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STUDY ON ALKALOID ESTIMATION BY HPLC IN *WITHANIA SOMNIFERA* (LINN) DUNAL WITH REFERENCE TO TREATMENT WITH GROWTH REGULATORS

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ABSTRACT

Many medicinal plants are used in modern medicine where they occupy a very significant place as raw material for important drugs and plants used in traditional system of medicine in pharmaceutical houses are collected from wild sources (Audu *et al*., 2007). Two varieties of Ashwagandha i.e. var WS-134 and var WS-20 were cultivated by farmers for herbal drugs are selected for present research. The level of withanolide-A was generally higher in the roots of treated plants than that in control plants of WS-134. GA and GA+IAA seem to be very effective treatments.

Keywords: medicinal plants, Ashwagandha.

INTRODUCTION

Ayurveda is the oldest system of Medicine in the world, its antiquity going back to the Vedas. It adapts a unique holistic approach to the entire science of life, health and cure. The areas of special consideration in Ayurveda are geriatrics, rejuvenation, nutrition, immunology, genetics and higher consciousness. Vagbhatta (300AD) and Sarangdhara (1300AD) described the time-bound sequential biological human aging in terms of sequential loss of certain biological qualities of life specific to different decades of life. If these bio losses are compensated in respective decades by appropriate life-style, nutrition and Ayurvedic rejuvenate Rasayana remedies described for this purpose the rate of biological aging may be retarded [1,2].

Hormones regulate the speed of growth of the individual parts and integrate these parts to produce the form that we recognize as plants [3]. There are commonly five recognized group of plants hormones- auxins, gibberellins, cytokinins (Cks), abscisic acid and ethylene. Of the five plant hormones, auxin and Cks are shoot and root hormone respectively, and GA, ETH and ABA are involved in both long term, seasonal and environmental responses of the plants. Auxins were the first plant hormones to be discovered. The principle auxin in plant is Indol-3-Acetic Acid (IAA). The initial discovery of IAA in plants and recognition of its role in growth and development stimulated the search for other chemicals with similar activity. Indole-Butaryc-Acid (IBA) is one of the examples, which was originally thought to the synthetic, but then has been isolated from seeds and leaves of maize and other species [4]. Auxins serve primarily to regulate cell growth and stem elongation. In addition to stimulate cell elongation, auxins are also involved in regulating cellular differentiation. GA stimulates stem elongation. Thus treated plant becomes taller than it would normally be. Exogenous application of IBA, GA, Cks is used to enhance growth and productivity of crop plants but such use of hormones is not so common in medicinal plants.

Withania somnifera L. (Ashwagandha in Sanskrit) is an evergreen tomentose shrub, belongs to the family Solanaceae. It grows in wild and it is also cultivated for medicinal purpose in several parts of India. This plant is attributed with curative properties against a number of diseases including cancer and finds mention in the ancient medical treatise in India [2]. It is especially reputed for its rejuvenating properties and is used in combination with other herbs. The wide range of its medicinal uses has earned it the name 'Indian Ginseng'. The major biochemical constituents of ashwaganda root are steroidal alkaloids and steroidal lactones in a class of constituents called withanolides. At present, 12 alkaloids, 35 withanolides, and several sitoindosides from this plant have been isolated and studied. A sitoindoside is a withanolide containing a glucose molecule at carbon 27. Much of ashwaganda's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D.

Looking to the importance of Ashwagandha it was

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selected for the study. Two varieties of Ashwagandha i.e. var WS-134 and var WS-20 were cultivated by farmers for herbal drugs. It was of interest to study the effect of PGRs on alkaloids.

MATERIAL AND METHOD

Ashwagandha (Withania somnifera) was selected for the study. Two varieties namely var WS-134 and var WS-20 were used for experimental purpose. Gibberellic acid (GA), Kinetin (KIN), Indole butyric acid (IBA) and Indole acetic acid (IAA) were used as plant growth regulators (PGRs). The PGRs singly, in combination of two and in combination of three (each having 10-5M conc) were used in the study.

The seeds of Ashwagandha var WS-134 and var WS-20 were sown in the prepared plots of 2M×2M (100seeds/plot). Two plots were kept for each treatment. Necessary watering was done and the plants were cultivated under natural condition. After 30 days of sowing, the plants were sprayed with DW, GA 10^{-5}M, KIN 10^{-5}M, IBA 10^{-5}M, IAA 10^{-5}M, GA+KIN and GA+IAA. Hand sprayer was used for the spray. Spray was given till both the sides of leaves become wet. Three such sprays were given at regular interval of three days. Procedure of spray treatment was repeated after 60, 90, 120 and 150 days of sowing. Growth data of sprayed plants was taken after 45, 75, 105, 135 and 165 days of sowing. The roots of 135 days old control and treated plants in replicates were estimated for HPLC (High performance liquid chromatography) analysis.

Procedure: Take dry root as sample then crush it and make fine powder.

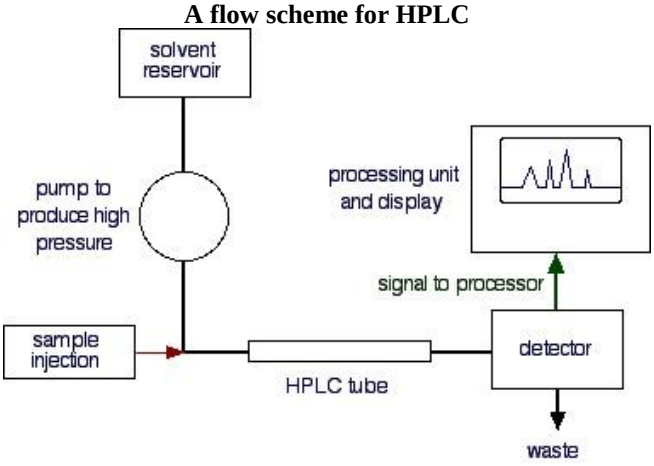
Add a few drops of ammonic solution and shake frequently. Wash the sample with methanol. Repeat the procedure 3 times and collect the extract. Take 1g of extract and evaporate in a water bath. Let it be completely dry. Dissolve 20 ml of HPLC grade methanol in the residue. Filter through 0.45µm nylon filter. Use the filtrate for HPLC analysis. For HPLC analysis deoxywithastromonolide and withanolide-A are used as standard solutions for alkaloid estimation.

General technique of HPLC analysis:

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. It also allows use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. HPLC is achromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying and purifying the individual components of the mixture. Some common examples are the separation and quantitation of performance enhancement drugs (e.g. steroids) in urine samples, or of vitamin D levels in serum. The other major improvement over column chromatography concerns the detection methods which can be used.

The sample to be separated and analyzed is introduced in a discrete small volume, into the stream of mobile phase percolating through the column. The components of the sample move through the column at different velocities, which are functions of specific physical or chemical interactions with the stationary phase. The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called the retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte. Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrileand methanol). In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (=short retention times). A typical gradient profile in reversed phase chromatography might start at 5% acetonitrile (in water or aqueous buffer) and progress linearly to 95% acetonitrile over 5–25 minutes.

