SRI LANKAN MEDICINAL PLANT

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VOL - 4

WITHANIA SOMNIFERA

LAKSHMI ARAMBEWELA & RUVINA SILVA

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PREFACE

The medicinal plants of Sri Lanka have been investigated in the Natural Products Development Group for nearly two decades. This monograph which is the fourth in this series is the result of the literature surveys, researches and experiences of the Natural Products Development Group of Industrial Technology Institute (Ceylon Institute of Scientific & Industrial Research). This is intended for varied reading public the herbal drug manufactures who need to identify and standardise their herbal raw materials, the Ayurvedic physicians who need some scientific information on medicinal plants, the research workers requiring a quick background on a plant, the industrialist or entrepreneur pondering on commercial ventures and the inquiring lay readers. We hope this monograph fulfils some requirements of each of these.

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WITHANIA SOMNIFERA

Synonyms - *Physalis somnifera*
Physalis flexuosa
Physalis arborescens
Physalis somentosa[1]

Sinhalese name - Amukkara, Asgondu, Ashwaganda

Other names - Tamil – Amukkiray, Asubam, Asuvagandi, Achmaganda,
Hindi – Asgand, Punir, Kushthagandha, Kushthagandhini, Palashaparni, Priyakari, Punya,
Sanskrit – Ashvagandha, Gandhapatri, Ashvakandika, Baluda, Balaja, Haya, Hayapriya, Kala, Kambuka, Kanrupini, Pushtida, Pushtipavira, Shyamala, Turagagandha, Turagi, Vajigandha, Vajikari, Vajini, Varada, Varagatrakari, Varahakarni, Varahapatri, Vataghni,
Bengal – Ashvaganda,
Teligu – Pulivendram,
Marati – Askandhatilli,
Panneru – Gadda,
Gujarati – Ghodakun, Ghoda, Asoda, Asan,
Kannada – Viremaddlinagadde, Pannaeru, Aswagandhi, Kiremallinagida,
Punjab – Asgand, Isgand,
Bombay – Asgund, Asvagandha,
Rajasthan – Chirpotan[1].

Family - Solanaceae

Parts Used - Leaves and Roots.

Pharmacopoeia - Ayurveda Pharmacopoeia[2]
Sinhalese Materia Medica[3]
Materia Medica of Ayurveda[4]
The Indian Pharmaceutical Codex[5]
Indian Materia Medica[6]

Official drugs - Root Powder, Ghee, Wines, Paste, Medicated oil[2]

Uses in Traditional Medicine - Dried entire plant is used in rheumatism, tuberculosis, tumors, inflammations, bronchitis, ulcers, scabies, dyspepsia, ophthalmia, syphilis and as a diuretic in Sri Lanka[7].

In India *Withania* is used to improve male sexual function and as an emmenagogue. Leaves are used for tumors, dried leaves and roots are used as narcotic, diuretic deobstruent, and to fester boils. Root is used as an abortifacient, aphrodisiac, dried root is used as a nervine tonic and certain forms of hypertension[8-13].

The dried entire plant is used to treat snakebite in Saudi Arabia [14].

In Tanzania fresh entire plant is used in traditional medicine[15].

In Nigeria *W. somnifera* dried leaves and dried roots are used to teat arthritis [16,17].

In South Africa root is used to induce abortion, alleviate labour pains and as a sexual stimulant in Africa. Root is used to remove retained conception products by "Transvaal sotho" [18].

In Nepal root is used as an abortifacient, narcotic and aphrodisiac [19,20].

In Pakistan the roots are used to cause abortion, tone up uterus in habitual abortion cases, remove retained contraception products, as an aphrodisiac, a tonic and alterative [21].

In Tanzania root of *W. somnifera* is used as an abortifacient and sexual stimulant [19].

W. somnifera dried root is used as a narcotic, diuretic, deobstruent, nervine tonic, sedative for insanity, sedative for epilepsy and for certain forms of hypertension[11,13].

W. somnifera plant is used as a central nervous system depressant in Egypt[22].

In Rwanda dried root of *W. somnifera* is used for gonorrhea[23].

In Ethiopia *W. somnifera* dried stem is used for rheumatism and dried wood is used as a diuretic, diaphoretic and purgative[24,25].

Distribution - Occurs in Sri Lanka, India, Mediteranean region, Cape of Good Hope, South Africa and Middle East. It grows well in sunny dry places[1].

Morphology - Erect hoary perennial or semiwoody shrub 1m high. Stems terete, branched, covered with a fine mealy stellate pubescence. Leaves ovate, 2-11 cm long, 1.5-9 cm wide, subacute, unequal at base, entire, finely stellate-pubescent especially beneath; petiole 3-17 mm long, channelled above. Flowers fascicled, 3-6 together, pedicels 2-4 mm long. Calyx in flower 2 mm long, rapidly enlarging after flowering to 1-2 cm long, ovoid, scarious when mature with linear teeth at apex. Corolla greenish, 10 mm long, clearly 5-lobed, lobes lanceolate, erect but recurved in upper part, pubescent outside. Ovary glabrous; berry globose, 6 mm diameter., bright red. Seeds 2.5 mm diameter., compressed[26].

