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Study of phytochemical analysis of *Withania somnifera* and *Rauwolfia serpentina* plant extract

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Abstract
Withania somnifera (family-*solanaceae*) and *Rauwolfia serpentina* (family- *Apocynaceae*) is a highly acclaimed genus in the Indian ayurveda, *Withania* is known to promote physical and mental health and used to treat almost all the disorder that effect human health. *Withania somnifera* and *Rauwolfia serpentine* are the two most esteemed species having high medicinal significance. In the present study the phytochemical analysis of organic extracts of different parts of *Withania somnifera* and *Rauwolfia serpentina* was carried out. The qualitative phytochemical study was performed to ascertain the presence of alkaloids, carbohydrate, saponin, glycosides, protein, phytosterol, phenolic compounds, flavonoids, terpenoids, tannins, anthroquinone, emodine etc. Both the plant species were collected and processed from Chhattisgarh region. Important phytochemicals like vinblastine, taxol and vindolin etc, potentially useful in cancer treatment were extracted from the plants.

Keywords: Phytochemical, *Withania somnifera, Rauwolfia serpentine.*

Introduction
Medicinal and aromatic plants (MAPs) have been the focus of study in terms of their conservation and traditional usage in herbal medicines [1]. Throughout the history of mankind, many infectious diseases have been treated with herbs. Medicinal and aromatic plants possess aromatic compounds in the form of oils, which are volatile at the room temperature and the specific property found among them act as a cure for several diseases. These plants have traditionally been used as raw materials for extraction of essential oils, as well as source of spices and other natural products such as traditional herbal medicines, pharmaceuticals, cosmetics, botanical pesticides, insect repellents, other herbal products etc. More than 30% of the entire plant species, at one time or other was used for medicinal purposes [2]. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in the developing countries such as China and India, the contribution is as high as 80% [2]. Thus, the economic importance of medicinal plants is much more in India than the rest of the world and the Indian subcontinent constitutes a rich repository of medicinal plants that are used by various indigenous health care systems. As per the estimate, over 7000 species of medicinal plants are used for medicinal purposes [3].
Medicinal plant, *Withania somnifera* is a small, woody shrub of 60-200 cm height in the Solanaceae family. *Withania somnifera* is locally known as Ginseng and Ashwagandha. It can be found in the India, Mediterranean, and Africa. The roots of this plant are mainly used therapeutically [4-5]. Withanolides, which are the active pharmaceutical ingredients, are isolated from the root and leaves of *Withania somnifera*. Recently, the plant was found to show antibacterial activities [6-8]. Besides antibacterial activities it also exhibited immune modulatory and antitumor activity [9-11]. Root extract of *Withania somnifera* can reverse Alzheimer's disease pathology via the peripheral clearance of *β*-amyloid [12]. The water extract from the leaves of *Withania somnifera* protect RA differentiated C6 and IMR-32 cells against glutamate-induced excitotoxicity [13].
Ashwagandha was found to have anti-carcinogenic effects. Research on animal cell cultures has revealed that the herb reduces the intercellular tumor necrosis factor, decreases the levels of the nuclear factor kappa B and potentiates apoptotic signaling in cancerous cell lines [14]. Ashwagandha also has capacity to fight cancers by reducing tumor size [15, 16].
Rauwolfia serpentina is an important medicinal plant of the world. There is a great demand for its roots, and indiscriminate uprooting from wild sources brought the plant to the verge of extinction. Presently all supplies of *Rauwolfia serpentina* roots are being met from the natural resource, which is declining due to overexploitation by tribal and local collectors. This has led to listing of this species as "endangered" by the International Union for Conservation of Nature and Natural Resources (IUCN) [17]. In India, it has also become an endangered species due to overexploitation and Government of India has prohibited the collection of plants growing in wild in forests and its export since 1969.

In this scenario, there is urgent need for developing its conservation strategy.

Rauwolfia serpentina plant commonly known as Sarpaghandha is widely used medicinally both in the modern medical system and also in Ayurveda, unani and folk medicine. Roots are main source of drug. *Rauwolfia serpentina* is a rich source of indole alkaloids of medicinal value such as reserpine, ajmaline, ajmalicine and serpentine which are used in the treatment of circulatory disorders. It helps to reduce blood pressure by dilating blood-vessels, depresses activity of central nervous system and acts as a hypnotic. In Ayurveda, its roots and whole plants are used for the treatment of cardiovascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy and leaves are used in removal of opacities of cornea [18, 19]. It is used in traditional medicine in India, Root of *Rauwolfia serpentina* is bitter, acrid, laxative, anthelmintic, thermogenic, diuretic and sedative. The root barks has more than 90% of the total alkaloids in roots. The alkaloids are accumulated in the roots over a period of 1-3 years and total content varies from 1.0-3.0% of the dried roots. More than 50 alkaloids have been reported from *Rauwolfia serpentina*. The alkaloids are classified into 3 groups, viz, reserpine, ajmaline and serpentine groups. Reserpine group comprises of reserpine, rescinnamine, deserpine etc. Ajmaline, ajmalicine, ajmalinine, iso-ajmaline etc. are of the ajmaline group. Whereas, serpentine group includes serpentine, sepentinine, alstonine etc. Quantitative analysis of reserpine–rescinnamine group of alkaloids is performed by spectrometric analysis or by high-performance liquid chromatography. The roots also contain ophioxylin, resin, starch and wax [20]. So the present study was undertaken to showcase the presence of various phytochemicals in *Withania somnifera* and *Rauwolfia serpentina*.

