Original Research Paper

Volume-9 | Issue-2 | February-2019 | PRINT ISSN - 2249-555X

Science

PHARMACOLOGICAL AND PHYTOMEDICINE ASSESSMENT OF *WITHANIA SOMNIFERA*

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ABSTRACT The composition of bioactive compounds from *withania somniferous* (Aswagandha) was determined by UV spectroscopy and HPTLC photodocument. Qualitative and quantitative screening revealed that tannins, saponins, alkaloids, steroids, protein, carbohydrates were present in extract of all the parts of the plant that claimed to possess medicinal activities on various ailments. The presence of these phytochemicals in *withania somnifera* could justify the applications of the plant in management and curing of various diseases.

KEYWORDS : *Withania* somniferous, phytochemicals, alkaloids, HPTLC

INTRODUCTION

1.1 Classification and Nomenclature

Herbal medicine is natural source of drug. Ayurveda is a traditional medicine system that have lot of knowledge, secret, treasure house from classical to modern medicine. From chemistry point of view *withania somnifera* phytochemistry comprises bunches of phytochemicals. According to literatures alkaloids and its subcompound like cocain, atropine, morphin etc. were mainly present in the *withania somnifera* **[1 & 2].**

Table 1: Scientific classification of Aswagandha

Kingdom	Plantae (Plants)
Sub kingdom	Tracheaobionta (Vascular Plant)
Super division	Spermatophyta (Seed Plants)
Division	Magnoliophyta (Flowering Plants)
Class	Magnoliopsida (Dicotyledone)
Sub class	Asteridae
Order	Solanales
Family	Solanaceae
Genus	*Withania*
Species	Somnifera

1.2 Botanical Description of Aswagandha

There are so many names of withania somnifera like Indian ginseng, poison gooseberry deserves to nightshade family and it is popular herb of traditional drugs **[3 & 4].** Active phytochemical like alkaloids, steroidal and lactone is sub containing parts are tropins and cuscochgrine is a pyrrolidine alkaloid found in coca. It can also be extracted from plants of the family Solanaceae, including Atropa belladonna **[5].** Tropine contains hydroxyl groups at third carbon.

Some time as in the case of alkaloids we may try to predict the activity of some molecules on the base of their structure, even if we do not have direct data. As some substances can exhibit several pharmacological activities at the same time, and in the same series of compounds these can be substances endowed with different activities. We shall group the various substances according to their chemical structure.

The antibiotic activity of the roots as well as leaves has recently been shown experimentally. *Withania somnifera* in concentration of 10g/ml inhibited the growth of various Gram-positive bacteria, acid-fast and aerobic bacilli, and pathogenic fungi. It was active against *Micrococcus pyogenes var aureus* and partially inhibited the activity of *Bacillus subtilis* glucose-6-phosphatedehydrogenase **[6 & 7].**

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, *chamase*, in the berries may be responsible for the high content of the amino acid **[8 & 9].**

1.3 Economic Importance and Nutritional Composition

Aswagandha is one of the most important medicinal plants of India.

Table 2: Nutritional composition of mature Aswagandha

Nutritional value per 100 g	
Carbohydrates	62.78 g
Fat	1.49 g
Thiamine (vit. B1)	0.643 mg (56%)
Niacin (vit. B3)	2.965 mg (20%)
Vitamin B6	0.283 mg (22%)
Calcium	130 mg (13%)
Magnesium	183 mg (52%)
Phosphorus	367 mg (52%)
Sodium 17 mg	(1%)

The flavones (apigenin, luteolin, chrysoniol) occurred either as 7-0-glycoside or 7-0-apiglycosides while the flavonols (Querectien, isorhamnetin) are 3-0-monoglycoside or 3-7-diglycosides. β-glycosidase enzyme for degradation of flavonol-3-glycoside but each cell has not this enzyme so degradation of flavonoid is not so common. Based on the number of carbon atoms in their skeleton, phenolics are divided into several major groups (Hess, 1975), Phenol carboxylic acid Flavan derivatives. Hence, the present investigation was taken up for qualitative and quantitative analysis of in vitro produced flavonoids through TLC and HPTLC **[10].**

Withania somniferous is one of special versatile characteristics plants it is king of solnacae family.in research studies shows the such alkaloids parts of withania somnifera in roots ,but in this region plants all parts showed appriciable activity of alkaloids

These phytochemicals conclude that alkaloid, flavanoids, saponin, tannin, phenol content useful for work against many diseases by its own anti-inflammatory, anticancerous, antidiabetic, antibacterial activity etc.