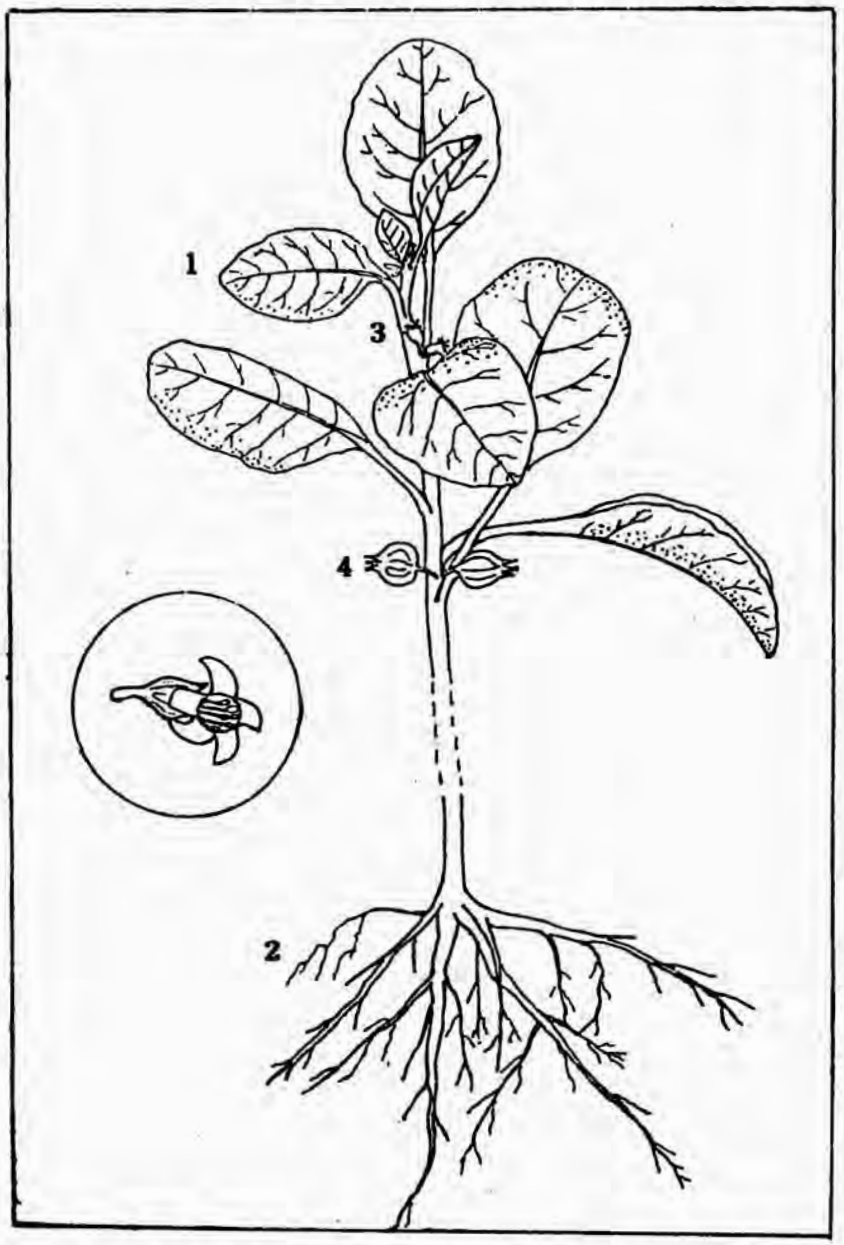


Fig 1 : 1. Leaf 2. Root 3. Flower 4. Pod

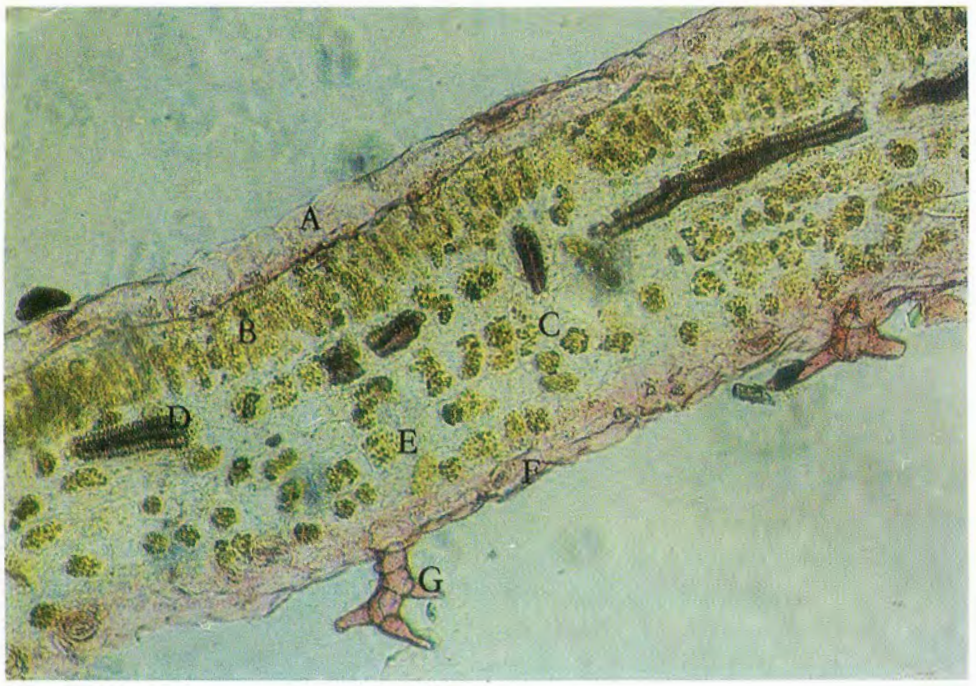


Fig 2 : Cross section of *Withania somnifera* leaf

A. Upper epidermis B. Palisade parenchyma cells C. Chloroplast
D. Vessel E. Spongy parenchyma cells F. Lower epidermis G. Dendroid hair

