Review of Literature

2.0 REVIEW OF LITERATURE

Withania somnifera is one among the top ranking medicinal plants of India and is highly valued for its medicinal and neutraceutical properties. It holds a position of importance similar to ginseng in China. *Withania somnifera*, also known as ashwagandha, Indian ginseng and winter cherry, has been an important herb in the ayurvedic and indigenous medical systems for more than 3,000 years. It is a reputed small or middle sized medicinal shrub distributed throughout the drier parts of India grows up to 1.5m high with ovate leaves and greenish yellow flowers.

In vitro and *in vivo* molecular pharmacological investigations have elucidated association of pharmacological activities of the herb, *Withania somnifera* with its specific secondary metabolites known as withanolides, a class of phytosteroids named after *Withania somnifera (*Kaileh *et al., 2007*). Although the leaves and fruit of *Withania somnifera* are therapeutic, most of the herbal medicine available is derived from the roots of this shrub (luvone *et al.*, 2003).

Presently, withanolides have been commercially obtained by solvent extraction of roots of the plant. Kulkarni and Dhir (2008) indicated that the drug prepared from *Withania somnifera* is very effective without having any serious toxicity or side effects known till date and thus can be safely used in humans for acute and chronic treatment regime

The literature relevant to the **"Extraction and purification of withanolides from dry roots of *Withania somnifera*"** is reviewed in this chapter.

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2.1 Growth characteristics of *Withania somnifera*

Withania somnifera is a small or middle sized under shrub grows throughout the drier parts of sub-tropical India, typically north-western India. It is a weed of the drier warm countries. The morphological and physiological variations based on extremely diversified geographical distribution of *Withania somnifera* were identified (Atal and Schwarting, 1962).

Sundari *et al., (*1999) analyzed the morphological parameters of *Withania somnifera* and found that it grows as an erect, branched, hairy-tomentose perennial sub shrub to a height 120 (60-150) cm with densely velvety stems and leaves.

Flowers are perfect, actinomorphic with erect stigma. Fruits when ripe are red or yellow-coloured berries with numerous seeds. Flowers are small, lurid-yellow, odourless and visited by both oligolectic bees and anthophagous beetles.

Withania somnifera is well known for its fruiticose habit, urceolate calyx with long setiform teeth, enlarging with the growth of the fruit into campanular shape, with a wide, open mouth, and becoming coriaceous in substance, not resolving itself into a globular bladder-like form of thin reticulated texture, and concealing the berry.

The fruits of *Withania somnifera* are tiny orange berries and reported to contain saturated and unsaturated fatty acids. The fruit of this plant has been also used in folk medicine as febrifuge, diuretic, and anti rheumatic under the name "Morgan" in Egypt.

2.2 Active principles in *Withania somnifera*

The roots of *Withania somnifera* contain several alkaloids, withanolides, a few flavanoids and reducing sugars (Ganzera *et al.,* 2003). Baldi *et al.,* (2008) indicated that at present 12 alkaloids, 35 withanolides, and several sitoindosides from *Withania somnifera* have been isolated and studied.

2.2.1 Alkaloids

Many biochemically heterogenous alkaloids have been reported in this plant, especially in its roots. The various alkaloids have been reported in *Withania somnifera* include withanine, somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudotropine, 3-α-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine and anahydrine (Tyler *et al.,* 1981).

Alkaloids stimulate the generation of cytotoxic T lymphocyte thus reduces tumor growth and enhances the proliferation of lymphocytes, bone marrow cells and thymocytes. It enhances the phagocytic activity of peritoneal macrophages. It enhances cytokine production and stem cell proliferation and its differentiation.

Studies found that alkaloids from ashwagandha. caused relaxant and antispasmodic effects during induced muscle contraction. This data supports the use of Ashwagandha as a relaxant due to its alkaloid content.

