5. INTRODUCTION TO THE HERBS AND REVIEW OF LITERATURE:

5.1 Eclipta prostrata (L.) L.:

5.1.1. INTRODUCTION:

FAMILY: Asteraceae (Compositae)

PARTS USED: Entire plant is used in medicine.

COMMON NAMES:
- Gujarati: Bhangra, Dodhak, Kalobhangaro, Kaluganithi
- Hindi: Babri, Bhangra, Mochkand.
- Kannad: Garagadasoppu
- Malayalam: Kyonni
- Marathi: Bangra, Maka
- Sanskrit: Ajagara, Bhringaraja, Ekaraja, Keshraja, Keshya, Kuntalavardhana, Mahabherringa, Mahanila, Makara, Nilbherringaja, Pitrirpriya, Shymala
- Tamil: Kayanthakoru, Kaikeshi
- Telugu: Guntagairo, Guntalijeru
- Urdu: Bhangra
- Uriya: Kesarda

DESCRIPTION:
Herb is annual, erect or prostrate, branched, often rooting at nodes; Stem & branches strigose with appressed white hairs, leaves sessile, 2.5 - 7.5 cm. long, variable in breadth, usually oblong-lanceolate, subentire, acute or subacute, sparsely strigose with appressed hairs on both sides, base tapering. Heads 6 - 8 mm. diameter, solitary or 2 together on unequal axillary peduncles. Involucral bracts about 8, ovate, obtuse, herbaceous, strigose, with appressed white hairs. Ray flowers ligulate, the ligule small, spreading, scarcely as long as the bracts, not toothed, white. Disc flowers tubular, the corollas often 4 toothed. Pappus 0, except occasionally very minute teeth on the top of the achene. Achenes cuneate, compressed and with a narrow wing, covered with warty excrescences.

DISTRIBUTION:
Bengal, Central India, Rajasthan, Gujarat, South India, Burma, Sri Lanka, etc.
Cosmopolitan in warm climate.
The herb popularly known as Bhringraj or Bhangra is reputed in many Indigenous systems of medicine. The entire herb or its leaf and root parts are found to be incorporated in many Ayurvedic and Unani preparations.

The plant has a bitter, hot and sharp taste and is used in Ayurveda for the treatment of "Kapha" and "Vata". It is found to possess antihypertoxic, anti-inflammatory, anti-ulcerogenic, antibiotic, and antihepatotoxic properties. In traditional medicine, the decoction of the herb is much valued as a tonic and is also used in liver and spleen enlargements. An Ayurvedic alcoholic preparation "Bhringraj Asawa" is highly reputed for its general tonic properties. It is said to be blood purifier and is used in various skin diseases like itching, elephantiasis and for curing sores on shoulders caused by carrying heavy loads. An Ayurvedic herbomineral preparation, "Gandhak Rasayana" is highly valued in all types of skin diseases.

It is used as anthelmintic, antiseptic, alexipharmic, expectorant, antipyretic, stomachic, antispasmodic, uterine pain reliever after delivery, antiasthmatic and antibronchitis.

It is astringent and is used in cases of stomatitis, toothache and for strengthening the gums. In China, the plant is rubbed on gums for toothache and pounded leaves are prescribed in cases of haemorrhages and fluxes.

It is used in cases of night blindness, hernias, syphilis, headache, hemorrhania, vertigo and heart diseases.

It is highly valued in cosmetics. The paste of the herb is rubbed on the discoloured skin for improving its complexion. The extract of the herb is used internally and externally to give the natural black colour to the hair. The fresh juice or the "Bhringraj hair oil" is rubbed on the shaven scalp to promote the growth of the hair. Due to these properties, the herb is commonly named as hair promoting herb or "Keshraj". It is also used as a dyeing herb in tattooing.

The root is emetic and purgative. It is used in veterinary practices. It is applied externally as an antiseptic to ulcers and wounds of the cattle. The root is applied in conjunctivitis and galled necks of the cattle. In Chota Nagpur, the roots are merely tied to the belly of the cattle to remove its diseased condition. The root is also used for relieving scalding urine.

**ETHNOMEDICAL USES**:

The plant is valued not only in India but also in number of other neighbouring countries like Indonesia, China, Taiwan, Thailand etc. As per the disease its ethnomedical uses are listed below, along with name of the country and part of plant used in the bracket.