A flow scheme for HPLC



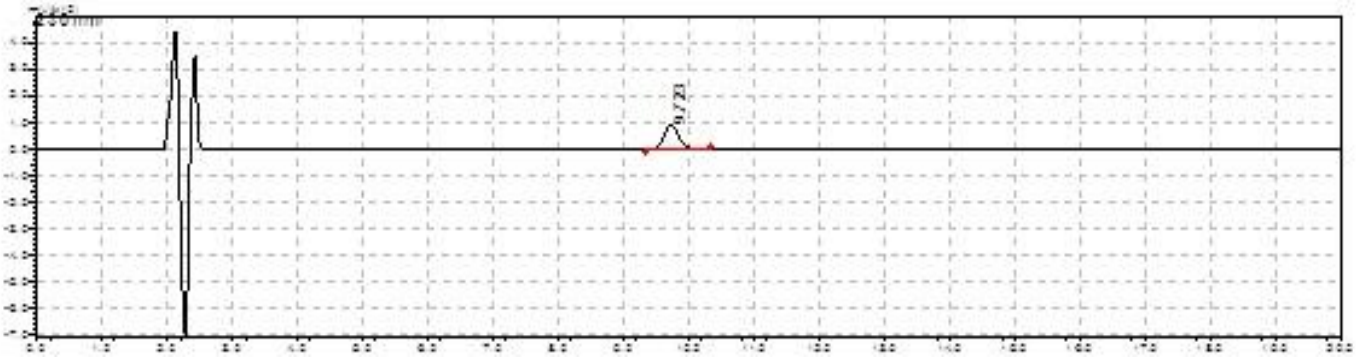
RESULT AND DISCUSSION

Withanolides are a group of at least 300 naturally occurring chemical compounds. They occur as secondary metabolites. Structurally, withanolides consist of a steroid backbone bound to a lactone or one of its derivatives; they are produced via oxidation of steroids. This class of steroidal lactones involves an ergostane-type framework which C-22 and C-26 are appropriately oxidised to form a (delta)-lactone ring. They are subdivided into nine groups: withanolides, withaphysalins, physalins, nicandrenones,

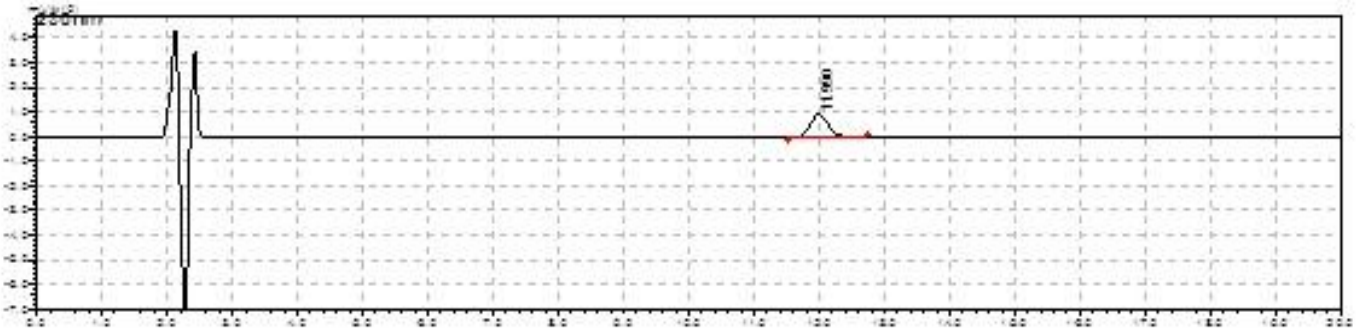
jaborols, ixocarpalactones, perulactones, acnistins and miscellaneous with asteroids. The exogenous application of PGRs to the leaf of WS-134 generally enhanced the amount of 12-deoxy with astromonolide (Graph -1). The increase was more or less similar in PGRs treated plants. The amount was slightly lowered in DW treated plants than that in control. Foliar spray of PGRs to the leaf of WS-20 also increased the amount of 12-deoxywithastromonolide. Maximum amount was found in GA treated plants. DW slightly increased it. Data suggest that by using simple technique one can increase the economic value of Ashwagandha varieties. It is interesting to note that WS-20 consists higher amount of 12-deoxywithastromonolide than that in WS-134. Out of all PGRs foliar spray of GA to WS-20 may be recommended.

The level of withanolide-A was generally higher in the roots of treated plants than that in control plants of WS-134. GA and GA+IAA seem to be very effective treatments (Graph-2). The amount of withanolide-A was more or less similar in control and DW treated plants. The exogenous application of PGRs increased the withanolide-A content in the roots of WS-20. The increase was similar and highly significant in GA, KIN, IBA, GA+KIN and GA+IAA treated plants. DW did not influence the amount of withanolide-A. The data suggest that for getting maximum amount of withanolide-A WS-20 variety instead of WS-134 may be used and the content of withanolide-A may be enhanced by foliar spray of PGR like GA, KIN, IBA, GA+KIN and GA+IBA (Graph-2). Withanolides are currently being explored for their brain regenerative properties.

Withaferin-A



12-Deoxywithastromonolide



Withanolide-A

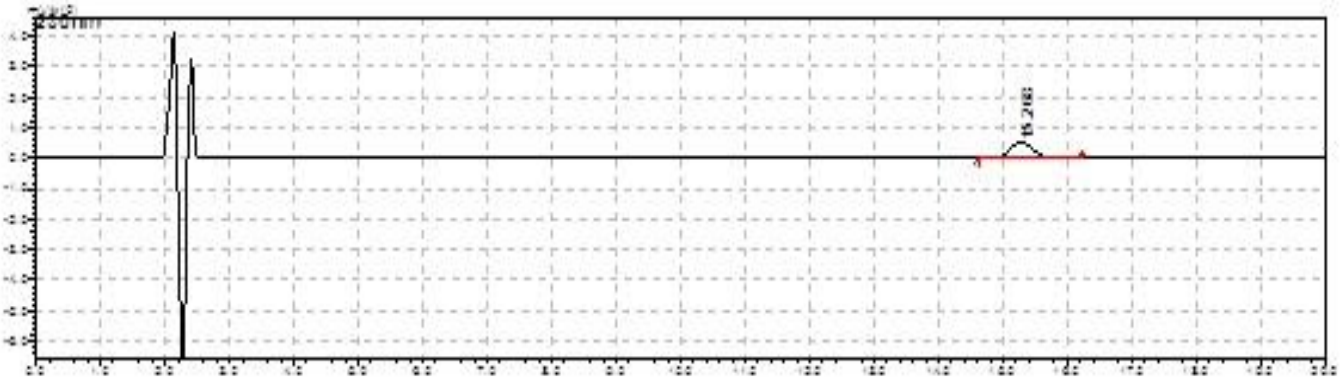
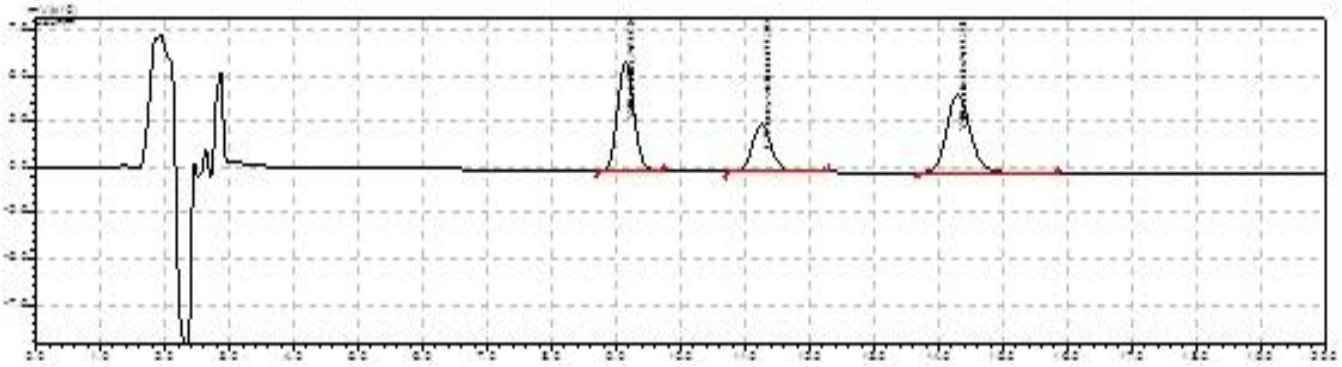
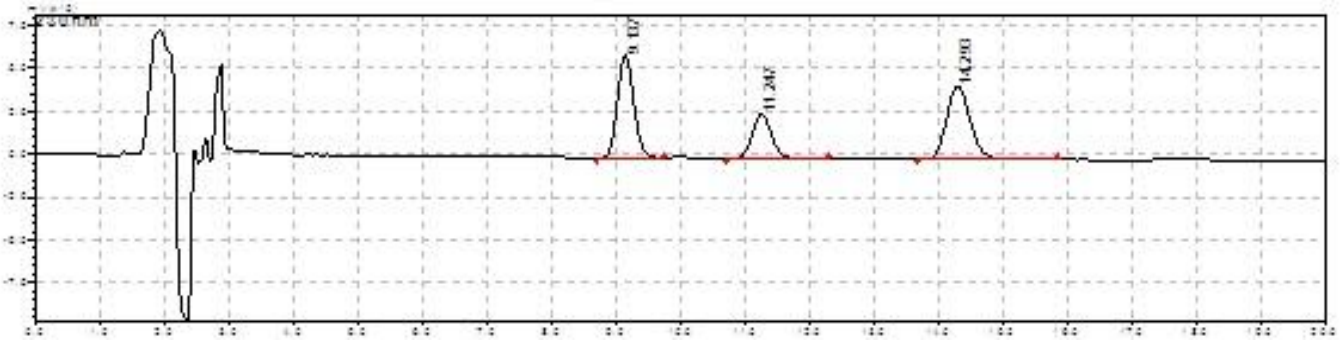


