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Preliminary phytochemical screening of various extracts of Valeriana wallichii root

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Worldwide herbal medicines have been used traditionally for the prevention and treatment of various diseases as these are rich source of bioactive phytochemicals or bionutrients. Past three decades, studies carried out had shown that these phytochemicals have an important role in preventing chronic diseases like cancer, diabetes and coronary heart disease. The disease – preventing functions that constitute the major classes of phytochemicals are dietary fibre, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents. Some of the phytochemicals possess more than one function. In this aspect to reveal the phytochemical constituent of the plant Valeriana wallichii was carried out. V. wallichii is widely used in the indigenous system of medicine. In the present study preliminary phytochemical screening was carried out for alkaloids, flavonoids, glycosides, steroids, saponins and terpenoids. The tests revealed for the presence of flavonoids, reducing sugar, tepenoids and tannins and phenolics. It is also known as Valeriana jatamansi. Different parts of the plant root, rhizome, seed and flower have medicinal values. The therapeutic action of the plant is due to presence of major chemical constituent flavonoids. The herb is beneficial in treating digestive, spleen, liver and abdominal pains. Many pharmacological activities namely antiulcer, myorelaxant, antispasmodic, anti inflammatory, anti emetic, antioxidant, and cytoprotective and spasmolytic activity had been reported in different plant extracts. Based on many scientific researches this article reveals the therapeutic aspects of the herb.

Keywords: Valeriana wallichii, hypnotic, flavonoids, ethanolic extract.

INTRODUCTION

In plants, phytochemical (In Greek “phyto”, meaning plant) are biologically active, naturally occurring chemical compounds, which provide health benefits for humans that are attributed to macronutrients and micronutrients (Saxena et al., 2013). The phytochemicals present in Valeriana wallichii are responsible for preventing disease and promoting health. To extensively establish their efficacy and to understand the underlying mechanism of their action the phytochemical constituents of the plant V. wallichii were determined. V. wallichii is a small 14-45 cm height perennial herb (Katoch et al., 2012). Valerian is a widely used plant – based medicine. It belongs to the Valerianaceae family consisting of about 250 species of Valeriana occurring throughout the world (Ghosh et al., 2011 Lefebvre et al., 2004). V. wallichii are generally found to grow mostly in the mountainous (1,300-3,300m) terrain of the Himalayas (Ghosh et al., 2011). Valeriana (Valerianaceae) originated from the Latin word “Valere” meaning to be in good health, a source of important phytomedicines. V. wallichii is an ingredient of herbal

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medicines in Indian systems of medicine (Sah et al., 2010). The plant is popularly known as Indian valerian. The rhizome is hard and tough internally, it is greenish-brown in colour. The odour is powerfully valerianaceous. It is well distributed in all the temperate regions of the world. In India, it is grown in Himalayas. The plant was identified and its active principles of this herb are valerianate, dihydraldratrate, isovalerianate, 6-methylapigenin, hesperidins and sesquiterpenoids. Its rhizome and root contains volatile oil (valerianic oil) which is composed of aboryl isovalerianate, chatinine, formate, glucoside, isovalerenic acid, 1- camphene, 1-pinene, resins, terpineol and valerianine. From the rhizomes, some important compounds, such as citric acid, malic acid, malol, succinic acid and tartaric acid have been isolated (Katoch et al., 2012). Valerian roots have been used for hundreds of years for its sedative and antispasmodic properties. The plant is widely known for its use in anxiety, insomnia, epilepsy and hysteria. It is considered useful as a potent tranquilizer, antispasmodic and hypotensive, stimulant and also to improve liver function in gastrointestinal disorders (Sahu et al., 2012). Historically too, Valerian roots were known to alleviate less severe forms of fever (Ghosh et al., 2011). Valerian has continuously being a safe sedative / hypnotic choice for patients with mild to moderate insomnia and also has depressant effect on the central nervous system (CNS) (Kalim et al., 2010; Sah et al., 2010). The plant is also used as a mild sedative (Leathwood et al., 1982). The plant essential oil exhibited antimicrobial activity against large number of pathogenic bacteria and potent antifungal activity against different human plant fungal pathogens (Katoch et al., 2012). Therapeutic purposes are the roots and rhizomes which improve subjective experiences of sleep when taken rightly over one to two periods but long-term safety studies are lacking (Lefebvre et al., 2004). One of the most widely used traditional remedies for various complications associated with nervous system and digestion is Valeriana wallichii (Khuda et al., 2012). Valerian is one of the most widely used herbal medicines in the world. It was first used as a treatment of epilepsy in the late 16th century. Numerous reports by a wide variety of writers followed and Valerian subsequently became routinely used for the treatment of various disorders. Uses reported on and related its usefulness for hysteria, antispasmodic, anthelmintic, diuretic, diaphoretic and emmenagogue actions to Valerian (Joseph et al., 2013). One of the most widely used traditional remedies for various complications associated with nervous system and digestion is Valeriana wallichii (Khuda et al., 2012). From the underground organs of several Valeriana species, Valerian is an ancient tranquilizing drug obtained (Marder et al., 2003). In hospital based clinical setup of Valeriana wallichii an Indian medicinal plant, has been on trial for its role in stress disorders (Bhattacharya et al., 2007).

