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Phytochemicals Profile of Methanolic Extract of *Withania somnifera* (L.) Dunal roots by Validated HPLC Technique.

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ABSTRACT

To performs phytochemicals profile of methanolic extract of *Withania somnifera* (L.) Dunal roots by validated HPLC technique. Methanolic Extract was subjected to HPLC investigation, the Phenomenex Luna C18 column (250 mm *4.6 mm, 5 m) was used, with a flow rate of 1.5 ml/min and a UV Wavelength of 227. This Chromatographic study revealed the major Bio active Phytochemicals has found with Retention time of Withanoside IV (14.78), Physagulin D (15.96), 27-Hydroxywithanone (17.22), Withanoside V and Withanoside VI (19.43), Withaferin A (19.85), Withastramonolide (21.17), Withanolide A (22.04), Withanone (22.28), Withanolide B (25.36) are found in Retention Time range between 14.78 to 25.36. linearity range was plot between 10 to 30 mg/ml , LOD was found to be approximately 0.36 ppm and LOQ is 1.18 ppm, Intermediate Precision intraday shows 0.9 %, Interday is 0.25 %, Average Recovery is 94.38 % when planed at 3 different concentration level 50 %, 100 % and 150 %. The result obtained from these studies shows the present HPLC method was accurate, Precise and Validate for Phytochemicals analysis and this approach is recommended for routine quality control analysis of Active Phytochemicals in a wide variety of herbal plants.

Keywords: HPLC, *Withania somnifera* (L.) Dunal, Withanoside, Withanolide, Withaferin.

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INTRODUCTION

Almost 80% of the world's population believed in the use of traditional medicines for primary health care. In India; almost 95% of the doctor prescriptions have been reported to be plant based in the traditional systems of Ayurveda, Unani, Homeopathy and Siddha [1]. The toxicity of medicinal plants is less as compared to synthetic drugs [2]. More than 30% of the pharmaceutical preparations are based on plants [3].

Plant Description: *Withania somnifera* Dunal (WS) (Family-*Solanaceae*) is generally known as Ashwagandha, Indian ginseng is a one of the most important medicinal plant in Indian traditional medicine [4]. WS indicates for "horse smell" in Sanskrit [5]. It has been utilised for thousands of years in Ayurvedic medicine and categorised as a Rasayana (chemical) plant that enhances body- and brain-health by numerous ways including, raising the stress tolerance and boosting the immune system [6].

Taxonomical Classification: The plant is significantly close to 2 feet tall at complete height [7]. It form small, green flowers that contain the red fruit in the center, look like a small berry and Leaves are simple, ovate, glabrous, and length up to 10 cm [8]. The roots are the most important part of the plant for medicinal purposes [9].

Table 1: Withania somnifera Dunal Taxonomic Classification [7]

Kingdom	Plantae,
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Angiosperma
Class	Dicotyledons
Order	Tubiflorae
Family	Solanaceae
Genus	Withania
Species	somnifera Dunal

Figure 1: *Withania somnifera* (L.) Dunal plants have been found and collected in the Botanical garden, Shreeyash campus, Aurangabad.

Distribution: In India, Pakistan, and Afghanistan, as well as Congo, South Africa, Morocco, Egypt, Israel, and Jordan, the plant is grown extensively and economically. Madhya Pradesh, Uttar Pradesh, Punjab, Gujarat, and Rajasthan are among the Indian states where the plant is grown [10,11]. It thrives in dry, subtropical climates. Ashwagandha is a drought-tolerant and resilient plant. The biggest producers of this crop in the nation are Madhya Pradesh, Gujarat, Haryana, Maharashtra, Punjab, Rajasthan, and Uttar Pradesh [12].

Phytochemistry: The major chemical constituents of *Withania somnifera* are alkaloids and steroidal lactones and various alkaloids like withanine. Other alkaloids are somniferine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, cuscohygrine, anferine and anhydrine. Two acyl sterol glycoside viz. sitoindoside VII and sitoindoside VIII have been Present and isolated from root [13]. Also withanolide D, Anaferine (alkaloid), anahygrine (alkaloid), beta-sisterol, chlorogenic acid (in leaf only), cysteine (in fruit), cuscohygrine (alkaloid), iron, scopoletin, somniferinine (alkaloid), tropanol (alkaloid) and withanolides A-Y (steroidal lactones), 3-a-gloyloxytropane, isopelletierine, isolettetierine, 3-alpha-gloyloxy tropane have been found in WS [14,15]. The leaves and roots of WS contain different types of alkaloids withanolides, withaferin A. In various research papers Reported that withaferin A and withanolide A have to be Controlling Concentration in various tissues metabolite distribution [16, 17, 18].

