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Phytochemical screening and qualitative analysis of Withaniasomnifera

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ABSTRACT. Medicinal plants have been playing an essential role in the development of human culture. As a source of medicine, Medicinal plants have always been at forefront virtually all cultures of civilizations.. For thousands of years medicinal plants have been used to treat health disorders, to add flavor and conserve food and to prevent diseases epidemics. The secondary metabolites produced by the plants are usually responsible for the biological characteristics of plant species used throughout the world. The objective of the present study was to find out the presence of photochemical and to determine the antioxidants activity of Withaniasomnifera . Withaniasomnifera also known as Ashwagandha, has been an important herb in the ayurvedic and indigenous medical systems. To validate this use, leaves of the plant was subjected to preliminary phytochemical analysis. Preliminary phytochemical analysis revealed the presence of carbohydrates, glycosides, alkaloids, phenolic compounds and flavonoids in extracts.Theaqueous extract was prepared with triple distilled water .The comparative study of the phytochemicals represented here shows that the flavonoids and alkaloid presence was higher in the aqueous extract of Withaniasomnifera which also proves that the extract can be further analysed for certain type of pharmacological activities..

Index terms: phytochemicals, extract, medicinal, flavonoids.

I. INTRODUCTION

Medicinal plants have provide mankind a huge range of strong drugs to alleviate or eradicate infections and suffering from disease in spite of development in synthetic drugs, some of the plant-derived drugs still retain their importance and relevance. Therefore, plants can be described as a major source of medicines, not just as isolated active principles to be dispensed in standardized dosage form but also as crude drugs for the population. The traditional medicine practice is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand.

About 40% of the total medicinal consumption is attributed to traditional tribal medicines alone byChina. Medicinal plants have proved their sole role in coping with anumber of deadly diseases including cancer and the diseases associated with viral onslaught viz.Hepatitis, AIDS etc Even today, plants are not only indispensable in health care, but formthe best hope of source for safe future medicines

Most of the important drugs of the past 50 years, which haverevolutionized modern medicinal practice, have beenisolated/derivatized from plants. These chemical ingredients exhibittherapeutic properties of plant and animal drugs. The WHO endorsesand promotes the addition of herbal drugsin national health careprograms because they are easily accessible at a price within the reachof a common man and are time tested and thus considered to be muchsafer than the modern synthetic drugs. Thus, the research ofpharmacologically/ biologically active agents obtained by screeningnatural sources such as plant extracts had led to the detection of manypharmaceutically valuable drugs that play a key role in the treatment of human diseases . The photochemical-pharmacological researchwork has recently yielded effective solutions to certain diseases whichsynthetic drug industry has failed to afford.

spp. etc. Suchplants were earlier considered as poisonous or useless, but now havebeen found to contain molecules of high drug values and are considered as medicinal herbs of great significance. Modern searches for bioactive molecules typically make use ofsophisticated bioassays and bioassay-guided fractionation ofmedicinal plants used by traditional healers. This has led to theisolation of several new therapeutically important compounds.

A good number of potent drugs and a large number of therapeutic leads and many new

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pharmacologically active constituents have been developed from herbal drugs due to the dedicated efforts of researchers.

Plant description :

Withaniasomnifera (WS), also known as ashwagandha, Indian ginseng, and winter cherry, it has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. The roots of the plant are categorized as rasayanas, which are reputed to promote health and longevity by augmenting defense against disease, arresting the ageing process, revitalizing the body debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing .It is in use for a very long time for all age groups and both sexes and even during pregnancy without any side effects .

Historically, the plant has been used as an antioxidant,adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. Clinical trials and animal research support the use of WS for anxiety,cognitive and neurological disorders, inflammation,hyperlipidemia and Parkinson's disease.

Numerous photochemical with potential or established biological activity have been identified. However, since a single plant contains widely diverse photochemical, the effects of using a whole plants medicine are tentative. Further, the phytochemical content and pharmacological actions, if any of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety. The compounds found in plants are of many kinds, but most are in four major biochemical classes: Flavonoids, alkaloids, polyphenols and terpenes.