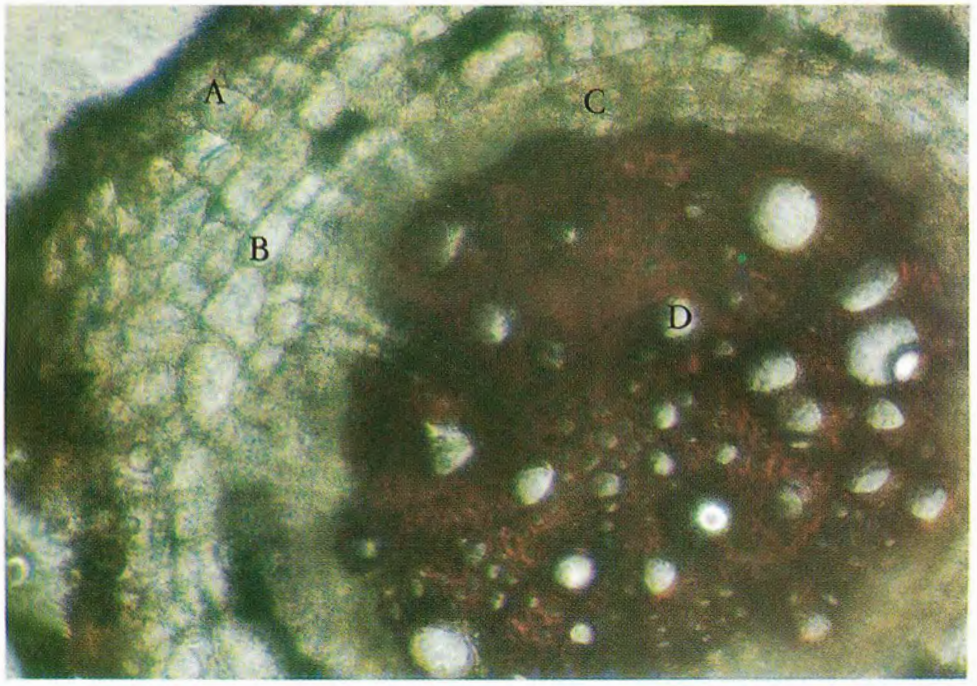


Fig 3 : Cross section of *Withania somnifera* root

A. Cork cells B. Cortex C. Phloem D. Xylem

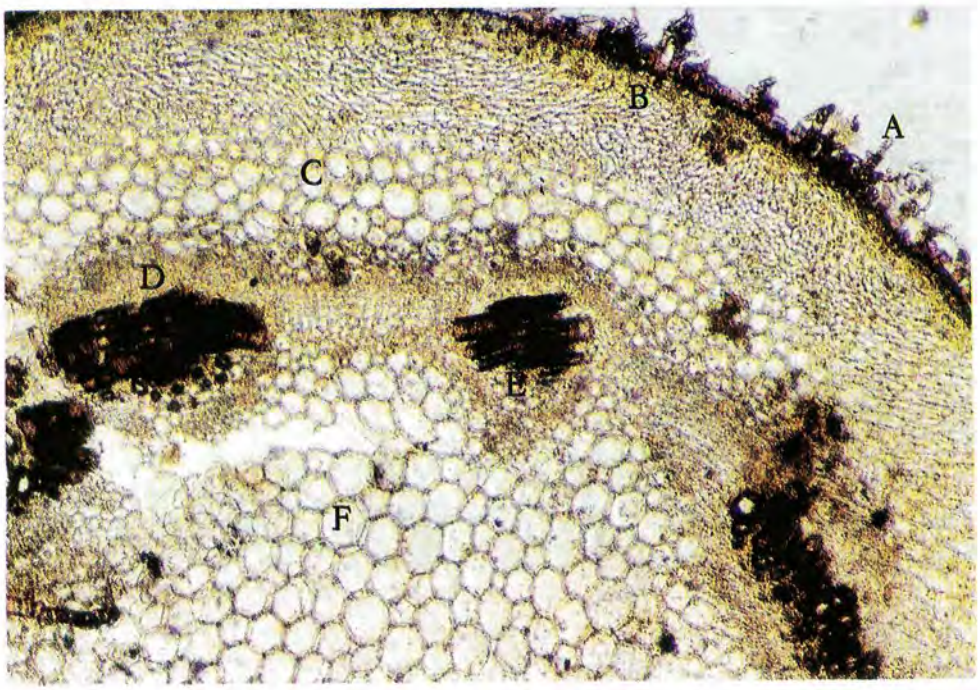


Fig 4 : Cross section of *Withania somnifera stem*

A. Hairs B. Chlorenchyma C. Cortex
D. Phloem E. Xylem F. Pith

ANALYTICAL SPECIFICATION

Powder Analysis : Aerial Part

Macroscopic characters

Colour	-	Brownish yellow
Odour	-	No odour
Taste	-	Bitter taste

Microscopic character

The powder of *Withania somnifera* aerial parts shows-

Thin walled parenchyma cells.

Fragments of thick walls of tracheids / vessels and parts of tracheids / vessels, wilt irregular shape parenchyma cells and their parts.

Parts of trichomes and guard cells with stomata.

Large numbers of droplet like granules that have come out from the cells.

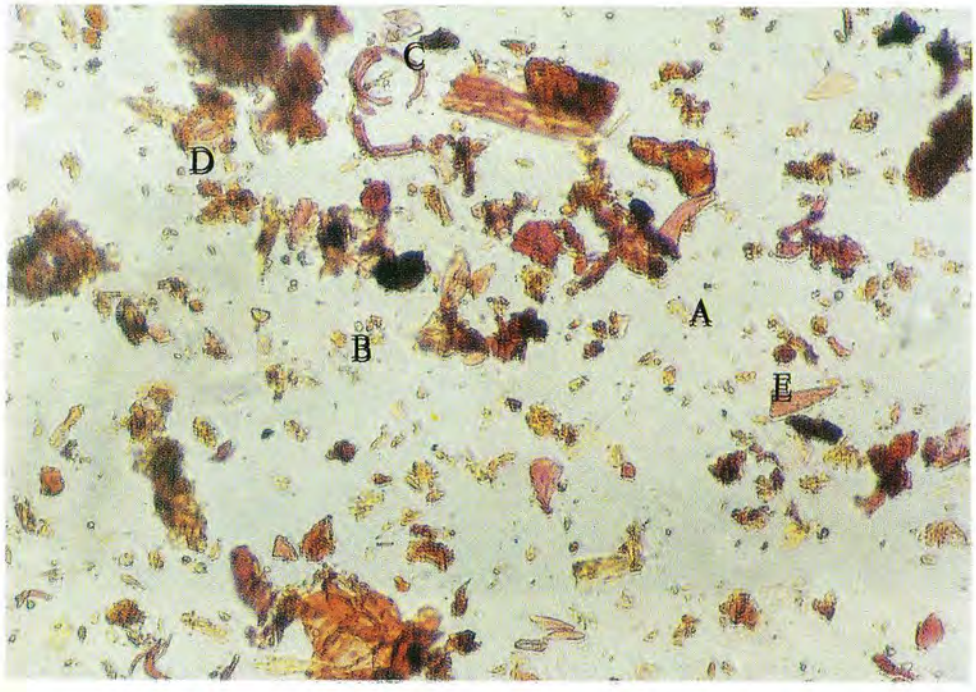


Fig 5 : Powder of *Withania somnifera* aerial parts under the microscope

A. Part of parenchyma cells B. Cell content

C. Fragment of a tracheid / vessel D. Guard cells with stomata

E. Part of trichome

Powder Analysis : Roots

Macroscopic characters

Colour	-	Brownish yellow
Odour	-	Woody smell
Taste	-	No taste

Microscopic character

The powder of *Withania somnifera* roots shows-

Thin walled parenchyma cells.

Fragments of thick walls of tracheids / vessels and parts of tracheids / vessel, wilt irregular shape parenchyma cells and their parts.

Number of acentric starch granules.

Large numbers of droplet like granules that have come out from the cells.