Pharmacological activity: Withania somnifera is used to treat wounds, cough, asthma, diabetes, tumours, hemiplegia, dyspepsia, diarrhoea, rheumatoid arthritis, lumbago, stress, insomnia, sexual debility, menstrual difficulties, leucoderma, scabies, and leucorrhoea [19,20]. This plant has been shown to be effective in the treatment of Alzheimer's disease, Parkinson's disease, anxiety, attention deficit hyperactivity disorder (ADHD), and cerebral ischemia in clinical trials and pre-clinical studies [21,22]. WS has medicinal characteristics, making it a potentially effective additional herb for radiation and chemotherapy patients.WS is also utilised as an immunological stimulant in patients with low white blood cell counts, as well as an adaptogen in patients suffering from nervous weariness, sleeplessness, and debility as a result of stress [23].

Market Formulation: In India, *Withania somnifera* is sold as a churna, a fine powder that can be blended with water, ghee, or honey [24]. A novel branded herbomineral formulation containing the herbal ashwagandha root extract as well as zinc, magnesium, and selenium minerals was developed for the pharmaceutical market [25,26,27]. WS having Important Pharmacological activity like antioxidant, antialzheimer's, antiparkinsonian, anti-inflammatory, anti-diabetic, hypolipidaemic, cardioprotective, anxiolytic, potent immunomodulatory, anticancer activity [28]. So in the present study has aim to performs phytochemicals profile of methanolic extract of *Withania somnifera* (L.) Dunal roots by validated HPLC technique.

MATERIAL AND METHODS

Collection of Plant material: *The Withania somnifera* was found and collected from the Medicinal Garden of Shreeeysh Instititute of Pharmaceutical Education and Research, Satara Parisar, Aurangabad, Maharashtra and voucher specimen (Voucher No. 0727) was deposited in the herbarium in this Department and authenticated by Professor and Head Dr. Arvind Dhabe Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra.

Extraction of Root Powder: The air dried Sample of *Withania somnifera* roots were powdered and pass through the sieve number 20 then Powder of having weight of 10 g was extracted using 50 ml methanol (99%) by shaking for Approximately 60 minutes and then sonicated for 40 minutes at room temperature. The extract was kept for drying by removing the solvents using rotary vacuum evaporator. Filtered through 0.45 micron filter paper and stored in air tight container until used for HPLC Studies.

Chemicals and reagents: Methanol, Acetonitrile of HPLC grade were used. Potassium dihydrogen phosphate, Phosphoric acid, distilled. Water and other chemicals were analytical grade.

Instrumentation: Phytochemical HPLC analysis was carried out using a Shimadzu LC-20AD pump system with a Shimadzu SPD-20AT UV- Visible detector and Rotatory evaporator throughout the study activity. A Shimadzu electronic balance was also employed.

Selection Chromatographic condition and mobile phase: Preparation of the standard/sample solution: dissolve 20 mg/mL powdered Withania somnifera Root Extract in methanol. Sonicate for ten minutes, centrifuge, and the supernatant is subsequently filtered by a 0.45-micron membrane filter.

Mobile phase: for the mobile phase 2 solutions were selected as follows-

- Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL and mix.

- Solution B: Filtered Acetonitrile was selected.

Methanol was used as Diluents for stock solutions. For this HPLC analysis Phenomenex , Luna C18 (250 mm *4.6 mm, 5 µm) column was used. from the UV spectrum wavelength was found to be selected 227 nm. Column temperature 27 °C was selected. Flow Rate 1.5 mL/min was selected.20µL volume of injection was selected.

Table 2: Gradient program of HPLC analysis of WS root extract.

Sr.no	Time (min)	Flow Rate (mL/min)	%Solution A	%Solution B
1	0	1.5	95	5
2	18	1.5	55	45
3	25	1.5	20	80
4	28	1.5	20	80
5	30	1.5	95	5
6	40	1.5	95	5

Validation of Present method

It was carried out with help of checking linearity and range, limit of detection (LOD), limit of Quantification (LOQ), precision and recovery.

Calibration Curve & Linearity: Different 5 concentrations of Methanolic WS Root Extract as stock solution after dilution (10, 15, 20, 25 and 30 mg/ml) with mobile phase were injected in 3 times. Calibration Curve was plotted as concentration versus peak area response.

Limit of detection (LOD), Limit of Quantification (LOQ): To study the linearity of Phytochemicals using dilutions series (60, 80, 100 µg/ml) were made from Methanolic WS Root Extract as stock solution LOD, LOQ were calculated by following formula,

$$LOD = 3.3 * \sigma/S$$
$$LOQ = 10 * \sigma/S$$

Where, σ = Standard deviation of the y-intercept, S = Slope of the calibration curve (m)

Precision or Repeatability: Precision of the method was expressed in terms of %RSD calculated from the calculation of peak area response.

Accuracy or Recovery study: Accuracy was evaluated in triplicate by addition of known concentration of Sample solution to the pre-analyzed sample of roots. Three samples of each concentration level 50 %, 100 % and 150 % were prepared.