METHODS AND MATERIAL

Accumulation of plants:

Fresh plant specimens of *withania somnifera* were collected from herbal garden of Arogyadham campus, Deendayal Research Institute Chitrakoot, Satna, MP, India and authenticated by taxonomist. A voucher specimen no. 104 is kept at herbarium of research lab in University campus, Chitrakoot for further reference. The All chemicals and reagents used including the solvents were of analytical grade.

Extraction

All the plant specimens were washed thoroughly with normal tap water followed by sterile distill water and air dried at room temperature for one week to get consistent weight. The dried samples were crushed to powder using grinder. Powder was stored in air tight container bottle. 10 gm of each sample were extracted with 100 ml of methanol and water for using soxlet extraction. The mixtures were evaporating under reduced pressure using rotary vacuum evaporator (Buchi type) and stored in refrigerator. The condensed extracts were used for

preliminary screening of phytochemical.

Physico-chemical analysis
Air dried plant material was used for the quantitative determination of loss on drying, total ash, acid insoluble ash, alcohol and water soluble extractive values, according to standard procedure of Indian Pharmacopoeia and WHO/QCMMPM **[11].**

Preliminary Phytochemical Screening
The phytochemical tests of the ethanol and water extracts of *Withania somnifera* were carried out to identify various phyto-constituents using with standard procedures for determining the presence and/or absence of phytochemicals**[12].**

Qualitative phytochemical screening
1. Alkaloid
Dragendorff's test: Take 2ml of alcoholic or aqueous extracts of sample in 5ml of distilled water in a test tube. Now add 2ml of 1N HCL until a complete acidic reaction occurs. Add few drops of dragendorff reagent. Appearance or formation of orange colour indicates the presence of alkaloid.

Wagner test: Take 1ml of alcoholic extract of sample in a test tube and add 3-4 drops of wagner reagent. Brown colour indicates the presence of alkaloid.

Mayer's test: Take 1ml of alcoholic extract of sample and add few drops of Mayer's reagent in a test tube. Pale yellow colour indicates the presence of alkaloid.

2. Carbohydrate
Anthrone test: Add 0.5ml of aqueous extract of drug in 2ml of Anthrone reagent. Green or blue colour indicates the presence of carbohydrate.

Fehling test: To 1ml aqueous extract of drug, add 1ml of each of equal part of Fehling solution A and Fehling solution B. Boil the content for 5 minutes. Formation of brick red colour indicates the presence of carbohydrate.

Molisch test: To 1ml of aqueous extract of drug, add 2-3 drops of alpha napthol after it add few drops of conc. sulphuric acid. Red violet colour ring appears which gradually disappear on addition of excess of alkali which shows the presence of carbohydrate.

3.Flavonoid: To 0.5ml of an alcoholic extract of drug, add 5-6 drops of dilute HCl and few pieces of Magnesium metal. Pink or brown colour indicates the presence of flavonoid.

Protein:
Bieuret test: To 1ml of alcoholic extract of drug, add 1.5% sodium hydroxide solution and add 1or 2 drops of 5% copper sulphate solution. Violet colour indicates the presence of protein.

Millons test: To 1ml of alcoholic extract of drug add 5-6 drops of millons reagent which result in formation of white precipitate which turns red on heating.

4.Resin:
Add 1ml of alcoholic or aqueous extract in 2ml of acetone and add 1ml of distilled water. Turbidity indicates the presence of resins.

5.Saponin:
Pour 1ml of alcoholic or aqueous extract of drug in 1ml of sodium bicarbonate. Honey comb like froth forms indicate the presence of saponin.