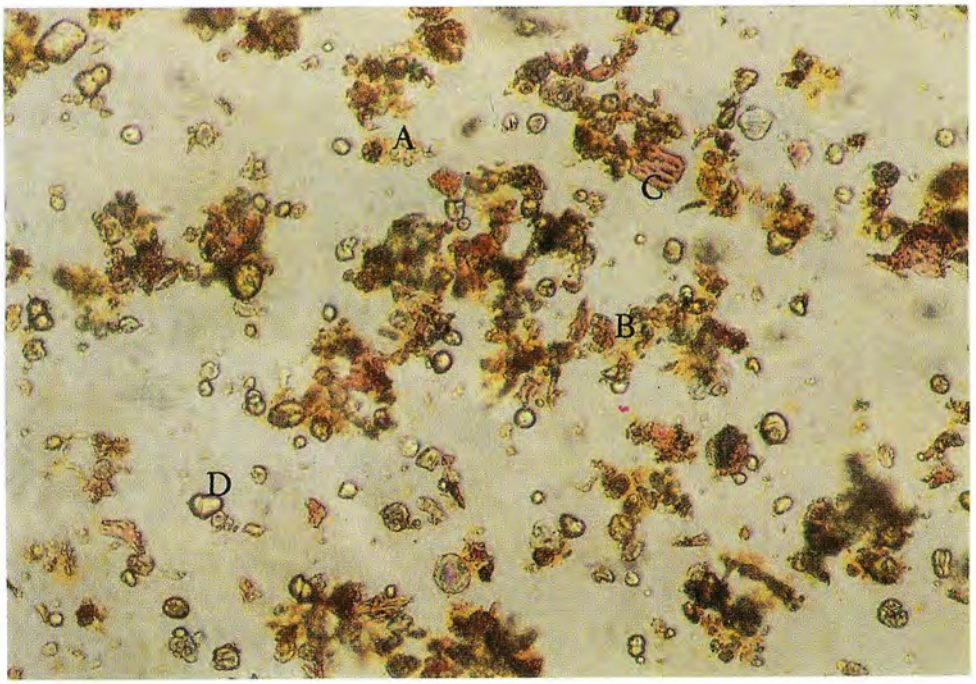


Fig 6 : Powder of *Withania somnifera* roots under the microscope

A. Part of parenchyma cells
B. Cell content
C. Fragment of a tracheid / vessel
D. Starch granules

Table 1 : Physico – Chemical Analysis

Test	Limits
Ethanolic extract of aerial part	17.5-21.5%
Ash content of aerial part	<9.9%
Acid insoluble ash of aerial part	<0.26%
Ethanolic extract of root	8-12%
Ash content of root	<5.25%
Acid insoluble ash of root	<1.58%

PHYTOCHEMISTRY

W. somnifera entire plant contains steroids, cholest-5-22-dien-3-β-ol-24-ethyl, cholest-5-en-3-β-ol-24-ethyl, cholesta-5-(z)-24-(28)-dien-3-β-ol,24-ethyl, cholesta-5-24(25)-dien-3-β-ol-24-methyl, witha-2-5-8(14)-24-tetraenolide-20-28-dihydroxy-1-oxo, withaferin A, withaferinil, withanolide D, withanolide F, withanolide E, withanone 14-α-hydroxy, and withanone-14-β-hydroxy, 17β,27-dihydroxy-14-20-epoxide-1-oxo-(22R)-witha-3-5-24-trienolide, withasomidienone, 27-hydroxy-3-oxo-(22R)-witha-1-4-24-trienolide, 5-dehydroxy withanolide-R(23β-hydroxy-6α-7α-epoxy-1-oxo-22R-witha-2-24-dienolide) and withasomniferin-A (17-hydroxy-6α,7α-epoxy-1-oxo-22R-witha-4,24-dienolide) somnifericin, 2,3-dehydrosomnifericin, withaoxylactone, Withalactone, quresimine A(4β-hydroxy-3β-methoxy-5β,6β-epoxy-(22R)-witha-24-enolide), quresimine B(4β,27-dihydroxy-3β-methoxy-5β,6β-epoxy-(22R)-witha-24-enolide) alkane, hentriacontane,N, ipuranol, lipid, linoleic acid and stearic acid, and vitamin C[1,10,27-37].

Stem bark contains withasomnilide{(20S, 22R)-oxo-5α,8β-dihydroxywitha-6α,7β-epoxide-2,24-dienolide}, withasomniferanolide{(20S,22R)-1oxo-8β,11β,16β-trihydroxy-witha-2,5,24-trienolide}, somniferawithanolide{(20R,22R)-1-oxo-8β,18,20β-trihydroxy-witha-2,5,24-trienolide}, somniwithanolide{(20R,22R)-1-oxo-7β,18,20β,27-tetrahydroxy-witha-2,4,24-trienolide}[38].

W. somnifera root contains alkaloids, anaferine, anahygrine, choline, cuscohygrine, iso-pelletierine, tigloate 3-tropyl, 3-tropyltigloate, tropane 3-α-tigloyl-oxy, tropine, and withasomnine, steroids, sitoindoside vii, sitoindoside viii, witha-2-24-dienolide,22(R) 5-α-20-dihydroxy –1-oxo-6-α-7-α-epoxy, withaferin A, visamine, 5-20-α-(R)-dihydroxy-6-α-7-α-epoxy-1-oxo-(5α)-witha-2-24-dienolide, witha-2-24-dienolide-22(R)-5-α-27-dihydroxy-1-oxo-6-α-7-α-epoxy, 3-tropyltigloate, β-sitosterol, withasominiferol A(5α-20α_F(R)27-trihydroxy-6α,7α-epoxy-1-oxowitha-2,24-dienolide), withasominiferol B(5α,20α_F(R)-dihydroxy-6α,7α-epoxy-1-oxowitha-2-enolide), withasominiferol C(5α-14α,20α_F(R)-trihydroxy-1-oxowitha-2,7,24-enolide) starch, reducing sugar, hentriacontaine, dulcitol, withaniol, free amino acids such as aspartic acid, glycine, tyrosine, alanine, proline, tryptophan, glutamic acid and cystine [15,39,40,41].