RESULT AND DISCUSSION

The chemically active content was extracted and analyzed by HPLC. The key Bio active Phytochemicals has found in found in Retention Time range between 14.78 to 25.36 Withanoside IV, Physagulin D, 27-Hydroxywithanone, Withanoside V and VI, Withaferin A, Withastramonolide, Withanolide A, Withanone, and Withanolide B are all present in this extract. in validation parameter linearity range was plot between 10 to 30 mg/ml , LOD was found to be approximately 0.36 ppm and LOQ is 1.18 ppm, Intermediate Precision intraday shows 0.9 %, Interday is 0.25 %, Average Recovery is 94.38 % when planed at 3 different concentration level 50 %, 100 % and 150 %.

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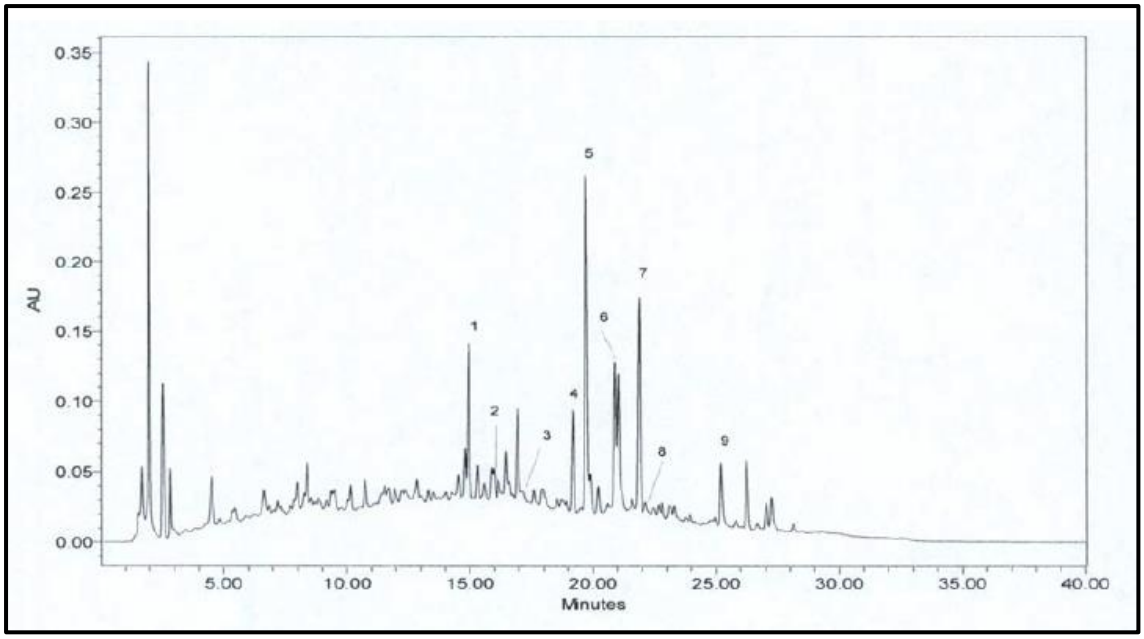

Figure 2: HPLC chromatogram of different Phytochemicals of WS root extract

Table 3: Following valuable Phytochemicals was found from HPLC analysis

Sr.no	Name	Retention time (min)	Area (MV*S)	Height (MV)
1	Withanoside IV	14.78	125.311	14.561
2	Physagulin D	15.96	35.264	6.023
3	27-Hydroxywithanone	17.22	18.845	3.658
4	Withanoside V and Withanoside VI	19.43	84.932	9.357
5	Withaferin A	19.85	346.746	27.554
6	Withastramonolide	21.17	138.147	12.843
7	Withanolide A	22.04	294.385	17.710
8	Withanone	22.28	13.177	3.486
9	Withanolide B	25.36	62.592	5.940

Table 4: Validation parameter of developed HPLC method for detection of Phytochemicals.

Sr.no	Validation parameter	Results
1	Linearity range	10 to 30 mg/ml
2	LOD (ppm)	0.36 ppm
3	LOQ (ppm)	1.18 ppm
4	Precision-(RSD %) a) Intraday (%)	0.9 %
	b) Interday (%)	0.25 %
5	Average Recovery (%)	94.38 %

The result obtained from these studies shows present HPLC method was accurate, Precise and Validate for Phytochemicals analysis. Some important Phytochemicals was absent due to Variations in environmental condition one place to another.

CONCLUSION

The Active Phytochemicals of Withania somnifera (L.) plants growing in Maharashtra, India, were determined using an HPLC Analysis technique. It is a programmed approach for analysis that is easy, accurate, and exact. In the absence of HPTLC, the purity of this method is suitable on a laboratory scale. As

an outcome, systematic quality control analysis of Active Phytochemicals in a wide range of herbal plants is suggested using this method.

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ABBREVIATIONS

HPLC: High Performance Liquid Chromatography; **WS**-Withania somnifera, **LOD-** Limit of detection, **LOQ-** Limit of Quantification

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