2.2.2 Withanolides

The withanolides are a group of naturally occurring oxygenated ergostane – type steroids generally having a δ lactone in the side chain and a 2-en-1-one system in ring A. *Withania somnifera* contains many structurally diverse withanolides in its leaves as well as in roots. More than 35 withanolides have been isolated and characterized from its roots and leaves. Much of Withania's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D. Thus the principal withanolides in *Withania somnifera* are withaferin A and withanolide D (Kirson *et al.,* 1971).

Genuine interest arose in withanolides of *Withania somnifera* when it was found that they show anti-tumour activity in a number of animal studies. In addition, cytotoxicity, immunosuppressive, antimicrobial, hepatoprotective, insect antifeedant and anti-inflammatory properties were observed (Bessalle and Lavie, 1992).

Withanolides significantly up-regulate the activity of T-helper cell. It inhibits the growth of human tumor cell line. It improves bone calcification and act as potential agent in the treatment of osteoporosis. It increases the levels of corticosterone in the adrenal glands of stress.

Withanolides were first isolated from *Withania somnifera* from which they derived their name. The withanolides have C28 steroidal nucleus with C9 side chain, having six membered lactone ring similar to that of ginsenosides in *Panax ginseng*. Withanolides are structurally distinct from tropane/nortropane alkaloids.To date, a few other withanolides have been isolated from *Ajuga parviflora* and *Tacca chantrieri* which belonging to the families Labiatae and Taccaceae (Su *et al.*, 2004).

Two glycosides, sitoindoside VII and sitoindoside VIII isolated from the roots of *Withania somnifera* showed significant anti-stress activity when tested in diverse spectrum of stress-induced paradigms (Bhattacharya *et al.*, 1987).Ghosal *et al.*, (1988) reported the occurrence of O-glycosylated withanolides, named sitoindosides in extracts from the roots of several cultivated varieties of *Withania somnifera*.

Sitoindoside IX and sitoindoside X in a dose range of 50–200 mg/kg produced significant antistress activity in mice and rats and augmented learning acquisition and memory retention in both young and old rats (Ghosal *et al.*, 1989).

Sitoindosides from *Withania somnifera* were shown to reverse cognitive deficits induced by ibotenic acid, concomitant with attenuation of cholinergic deficits induced by these agents (Bhattacharya *et al.*, 1995).

Sitoindosides VII–X and withaferin-A isolated from aqueous methanol extract from the roots of cultivated varieties of *Withania somnifera* is used in Indian medicine to attenuate cerebral functional deficits including amnesia in geriatric patients (Schliebs *et al.*, 1997).The

glycosylated withanolides present in medicinal plants of *Withania somnifera* are reported to have antioxidant and immunomodulatory activities (Matsuda *et al.*, 2001).

Investigation by Tohda *et al.*, (2005) indicated that withanolide A, withanoside IV and withanoside VI facilitated the reconstruction of both post-synaptic and pre-synaptic neurons. Studies on the administration of withanosides extracted from *Withania somnifera* roots significantly improved memory deficits in β amyloid-injected mice and prevented loss of axons, dendrites, and synapses in the cerebral cortex and hippocampus (Kuboyama *et al.*, 2006).

2.3 Pharmacological activities of withanolides from *Withania somnifera* roots

Withaferin A, chemically characterized as 4β, 27-dihydroxy-5β-6β-epoxy-1-oxowith-2, 24-dienolide is one of the main withanolidal active principles isolated from *Withania somnifera.* Withaferin A and 3β–hydroxy-2, 3-dihydro withanolide F show promising antibacterial, antitumour, immunomodulating and anti-inflammatory properties (Budhiraja and Sudhir, 1987).

Of the various withanolides, withaferin A and its 5-hydroxy-6-chloroderivatives have been reported to exhibit marked cytostatic activity against cells derived from human carcinoma, experimental mouse tumours and HeLa 229 cells *in vitro* (Sharada *et al.*, 1993).