W. somnifera leaves contain steroids, physalolactone 4-deoxy, β-sitosterol, witha-2-enolide- 4-β-20-α-(R)-dihydroxy-1-oxo-5-β-6-β-epoxy, witha-2-enolide-4-β-hydroxy-1-oxo-5-β-6-β-epoxy, witha-24-enolide-22(R)1-α-3-β-5-α-trihydroxy-6-α-7-α-epoxy, witha-2-14-24-trienolide-22(R)4-β-hydroxy-1-oxo-5-β-6-β-epoxy, witha-2-24-dienolide 22(R)5-α-17-α-dihydroxy-1-oxo-6-α-7-α-epoxy, witha-2-24-dienolide22(R)5-α-27-dihydroxy-1-oxo-6-α-7-α-epoxy, witha-2-24-dienolide-4-β-20-α-dihhydroxy-1-oxo-5-β-6-β-epoxy, witha-2-5-24-trienolide22(R)17-α-27-dihydroxy-1-oxo, witha-2-5-24-trienolide 22(R)7-α-27-dihydroxy-1-oxo, witha-2-6-24-trienolide-22(R)5-α-17-dihydroxy-1-oxo, withacnistin, withaferin, withaferin A, withaferin A-14-hydroxy-27-deoxy, withaferin A-2-3-dihydro, withaferin A-27-deoxy, withaferin A-27-deoxy-14-α-hydroxy, withaferin A-27-deoxy-17-α-hydroxy, withaferin A-dihydro, withanicandrin, withanolide D, withanolide D-2-3-dihydro, withanolide D-27-hydroxy, withanolide D-7-β-hydroxy, withanolide E, withanolide E-4-β-hydroxy, withanolide F, withanolide G, withanolide H, withanolide J, withanolide K, withanolide L, withanolide M, withanolide N, withanolide O, withanolide P, withanolide Q, withanolide R, withanolide S, withanolide-14-α-hydroxy, withanolide-17-α-hydroxy, withanone, witha-2-24-dienolide-20-20-(R),5-α-17-α-dihydroxy-1-oxo-6-α-7-α-epoxy, witha-2-24-dienolide-5-α-17α-dihydroxy-1-oxo-6-α-7-α-epoxy-20(R)-22(R), withanolide D-14-α-hydroxy, withanolide D-17-α- hydroxy, withanolide D-27-α- hydroxy, withanolide I, withanolide Y, withanolide D-6-α-chloro-5-β-hydroxy, withanolide D-24-25-dihydro, withanolide D-4 –dehydro, withanolide D-4-dehydro-24-25-dihydro, withanolide T, withanolide U, withanone-20-α- hydroxy-17-deoxy, witha-2-24-dienolide-5-α-ethoxy-1-oxo-6-β-14-α-17-β-20-α-F-tetrahydroxy-20(S)-22(R), witha-3-5-24-trienolide-1-oxo-14-20-α-F-27-trihydroxy- 20(R)-22(R), witha-5-24-dienolide-1-α-3-β-20-α-F-trihydroxy-20(R)-22(R), withanone-17-deoxy-20-hydroxy, chlorogenic acid 3β-methoxy-2,3-dihydro-27-deoxywithaferin A, 2,3-dihydrowithaferin A, 3β-methoxy-2,3-dihydrowithaferin A, 27-deoxywithaferin A[40-54].

W. somnifera, seeds contain linoleic, oleic, palmitic, stearic and myristic acids[55].

W. somnifera germinating seeds contain sitosterol, stigmasterol, campesterol, 24-ethylidenecholesterol and 24-methylenecholesterol[56].

PHARMACOLOGY

The extracts of *W. somnifera* plant, alcoholic extracts of roots and extracts of aerial parts showed tumor and granuloma-tissue formation inhibitor effects, It has also acted as a radiosensitizer, by lowereing the plasma and blood glutathione(GSH) levels by increasing the total white blood cells count, increasing the bone marrow cellularity and by exhibiting antileukopenic activity in cancer patients treated with sublethal doses of gamma radiation or cyclophosphamide[57-66].

Dried plants of *W. somnifera*, root powder and seeds produced dose-dependent significant antiulcerogenic effect on gastric ulcers[67-70].

W. somnifera, roots, its extracts, methanolic extracts of aerial parts , dried leaves, extracts of leaves and dried fruits have produced significant anti-inflammatory activity against carrageenan induced paw oedema comparable to hydrocortisone sodium succinate in rats[21,62,63,78,71-79].

W. somnifera dried plants, dried aeriel parts dried leaves, roots, fruits and stems showed antibacterial activity against *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* spp, *Staphylococcus aureus Staphylococcus epidermidis Bacillus subtilis*, *Candida albicans*[23,63,80-84].

W. somnifera plant, root and leaf extracts showed antiviral activity and antitoxic activity against Ranikhet virus, Vaccinia virus, Tobacco mosaic virus and Spinach mosaic virus[63,85-87].

W. somnifera dried aerial parts, dried stems and roots, showed anti-yeast and anti fungal activity[81-83,23,87,88,89].

Dried entire plants and alcoholic leaf extracts of the plant exhibited antihepatotoxic activity in rats[69,74].

The protective effect of various doses of the MeOH extract of *W. somnifera* against pentylenetetrazole(PTZ)-induced convulsions in mice has been reported[104].

Intake of *W. somnifera* capsule with milk showed enhancement of certain aspects of psychomotor performance such as strength, vitality process and endurance time[106].

A methanolic extract of *Withania somniferea* possesses Gamma Amino Butyric Acid (GABA) – mimetic activity[107].

Ashwagandha rasayana when given to patients with keratitis and rough skin with xerosis of eyes, the results were encouraging. It also stopped falling of hair[109].

The dried aerial parts, roots and seeds of *W. somnifera* showed barbiturate potentiation[13, 94,95,99].

Aqueous suspension of root extract of *W. somnifera* prevented the rise in lipid peroxidation in rabbit and mice[129].

W. somnifera seeds, roots and their extracts showed non-specific resistance stimulation, and antistress activity [70,74,93,97,98].

W. somnifera plant showed –
Antiamoebic activity against *Entameoba histolitica*[86].
Insect antifeedant effects[63].
Antiaggression effect[74].
Anticonvulsant activity against audiogenic seizure in rats[74].
Psychotropic activity on human[100].
Altered behavioural patterns, responsiveness, loneliness, state of awareness along with physical capabilities[105].
Inhibition of the development of tolerance to morphine-induced analgesia in Swiss mice[108].

The aerial parts of *W. somnifera* showed –
Anti-tuberculosis activity[23].
Catecholamine inhibitory effects on brain of rat[90]

W. somnifera root exhibited –
Glycosaminoglycan syntheis inhibition[101].
Oxidative phosphorylation uncoupling effects[101].
Protein synthesis stimulation[75].
Membrane stabilization effects[102].
Antihyperglycemic activity [103].
Central nervous system depressant properties[13,22,95,99].
Hypotensive activity[96].
Antifertility activity[21].
Nematocidal activity[90,91].

The seeds of *W. somnifera* showed –
Antimalarial activity against *Plasmodium berghei*[69].
Adaptogenic activity[93].
Anabolic activity[93].

Withaferin A is the most important of the withanoloids isolated so far, to which the curative properties of the leaves are attributed. Withaferin A inhibited tumour growth and showed radiosensitizing effects, anti-inflammatory activities and antibiotic activity[13, 58,60,110-113].

Withaferin A was more tolerated than plumbagin when screened for acute toxicity in Swiss mice[114].

4 β-Hydroxy withanolide E and withaphysacarpin from the leaves of *Withania somnifera* showed significant anti-bacterial activity against Gram-positive bacteria[113,115].

The alkaloids of *W. somnifera* are reported to possess anthelmintic action[113].