Materials and Methods

2.1 Collection of the Plant Samples

The experiment was carried out on two kinds of *Withania somnifera* samples, one is *Withania Somnifera* Root extract (WSR) and other is *Withania Somnifera* Stem (WSS). WSR and WSS *Rauwolfia serpentine* Root extract (RSR) and *Rauwolfia serpentine* Stem (RSS) were collected from Mahasamund District, Chhattisgarh and Raipur, Chhattisgarh, India respectively during winter season.

2.2 Chemicals

All the chemicals used for the experiments are of analytical grade (Merck, Germany).

2.3 Extract preparation

Withania somnifera Root & Stem **(**100 g**)** and *Rauwolfia serpentine* Root and Stem (100 g) was defatted with Acetone, Methanol, and Chloroform (250 ml) with the help of soxhlet extraction unit. The sample was collected and concentrated in water bath at 40-50 ^{o}C and dried in hot air oven at 40 ^{o}C. The dried powder was kept in air tied box.

2.4 Phytochemical screening of the extract

The portion of the dry extract was subjected to the Phytochemical screening using the method adopted by Trease, Evans and Harbourne. Phytochemical screening was performed to test for alkaloids, carbohydrates, saponins, glycosidal sugars, proteins, phytosterols, phenols, flavonoids, triterpenoids, tannins Anthraquinone, Phlobatanins, Coumarins, Emodins, fixed oil and Fats.

2.4.1 Test for Alkaloids

A small portion of the alcoholic extract was stirred separately with 1 ml of dilute Hydrochloric acid and filtered. The filtrate was treated with Dragandroff's reagent. Appearance of organic precipitate shows the presence of alkaloids.

2.4.2 Test for Carbohydrates

A 1 mg of extract was dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates. Appearance of violet colored ring at the junction of two liquid shows the presence of carbohydrates.

2.4.3 Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate 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, then observed for the formation of emulsion.

2.4.4 Test for Glycosides

1 gm of the alcoholic extract was hydrolyzed with 5ml Hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Fehling's test. To 2ml of Fehling's solution (1ml of Fehling's A and 1 ml of Fehling's B solution), 2ml of extract was added, mixed well and boiled. Appearance of yellow or red color precipitate indicates the presence of reducing sugars.

2.4.5 Test for Proteins

Small quantity of the extract was dissolved in 5 ml of water and subjected to Xantho protein test. To 3 ml of the extract, 1ml of concentrate Nitric acid was added. A white precipitate was obtained. The solution was heated for 1minute and cooled under tap water. It was made alkaline by excess of 40% NaOH. Appearance of orange precipitate indicates the presence of protein.

2.4.6 Test for Phytosterol

Salkowski test was done for the detection of phytosterols. In this test, 1 ml of concentrated Sulphuric acid was added to the 1g plant extract and allowed to stand for 5 minutes. After shaking, formation of golden yellow color in the lower layer indicates the presence of phytosterols.

2.4.7 Test for Phenolic Compounds

A small quantity of the extract was dissolved in few ml of water and subjected to $FeCl_3$ test. The dilute extract was treated with dilute $FeCl_3$ solution (5%) and appearance of violet colour shows the presence of phenolic compound and tannins.

2.4.8 Test for Flavonoids

The extract was treated with concentrated Sulphuric acid. Appearance of yellowish orange show the presence of anthocyanins, yellow to orange color show the presence of flavones, and orange to crimson show the presence of flavonones.

2.4.9 Test for terpenoids (Salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.4.10 Test for tannins
About 0.5 g of the dried powdered samples was boiled in 20 ml 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 colouration.

2.4.11 Anthraquinone
0.5g plant extract boiled with 10% HCl (5 ml) for few min. in water bath then the reaction mixture was filtered and allow for cooling. 2ml 10% ammonia was added and the mixture then heated, rose pink color formation indicates the presence of anthraquinon.

2.4.12 Phlobatanins
0.5g Extract dissolved in 1 ml distil water and filter, boil with 1ml of 2% HCl solution, red color precipitate show the presence of phlobatanins.

2.4.13 Coumarins
3ml of 10% NaOH was added to 2ml aqueous extract, formation of yellow color indicate the presence of Coumarin.

2.4.14 Emodins
2ml of NH_4OH and 3ml benzene was mixed with 1 ml extract, appearance of red color indicates the present of Emodins.