The present study is designed to explore the preliminary phytochemical constituents analysis of Valeriana wallichii which is responsible for the pharmacological activities.

MATERIALS AND METHODS

Plant material

The plant material used for this study consists of dried rhizomes of Valeriana wallichii belonging to the family Valerianaceae.

Collection and authentication of plant

The rhizomes of Valeriana wallichii used for this study were procured from Bangalore, Karnataka, India, during the month of June. The plant was identified and authenticated by Dr. Satyanarayana, Scientist, Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Preparation of plant extracts

Valeriana wallichii roots were dried under shade separately powdered in mechanical grinder. The powdered plant materials were then passed through a sieve No. 22 and stored in an air tight container until the time of use.

Method of extraction

Continuous hot percolation (successive solvent extraction) process by using Soxhlet apparatus, chloroform, ethanol, ethyl acetate and shade dried coarse powder of Valeriana wallichii was carried out.

Extraction procedure for Valeriana wallichii

Extravst of dried roots of Valeriana wallichii were obtained with different solvents of increasing polarity by sequential Soxhlet extraction. Two hundred grams of coarse powdered dried roots were sequentially extracted in a Soxhlet extractor using 1500 ml of chloroform, ethyl acetate and ethanol. For each solvent the extraction time was four hours. The resulting extracts were evaporated using rotary evaporator and 2 ml of each were stored at 4°C. Portions of these extracts were treated with small amount of activated charcoal and incubated at 40°C for 10 min, filtered through a fluted filter paper and concentrated by rotary evaporator.
Identification of phytochemical constituents

Since ancient times the therapeutic potentials of plant and animal origin are being used by simple process without isolation of pure compounds in the form of crude drugs. Based on the nature of its constituents the pharmacological action of crude drugs is determined. Inspite of acting as a biosynthetic laboratory for chemical compounds, carbohydrates, proteins and fats as food by humans they are used for a multitude of compounds including alkaloids, flavonoids, glycosides etc. which exert definite pharmacological activity.

By using suitable solvents the plant materials are extracted to take out the desired components and the resulting principle being employed as therapeutic agents to obtain the pharmacological activities. The chemical entities present in the phytochemistry of herbal drugs are termed as constituents. Even though numerous chemical compounds are present in the herbal drugs, it is essential to single out those responsible for therapeutic effect that are called as active constituents (Palanisamy et al., 2012).

Thus, by considering the above facts, before evaluating the biological activity it is necessary to evaluate the nature of extract. The plant *V. wallichii* which contain large number of chemical constituents had been selected for the pharmacological activity. Hence for this purpose, qualitatively, it is necessary to perform the preliminary tests to evaluate chemical nature of extracts.

Preliminary phytochemical tests

All the extracts of *V. wallichii* were subjected to qualitative tests for the identification of various active constituents. The following are the reagents used for the phytochemical screening (Table 1):

**Dragendorff's reagent**

Accurately 1.7 g basic bismuth nitrate and 20 g tartaric acid are dissolved in 80 ml of water. This solution was mixed with a solution containing 16 g potassium iodide and 40 ml of water.

**Mayer's reagent**

An amount of 1.36 g of mercuric iodide in 60 ml of water mixed with a solution, which contains 5 g of potassium iodide in 20 ml of water.

**Libermann-burchard's reagent**

About 5 g of acetic anhydride was carefully mixed under cooling with 5 ml of concentrated sulfuric acid; this mixture was added continuously to 50 ml of absolute ethanol with cooling.

**Fehling's solution A**

A weight of 34.64 g copper sulphate was dissolved in a mixture of 0.5 ml of sulphuric acid and sufficient water to produce 500 ml.