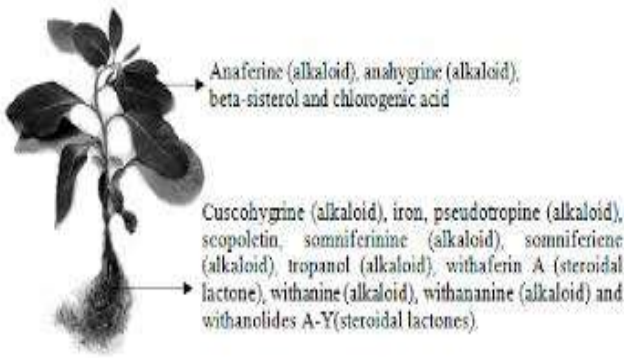

FIGURE 1: DISTRIBUTION OF MAJOR PHYTOCHEMICALS IN WITHANIA SOMNIFERA

II. PHYTOCHEMICAL STUDIES

A review of literature reveals the presence of various chemical constituents in the different parts of the plant which are as follows:

Root :-

The roots are reported to contain alkaloids, amino acids, steroids, volatile oil, starch, reducing sugars, glycosides, hentriacontane, dulcitol, withaniol, an acid (m.p. 280-283° decomp.), and a neutral compound (m.p. 294-296°). The total alkaloidal content of the Indian roots has been reported to vary between 0.13 and 0.31 percent, though much higher yields (up to 4.3%) have been recorded elsewhere (Anonymous, 1982, Anonymous, 2007). Identity, purity, strength and assay of the dried roots of the plant are given in (Table 2).

Table. 2: Identity, purity, strength and assay

Foreign organic matter Not more than 2%	
Physicochemical constants	
Ash values (%)	
Total ash	6.0
Acid insoluble ash	1.5

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Water soluble ash	3.0
pH values	
1% solution	5.5
10% solution	5.5
Loss on drying at 105□ C	8.7%
Solid contents	91.3%
Successive extractive values (%)	
Pet. Ether	0.348
Chloroform	0.304
Acetone	0.305
Alcohol	0.184

Many biochemically heterogeneous alkaloids have been reported in the roots. Basic alkaloids include cuscohygrine, anahygrine, tropine, pseudotropine, anaferine, isopelletierine, withananine, withananinine, pseudo-withanine, somnine, somniferine, somniferinine. Neutral alkaloids include 3- tropyltigloate and an unidentified alkaloid. Other alkaloids include withanine, withasomnine, and visamine. Withanine is sedative and hypnotic .Withasomnine has been separated from the roots of the plant grown in West Germany. Visamine is a new alkaloid which has been separated from the roots of the plant grown in Soviet Union. It prolonged hexanal-induced sleeping time and showed hypothermic and nicotinolytic effects in mice .The free amino acids identified in the root includeaspartic acid, glycine, tyrosine, alanine, proline, tryptophan, glutamic acid, and cystine .

Leaf :-

The leaves of the plant (Indian chemotype) are reported to contain 12 withanolides, 5 unidentified alkaloids (yield, 0.09%), many free amino acids, chlorogenic acid, glycosides, glucose, condensed tannins, and flavonoids . The leaves of the plant from different habitats contain different withanolides–a group of C28 steroids characterized by a 6-membered lactone ring in the 9-carbon atom side chain.

Withaferin A, a steroidal lactone is the most important withanolide isolated from the extract of the leaves and dried roots of Withaniasomnifera. It is thermostable and slowly inactivated at pH 7.2. It is insoluble in water and is administered in the form of suspension. For its separation, the leaves are extracted with cold alcohol; the extract is purified and dried, and finally crystallized from aqueous alcohol (yield, 0.18% air dry basis). The yield of this compound from the South-African plants is reported to be as high as 0.86 percent. The curative properties of the leaves and roots are attributed to Withaferin A.

Fruit:-

The green berries contain amino acids, a proteolytic enzyme, condensed tannins, and flavonoids. They contain a high proportion of free amino acids which include proline, valine, tyrosine, alanine, glycine, hydroxyproline, aspartic acid, glutamic acid, cystine and cysteine. The presence of a proteolytic enzyme, chamise, in the berries may be responsible for the high content of the amino acid.

Shoots :-

The tender shoots are rich in crude protein, calcium and phosphorous, and are not fibrous. They are reported to contain scopoletin.

Stem :-

The stem of the plant contains condensed tannins and flavonoids.

Bark :-

The bark contains a number of free amino acids .

III. QUALITATIVE ANALYSIS

3.1. MATERIAL ANDMETHOD

3.1. Collection of plant samples:-

The leaves of Withaniasomnifera commonly known as ashwagandha was collected from Botanical garden Noida, Sector-37.

3.2. Processing of plant sample:-

The leaves of the plant were allowed to naturally under shade drying. Then the leaves were ground in grinder in powdered form and were kept in plastic bottle.