The total alkaloid extracts of roots exhibited prolonged hypotensive, bradicardiac and respiratory-stimulating action in dogs. The alkaloids produced a mild depressant effect (tranquillizer - sedative type) on the central nervous system in several experimental animals[113].

The total alkaloids also showed relaxant and antispasmodic effects[113].

Steroids isolated from *Withania somnifera*, have shown antifeedant activity against larvae of *E.varivestis*[116].

Anti-inflammatory effects, relief in symptoms and healing of simple fractures were shown by compound drug *Withania somnifera, Apium graveolens, Achillea santolina, Matricaria chamomilla* and *Myrtus communis,* Laksha Guggulu containing *Withania somnifera, Commiphora mukul, Terminalia arjuna, Vitis quadrangularis Grewia hirsuta*, and the herbal compound containing *Withania somnifera, Boerhaavai diffusa, Tribulus terrestris* and *Aconitum ferox*[78,117,118].

Antiarthritic activity was shown by the herbomineral formulation, Articulin-F (containing roots of *Withania somnifera*, stem of *Boswellia serrata,* rhizomes of *Curcuma longa* and a zinc complex)[77,119].

Adaptogenic activity was shown by Ayurvedic formulation *Withania somnifera, Ocimum sanctum, Tinospora cordifolia, Picrorrhiza kurroa, Eclipta alba* and Shilajit. The results exhibited a similar profile as *Panax ginseng*, a standard adaptogen, but less toxic than ginsang[120].

A herbal combination of *Withania somnifera*, *Bergenia ligulata*, *Tinospora cordifolia*, *Eclipta alba*, *Asperagus racemosus*, *Myristica fragrans* was successfully used for the treatment of renal calculi[121].

Two herbal drugs Ashwagandha (*Withania somnifera*) and Kapikacchu (*Mukuna pruriens*) clinically tried in depressive illness, showed notable symptomatic improvement, decrease in the degree of anxiety and depression[122].

Jivak containing *Withania somnifera*, *Asparagus racemosus*, *Conscora decussata*, *Ipomoea digitata*, *Hemidesmus indicus*, *Mucuna pruriens*, *Tribulus terrestris*, *Eclipta alba*, showed Improvement in appetite, increase in weight, feeling of freshness in treated patients. It was found useful in debility, indigestion and lowered body resistance[123].

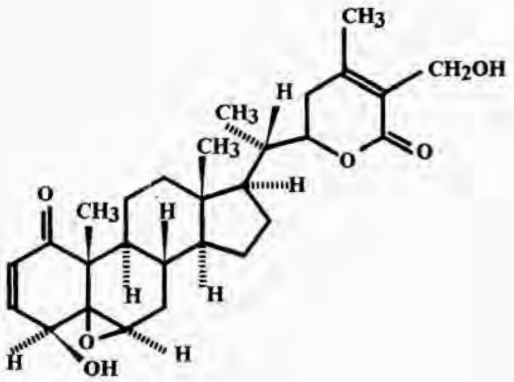
Withania somnifera and Ginseng (*Panax ginseng*) are widely used as geriatric tonic[124].

The lactation was improved and stimulated by treating with Lactare capsules containing *Withania somnifera*, *Asparagus racemosus*, *Glycyrrhiza glabra*, *Trigonella foenumgra* and *Allium sativum*[125].

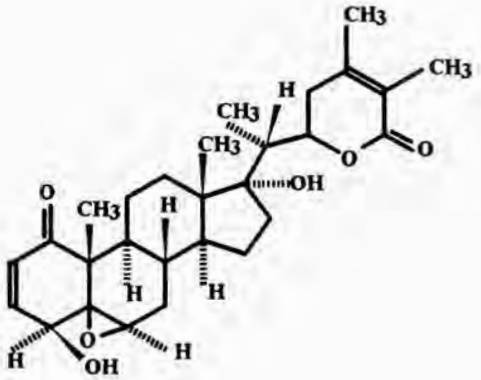
Aqueous extracts derived from *Withania somnifera, Convolvulus mycrophyllus*, *Hydrocotyle asiatica*, *Glycyrrhiza glabra*, *Saussurea lappa*, *Acorus calamus*, *Rauwolfia serpentina*, *Myristica fragrans* was effective in the treatment of refractory cases and untreated cases of schizophirenia[126].

Withania somnifera and *Glycyrrhiza glabra* root powders were found to be more potent drugs in the management of 'amla pitta' with least untoward side effects[127].

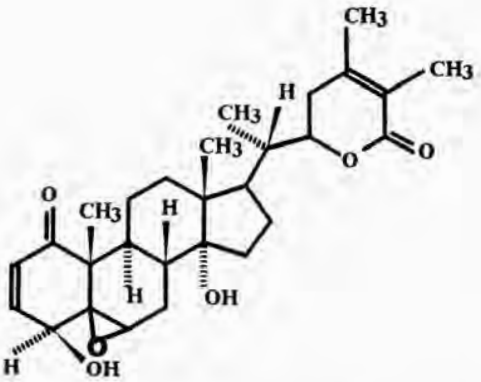
Trasina, an Ayurvedic herbal formulation comprising of *Withania somnifera*, *Tinospora cordifolia*, *Eclipta alba*, *Ocimum sanctum*, *Picrorrhiza kurroa* and shilajit, decreased hyperglycaemia and increased pancreatic islet superoxide dismutase activity in hyperglycaemic rats[128].