**Fehling's solution B**

A weight of 176 g of sodium potassium tartarate and 77 g of NaOH are dissolved in sufficient water to produce 500 ml. Equal volumes of above solutions was mixed at the time of use.

**Benedict's reagent**

Approximately 1.73 g of cupric sulphate, 1.73 g of sodium citrate and 10 g anhydrous sodium carbonate are dissolved in water and the volume was made up to 100 ml with water.

**Molish's reagent**

About 2.5 g of pure α-naphthol was dissolved in 25 ml of ethanol (Kokate. 1994).

**Test for alkaloids**

A small portion of the extracts viz, chloroform, ethyl acetate and ethanol was stirred with few drops of dil. Hcl and filtered. To the filtrate Dragendorff’s reagent (Potassium bismuth iodide solution) was added and an orange brown precipitate indicates the presence of alkaloids.

To the filtrate, Mayer’s reagent was added and a cream precipitate indicated the presence of alkaloids.

Measured 2 ml of various extract solutions was then mixed with dilute hydrochloric acid and 0.1 ml of Wagner’s reagent. Formation of reddish brown precipitate indicated the positive response for alkaloid.

About 2 ml of various extract was allowed to react with 0.2 ml of dilute hydrochloric acid and 0.1 ml of Hager’s reagent. A yellowish precipitate suggested the presence of alkaloid.

**Tests for flavonoids**

To a portion of the various extracts, concentrated sulphuric acid was added. A yellow coloration indicated
Table 1. Preliminary Phytochemical screening of the various extracts of *Valeriana wallichii* (VW).

<table>
<thead>
<tr>
<th>S/No</th>
<th>Constituents</th>
<th>VWchE</th>
<th>VWEAE</th>
<th>VWEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
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<tr>
<td></td>
<td>Dragendorff’s test</td>
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<tr>
<td></td>
<td>Hagner’s test</td>
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<td>Wagner’s test</td>
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<td></td>
<td>Mayer’s test</td>
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<td>2.</td>
<td>Flavonoids</td>
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<tr>
<td></td>
<td>Concentrated sulphuric acid test</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td></td>
<td>10% lead acetate test</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>3.</td>
<td>Glycosides</td>
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<td></td>
<td>Fehling’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td></td>
<td>Cardiac glycosides</td>
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<td>4.</td>
<td>Reducing sugar</td>
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<td></td>
<td>Benedict’s test</td>
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<td>Fehling’s test</td>
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<td>5.</td>
<td>Steroids</td>
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<td>Salkowsky test</td>
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<td>6.</td>
<td>Saponins</td>
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<td>Foam test: Distilled water</td>
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<td>7.</td>
<td>Terpenoids</td>
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<tr>
<td></td>
<td>Concentrated sulphuric acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
<td>10% Sodium hydroxide</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>8.</td>
<td>Tannins and Phenolics</td>
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<tr>
<td></td>
<td>Ferric chloride test</td>
<td>+</td>
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<td></td>
<td>Pot. dichromate</td>
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<td></td>
<td>Lead acetate</td>
<td>+</td>
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<td></td>
<td>Gelatin test</td>
<td>-</td>
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<td></td>
<td>Phlabotannins</td>
<td>-</td>
<td>-</td>
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<td>9.</td>
<td>Amino acids</td>
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<td></td>
<td>Ninhydrin test</td>
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<td></td>
<td>Aromatic amino acid</td>
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<td>Concentrated nitric acid</td>
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<td></td>
<td>Coumarins</td>
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<td></td>
<td>NaOH</td>
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<td>10</td>
<td>Quinones</td>
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<td></td>
<td>Concentrated sulphuric acid</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

VWchE – *Valeriana wallichii* chloroform extract, VWEAE – *Valeriana wallichii* ethyl acetate extract, VWEE – *Valeriana wallichii* ethanolic extract, + denotes present, - denotes absent.

The presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% AlCl₃ solution were added to a portion of each fraction a yellow coloration indicated the presence of flavonoids.

**Test for glycosides**

To 1 ml of all the plant extracts, equal volume of Fehling’s solution A and B was added. The mixture heated in a water bath. Brick red coloration indicated the presence of glycosides.

**Test for reducing sugars**

Five (5) ml of the extracts, mixed with 5 ml of Fehling’s
solution was boiled for 5 min. Formation of brick red colored precipitate demonstrated the positive test for reducing sugars.

To 5 ml of the extracts, 5 ml of Benedict’s solution was added in a test tube and boiled for few minutes. Development of brick red precipitate confirmed the presence of reducing sugars.