Figure 1. HPLC Chromatogram of reference Standards

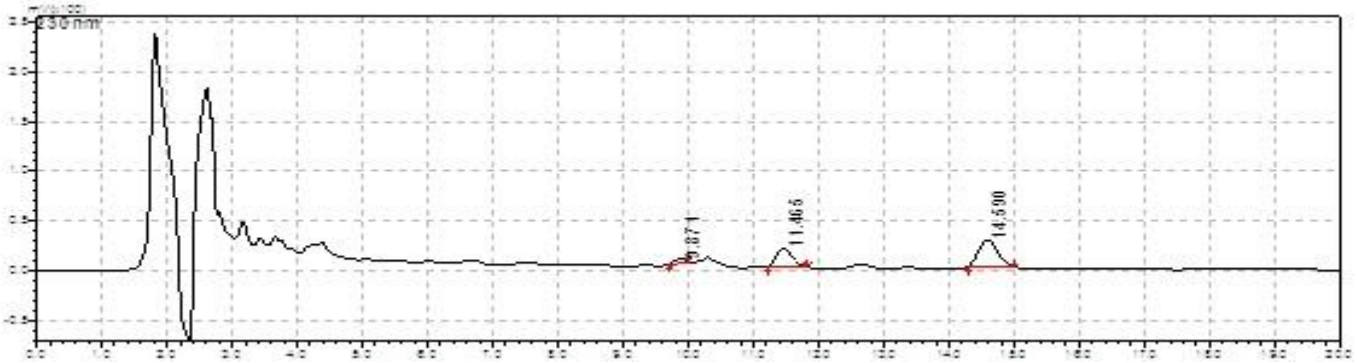
WS-134



CON



DW



GA

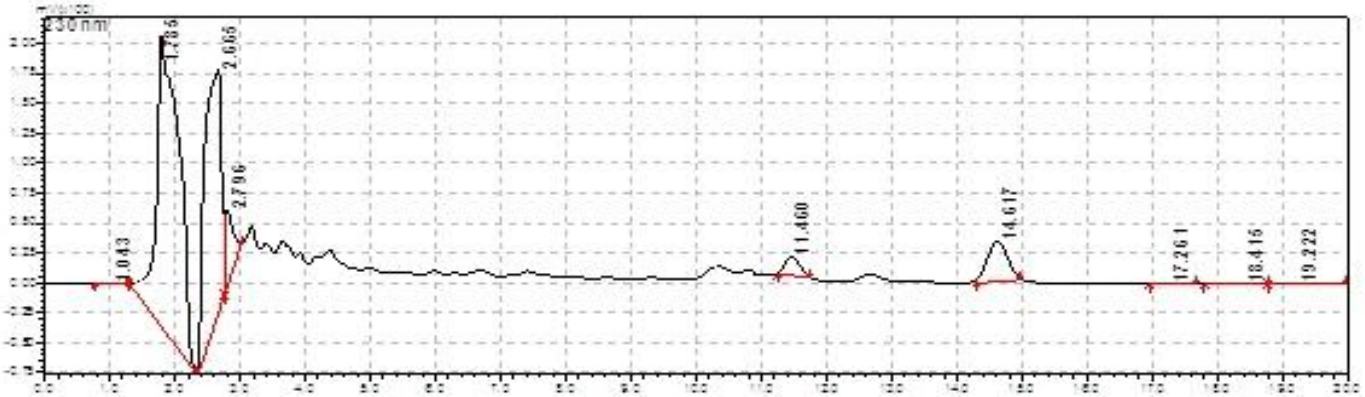
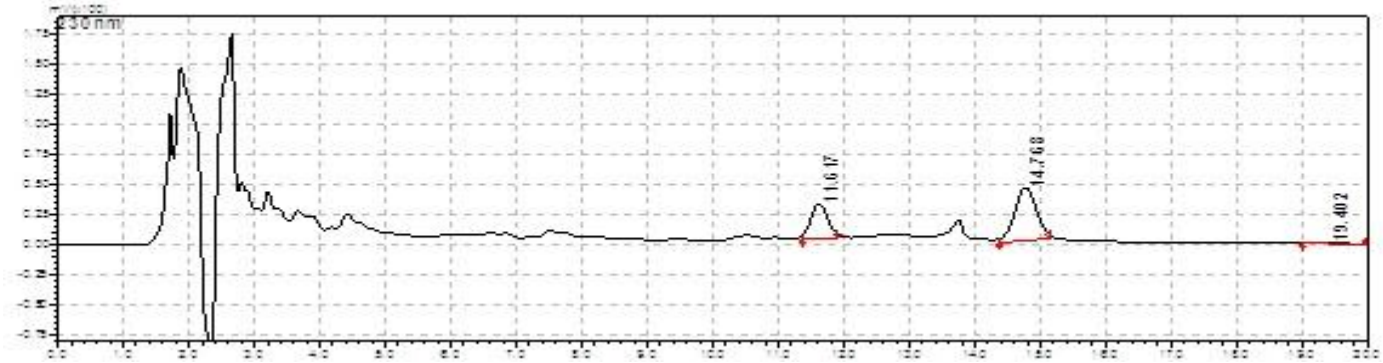
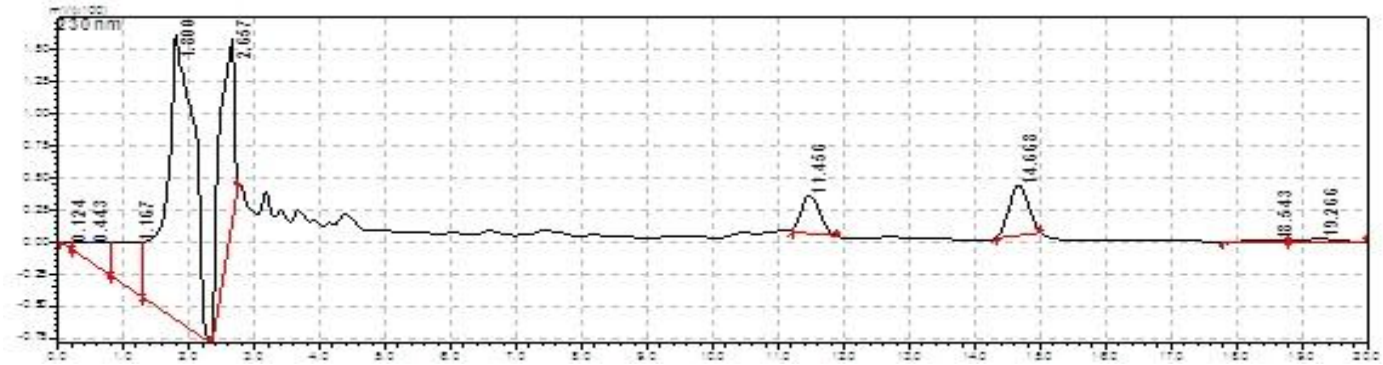