Withania somnifera possesses therapeutic value against a large number of ailments and it is an ingredient of many formulations prescribed for a variety of musculoskeletal conditions, and as a general health tonic for elderly persons and lactating mothers (Bone, 1996).To authenticate its use as a multipurpose medicinal plant, a battery of pharmacological investigations has been reported.

Withaferin A inhibits cyclooxygenase 2 (Sethi *et al.*, 1970) but not cyclooxygenase1 desired for a non-ulcerating anti-inflammatory/chemotherapeutic drug. Withaferin A has also been reported to have immunosuppressive action on β-lymphocyte proliferation (Jayaprakasam and Nair, 2003).

Major withanolides, like withaferin A and withanolide A of the plant have been demonstrated to possess significant and specific therapeutic action in carcinogenesis, Parkinson's disease and Alzheimer's disease (Choudhary *et al.*, 2005).

2.4 Biosynthesis of Withanolides in *Withania somnifera*

To date, there has been little biosynthetic or metabolism-related research on withanolides. It is thought that withanolides are synthesized in leaves and transported to roots like the tropane alkaloids, a group of bioactive secondary metabolites in Solanaceae members known to be synthesized in roots and transported to leaves for storage. Sangwan *et al.*, (2008) confirmed that the withanolides are synthesized in different parts of the plant (through operation of the complete metabolic pathway) rather than being imported.

Administration of 24-methylene-cholesterol-[28-^{3}H] to *Withania somnifera,* yielded [^{3}H] radioactivity in the isolated withaferin A and withanolide D indicating that 24-methylene-cholesterol as the sterol precursor of the withanolides.

2.5 Extraction and Purification of withanolides from *Withania somnifera* roots

There occurs a need for isolation of withanolides using chromatographic techniques such as silica gel column, preparative TLC, centrifugal adsorption chromatography and preparative HPLC analysis in order to study the effect of each withanolides in bioassay studies.

The extracts from the root of medicinal plant *Withania somnifera* have been used in particular for the treatment of disorders of the uterus, menstrual cycle and arthristis. In recent years, herbal formulation containing substantial amounts of *Withania somnifera* root extract have been employed in small clinical trials and shown to have efficacy for the treatment of osteoarthritis.

Extensive studies have also been carried out in order to study the structure of withanolides that has contributed to the efficient pharmacological properties in traditional medicines as well as in the ayurvedic drugs.

Abraham *et al.*, (1975) isolated various withanolides from two different chemotypes namely I and II on subjecting their ethereal extracts upon silica gel column chromatography. On elution with $CHCl_3$: Ethyl acetate =1:1 various withanolides namely 27-deoxy withaferin A, withanolide P, withanolide O, 27-deoxy 14α-hydroxyl withaferin A and withaferin A were obtained in the fractions of chemotype I whereas from chemotype II Withanolide G and withanolide D were obtained.

Velde and Lavie (1981) subjected the methanol extracts of chemotype III on silica gel column and identified three new withanolides along with withanolide E. The structures of the three new withanolides was determined from ^1H NMR and ^{13}C NMR and it was found to be 1α, 3β, $20_{\alpha F}$-trihydroxy-20R 22R-witha-5, 24-dienolide, 1-oxo-14α, 20αF, 27-trihydroxy-20R, 22R-witha-3, 5, 24-trienolide and 5α-ethoxy-1-oxo-6β, 14α, 17β, 20-tetrahydroxy-20S, 22R-witha-2,24-ienolide.Withanolide E obtained from the fractions has been used for the biological testing as this compound exhibits antineoplastic activity and has immunosuppressive properties.

Dried leaves from chemotype III of *Withania somnifera* was defatted using n-hexane followed by extraction with 50% methanol. $CHCl_3$ fractionation of 50% methanol extract obtained was subjected to silica gel column chromatography and elution was carried out with various gradients of hexane and ethyl acetate mixtures while isolated a chlorinated withanolide and was found to be 6α-chloro-5β-hydroxy withanolide D by ^1H NMR analysis (Nittala *et al.*, 1981).