6.Test for Tannin:
a.Ferric chloride test-
The extract (50 mg) is a dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

b. Lead acetate test-
The extract (50 mg) is dissolved in distilled water then 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Quantitative Analysis
1. Quantification of Total Saponin
Determination of total saponin was done using anisaldehyde reagent. Sample solution was prepared in water. For total saponins estimation 500 µl of sample, 500 µl of 0.5% anisealdehyde reagent were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60°C. After 10 min tubes were cooled and absorbance was taken at 435 nm by UV spectrophotometer. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard sponging (100-1000 µg/ml) **[13].**

2. Quantification of total flavonoid content
The amount of total flavonoid content was determined with aluminium chloride ($AlCl_3$) according to a known method **[14]**. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by $NaNO_2$ (0.03 ml, 5%). After 5 min at 25°C, $AlCl_3$ (0.03 ml, 10%) was added. After a further 5 min, the reaction mixture was treated with 0.2 ml 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. All tests were performed six times. The flavonoid content was calculated from a quercetin standard curve.

3. Quantification of carbohydrate content
Quantification of carbohydrate content was carried out method **[15]** with slide modification. 100 mg of the 70% methanolic extract of root was weighed into a test tube, hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5N HCl and cooled to room temperature. The volume was made to 100 ml and centrifuged. 0.25 ml supernatant was made up to 0.5 ml with distilled water and mixed with anthrone reagent (4 ml) and was incubated at 95°C for 8 min. After incubation, cooled rapidly and absorbance was measured of green to dark green colour at 630 nm. All tests were performed six times. The carbohydrate content was evaluated from a glucose standard curve.

4. Quantification of alkaloid content
Quantification of alkaloid content was carried out method **[16]** with slide modification. To the 1 ml of extract (1 mg/ml) in water 0.1 ml of $FeCl_3$ (2.5 mM $FeCl_3$ in 0.5 M HCl) was added followed by addition of 0.1 ml 1,10 phenanthroline. After incubation for 30 min at 70°C the absorbance was taken at 500 nm. All tests were performed three times. The alkaloids content was evaluated from the bismuth nitrate standard graph.

5. Determination of total proteins
Extraction of Protein from sample extraction is carried out with buffers used for the enzyme assay. Accurately weighed 500 mg of the sample were ground well with a pistle and mortar in 5-10 ml of the buffer. Centrifuged (SORVALL RC 5B plus) 20 minutes at 10,000 per 25 rpm and collected supernatant used for protein estimation.

Standard Protein solution weighed accurately 50 mg of bovine serum albumin (fraction V) and dissolved in distilled water and the volume was made up to 50 ml in a standard. Diluted 10 ml of the stock solution to 50 ml with distill water in a standard flask. 1ml of this solution contains 200 µg protein **[17].**

Estimation of protein pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes. The volume was made to 1ml in all the test tubes. A tube with 1 ml of water serves as the blank. Added 5 ml of Alkaline copper solution to each tube including the blank. Mixed well and allowed standing for 10 minutes. Then added 0.5 ml of freshly prepared Folin-Ciocalteau reagent mixed well and incubated at room temperature, kept in the dark for 30 min. A blue color is developed. The absorbance was measured at 660 nm by UV spectrophotometer and calculated the amount protein in the sample. By plotting a standard graph and expressed the amount of protein in mg/gm of sample **[18-19].**

High performance thin layer chromatography (HPLC)
The methanolic extracts of the samples T1 (leaf) T2 (stem bark) and T3 (root) of *withania somnifera* were subjected to HPTLC fingerprint profile to check the presence of different phyto-constituents. HPTLC Fingerprinting was carried out on pre-coated silica gel HPTLC aluminium plates 60F254 (10 cm×10 cm, 0.2mm thickness, 5-6 µm particle size, E-Merck, Germany) by using CAMAG HPTLC System (Switzerland). 4 µL and 6 µL of each of the extract were spotted as

bands of 8 mm width by using a LINOMAT 5 Sample Applicator fitted with a 100 µL Hamilton Syringe. The plates were developed using *Tolune: ethyl acetate* (7:3) as a mobile phase in CAMAG twin-trough chamber lined with filter paper and pre-saturated with 10 ml mobile phase. The resulted plates were dried in air and photo documentation was done at ultraviolet light at 254nm, 366nm and day light using CAMAG REPROSTAR 3 equipped with WINCATS Software. Numbers of bands, color of separated compound and Rf values were recorded **[20].**

RESULTS AND DISSCUSION

In this present study we take all parts of *Withania somnifera* (L.) leaves, stem bark, seed and root of Linn were evaluated for its physicochemical aspects. Here we found that total ash, solubility parameter, alcohal soluble and acid insoluble. Physicochemical parameters results given in the fig.1 .showed water solubility is more than methanol solubilty, root represent less total ash value, where stem bark is less insoluble in acid pH is suitable as for pharmacopeia of India. It is important that plant should be properly identified and characterized for their physical and chemical characteristics. Therefore a control on their quality should be enforced. The aim of our study is to provide scientific evidence concerned to the medicinal values of these unexplored plants.

