenyl-1-picryl-hydraxyl radical (DPPH) which was used to determine the free radical scavenging activity.

Keywords: Antioxidants, Free radicals, Phytochemical Screening, DPPH

INTRODUCTION

Numerals of notorious antioxidants as well as so far indefinite antioxidants are hypothetically present in plants. These antioxidants are obtainable to do a lot of good to human health by sequester the dangerous free radicals which are generated owing to physiological errors in the cells. It can forage oxygen radicals in that way preventing harmful affects on DNA, intracellular proteins in addition to membrane lipids. There is vibrant stability flanked by the amount of free radicals generated in the body furthermore antioxidants aligned with their harmful effects[1]. Though, the amounts of these defensive antioxidant principles present beneath the ordinary physiological conditions are adequate only to deal with with the physiological rate of free radical production. It is hence obvious that any supplementary burden of free radicals, whichever from environment or formed within the body can tip the free radical (pro-oxidant) and anti free radical (antioxidant) equilibrium leading to oxidative stress; which may perhaps consequence in tissue injury along with subsequent diseases[2].

Presently, there is a sturdy concern in plants as pharmaceuticals, particularly from edible plant parts, for the reason that these compounds engage in recreating an imperative role, preventing free radical induced diseases like as cancer moreover atherosclerosis[3]. The blow of a number of diseases might be prohibited by recuperating the nutritional intake of ordinary nutrients in the midst of antioxidant properties such as vitamin E, vitamin C, β -carotene as well as plant phenolics such as tannins and flavonoids[4]. The occurrence of phytochemicals in therapeutic plants has concentrated on their role in preventing diseases. Therefore, in current study attempts will be prepared to screen Indian Medicinal Plant viz. *Valeriana wallichi* for their antioxidant activity moreover their phytochemical evaluations. The plants will be screened for the presence of phyto-chemicals viz. alkaloids, flavonoids, tannins, saponins, glycosides etc and their effect on 2,2-Diphenyl-1-picryl-hydraxyl radical (DPPH) will be used to determine their free radical scavenging activity.

Valeriana wallichii normally known as Indian valerian is one of the significant plant species which belong to the family Valerianaceae. It is inhabitant to India (Himalayas). It is used in an assortment of pharmaceutical preparations for the cure of migraine. The active constituent of *Valeriana wallichi* is the root which is valerenic acid, valerenol, valerenone, valtrate, Isovaltrate. The plant root occurs in short, irregular pieces about 5 cm long and 6-12 cm in diameters discernible with oblique ridges along with bearing abundant, outstanding, spherical tubercles, to a number of of which on the beneath the surface, broad rootlets are attached. The upper surface bears the remnants of leaves. The rhizome is solid also tough internally, it is greenish-brown in colour along with the odour is effectively valerianaceous[5,6].

MATERIALS AND METHODS

Collection

Authentic samples: Various market samples of *Valeriana wallichi* were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

Identification

All the samples were authenticated and were given identification number. The identification was as follows:

These samples were authenticated and submitted in Ethno-medicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Processing of plant materials

During the course of the study each sample was screened for its foreign matter and milled, before use.

Experimental details

Present studies were performed on *Valeriana wallichi* for the following studies-.

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant

1. Phytochemical Screening

Phytochemical screening was performed using standard procedure:

Test For Reducing Sugars (Fehlings Test)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test For Terpenoides (Salkowski Test)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

Test For Flavonoides

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

Test For Tannins

About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test For Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously. It was observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test For Alkaloids

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent.

The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

2. Antioxidant Activity

Preparation of test extracts

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

Preparation of DPPH

DPPH (2, 2'-diphenyl-1-picrylhydrazl, $C_{18}H_{12}N_5O_6$; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08 mg/ml for antioxidative (qualitative and quantitative) assay.

Qualitative assay

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25µl was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517 nm on a UV spectrophotometer (Varian Cary PCB 150, Water Peltier System)

Quantitative assay

A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations (10^2µg to 10^{-3} µg/ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance (RC_{50}) was calculated. The same procedure was also followed for the standards- quercetin and ascorbic acid.

RESULTS AND DISCUSSION

Table 1: Showing Optical density of *Valeriana wallichi* on different concentrations.

Concentration (µg/ml)	O.D (nm)
0.001	1.578
0.01	1.439
0.1	1.321
1	1.226
10	1.472
100	1.638
1000	0.487

In the present investigation it was showed that the maximum optical density comes out to be 1.638 nm which is at the concentration 10^2 µg/ml and the smallest optical density is 0.487 nm which is at the concentration 10^3 µg/ml where as the other shows comparable O.D at different concentrations i.e. 1.578 nm at 10^{-3} µg/ml, 1.439 nm at 10^{-2}µg/ml, 1.321 nm at 10^{-1} µg/ml, 1.226 nm at 1 µg/ml, 1.472 nm at 10^1 µg/ml.

