IJPSR (2013), Vol. 4, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 March, 2013; received in revised form, 17 April, 2013; accepted, 26 June, 2013

PHYTOCHEMICAL SCREENING OF ACTIVE SECONDARY METABOLITES PRESENT IN *WITHANIA SOMNIFERA* ROOT: ROLE IN TRADITIONAL MEDICINE

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Keywords:

Withania somnifera, Secondary Metabolites, Traditional Medicine, Phytochemical Screening

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ABSTRACT: Medicinal plants are a source of naturally active compounds used extensively by tribal people worldwide for many ailments. *Withania somnifera* (WS) is one such plant used to treat many ailments from the time of Ayurveda. Extraction of the bioactive plant constituents from the whole plant or from the different parts of the plant has always been a challenging task. As the dried roots of WS are widely used in the treatment of many disorders, the current investigation aimed at extraction and detection or screening of active phytochemical compounds from different extracts of WS root. Phytochemical screening of different extractions revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, glycosides and reducing sugars which could account for its varied medicinal properties like anti-inflammatory, anti-spasmodic, anti-analgesic, neuroprotective and diurectic effects.

INTRODUCTION: For over a decade, interest has been revived in the study and use of traditional medicine in different parts of the world. As a result, countries have sought cooperation in identifying and using safe positive components of traditional medicine in their national health systems [1].

Since ancient times, people have been exploring nature particularly plants, in search of new drugs, and this has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(7).2770-76

Article can be accessed online on: www.ijpsr.com

Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [3]. In India, almost 95% of the prescriptions have been reported to be plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha [4].

Plants produce primary and secondary metabolites with divergent functions [5]. The primary metabolites, amino acids, simple sugars (glucids), proteins and lipids are involved in cellular processes. Secondary metabolites are chemically active compounds (flavonoids, alkaloids, terpenoids, steroids, saponins, etc.), which are produced in response to stress with complexity in structure and more restriction in distribution than the primary metabolites [6].

Plants can produce different kind of secondary metabolites also known as natural products as they elicit effects on other organisms [7].

Withania somnifera (L. Dunal) (WS), known as Indian ginseng, is a dense pubescent shrub, which grows about 2 feet in height and belongs to the family of Solanaceae. It is a popularly known medicinal plant for its therapeutic use in Unani and Ayurvedic systems of traditional medicine of India and locally known as Ashwagandha [8, 9]. It is capable of growing widely not only in all the drier parts of India but also in Bangladesh, Congo, South Africa, Egypt, Morocco, Jordan, Pakistan and Afghanistan, which offers tremendous potential as an energizing medicinal herb [10]. The roots are the main portions of the whole plant, as they possess wide number of therapeutic agents. The herb is termed as rasayana in Ayurvedic practice, which means it acts as a tonic for vitality and longevity [10]. WS stimulates the immune system cells, such as lymphocytes and phagocytes, which also counteract the effects of stress and generally promote wellness [11-13].

In order to promote Indian herbal drugs, it is imperative to initiate urgent steps for screening of plants for secondary metabolites. Many workers have reported the phytochemical screening in various plants [14-16]. Many pharmacological studies have been conducted to investigate the properties of ashwagandha and to authenticate its use as a multi-purpose medicinal agent [17].

The present communication helps to assess the status of phytochemical properties of roots of WS, which could account for its varied medicinal properties.

MATERIALS AND METHODS: Plant Material: Roots of WS was purchased from an Ayurvedic products distributor (Indian Drugs and Stores, Bangalore) and authenticated by a botanist, Department of Botany, S.V.University, Tirupati, Andhra Pradesh. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V. University, Tirupati (Voucher No. 2437).

Extraction: The dried roots were ground into a coarse powder using a pulverizer. Fine ground pulverized material was dissolved in different solvents as described in **figure 1**.