The results of preliminary phytochemical screening in the methanolic and water extracts of the drugs showed the presence of carbohydrates, flavonoids, alkaloids, resin, saponins and tannins, results were summarized in Table-1. The various phytochemical compounds detected are known to have importance in medicinal sciences. For instance, Alkaloids, saponin, protein and carbohydrates have been associated with different kinds of biologically active compound that have properties for centuries and no one side effect.

The quantitative analysis of phenolic contents, protein, saponin, carbohydrates, flavanoids and alkaloid were carried out using spectrophotometer and results are given in Table-2. The total phenol contents in the selected plant extracts is expressed in terms of gallic acid equivalent (the standard curve equation: Y=1.77186x+0.07036, r^2=0.99989). The concentration of total phenols was measured 14.022, 12.274, 13.538, 13.752, 4.50 ug/ml for leaf, stem bark, seed, flower and root respectively while fruit contain less phenol than other part of plant. The protein contents in the examined plant extracts is expressed in terms of BSA equivalent (the standard curve equation: Y=0.05346x + 0.1614, r^2=0.99843).The concentration of protein was measured 28.680, 18.36, 16.30, 19.00, 27.00 ug/ml for leaf, stem bark, flower and root, seed, flower respectively. The saponins contents is expressed in terms of saponins equivalent (the standard curve equation: Y=0.07150x+0.01277, r^2=0.97804). The concentration of saponins was found 25.60, 14.50, 24.23, 18.86, and 16.46 ug/ml for leaf, stem bark, seed flower and root respectively, where stem bark of *W. somnifera* has composes less saponin and leaf has much more. Similarly the alkaloid contents is expressed in terms of mg of Bismuth nitrate/g of extract equivalent (the standard curve equation: Y=0.99892x + 0.19872, r^2=0.99693).The concentration of alkaloids was found 14.864, 13.42, 3.89, 18.69, 17.35 ug/ml for leaf, stem bark, seed, flower and root respectively.

The carbohydrates contents is expressed in terms of D-glucose equivalent (the standard curve equation: Y=0.99892x + 0.019872, r^2=0.99693). The concentration of carbohydrates was found 8.703, 6.980, 5.342, 5.652, 8.965 ug/ml for leaf, stem bark, seed, flower and root respectively.

The flavanoids contents is expressed in terms of quercitine equivalent (the standard curve equation: Y=0.0.053462x +0.16149 r^2=0.98843). The concentration of flavanoids was found 16.343, 18.09, 17, 89, 12.74 and 12.910 ug/ml for leaf, stem bark, seed, flower and root respectively.

Standard graph using BSA for protein and bismuth nitrate for alkaloids, D-glucose for carbohydrates, using saponin standard for saponin, and finally for phenol using catechol, quercitine using for standard.

Qualitative HPTLC analysis was simply performed and Rf values were recorded. HPTLC Plate shows four bands at Rf 0.1 (pink), 0.25(yellow), 0.8(blue), 0.9 (red) for test sample T1; six bands at Rf, 00.2 (blue), 0.3 (blue), 0.45 (yellow), 0.55 (blue), 0.8(blue), 0.91(red) for test sample T2 at 366 nm., 0.12 (pink), 2.8 (red), 0.31 yellow), 0.6(blue), 0.71 9(pink), 0.79 (blue), 0.91 (red) observed for T3 six spots at 366nm 0.12 (pink), 2.8 (red), 0.31 yellow), 0.6(blue), 0.71 pink, 0.79 blue, 0.91 red & T4 only 2 spots 0.79 blue, 0.91 red were observed .For test sample T5 three spot at 0.6,(blue) 0.8,(blue), 0.9 (pink under 366nm. The results of Rf values and colors of the resolved bands were shown in Table -3 & Plate-1 and plate 2 (Fig- a, b & c). In present study we can be used as a reference standard for identification of veritable medicine or traditional drug with same ingredients. Further marker based identification and pharmacological studies enhance its therapeutic potential for making low side effect easily available drug.