Figure 2. HPLC Chromatogram of alkaloids in Ashwagandha Var WS-134 (CON, DW, GA)

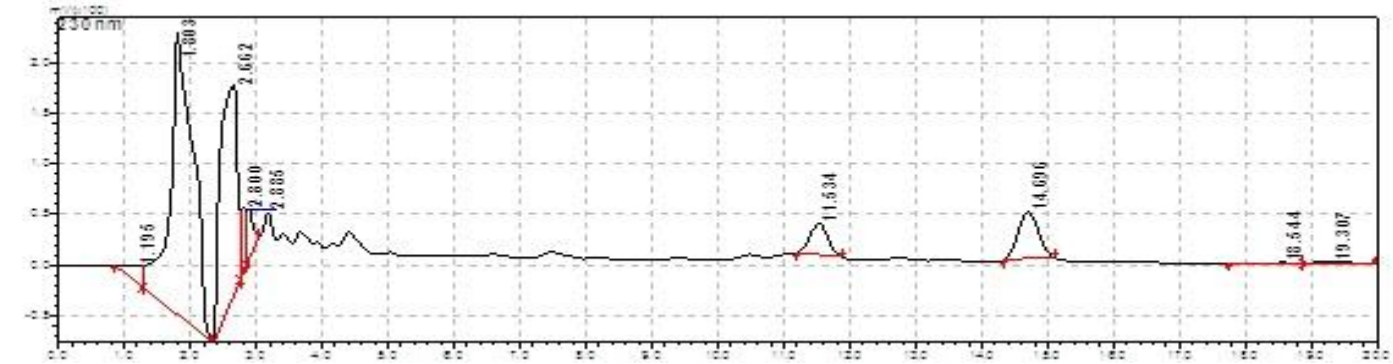
WS-134



KIN



IBA



IAA

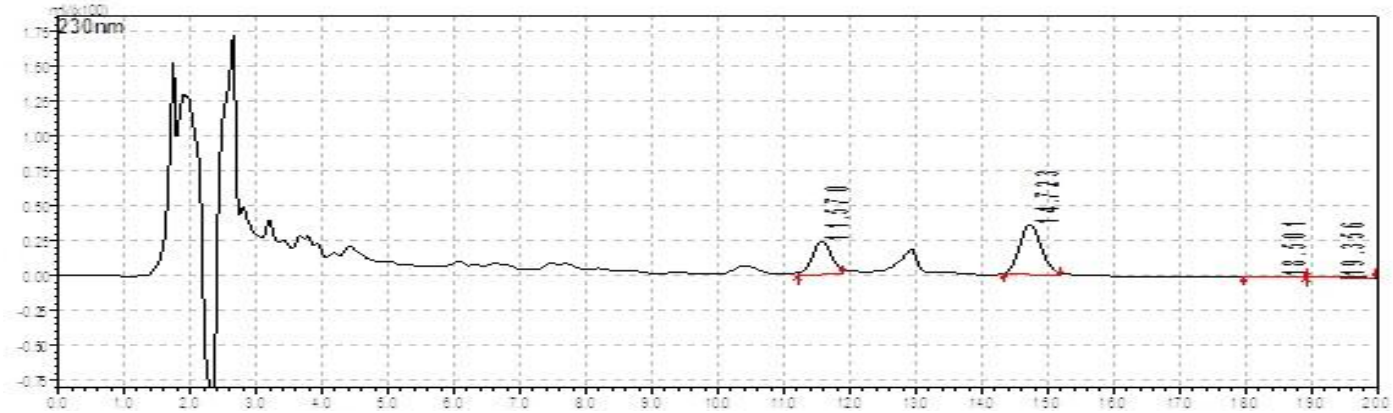
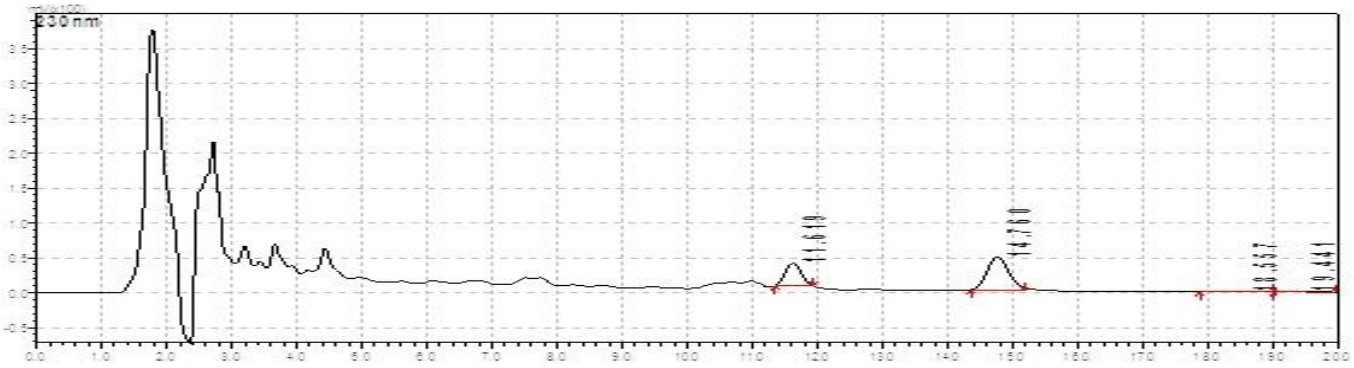
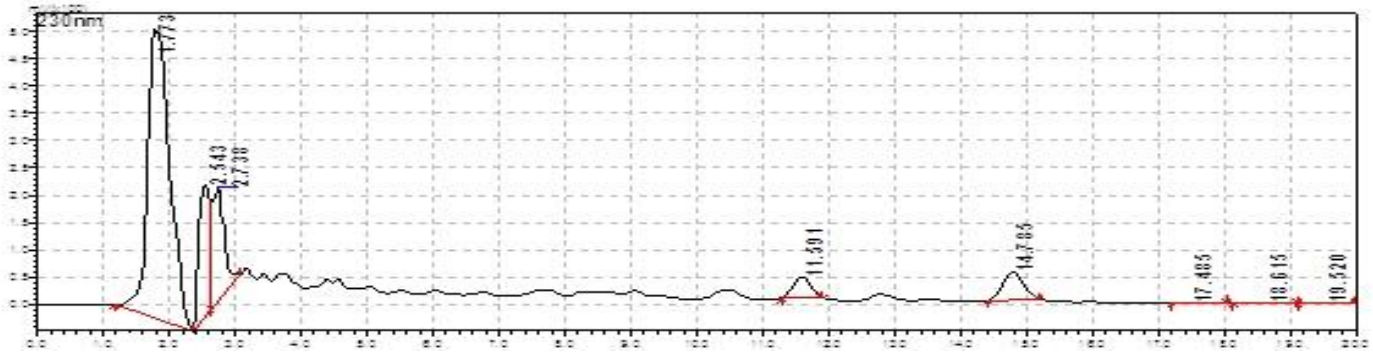