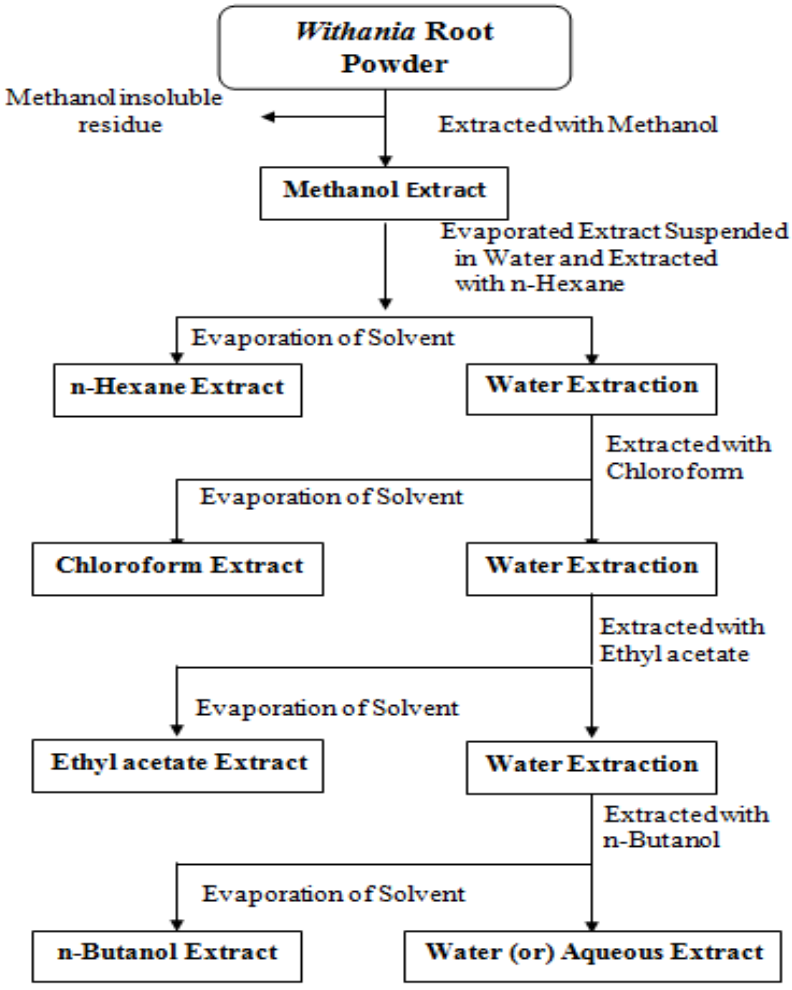

FIG. 1: PREPARATION OF DIFFERENT EXTRACTS FROM THE ROOT OF WS USING THE DIFFERENT SOLVENTS

Powdered plant material was soaked in methanol for 24 hrs. at room temperature and the solvent was filtered with Whatman filter paper (125mm). This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi Rotavapor R-114 yielding a gum-like residue.

The same was repeated with organic solvents and distilled water of increasing polarity (starting with lipophilic solvent n-Hexane, ending with the more hydrophilic n-Butanol). The solvent from each extract was filtered and concentrated under reduced pressure in Buchi rotavapour. Finally the crude extracts were collected, weighed and stored in refrigerator at 4^oC for further phytochemical analysis.

Qualitative Detection of Phytochemical Constituents: Detection of active phytochemical constituents was carried out for all the extracts using the standard procedures [18].

Detection of Alkaloids: Extracts were dissolved individually in dilute HCl and filtered.

1. **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

2. **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of Flavonoids:

1. **NaOH test:** A small amount of extract was treated with aqueous NaOH and HCl, and observed for the formation of yellow orange color.

2. **H_2SO_4 test**: A fraction of the extract was treated with Conc.H_2SO_4 and observed for the formation of orange color.

Detection of Steroids and Terpenoids:

1. **Liebermann - Burchard test**: 4mg of extract was treated with 0.5ml of acetic anhydride and 0.5ml of acetic acid. Then concentrated H_2SO_4 was added slowly and blue green color was observed for terpenoids and reddish brown color for steroids.

Detection of Saponins:

1. **Foam test**: About 2g of the plant extract was mixed with 10ml of distilled water and shaken vigorously for a stable persistent froth. Appearance of froth indicates the presence of saponins.

Detection of Tannins:

1. **Ferric chloride test**: 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ was added and observed for brownish green-black or a blue-black coloration.

2. **Lead acetate test:** 2ml of plant extract was combined with 2ml of distilled water. 0.01g lead acetate was added to this combined solution and shaken well. Development of white turbidity and precipitate indicates the presence of tannins.

Detection of Phenols:

1. **Ferric chloride test**: About 2ml plant extract was taken to water and warmed at $45\text{-}50^0C$. Then 2 ml of 0.3% $FeCl_3$ was added. Formation of green or blue color indicates the presence of phenols.

Detection of Glycosides:

1. **Fehling's test:** Fehling's solution A and B was diluted with distilled water and boiled for 1min. To this clear blue solution, 8 drops of plant extract was added. After that it was mixed with 1ml of Fehling's solution and boiled in a water bath for 5 min. The formation of brick red precipitation indicates the presence of glycosides.

RESULTS AND DISCUSSION: The concentration of extraction yield differed among the solvents depending on their polarity. The quantity of extraction yielded was high from high polarity solvents like methanol and water and low from the low polarity solvents like n-hexane, chloroform, ethyl acetate and n-butanol.

The concentrations of extractions obtained from the root of WS are shown in **figure 2**.