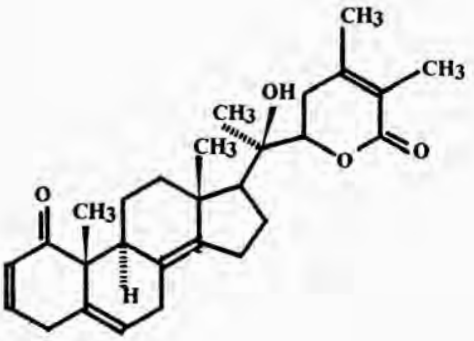
Withaferin A



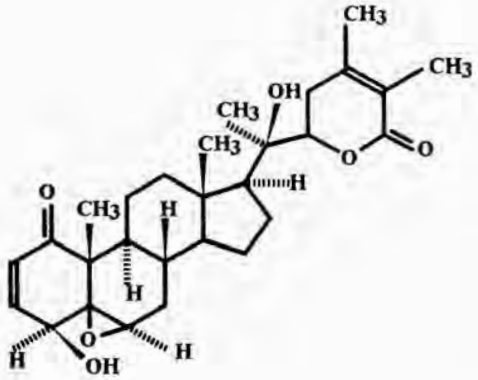
27- deoxy-17 - hydroxy withaferin A



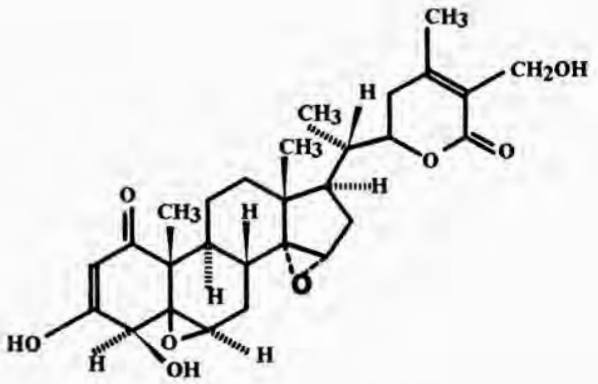
27- deoxy-14 - hydroxy withaferin A



Withanolide G

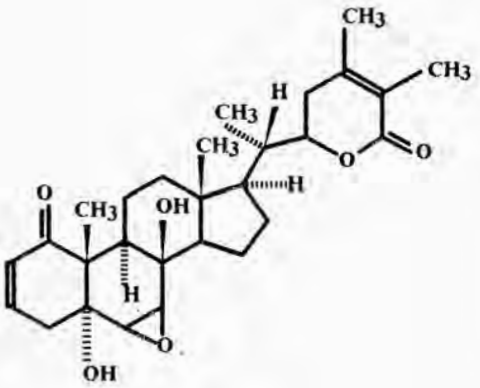


Withanolide D

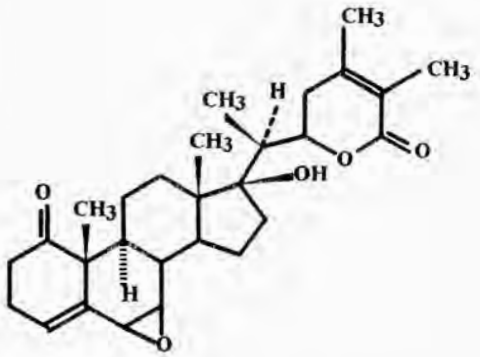


Withaoxylactone

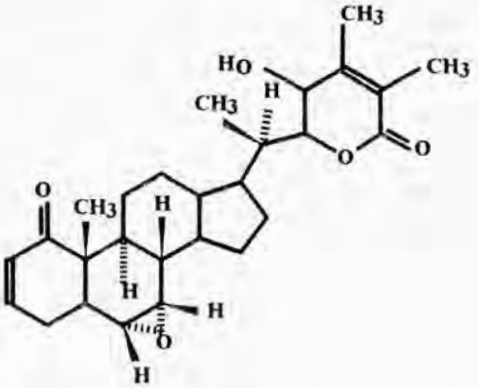
Fig. 7 Compounds present in Withania somnifera



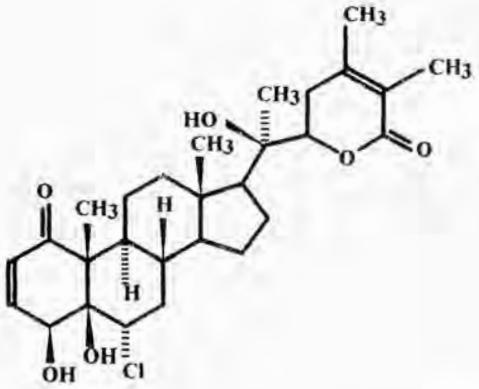
Withasomnilide



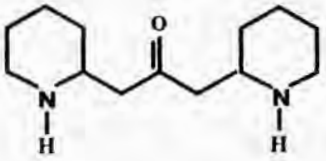
Withasomniferin A



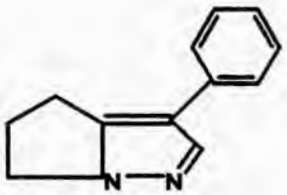
5- dehydroxy withasomnilide



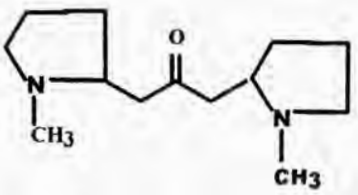
6 - chloro- 5 - hydroxy withanolide D



Anaferine



Withasomnine



Cuscohygrine

Fig. 8 **Compounds present in Withania somnifera**

TLC PROFILE

Sample preparation

Sample detail	-	*W. somnifera* aerial part
ample preparation	-	*W. somnifera* aerial part (5.0g) was extracted with ethanol and concentrated (87.4mg/10ml) 15µl of extract was applied to TLC plate.
Adsorbent	-	Silica gel GF_{254}
Solvent system	–	Benzene : ethyl acetate (3 : 7)

Detection

Direct evaluation	-	UV_{254}nm, UV_{366}nm Rf values (UV_{254}) Extract : 0.76, 0.69, 0.43, 0.21, 0.01 Rf values (UV_{366}) Extract : 0.76, 0.69, 0.21
Scanning	-	Densitometer
Spray reagent	-	Vanillin – sulphuric acid

Fig 9
TLC chromatogram of
W. somnifera aerial part extract

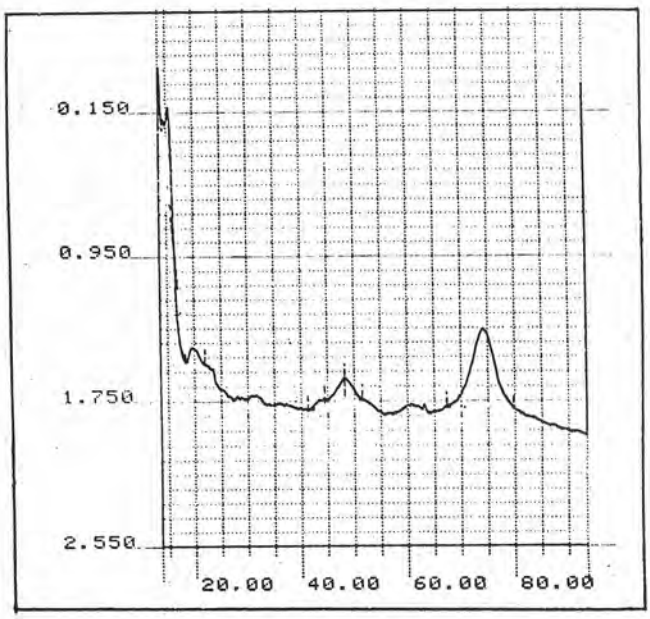


Fig 10
TLC Densitometer finger print

TLC PROFILE

Sample preparation

Sample detail	-	*W. somnifera* root
Sample preparation	-	*W. somnifera* root (5.0g) was extracted with ethanol and concentrated (46mg/10ml) 10µl of extract was applied to TLC plate.
Adsorbent	-	Silica gel GF_{254}
Solvent system	–	Benzene : ethyl acetate (3 : 7)