Velde and Lavie (1982) adsorbed the residue obtained from the dried leaves from chemotype III of *Withania somnifera* after the usual extraction procedure as that followed by Nittala *et al.* (1981) on Kieselgel 60 column. And the column on elution with ethyl acetate yielded withanolide E. Withanolide E on reduction resulted in increased concentration of Δ^{16}-withanolide indicating that the compound acts as a direct precursor of withanolide E. Δ^{16}-withanolide was identified as (20R, 22R)-14α, 20αF -dihydroxy -1-oxowitha -2,5,16,24-tetraenolide by ^1H NMR and ^{13}C NMR.

Bessalle and Lavie (1987) extracted *Withania somnifera* with methanol as described earlier by Nittala *et al.* (1981). The crude residue in methanol: water=1:1 after defatting using n-hexane was fractionated with chloroform. Fifteen withanolides were isolated from

chloroform fraction of 50% methanol extract when subjected to silica gel column chromatography and found that different combinations of substituents of withanolides which themselves appear in different quantities. During the isolation process of several withanolides, low concentration of a new withanolide Y was identified and the structure of it was elucidated as (20R, 22R)-5α, 6α-epoxy-7α, 17α, 20-trihydroxy-1-oxo witha-2, 24-dienolide by X-ray single crystal analysis.

Rahman *et al.*, (1991) isolated 5-dehydroxy withanolide R and withasomniferin A using methanolic extract from air dried whole plant of *Withania somnifera* after defatting wth n-hexane was dissolved in water. The aqueous extract obtained was then fractionated with chloroform at pH 7.0.The chloroform fraction obtained at pH 7.0 was then subjected to silica gel column followed by elution first with hexane and then with hexane-chloroform mixtures. Two new withanolides isolated were identified as 5-dehydroxywithanolide-R(23β-hydroxy-6α,7α-epoxy,1-oxo-22R-witha-2,24-dienolide and 17-hydroxy-6α,7α-epoxy,1-oxo-22R-witha-4,24-dienolide respectively.

Rahman *et al., (1*993) used the defatted plant material of *Withania somnifera* for extracting withanolides using the methanol as solvent. The residue of methanol extract was then redissolved in water and fractionated with chloroform at pH 7.0.The chloroform fraction thus obtained was then subjected to the silica gel column chromatography and eluted with petroleum ether and petroleum ether–chloroform mixtures. The fraction collected on elution with chloroform: petroleum ether (40:60) was purified by preparative TLC to afford withasomidienone. The isolated withasomidienone was then identified as 27-hydroxy-3-oxo-(22R)-with a-1,4,24-tienolide on the basis of spectroscopic studies namely ^{1}H NMR and ^{13}C NMR.

Jamal *et al.* (1995) obtained the concentrated methanolic extract from *Withania somnifera* as gum. And the gum was dissolved in methanol and defatted with petroleum ether (40-60°C). The defatted methanolic extract was again evaporated and the residue was dissolved in water. The aqueous extract was extracted with $CHCl_3$ at different pH values. The fraction obtained at pH 7 was loaded on a silica gel column and eluted first with hexane and then with hexane-chloroform, chloroform and chloroform-methanol mixtures. The fractions collected on elution with hexane-chloroform (3:7) were subjected to preparative

TLC (precoated silica gel, 0.25 mm) in methanol-chloroform (2.5:7.5) to afford Withalactone and Withaoxylactone .The chloroform fraction was loaded on a 3.5 cm diameter column packed with silica. The fraction obtained with methanol-chloroform (4: 96) system) was again subjected to a 14.5 cm long and 1.5 cm in diameter flash column chromatography, which resulted in the isolation of the compounds Quresimine-A and Quresimine-B by the preparative TLC in acetone-hexane system (30:70) and (35:65) respectively.