1. **Antiasthmatic**: Dry (Ar- Thailand) powder is taken.
2. **Anticancer**: Decoction (Lf-Indonesia) is used orally in stomach and matrix cancer.
3. **Antidiabetic**: Decoction (Hb-Taiwan) is used orally.
4. **Antidiarrhoeal**: Decoction (Ar- India; Lf- Nigeria) are used orally.

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5. **Antiepileptic**: Leaf pounded with garlic and pepper is taken orally (Anantapur, A.P., India) in conscious epileptic patient. Its extract is dropped in the nostrils of the unconscious patient\(^{397}\).

6. **Anti-inflammatory**: Decoction (Hb-Bihar, India) along with black pepper and raw sugar is taken orally as an anti-inflammatory drug\(^{398}\).

7. **Antileprotic**: Decoction (Hb-India) is used alone orally as antileprotic\(^{340}\) while in other parts (Bundelkhand, India) hot water extract with other four herbs is used orally\(^{427}\). In Somalia they crush the entire plant, mix it with oil and apply externally\(^{399}\).

8. **Antileucodermal**: In Bundelkhand (India), they use the plant along with four other herbs orally\(^{427}\).

9. **Antimalarial**: Chenchu tribal people (A.P., India) use plant with pepper orally\(^{401}\).

10. **Antioedemic**: Leaf paste along with salt is applied externally by the people of Anantapur (A.P., India) on oedema of the legs\(^{397}\).

11. **Antipyretic**: Decoction (Hb-India\(^ {402}\)) and paste (Lf- Chittoor, India\(^ {426}\)) are used orally.

12. **Antirabidies**: Tribal people of Bihar (India) use 2-3 pills of root paste with sugar candy orally in dog bite\(^ {398}\).

13. **Antitubercular**: Decoction (Hb-China) is taken orally\(^ {403}\).

14. **Antivenin**: Decoction (Hb-China) is taken orally in snake bite\(^ {316}\) while they use fresh herb in Brazil\(^ {404}\). Yanadi tribal people (S. India) use leaf juice with butter milk orally\(^ {405}\), while in N. Gujarat they use, orally, leaf juice as an antidote for snake bite\(^ {406}\).

15. **Bronchitis**: Decoction (Hb- India) is used orally by tribal people of Ujjain\(^ {407}\).

16. **Cholagogue**: Decoction (Ar-Arab countries) is used orally\(^ {408}\).

17. **Emetic**: Decoction (Ar- Arab countries\(^ {408}\); Lf- Nigeria\(^ {396}\)) are used orally.

18. **Eye diseases**: Decoction (Hb-Ujjain, India) is used externally in eye diseases\(^ {407}\). Leaf juice with honey is used orally by tribal people of Katra (J&K, India) in catarrhal disorders\(^ {409}\).

19. **Haemostatic**: Decoction (Hb-China) is used orally\(^ {340}\).

20. **Headache**: Tribal people of Rayalseema\(^ {410}\) (A. P., India) and others\(^ {402}\) apply extract of the bud and the herb respectively in sesame oil externally on forehead.

21. **Haircare**: Tribal people of Katra (J & K, India) apply leaf juice with neem oil for the growth of hair\(^ {402}\) while people (Japan) apply water extract of the herb externally for this purpose\(^ {411}\). Chenchu tribe (A.P., India) uses juice of plant with coconut oil externally as hair tonic\(^ {401}\). People in Tamilnadu (India) use fresh unripe fruit juice with other herbs in olive oil externally to prevent premature greying of hair\(^ {412}\). Kani tribal people (Kerala, India) use leaf extract boiled with coconut oil to deepen black colour of the hair and to promote their growth\(^ {413}\).

22. **Heart diseases**: Hot water extract of the herb with other herbs and minerals is used orally in India\(^ {412}\).