Figure 3. HPLC Chromatogram of Alkaloids in Ashwagandha Var WS-134 (KIN, IBA, IAA)
WS-134



GA+KIN



GA+IAA

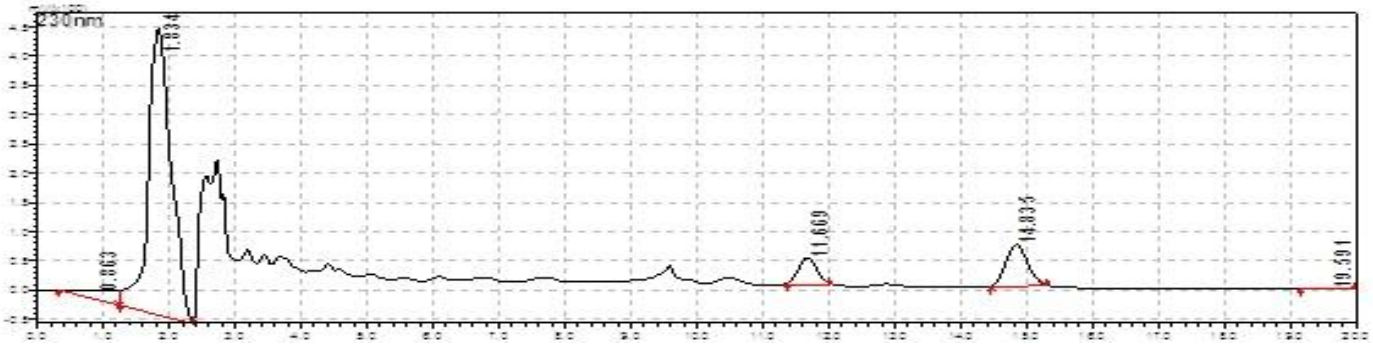
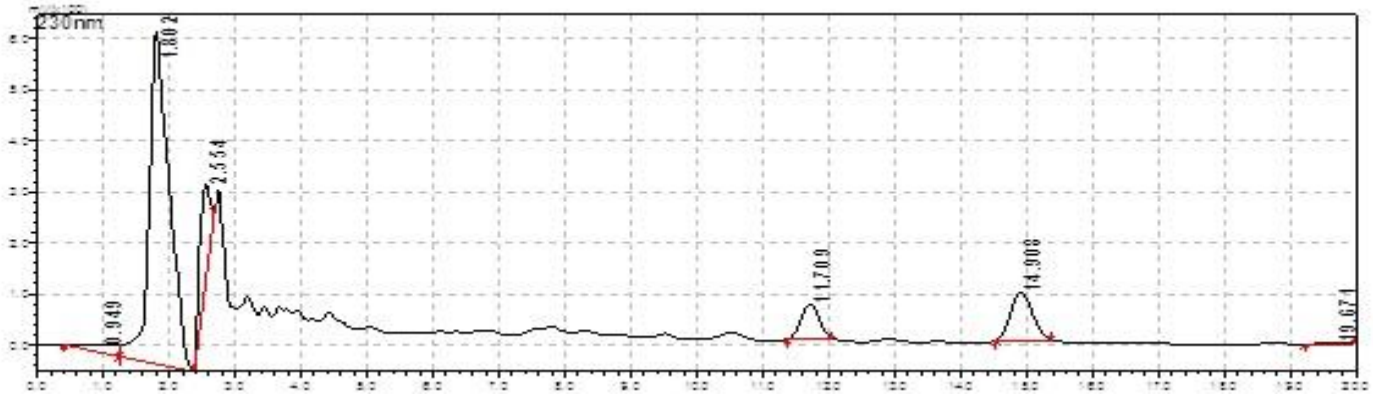
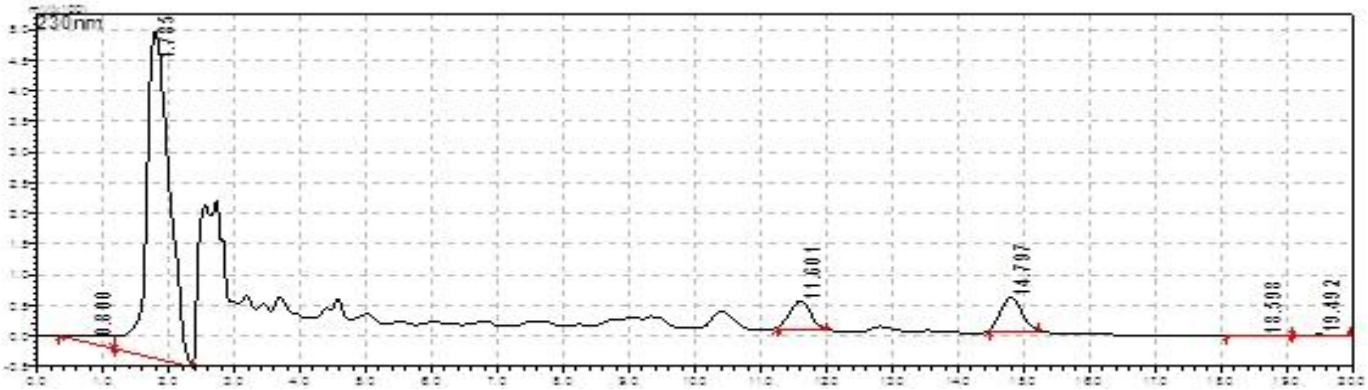


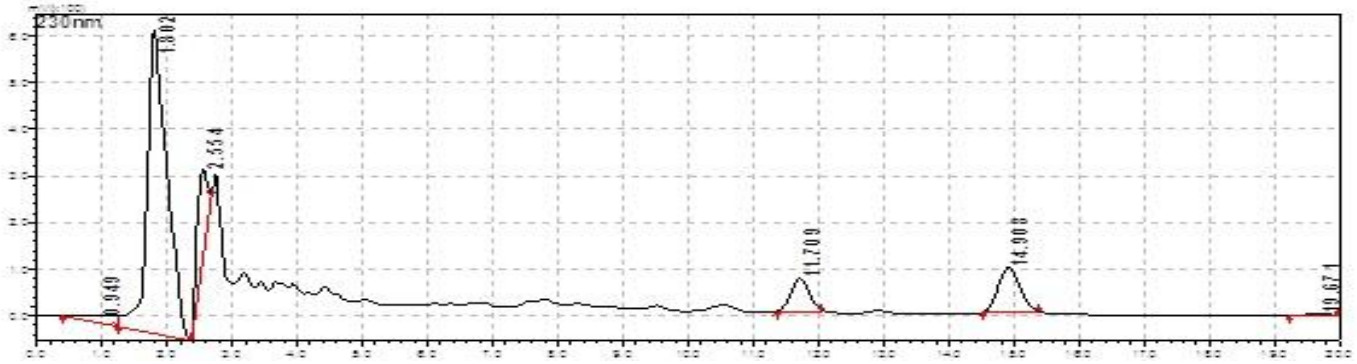
Figure 4. HPLC Chromatogram of Alkaloids in Ashwagandha Var WS-20 (GA+KIN, GA+IAA)
WS-20