The dried stem bark of *Withania somnifera* was extracted exhaustively in a soxhlet apparatus with 95% ethanol. The extract was concentrated in vacuo to yield a thick, viscous dark greenish mass. The residue obtained was then subjected to petroleum ether packed silica gel column chromatography followed by successive elution with petrol, chloroform and methanol in the order of increasing polarity which resulted in isolation of five new withanolides namely withasomnilide, withasomniferanlide, somniferanolide, somniferawithanolide and somniwithanolide elucidated structurally by ^{1}H NMR and ^{13}C NMR as (20R, 22R)-1-oxo-5α,8β-dihydroxywitha-6α,7β-epoxide-2,24-dienolide,(20R,22R)-1-oxo-8β,11β,16β-trihydroxy-witha-2,5,24-trienolide,(20R,22R)-1-oxo-8β,18,20β-trihydroxywitha-2,5,24-trienolide and (20R,22R) -1-oxo-7β,1820β,27-tetrahydroxywitha-2,4,24-trienolide (Ali *et al.*,1997).

Furmanowa *et al.*, (2001) isolated withaferin A from dried leaves of *Withania somnifera* extracted with ethanol at room temperature, stirred for 3 hours and then left overnight. The coloured extract was decanted from plant residue, and leaves were extracted with fresh ethanol two more times following the same procedure. The combined alcoholic extracts were then filtered and concentrated in vacuo to one third of its volume. Chlorophyll was then filtered off, an equal volume of water was added and the mixtures was extracted subsequently with hexane (four times) and ethylene dichloride (three times) and finally with chloroform/ethanol 7:3 (three times).Crude ethylene dichloride fraction (2 g) was subjected to column chromatography on silica gel (200 mesh) with gradient elution by means of methylene chloride-ethanol mixtures of increasing polarity and fractions of 50 cm^3 were collected. Homogenity of withaferin A in the fractions collected were confirmed by HPLC analysis.

Extracts from *Withania somnifera* are also known to have significantly inhibited tumour growth *in vivo* (Mohan *et al.*, 2004). Misra *et al.*, (2005) defatted the shade dried leaves of *Withania somnifera* three times with n- hexane by keeping at room temperature. The spent material was further extracted with methanol at room temperature overnight. The methanol extract was chromatographed over a column of silica gel with n-hexane as mobile phase and then elution was carried out in n-hexane and ethylacetate with solvent gradient.The polarity was increased by sequentially adding 5%, 10%, 25%, 50%, 75% ethylacetate,pure ethylacetate and finally 5%,10%,15% and 20% methanol was added in the ethylacetate. The fractions of column chromatography were collected and pooled into nine major fractions based on their TLC pattern.

Fraction 1 yielded oleic, linoleic and palmitic acids while fraction 2 and 3 were discarded as they contained chlorophyll and other pigments Fraction 4 yielded compound 5β,6β-epoxy-4β-hydroxy-1-oxo-witha-2,16,24-trienolide (R_f =0.72) in the solvent system $CHCl_3$:EtoAC:MeoH:C_6H_6=70:2:4:24] 24,25-dihydrowithanolide A and withanolide A. Fraction 5 on further column chromatography and crystallization yielded mainly withanone and withaferin A. Fraction 6 after further column chromatography gave withanone, withaferin A, 27-hydroxy withanone and 17-hydroxy withaferin A. Fraction 7 after column chromatography afforded 6α,7α-epoxy-3β,5α,17α-trihydroxy-1-oxo-witha-24-enolide (R_f=0.38,$CHCl_3$:EtoAC:MeoH:C_6H_6=70:2:4:24) while Fraction 8 after further column chromatography yielded sucrose and 5α,17α-dihydroxy -6α,7α-epoxy-1-oxo-3β-o-sulfate-witha-24-enolide(R_f=0.25,$CHCl_3$:EtoAC:MeoH:C_6H_6=70:2:8:20).Fraction 9 after acetylation and column chromatogaraphy yielded 27-acetoxy-3oxo-witha-1,4,24-trienolide(R_f=0.30,$CHCl_3$:EtoAC:MeoH:C_6H_6=70:2:4:24) glucose penta acetate and sucrose octa acetate. The structures of all these compounds were elucidated by spectroscopic methods and chemical transformations namely acetylation and solvolysis.