CONCLUSION

The drug Pharmacological studies consist of the dried powder of *Withania somnifera* which is official as a sedative in the pharmacopoeia of India. The pharmacological activity of the roots, stem bark, leaves,fruit parts of the withania somnifera is attributed to the presence of several alkaloids, polyphenol, saponin, flavanoids, glycosides in appropriate quantity etc. Based on this present study it may be concluded that physicochemical parameter showed that purity of sample for drug strandrization. The phytochemical analysis results of this study of *Withania Somnifera* has associated with presence of alkaloids, saponin, tannin,flavanoids, glycisides, total phenol. *Withania somnifera* revealed the maximum amount of poyphenol in leaf part among all them, additionally maximum protein content on root and leaf part of the *withania somnifera*,saponin present in maximum amount in leaf and fruit, alkalois present in maximum amount leaf and fruit. Carbohydrates present in maximum amount in root and leaf. And flavanoids present in maximum amount in leaf and root.

HPTLC performed that different colure bands showed different rf value give identification of That active compound that could therefore serve as source of bioactive agents for production of new drugs.

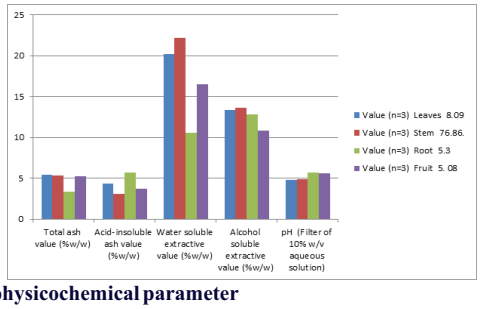

Fig.1 physicochemical parameter

Table-1. Phytochemical Analysis

Phyto-chemicals	Tests	Leaf		Bark		Seed		Flower		Root	
Carbohydrates		WE	ME	WE	ME	WE	ME	WE	ME	WE	ME
	Anthrone test	+	+	+	-	+	-	+	+	_	+
	Fehling test	+	-	-	-	+	-	-	+	_	-
Resin	Acetone	+	++	++	+	+	+	++	-	+	++
Saponins	Foam tests	+	+	+	+	++	+	+	+	+	++
Alkaloids	Dragendorff's test	++	++	-	-	+	-	-	+	++	++
	Wagner's	+	+		-	+	+		+	+	+
Steroids	Salkowski tests	+	-	-	-	+	-	-	+	+	-
Flavanoids	Magnesium test	+	+	+	+	+	+	+	+	+	+
Tannins	Shinoda test 5% FeCl3	+	+	+	+	+	+	+	_	+	++
Amino acids and Proteins	Biuratetest	+	+	+		+		+	+	+	+

ME= water extract, AE= alcohol soluble
[-]=absent, [+] =present, [++] =Appreciable amount
Fig.1. Stand