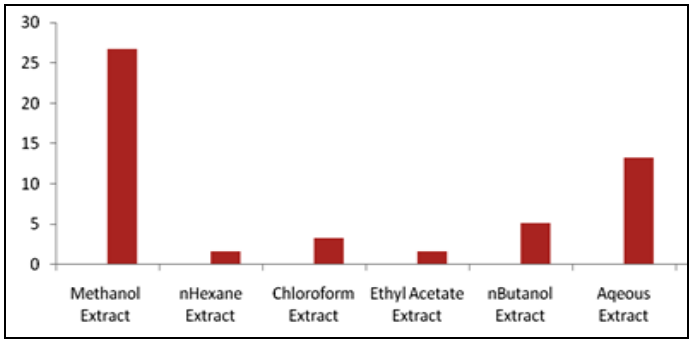

FIG 2: DIFFERENT EXTRACTIONS OBTAINED IN G/KG FROM WS ROOT POWDER

Preliminary phytochemical screening of the crude extracts of WS root revealed the presence of different kind of chemical groups (Summarized in **table 1**).

The presence of bioactive compounds indicate the medicinal value of various extracts of WS plant, because their possible use as natural additives emerging from a growing tendency to replace synthetic antioxidants and antimicrobials with natural ones [19].

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE CRUDE EXTRACTS OF *WS* ROOT REVEALED THE PRESENT OF DIFFERENT KIND OF CHEMICAL GROUPS THAT ARE SUMMARIZED IN BELOW TABLE

Phytochemical Constituents	Methanol Extract	Aqueous Extract	n-Butanol Extract	Ethyl acetate Extract	Chloroform Extract	n-Hexane Extract
Alkaloids	+	+	-	+	-	-
Flavonoids	+	-	+	-	+	+
Steroids	+	+	+	-	+	-
Terpenoids	-	-	-	+	-	+
Saponins	+	+	+	-	-	-
Tannins	-	+	-	+	-	-
Phenols	+	-	+	-	+	-
Glycosides	+	+	+	+	-	-

'+' indicates presence of compound; '-' indicates absence of compound

Methanol extract of WS root contain alkaloids, flavonoids, steroids, saponins, phenols and glycosides. Aqueous extract also possess all those phytoconstituents including tannins except flavonoids and phenols. n-Butanol extract of WS root contains flavonoids, steroids, saponins, phenols and glycosides.

Ethyl acetate extract contains alkaloids, terpenoids, tannins and glycosides. Chloroform extract contains flavonoids, steroids and phenols and n-hexane extract of WS root contains flavonoids and terpenoids.

Active chemical constituents and their medicinal values: The active chemical secondary metabolites present in the roots of WS are believed to account for its extraordinary medicinal properties as shown in the **table 2**.

Alkaloids: Alkaloids, which are chemically heterogenous group of natural substances and pharmacologically active compounds, compose more than 6000 basic nitrogen containing organic compounds, which occur in about 15% of all vascular terrestrial plants and in more than 150 different plant families.

Alkaloids are used for the treatment of tumors, nocturnal leg cramps caused by vascular spasms and diarrhea. These compounds possess anti-microbial activity and sedative effects. Many alkaloids are anesthetics and have calming effects on psychotic or hypertensive patients without inducing sleep. Alkaloids can also be used to treat psychiatric and palpitation [20].

It has been reported that total alkaloids extracted from extract of WS roots, caused relaxant and antispasmodic effects against various agents that produce smooth muscle contractions in intestinal, uterine, tracheal, and vascular muscles [21].

TABLE 2: ACTIVE CONSTITUENTS AND THEIR MEDICINAL VALUE OF DIFFERENT EXTRACTS OF WS ROOT

Active Constituent	Present in the Extract	Medicinal Value
Alkaloids	Methanol Extract Aqueous Extract Ethyl acetate Extract	Anti-microbial, sedative, relaxant, anti-spasmodic; used to treat tumors, nocturnal leg cramps, diarrhoea, psychiatric and palpitation
Flavonoids	Methanol Extract n-Butanol Extract Chloroform Extract	Anti-oxidant, strengthens capillary walls, reduces osteoporosis, improves blood cholesterol levels, and lowers risk of cancer and coronary heart diseases.
Steroids	Methanol Extract Aqueous Extract n-Butanol Extract Ethyl acetate Extract Chloroform Extract	Aphrodisiac, reduces cholesterol levels, affects immune system and tumor cells
Terpenoids	Ethyl acetate Extract n-Hexane Extract	Anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, anti-cancer; inhibits cholesterol synthesis
Saponins	Methanol Extract Aqueous Extract n-Butanol Extract	Anti-inflammatory, anti-hepatotonic, hypoglycemic, anti-microbial and anti-viral; used in detergents and molluscicides
Tannins	Aqueous Extract Ethyl acetate Extract	Anti-fungal, anti-biotic, anti-inflammatory, analgesic, astringent and wound healing
Phenols	Methanol Extract n-Butanol Extract Chloroform Extract	Anti-inflammatory, anti-oxidants, anti-cancer, anti-septic
Glycosides	Methanol Extract Aqueous Extract n-Butanol Extract Ethyl acetate Extract	Sedative, muscle relaxant, diuretic