In the present investigations antioxidant activity of *Valeriana wallichii* showed appreciable activity against the DPPH assay method where the regression line clear cut showed the effectiveness of it as it's have potentials which are comparable to ascorbic acid. The antioxidant activity of *Valeriana wallichii* in methanolic extract using DPPH assay method (Tahao, 1994) shows appreciable activity comparable to standard ascorbic acid. The straight line showed Y= -0.182x+1.776 & regression = 0.838 whereas, in above drug the straight line is Y= -0.097x+1.697 & regression = 0.292.

Table 2: Showing phytochemical screening results of *Valeriana wallichi*.

	Valeriana wallichi					
TEST	**Reducing Sugar**	**Saponin**	**Tannin**	**Terpenoides**	**Flavonoides**	**Alkaloides**
	-ve	**-ve**	**-ve**	**-ve**	**-ve**	**+**

The phytochemical screening of *Valeriana wallichi* shows the occurrence of alkaloids whereas it shows the absence of flavonoids, saponin, tannin, terpenoids and reducing sugar respectively. The screening of the *Valeriana wallichi* formulate only a a diminutive amount of differences in the ingredient of the hard-bitten plants. The drug shows the validation of strapping antioxidant activity corresponding or in a less important amount. The continuation of alkaloids in this plant is convincing to be painstaking for the free radical scavenging effects pragmatic.

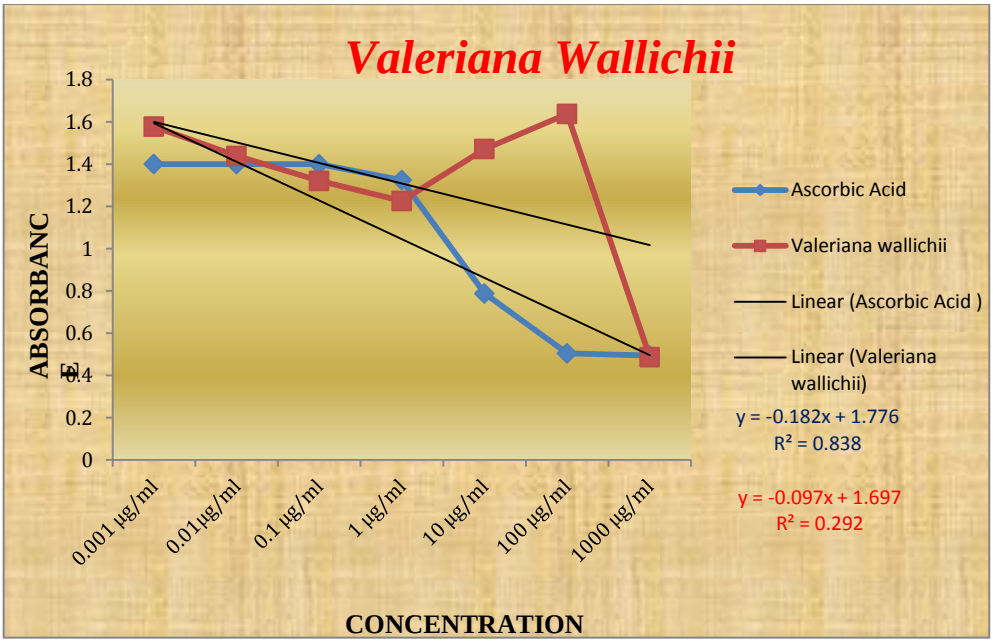

Fig 1: Graph showing Antioxidant Activity of *Valeriana wallichi* at different concentration.

CONCLUSION

Free radicals form the central part of contemporary hypothesis of disease. It is a contradiction that oxygen, which is indispensable to life, possibly will also put in the human ageing and illness. An antioxidant of peripheral source can put off oxidative damage by inhibiting the production of reactive species, scavenging free radicals or raising the echelon of endogenous antioxidant defense. Supplementation of normal antioxidants throughout a balanced diet can be more successful and also further economical than the supplementation of an entity antioxidant i.e. vitamin C or vitamin E, in shielding the body alongside oxidative damage below various conditions. The methanolic extract of *Valeriana wallichi* be experimental to screening. The ensuing test systems were worn for the compound analysis. There are momentous differences sandwiched between the constituent of the tested plant in which *Valeriana wallichi* acquire a large amount of alkaloids. The occurrence of these phytochemical compounds in huge quantity is realistically proportional to the antioxidant activity so it is manifestly demonstrate the occurrence of these compounds and they will prove the antioxidant activity furthermore endorse a drug for healing of infectious diseases caused by environment. The current conclusion on antioxidant properties shows that this medicinal plants have essential antioxidant properties also it is very important to categorize the antioxidant molecules.

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