CON



DW



GA

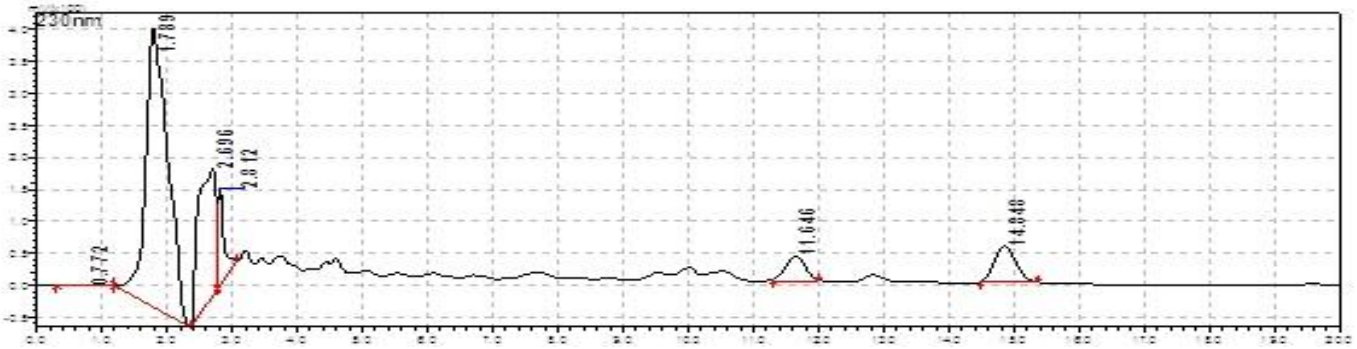
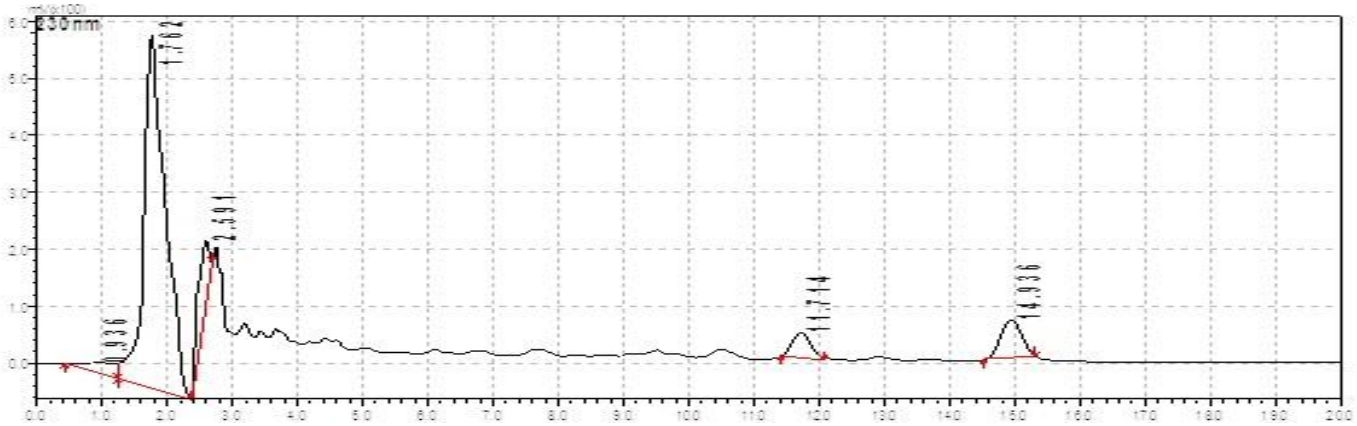
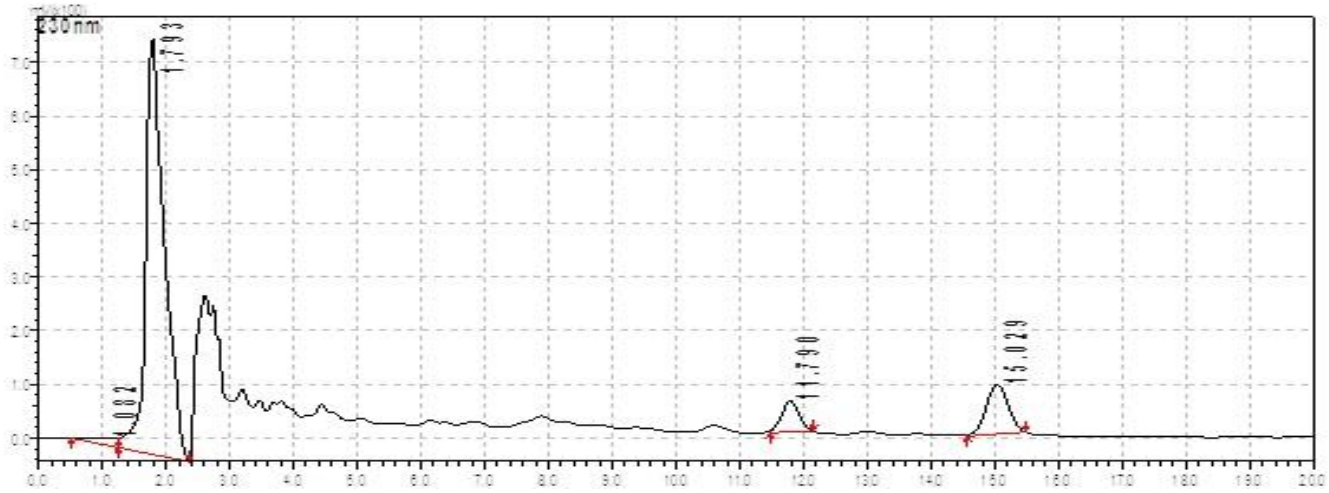


Figure 5. HPLC Chromatogram of Alkaloids in Ashwagandha Var WS-20 (CON, DW, GA)