23. **Hepatitis**: Decoction (Hb- Bangladesh\(^ {414}\) and Taiwan\(^ {415}\)) is used orally. Hot water extract with other three herbs is used orally in Tirupati (A.P., India), 4g thrice a day in divided doses for infective hepatitis in children\(^ {416}\).
24. **Insanity**: People in Bihar (India) use pills prepared from the root paste in insanity (orally), 4 to 5 pills twice a day for 7 days.\(^{398}\)

25. **Jaundice**: In Arab countries leaf juice is used orally as hepatic tonic.\(^{408}\) In Tamilnadu\(^419\) (India) people use fresh leaves, in Dandakaranya\(^428\) (India) they use herb orally in case of jaundice. People in India also apply paste of root and seeds of *Ricinus communis* near eyes in case of jaundice\(^{420}\). They use root juice orally in liver complaints in N. Gujarat\(^{406}\) (India). People in Cannanore (Kerala, India) use leaf with other herbs orally in case of jaundice\(^{418}\). Tribal people of Chittoor (A.P., India) use dry powder of the herb orally, 4 g for a week to cure jaundice and along with *P. amarus* to rejuvenate the liver\(^{417}\). In Rayalseema (A.P., India) they use juice of the herb with butter milk and curd twice a day orally, but no salt in case of jaundice\(^{410}\).

26. **Male sterility**: Tribal people of Gorakpur (U.P., India) use juice of the plant with *Terminalia chebula* fruit powder to treat male sterility\(^{429}\).

27. **Purgative**: Decoction (Ar-Arab countries\(^{408}\) and Lf- Nigeria\(^{396}\)) are used orally as purgative and laxative respectively.

28. **Safe delivery (Prevention of miscarriage)**: 10 to 15 ml juice of herb with cow’s milk per day is taken orally from early days of pregnancy to prevent miscarriage and to have safe delivery\(^{421}\).

29. **Skin diseases**: The roots of the plant with other herbs are used externally for cow as an antiseptic in Ujjain (M.P., India)\(^{407}\). Decoction (Hb-India) in combination with other plants is taken orally in elephantiasis\(^{425}\). A paste of the herb (India) with other plants in sesame oil is applied externally in elephantiasis\(^{402}\). People of Kondh tribe (Orissa, India) use paste of plant externally in case of ringworm lesions\(^{422}\). People of Rayalseema (A.P., India) use fresh juice of the plant externally in skin diseases\(^{424}\).

30. **Spleen disorder**: People in Katra (J & K, India) use leaf juice with honey orally\(^{410}\).

31. **Tonic**: Decoction (Hb- Arab countries\(^{408}\)) is taken orally.

32. **Toothache**: Paste of the plant (India) in sesame oil is applied externally\(^{402}\).

Abbreviations: **Ar**= Aerial parts; **Hb**= Herb; **Lf**= Leaf; **Rt**= Root.

### 5.1.3. PHARMACOGNOSTICAL REVIEW:

Mehra and Handa\(^{291}\) (1968) have compared the morphological and microscopical characters of *Eclipta alba* with its Ayurvedic substitute, *Wedelia calendulacea*, commonly known as Peeta (yellow flowered) Bhringraj.

Gupta\(^{1977}\) has reported two forms of *E. prostrata*, erect and prostrate. Erect forms grow luxuriantly in shady and moist habitat where as prostrate forms flourish in open sunlight, dry and disturbed areas irrespective of seasons. There is difference in size of roots, internodes, flowerhead etc. but no major difference in extractive values\(^{292A}\).

Satyavati\(^{292B}\) et al.\(^{1976}\) have mentioned morphological and microscopical characters of *E. alba*.
Linn et al. (1986) have described the pharmacognostical characters of a Chinese drug 'Han-lian-cao', i.e. *E. alba*.

Patel Kiran (1989) has described diagnostic microscopic characters of the powder of *E. alba*.

Indian Herbal Pharmacopoeia (1998) has mentioned the morphological and microscopic characters of leaf, stem and root of *E. alba* and its certain constants like foreign matter, ash values and extractive values.

Shrivastava et al. (1990) have discussed the morphological characters of three commercial adulterants of Bhringrajā namely, *Ageretum conyzoides* (Asteraceae), *Caesalia axillaris* (Asteraceae) and *Alternanthera sessilis* (Amaranthaceae) and compared the diagnostic microscopic characters of their powdered drugs of leaf, stem and root with that of *E. alba*.