Detection

Direct evaluation	-	UV_{254}nm UV_{366}nm Rf values (UV_{254}nm) Extract : 0.73, 0.66, 0.47, 0.42, 0.21 Rf values (UV_{366}) Extract : 0.71, 0.27, 0.20
Scanning	-	Densitometer
Spray reagent	-	Anisaldehyde and Vanillin – sulphuric acid

Fig – 11
TLC chromatogram of
W. somnifera root extract

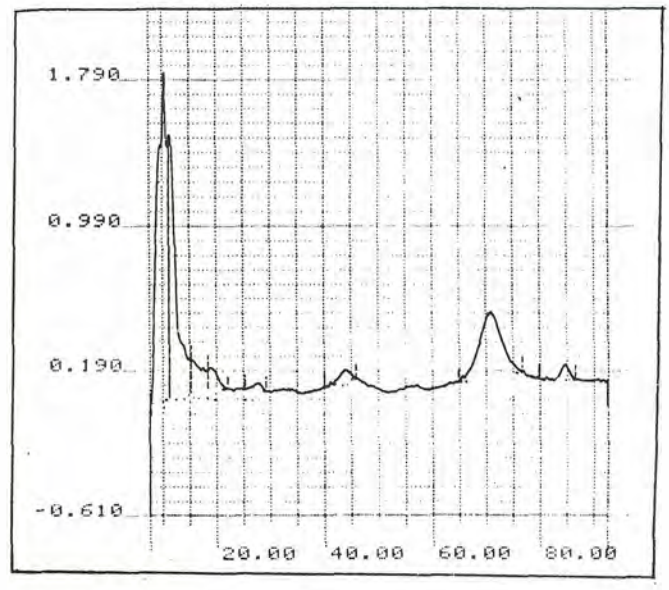


Fig – 12
TLC Densitometer finger print

HPLC PROFILE

W. somnifera aerial part,

Sample preparation - *W. somnifera* aerial part (5.0g) was extracted with ethanol (12.8mg/10ml) and purified using Sep-pak C18 cartridge.

Injection volume - 20µl

Apparatus - Waters 501 HPLC pump.
SPD 10AV Shimadzu uv-vis detector.

Column - µ Bondapak™ RP18

Solvent system - Methanol : water (1: 1)

Flow rate - 1ml/min

Detection - 254nm

Description of the HPLC – Chromatogram

Table 2 : Retention time of main peaks

Peak no	Rt (min)
1	1.66
2	3.10
3	3.92
4	13.36
5	14.86
6	17.88
7	24.21

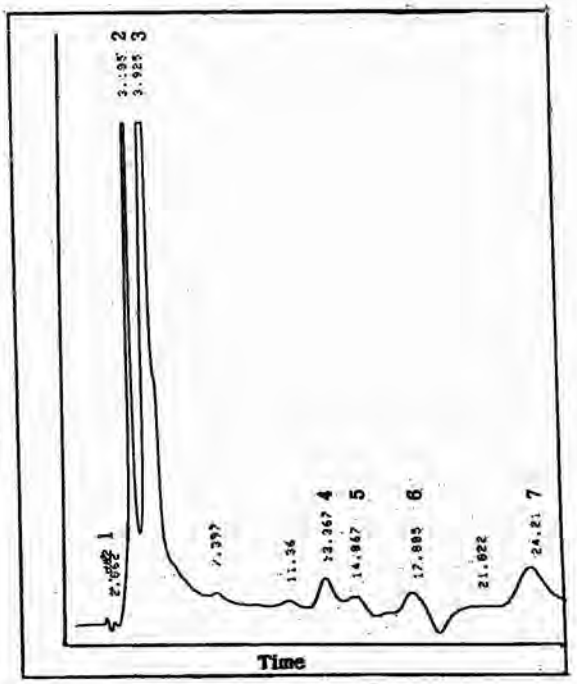


Fig – 13
HPLC chromatogram of aerial part

HPLC PROFILE

W. somnifera root,

Sample preparation	-	*W. somnifera* root (5.0g) was extracted with ethanol (4.8mg/10ml) and purified using Sep-pak C18 cartridge.
Injection volume	-	20µl
Apparatus	-	Waters 501 HPLC pump. SPD 10AV Shimadzu uv-vis detector.
Column	-	µ Bondapak™ RP18
Solvent system	-	Methanol : water (1: 1)
Flow rate	-	1ml/min
Detection	-	254nm

Description of the HPLC – Chromatogram

Table 3 : Retention time of main peaks

Peak no	Rt (min)
1	1.65
2	2.17
3	2.66
4	3.92
5	5.11
6	5.61
7	5.94
8	9.62
9	13.46
10	21.99

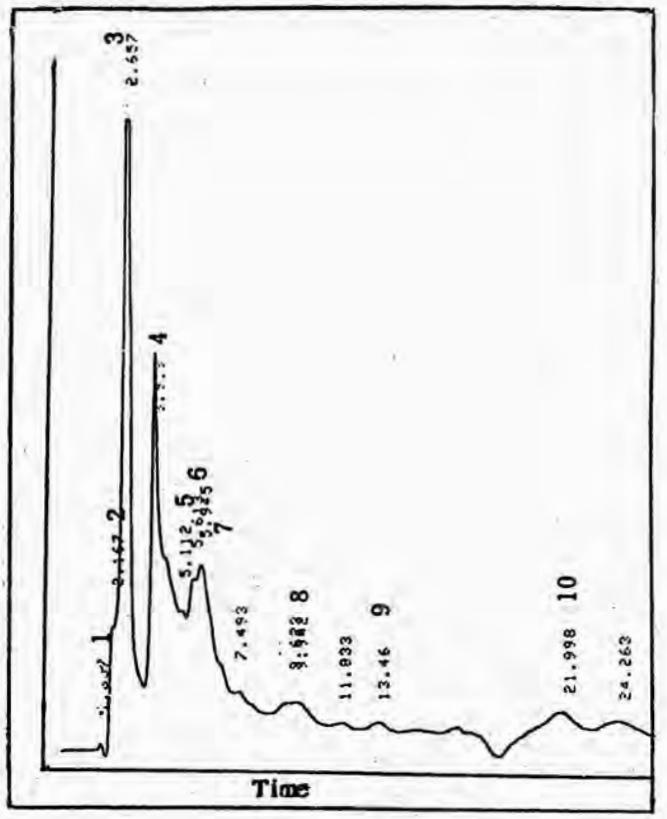


Fig – 14
HPLC chromatogram of root

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