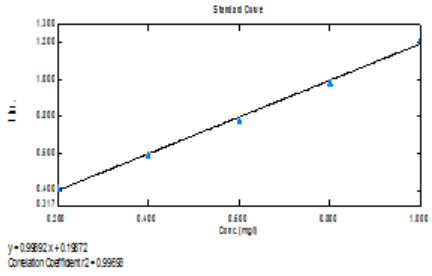

Fig.2. Standard curve of protien

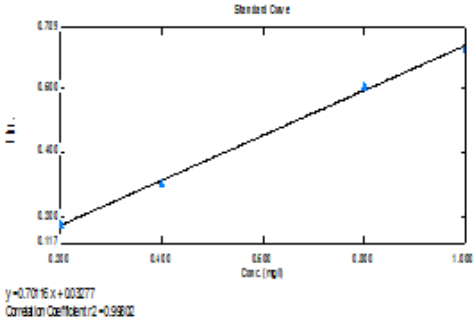

Fig.3. Standard curve of saponin

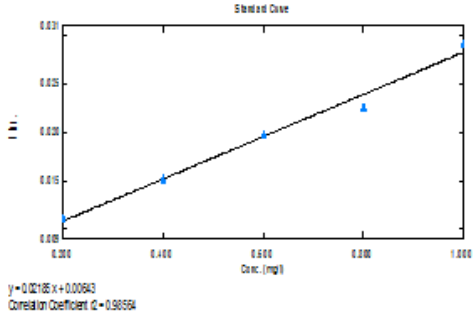

Fig. 4. Standard curve of alkaloids

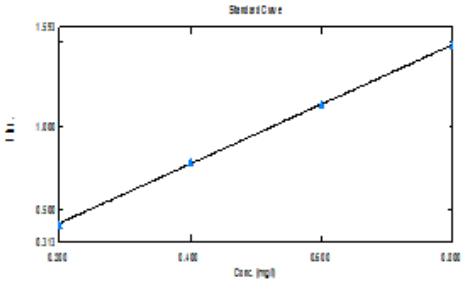

Fig. 5. Standard curve of polyphenol

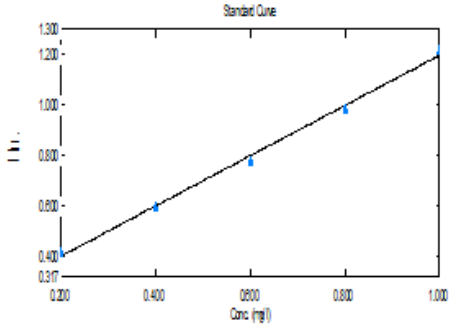

Fig. 6. Standard curve of carbohydrates

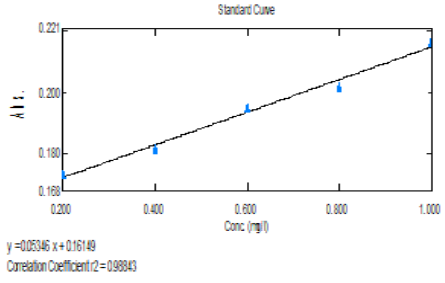

Fig. 7.Standard curve of flavanoids

Table-2.Quantitative estimation of Phytochemical Analysis

SN.	Phytochemical	Concentration in ug/ml					Unit
		leaf	Stem Bark	fruit	flower	Root	
1	Polyphenol	14.022	12.274	14.50	8.538	13.752	µg of GAE/ml of extract
2	Protein	28.68	18.36	16.30	19.20	27.30	µg of BSA/ml of extract
3	Saponin	25.60	14.50	24.23	18.86	16.46	µg of Saponins/ml of extract
4	Alkaloid	14.864	13.426	17035	13.02	3.89	µg of Bismuth nitrate/ml of extract
5.	Carbohydrates	8.703	6.980	5.342	5.654	8.965	µg of glucose nitrate/ml of extract
6.	Flavanoids	16.034	8.09	17.89	12.74	12.910	µg of quercitine /ml of extract

n=3 All study has been done triplicate

HPTLC FINGERPRINTS

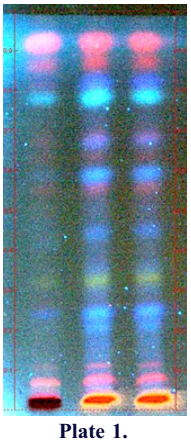

Plate 1.

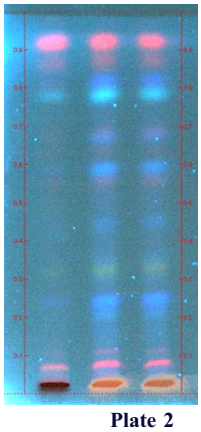

Plate 2

T1= ROOT, T2=LEAF, T3=STEM, T4=FLOWER, T5= FRUIT, T6=WHOLE PLANT

Table 3.Rf values color of the bands resolved in test solutions of withaniasomnifera

Mobile phase-Tolune: Ethyl Acetate (9:1) 366 nm					
T1	T2	T3	T4	T5	T6
0.1 (pink),.25(yellow),0. 8(blue),0. 9 (red)	0.2 (blue), 0.3 (blue), 0.45 (yellow), 0.55 (blue), 0.8(blue), 0.91(red)	0.12 (pink), 2.8 (red),0.31 yellow),0. 6(blue), 0.71 pink, 0.79 blue, 0.91 red	0.79 blue 0.91 red	0.14 pink 0.3 yellow 0.80blue 0.91 pink	0.6,(blue) 0.8,(blue) 0.9 (pink)

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