**Test for steroids**

**Salkowski test**

Concentrated sulphuric acid was added to 10 mg of different extracts dissolved in 1 ml of chloroform. A reddish blue colour exhibited by chloroform layer and green fluorescence by the acid layer suggested the presence of steroids.

**Test for saponins**

About 10 ml of the extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and then observed for the formation of an emulsion.

**Test for terpenoids**

About 5 ml of the extract was treated with 2 ml of chloroform and the concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

**Test for amino acids**

Different extract solution was treated with ninhydrin at the range of 4-8. Development of purple colour indicated the positive reply response for amino acids.

**Test for tannins and phenolics**

Around 5 ml of extract solutions was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicated the presence of tannins.

Approximately 5 ml of the extracts was treated with 1 ml of 10% aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.

Accurately 5 ml of the extracts was treated with 1 ml of 10% lead acetate solution in water. Yellow colour precipitation gave the test for tannins.

**RESULTS AND DISCUSSION**

The three extracts carried out revealed the following results:

**Valeriana wallichii chloroform extract:** The chloroform extract of *V. wallichii* revealed the presence of flavonoids, terpenoids, tannins and phenolics, coumarins and quinones.

**Valeriana wallichii ethyl acetate extract:** *V. wallichii* ethyl acetate extract revealed the presence of terpenoids, tannins and phenolics.

**Valeriana wallichii ethanol extract:** The ethanolic extract of *V. wallichii* revealed the presence of flavonoids, reducing sugar, terpenoids and tannins and phenolics.

Depending on their role in plant metabolism phytochemicals are classified as primary or secondary constituents (Wadood et al., 2013). Primary constituents include the amino acids common sugars, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids, flavonoids, terpenes, lignans, curcuminoids, steroids, saponins, phenolics and glycosides.

Flavonoids are the largest group of plant phenols. Flavonoids act as a potent water-soluble antioxidant and free radical scavenger that prevents the oxidative cellular damage (Yadav et al., 2014). Flavonoids are reported to have antibacterial, anti-inflammatory, anticancer, antifungal, anti-allergic and diuretic properties (Chic et al., 2014). Tannins possess amazing stringent properties and they bind to proline rich proteins and interfere with the protein synthesis (Radhika et al., 2013).

Another class of natural products is the terpenoids that have been derived from five-carbon isoprene units. These are natural lipids that can be found in every class of living things and occupy the largest group of natural products. Terpenes are widespread in nature, as constituents of essential oils. One such classification is the monoterpenoids. By biochemical modifications of monoterpenes either oxidation or rearrangement produces the related monoterpenoids. Monoterpenoids have two isoprene units. Monoterpenes may be of two types that is, linear (acyclic) or contain rings. Terpenoids had been found in the prevention and therapy of several diseases (Yadav et al., 2014).

The largest category of phytochemicals and the most widely distributed in the plant kingdom are the phenolic compounds. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and
polyphenols. Phenolic compounds are a large and complex group of chemical constituents present in plants.

In the above stated extracts, majority of the phytoconstituents were revealed by the ethanolic extract. Therefore, ethanolic extract can be selected for pharmacological studies. Ethanolic extract can be selected for the isolation of available active constituents because ethanol being a bipolar solvent it is possible to dissolve a wide range of phytoconstituents.

Conclusion

A unique source of structures of high phytochemical diversity is nature. Many of the medicinal plants possess interesting biological activities and medicinal properties. In the context of the worldwide spread of different diseases, it is extremely important for an intensive search for new lead compounds for the development of novel pharmacological therapeutics for the treatment of AIDS, chronic diseases and a variety of cancers. In this aspect, the plant V. wallichii was selected based on the traditional uses and literature review of earlier studies. The preliminary phytochemical studies on V. wallichii was carried out. The phytochemical constituents were extracted by chloroform, ethyl acetate and ethanol solvent extraction, and identified by various chemical tests. These tests showed the presence of various phytochemical constituents like flavonoids, terpenoids, tannins, phenolics and reducing sugars (gycosides). The pharmacological activity of the medicinal plant V. wallichii may be due to the presence of these active constituents. These phytoconstituents are merely responsible for the activity of the plant.

The present study on the evaluation of V. wallichii for the preliminary phytochemical could be used as the diagnostic tool for the standardization of medicinal plant. Thus our study may be an important landmark in correct identification of V. wallichii.

REFERENCES


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