3. Result
The phytochemical analysis of the *Withania somnifera* root in methanolic extract (Table: 1) showed that the major secondary metabolites present were alkaloid, carbohydrate, phenolic compound, terpenoid, phlobotanins, caumarins, and in present study secondary metabolites absent was protin, flavonoid.
The major secondary metabolites emodins present in chloroformic extract (Table: 2) and acetonic extract (Table: 3), but in methanolic extract emodins was absent.
In case of *Rauwolfia serpentine* the major secondary metabolites terpenoid was present in methanol (Table: 4), chloroform (Table: 5) as well as acetone extract (Table: 6).

- **Phytochemical Screening of *Withania Somnifera***

Table 1: Phytochemical screening of root and stem extract of *Withania Somnifera* in Methanol

Extract	Major Secondary metabolites (Methanolic Extract)													
	AL	C	SA	G	P	PS	PHC	FLA	TER	T	ATQ	PT	CUM	ED
Withania somnifera Root extract	+	+	+	+	_	_	+	_	_	+	+	+	+	_
Withania somnifera Stem extract	+	+	_	+	+	+	+	_	+	+	_	+	+	_

Table 2: Phytochemical screening of root and stem extract of *Withania Somnifera* in Chloroform

Extract	Major Secondary metabolites presence (Chloroformic Extract)													
	AL	C	SA	G	P	PS	PHC	FLA	TER	T	ATQ	PT	CUM	ED
Withania somnifera Root extract	+	+	_	_	+	+	+	+	+	+	+	+	+	+
Withania somnifera Stem extract	+	+	_	_	+	_	+	+	+	+	+	+	+	+

Table 3: Phytochemical screening of root and stem extract of *Withania Somnifera* in Acetone

Extract	Major Secondary metabolites (Acetonic Extract)													
	AL	C	SA	G	P	PS	PHC	FLA	TER	T	ATQ	PT	CUM	ED
Withania somnifera Root extract	+	+	+	_	_	+	+	+	_	_	_	+	+	+
Withania somnifera Stem extract	+	+	_	+	+	+	+	+	+	_	_	+	_	+

- **Phytochemical Screening of *Rauwolfia serpentine***

Table 4: Phytochemical screening of root and stem extract of *Rauwolfia serpentine* in Methanol

Extract	Major Secondary metabolites (Methanolic Extract)													
	AL	C	SA	G	P	PS	PHC	FLA	TER	T	ATQ	PT	CUM	ED
Rauwolfia serpentine Root extract	_	+	+		+	+	_	+	+	_	_	+	_	+
Rauwolfia serpentine Stem extract	_	+	_		+	+	_	+	+	_	+	+	_	+

Table 5: Phytochemical screening of root and stem extract of *Rauwolfia serpentine* in Chloroform

Plant Extract	Major Secondary metabolites (Chloroformic Extract)													
	AL	C	SA	G	P	PS	PHC	FLA	TER	T	ATQ	PT	CUM	ED
Rauwolfia serpentine Root extract	+	+	_	+	_	_	_	+	+	_	_	+	_	+
Rauwolfia serpentine Stem extract	+	+	_	_	_	+	_	+	+	_	+	_	_	+

Table 6: Phytochemical screening of root and stem extract of *Rauwolfia serpentine* in Acetone

Plant Extract	Major Secondary metabolites (Acetonic Extract)													
	AL	C	SA	G	P	PS	PHC	FLA	TER	T	ATQ	PT	CUM	ED
Rauwolfia serpentine Root extract	+	+	_	+	+	+	+	+	+	+	_	_	+	+
Rauwolfia serpentine Stem extract	+	+	+	+	+	+	+	+	+	+	+	_	_	+

Tannins- T, Terpenoids- TER, Flavonoids- FLA, Alkaloids- AL, Glycosides- G, Steroids, Carbohydrate- C, Saponin- S, Phytosterol- PS, Phenolic compound- PHC, Anthroquinon- ATQ, Phlobotanins- PT, Caumarins- CUM, Emodins- ED and Proteins- P, Present- (+), Absent- (-)

4. Discussion
Plant are source of large amount of drug which is having antibiotic properties in the traditional system and also used extensively by the tribal people worldwide *Withania somnifera* and *Rauwolfia serpentine* where chosen for the experiment as they have been known to have immense medicinal value. Extensive phytochemical analysis of the present study indicated that they presence of various secondary metabolites viz. alkaloid, terpenoid, carbohydrate, saponin etc. in methanolic, chloroformic and acetonic extract of root and stem of *Withania somnifera* and *Rauwolfia serpentine*. This study corroborated the fact that both plants have quality phytochemicals which are having immense medicinal importance as already exhibited by various researchers in different parts of the country [20, 4]. The present study also demonstrated that this part of the

country has rich natural resources of medicinal plants which can be explored further to showcase their full potential.

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