Flavonoids: The flavonoids are a large group of naturally occurring phenolic compounds found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine [22, 23]. Flavonoids present in WS are recognized as having beneficial properties to human health [24]. These compounds possess antioxidant elements and ensure healthy circulation.

Flavonoids help to strengthen capillary walls. These compounds, at times, are referred to as phytoestrogens. Phytoestrogens are associated with relief of menopausal symptoms, reduction of osteoporosis, improvement of blood cholesterol levels and lowering the risk of certain factors related to cancer and coronary heart diseases [22].

Steroids: Steroids are organic compounds with four cyclohexane rings. Steroids present in WS are also known as withanolides [10]. These steroidal compounds have been used to reduce stress, reduce cholesterol levels, activate immune system, enhance memory and learning and to treat tumor cells in cancer cases [25-27].

Terpenoids: Terpenoids are small molecular products synthesized by plants and are probably the most widespread group of natural products. Terpenoids show significant pharmacological activities, such as antiviral, antibacterial, antimalarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities [28].

Saponins: Saponins are heterogeneous group of natural products found in many plant-derived foods and medicinal plants. There are two types of saponins: triterpenoids and steroidal saponins. Many plants containing steroidal saponins have a marked hormonal activity while triterpenoids, saponins are often strong expectorant and aid the absorption of nutrients. Saponins extracted from plants show biological and pharmacological activities such as anti-inflammatory, anti-hepatotonic, wound healing, veinotonic, expectorant, spasmolytic, hypoglycemic, antimicrobial and antiviral [20]. Traditionally the saponins present in WS have been extensively used as detergents, pesticides and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects [29, 30].

Tannins: Tannins are members of polyphenol chemical family. Tannins are produced to a greater or lesser degree by all plants. They draw the tissues together and improve their resistance to infection. Tannin compounds presented in WS inhibits the growth of many fungi, yeast, bacteria and viruses [31].

Tannins are attributed with analgesic and anti-inflammatory activities. Apart from this, tannins promote the healing of wounds and inflamed mucous membrane with the property of astringency [32, 27].

Phenols: Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants [33]. Phenols are antiseptic and reduce inflammation when taken internally. These bioactive agents have an irritant effect when applied to the skin. Above of all, phenols have a high affinity to chelate metals and scavenge the free radicals in cells [34]. Polyphenols act as antioxidants, which protect cells and body chemicals against damage, caused by free radicals and reactive atoms that contribute to tissue damage in the body. It has been reported that these compounds deactivate the substances that promote the growth of tumors [20].

Glycosides: Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides can suppress and soothe irritant dry coughs. They have a helpful sedative and relaxant effect on the heart and muscles when taken in small doses. They are significantly diuretic [27].

CONCLUSION: Plants that are rich in secondary metabolites, called medicinal plants, are widely used in traditional medicine to combat and cure various ailments. WS is a plant used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The different extracts of root of WS contained many bioactive chemical constituents including, alkaloids, glycosides, steroids, terpenoids, saponins, tannins and reducing sugars. The anti-inflammatory, antispasmodic, antianalgesic and diuretic effects can be attributed to the high steroids, tannins, terpenoids, saponins and glycosides present in WS. It has been used as an aphrodisiac, neuroprotective, liver tonic, astringent, and to treat bronchitis, asthma, ulcers, emaciation, insomnia, and senile dementia. While WS has been used successfully in Ayurvedic medicine for centuries, more clinical trials should be conducted to support its therapeutic use.

The present study can be used in future for the economical formulation of the active chemical ingredients in natural drugs against a variety of neurological and inflammatory diseases.

ACKNOWLEDGEMENTS: The authors acknowledge the profound gratitude to the Science and Engineering Research Board (SERB), Govt. of India for providing the funds under "Fast Track Scheme for Young Investigators" (Ref No. SR/FT/LS-199-2009).

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How to cite this article:
Visweswari G, Christopher R and Rajendra W: Phytochemical screening of active secondary metabolites present in *Withania somnifera* root: Role in traditional medicine. *Int J Pharm Sci Res* 2013: 4(7); 2770-2776. doi: 10.13040/IJPSR. 0975-8232.4(7).2770-76