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3.3. Preparation of plant extract:-
The decoction of plant material was prepared by 4gm of sample in 20 ml deionized water and then boiled at 50 to 60◌ C for 30 minutes on water bath. The extract was filtered through Whatman no.1 filter paper and centrifuged filtrate at 2500rpm for 15 minutes. The extract was stored in sterile bottles at 4 to 8◌ C for further analysis.

3.2. PHYTOCHEMICAL ANALYSIS: -
Chemical tests were performed on extracted sample with standard methods for various secondary metabolites.

QUALITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS:-
3.2.1.TEST FOR FLAVONOIDS :-
Take 100µl of plant extract was treated with 2 ml of sodium hydroxide solution. Then formation of intense yellow colour was observed. This becomecolourless on addition of dilute HCL , indicating the presence of flavonoids.

3.2.2.TEST FOR TANNINS

FERRIC CHLORIDE TEST
Take 500 ul of plant extract was allowed to react with 5% alcoholic ferric chloride solution. Formation of blue or greenish color of the solution was observed. This was the
indication of the presence of the tannins.

CHLOROGENIC TEST
300 µl of the plant extract add about 500µl of NH_4OH. A green color is formed on exposure to air.

3.2.3.TEST FOR SAPONINS 1 ml of plant extract was taken in a test tube and 10 ml of distilled water was added to it.
The mixture was then shaken vigorously. The persistence of foam was observed that indicates the presence of saponins.

3.2.4 TEST FOR CARBOHYDRATES (BENEDICT'S TEST)
500 µl of extract when mixed with 500 µl of benedict's reagent and boiled. A reddish brown precipitate formed with indicated the presence of the carbohydrates.

3.2.5.TEST FOR PROTEINS
BIURET TEST
500 µl of extract equal volume of 40% sodium hydroxide solution and 500 µl copper sulfate solution was added. The appearance of violet color indicates that the presence of
protein.

NINHYDRIN TEST
Take 500 µl extract when boiled with 500 µl of 0.2% solution of ninhydrin (3 ml of ethanol with ninhydrin). The appearance of pink or purple color indicates that the presence of proteins, peptides or amino acids.

3.2.6. TEST FOR ALKAIOIDS
To 500 µl extract add 500 µl iodine potassium iodide solution. The formation of reddish z brown precipitate indicate the presence of alkaloids

3.2.7.TEST FOR TERPENOIDS (SALKOWSKI TEST)
Add 500 µl of chloroform to 300 µl of chloroform to 500 µl of each extract followed by few drops (500 µl of concentrated sulphuric acid). Production of a reddish brown precipitate immediately indicates the presence of terpenoids.

3.2.8.TEST FOR GLYCOSIDES
KELLER KILIANI TEST
To 500 µl extract add 500 µl glacial acetic acid, one drop of 5% ferric chloride and 500 µl of concentrated sulfuric acid. The production of reddish brown color appears at Junction of two liquid layers and upper layer appears bluish green, this indicates the presence of glycosides.

3.2.9. TEST FOR PHENOLS
To 500 µl of each extract add 500 µl to 5% aqueous ferric chloride were added. The formation of blue color indicates the presence of phenols in the sample extract

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IV. RESULTS

Table 1. Phytochemical screening of alcoholic and aqueous leaf extracts of Withaniasomnifera

S. No.	Qualitative test		Result
1.	**Flavonoids**		+
2.	**Tannins**	**Ferric Chloride test**	++
		Chlorogenic test	-
3.	**Test for saponins**		+
4.	Carbohydrates	**Benedict's test**	-
5.	Proteins	Biuret test	++
		Ninhydrin test	++
6.	**Alkaioids**		+++
7.	Terpenoids	Salkowski test	++
8.	Glycosides	Keller kiliani test	-
		Molish test	+
9.	Test for phenols		++

Where.,+++ =highly present , ++=moderately present , += slightly present ?, - = absent

V. CONCLUSION

The studies for Withaniasomnifera would be good natural source of potent and chemotherapeutic agent.Ashwagandha is a plant used in medicine form the time of Ayurveda, since long time in India.

Ashwagandha has been used as an aphrodisiac, anti-inflammatory agent, astringent, asthma, ulcers, and Insomnia. The comparative study of the phytochemicals represented here shows that the flavonoids and alkaloid presence was higher in the aqueous extract of Withaniasomnifera which also proves that the extract can be further analysed for certain type of pharmacological activities..Therefore for the future interpretation, we can say that the extracts can be used for the formulation of a herbal medicine

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