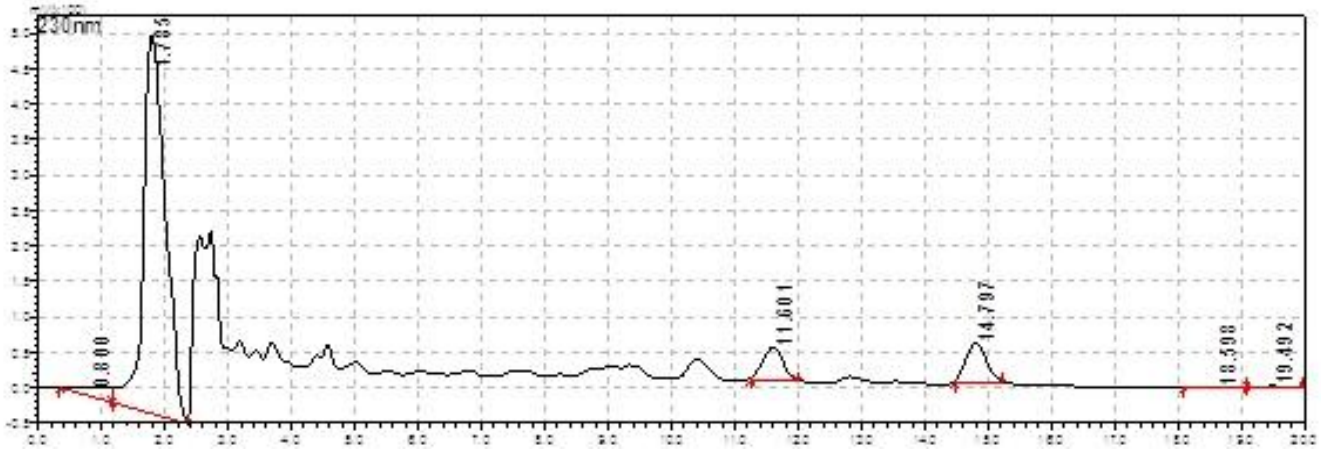
WS-20



KIN



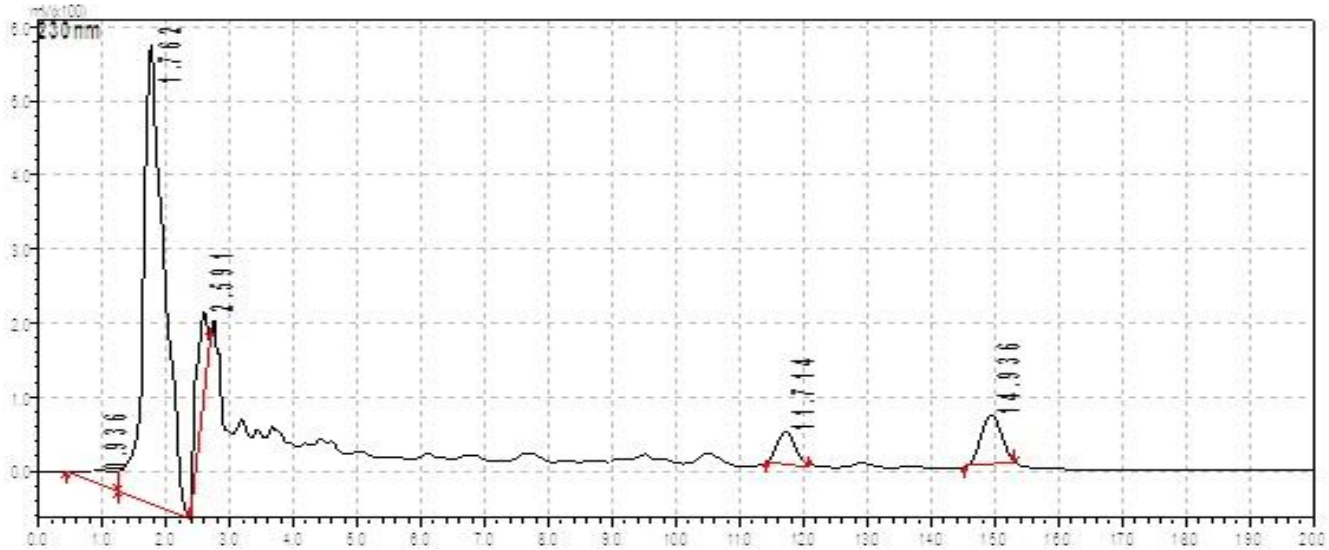
IBA



IAA

Figure 6. HPLC Chromatogram of Alkaloids in Ashwagandha Var WS-20 (KIN, IBA, IAA)

WS-20



GA+KIN

GA+IAA

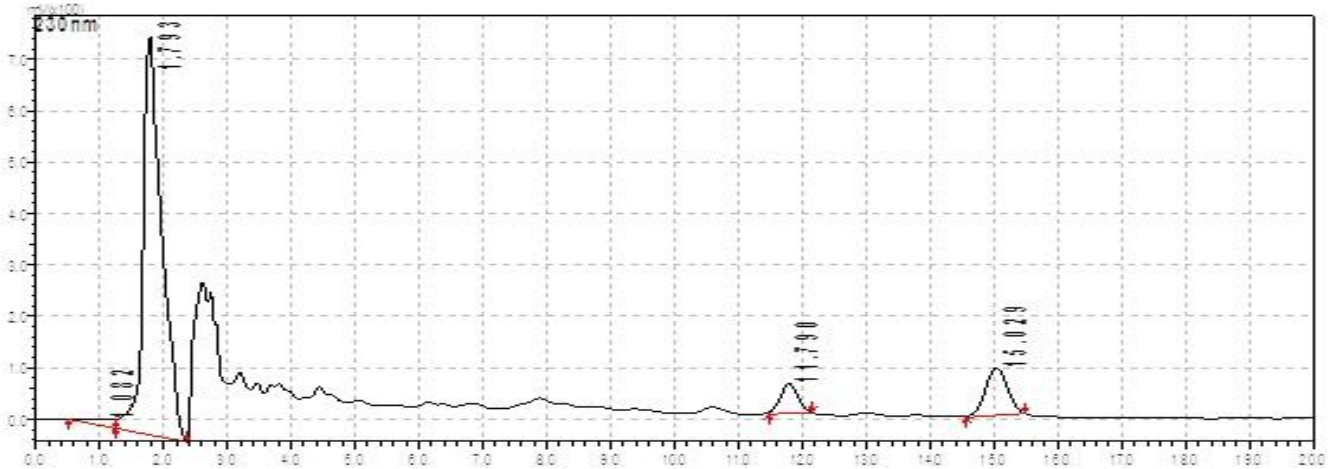
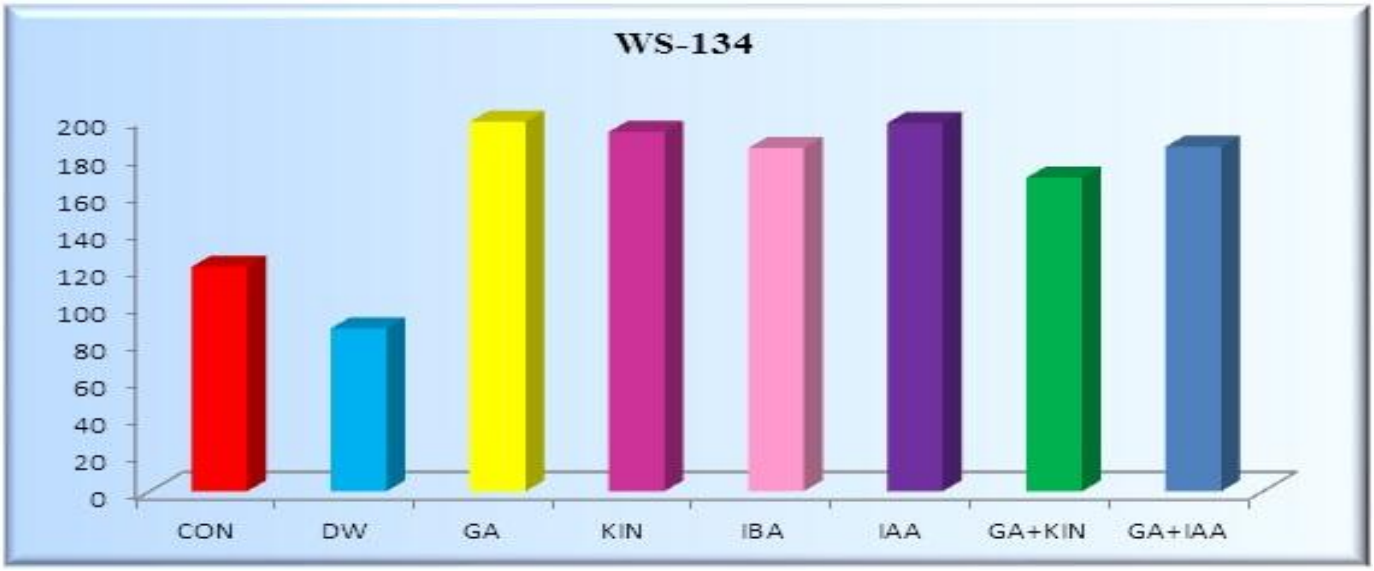
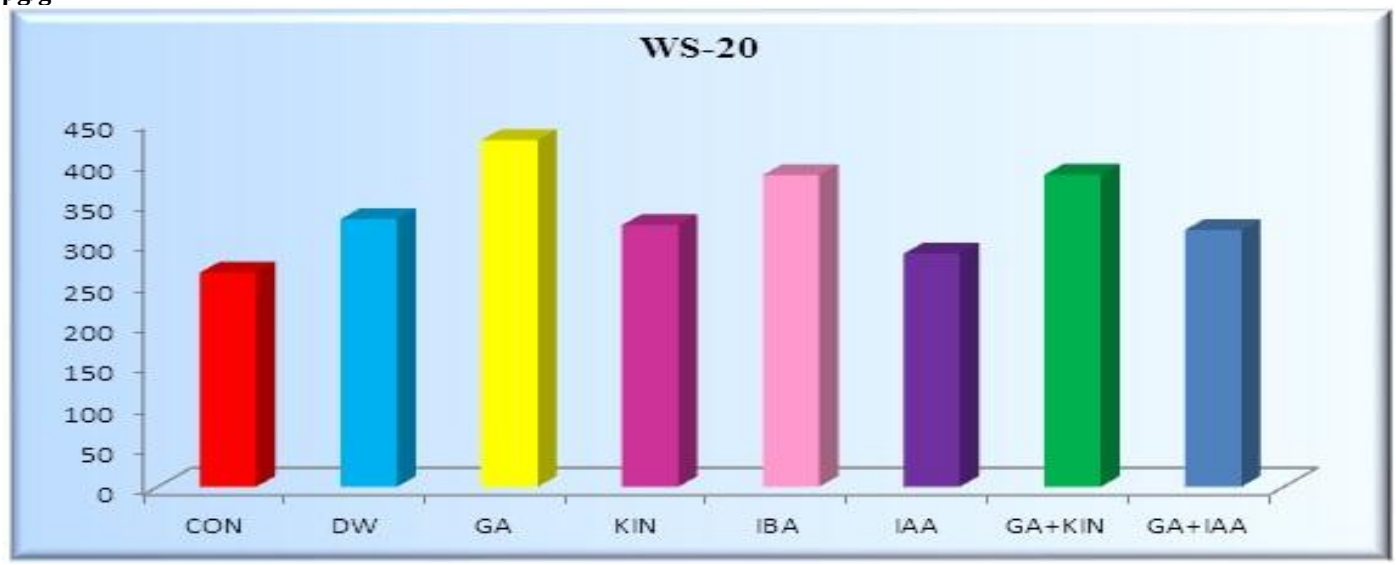