Abou-Douh (2002) isolated two new withanolides from the fruits of *Withania somnifera.* Air-dried and powdered fruit of *Withania somnifera* was defatted with petroleum ether (60–80°C) at room temperature for 48 hours. The defatted fruit was further extracted with acetone at room temperature for 48 hour. Acetone extract was subjected to silica gel column chromatography and the successive elution carried with *n*-hexane-acetone (10:1, 4:1, 2:1, 1:1) acetone and methanol afforded twelve fractions. Each fraction was further separated

by Sephadex LH-20 followed by preparative TLC [solvent: *n*-hexane-acetone (4:1) Benzene-Ethyl acetate (4:1, 7: 3) Benzene-acetone (4:1), Chloroform-acetone (9:1)] to give two new withanolides identified as 4-Deoxywithaperuvin; 5β, 6α, 14α, 17β, 20β-pentahydroxy-1-oxo-20S, 22R-witha-2,24-dienolide and 14α,17α-Dihydroxywithanolide R;6 α,7α -epoxy-5α,14α,17α,23β -tetrahydroxy-1-oxo-22R-witha-2,24-dienolide respectively.

Mulabagal *et al.*, (2009) used concentrated methanolic extract of *Withania somnifera* roots and is was then further stirred with ethyl acetate and this ethyl acetate extract obtained was subjected to column chromatography silica gel 100–200 mesh size using $CHCl_3$ and MeOH mixtures as eluants resulted in purification of withanolide sulfoxide.

Gupta *et al.*, (2008) isolated (20R, 22R)-6a, 7a-Epoxy-5a, 27-dihydroxy-1-oxowitha-2, 24- dienolide from the leaves of *Withania somnifera* by extraction with 95% ethanol while refluxing. The concentrated alcoholic extract was redissolved in water. Resulting suspension was extracted sequentially with chloroform, EtOAc and n-BuOH. The chloroform extract was subjected to silica gel column chromatography (60–120 mesh) and the elution was carried in increasing polarity with $CHCl_3$, 2% MeOH in $CHCl_3$, 5% MeOH in $CHCl_3$ and MeOH. Fractions got eluted in 5% MeOH in $CHCl_3$ were pooled,concentrated, the residue after crystallization from MeOH yielded 27-hydroxywithanolide B I.

Matsuda *et al.*, (2001) isolated seven new withanolide glycosides termed as withanosides I, II, III, IV, V, VI, and VII from the methanol extracts of Indian *Withania somnifera* roots. The residue obtained was subjected to Diaion HP-20 column chromatography, Water→MeOH→$CHCl_3$ to give the H_2O eluate, methanol eluate and $CHCl_3$ eluate. Normal-phase silica gel column chromatography $CHCl_3$–MeOH–H_2O of the methanol eluate provided four fractions. Fraction 1 was purified by reversed-phase silica gel column chromatography, Methanol–H_2O (50:50→70:30→80: 20 v/v) →MeOH] and HPLC analysis revealed the presence of withaferin A and 5α, $20\alpha_F$(R)-dihydroxy-6α, 7α -epoxy-1-oxowitha-2, 24-dienolide. Fraction 2 was separated by reversed phase silica gel column chromatography Methanol–H_2O (50:50→60:40→70:30, v/v) MeOH] and HPLC analysis to furnish withanosides I and III, physagulin D and coagulin Q. Fraction 3 was subjected to reversed-phase silica gel column chromatography MeOH–H2O (50:50→60:40→70:30, v/v)

→MeOH] and HPLC to give withanosides II IV, V, VI, VII respectively. The structure of withanosides I, III, IV, V, VI and VII were identified by spectroscopic analysis.