Figure 7. HPLC Chromatogram of Alkaloids in Ashwagandha Var WS-20 (GA+KIN, GA+IAA)

µg/g

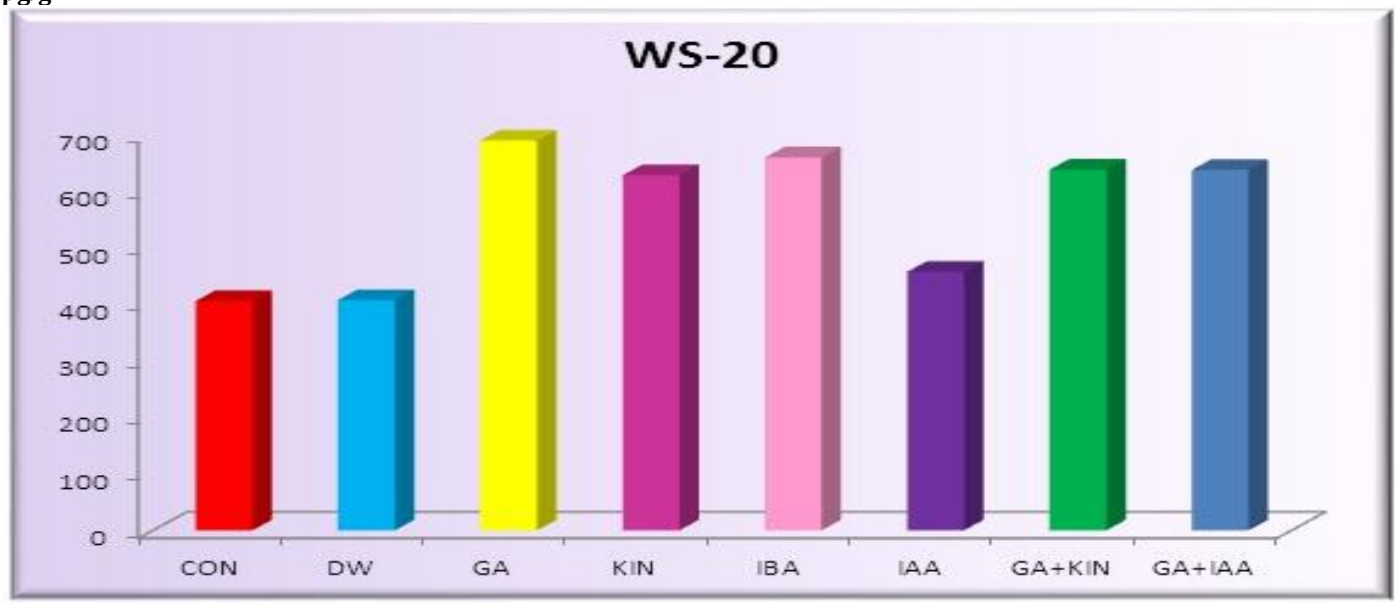


µg/g



Graph 1. Effect of Foliar Spray of PGRs ON 12-deoxywithastromonolide Content in the root of Ashwagandha Varieties µg/g

Graph 2. Effect of Foliar Spray of PGRs on Withanolide-A content in the root of Ashwagandha Varieties µg/g



SUMMARY AND CONCLUSION

Ashwagandha is one of the important medicinal plants. The root is herbal drug yielding organ. The root of this plant is used on large scale, thus now Ashwagandha is cultivated by the farmers.

Foliar spray of PGRs enhanced the amount of 12-deoxywithastromonolide and withanolide-A in both the varieties. WS-20 consists of higher amount of both the alkaloids than WS-134. GA highly enhanced the amount of both the alkaloids in both the varieties.

It is recommended that one can increase the biological yield of Ashwagandha var WS-134 and var WS-20 by foliar application of enhanced levels of PGRs. Among these two varieties WS-20 is much more influenced by PGRs than WS-134. Exogenous application of PGRs is very simple and not expensive also.

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