Zhao *et al.,* (2002) fractionated the methanolic extract from crushed roots of *Withania somnifera* with $CHCl_3$ and *n*-butanol successively to give $CHCl_3$ soluble and *n*-butanol soluble fractions. The *n*-butanol soluble fraction was chromatographed on a Diaion HP-20 column eluting with H_2O, MeOH–H_2O (3: 7 and 3: 2) and MeOH to furnish subfractions VI—IX. Sub fractions VII and VIII were further subjected to repeated chromatography on silica gel, Sephadex LH-20 and ODS. Compounds 27-*O*- β -D-glucopyranosyl pubesenolide 3-*O*- β-D- glucopyranosyl (1→6)- β -D-glucopyranoside (withanoside III),27-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosylpubesenolide3-*O*-β-D-glucopyranosyl(1→6)-β–glucopyranoside(withanosideIX),27-*O*-β-D-glucopyranosylpubesenolide 3-*O*- β –D glucopyranoside(withanoside X) and (20*R*,22*R*)-1 α,3 β,20,27-tetrahydroxywitha-5,24-dienolide 3-*O*- β -D-glucopyranoside (withanoside XI). RP-18 HPLC separation of subfraction VIII yielded compounds (20*S*, 22R)-4β, 5β,6α, 27-tetrahydroxy-1-oxowitha-2, 24-dienolide, withanoside IV, withanoside VI and coagulin Q.

Khajuria *et al.,* (2004) quantified the withanolides that were in trace quantities by LC-ES-MS analysis using reference compounds isolated from *Withania somnifera* roots. For qualitative and quatitative analysis of trace withanolides, liquid chromatographic separations were achieved using RP-18, Merck column (4-6* 250mm, 5μm).The mobile phase consisted of CH_3OH: water (60:40) delivered at a flow rate of 0.5ml/min.The samples were analyzed at 30°C to provide efficiency to the peaks. The UV chromatograms were recorded at 237nm.

2.6 Thin layer chromatographic analysis of Withanolides

Bhattacharya *et al.*, (1987) obtained the residue from aqueous: methanol (1: 1) extract of the roots of one-year old cultivated *Withania somnifera* and subjected it to Sephadex LH-20 filtration and the organic fraction was partitioned between diethyl ether: water (2: 1).The ether extract obtained was separated into neutral, phenolic and carboxylic fractions. The neutral fraction of ether extract, a hygroscopic solid on preparative thin layer chromatography (silica gel PF_{254} Merck) using hexane-ethyl acetate (1:2) as developer afforded two acylsterylglucosides namely Sitoindoside VII and Sitoindoside VIII.

Roja *et al.*, (1991) extracted 2.0g of dry powdered tissue from the plant *Withania somnifera* with methanol overnight at room temperature. The concentrated extract was then diluted with distilled water and further fractionated using chloroform. The solution was extracted first with petroleum ether followed by chloroform. The chloroform fraction was then analyzed by thin layer chromatography (TLC) on silica gel G plates using an Ethyl acetate: benzene: ethanol (90: 10: 1.5) solvent system and the withanolides were detected by spraying with anisaldehyde reagent and heating the plates at 100 °C for 5 minutes.

The concentrated crude methanol extracts from (Ray and Jha, 2001) the shoot cultures of *Withania somnifera* were spotted with reference compounds on Merck silica gel TLC plates and were developed in the solvent system $CHCl_3$: Ethyl acetate: CH_3OH: C_6H_6 (72:4:8:16). Liebermann-Burchard reagent (acetic anhydride: concentrated sulfuric acid: absolute alcohol, 5:5: 50) was used as the spraying agent and the major spots visualized were compared with standard samples of withaferin A (R_f= 0.34) and withanolide D (R_f= 0.51).

Ciddi (2006) analyzed the presence of withaferin A in the methanolic extract by using pre-coated silica gel plates of 250 μm layer, UV 254 by co-chromatography with authentic sample of withaferin A. The plates were developed in a solvent system of chloroform: methanol (95:5) and detected using a vanillin-sulfuric acid reagent