Gopalkrishnan & Johnson (1992) have compared the microscopic characters of leaves & stems of *E. alba* with its substitute *W. calendulacea*. Their certain physical constants (ash values, extractive values, loss of wt. on drying etc.) and other observations (fluorescence and chemical analysis) are also compared.

The substitutes and adulterants of *E. alba* can be differentiated by their habitat, and the morphological and microscopic characters of their leaves, stem and flowers. Some of the diagnostic characters have been summarized in the Table VII

<table>
<thead>
<tr>
<th>Character</th>
<th><em>E. alba</em></th>
<th><em>Ageratum conyzoides</em></th>
<th><em>Caesalia axillaris</em></th>
<th><em>Alternanthera sessilis</em></th>
<th><em>Wedelis calendulacea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Family</td>
<td>Asteraceae</td>
<td>Asteraceae</td>
<td>Asteraceae</td>
<td>Amaranthaceae</td>
<td>Asteraceae</td>
</tr>
<tr>
<td>2. Habit</td>
<td>Erect or spreading annual herb</td>
<td>Erect herb</td>
<td>Spreading herb</td>
<td>Erect herb</td>
<td>Erect perennial herb</td>
</tr>
<tr>
<td>3. Stem</td>
<td>Pubescent</td>
<td>Pubescent</td>
<td>Glabrous</td>
<td>Glabrous</td>
<td>Glabrous</td>
</tr>
<tr>
<td>4. Leaf</td>
<td>Linear to oblong-lanceolate, turning black on drying</td>
<td>Broadly ovate</td>
<td>Lanceolate, elliptical, linear oblone or oblanceolate</td>
<td></td>
<td>Ovate to ovate lanceolate</td>
</tr>
<tr>
<td>5. Inflorescence</td>
<td>Head, 7-9 mm. in</td>
<td>Head, 4-6 mm. in</td>
<td>Head globose, 10-</td>
<td>Spikelets globose-</td>
<td>Head, 2-3 cm in diameter,</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>6. Fruit</th>
<th>Achene, black, 2-2.5 mm long, obconical, compressed, narrowly winged, ridges on one side</th>
<th>Achene, 1.6-1.8 mm long, cuneate, sharply trigonous</th>
<th>Achene, 2.8-3 mm long, obovate, faintly ribbed, winged, notched at apex</th>
<th>Urticle, 1.5-2 mm long, obcordate-obreniform, deeply notched</th>
<th>Achene, obovate, triangular, glabrous, faintly ribbed.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Stomata</td>
<td>Anisocytic</td>
<td>Anisocytic</td>
<td>Anomocytic</td>
<td>Diacytic</td>
<td>Anisocytic</td>
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<td>8. Stomatal Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper surface</td>
<td>30-34</td>
<td>30-34</td>
<td>19-22</td>
<td>13-21</td>
<td>Not reported</td>
</tr>
<tr>
<td>Lower surface</td>
<td>33-38</td>
<td>31-35</td>
<td>23-28</td>
<td>19-25</td>
<td>Not reported</td>
</tr>
<tr>
<td>9. Trichomes of leaf, stem and flower</td>
<td>Small, 70-100μ long, thin walled, multicellular, uniseriate.</td>
<td>570μ-1222μ long, multicellular, uniseriate.</td>
<td>40-60μ long, thin filamentous, unicellular (flower).</td>
<td>Small, 170-300μ long, multicellular, uniseriate, thin-walled.</td>
<td>Absent.</td>
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<tr>
<td></td>
<td>-Large 300-800μ multicellular, tips pointed, thick-walled, wall rough, terminal cell very small (Leaf and stem).</td>
<td>-Glandular, 80-90μ in diameter (Leaf and stem).</td>
<td>-30-50μ, thin-walled, nucleated, multicellular, multiseriate (flower).</td>
<td>-Large, 330μ-1300μ, multicellular, uniseriate, tips sometimes barbed (Leaf and stem).</td>
<td></td>
</tr>
<tr>
<td>9. Stem fibres</td>
<td>325 to 500μ long</td>
<td>Absent</td>
<td>300 to 550μ long</td>
<td>Absent</td>
<td>Present but not measured</td>
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<tr>
<td>10. Calcium-oxalate crystals</td>
<td>Needle</td>
<td>Absent</td>
<td>Rosettes, 8-25μ</td>
<td>Rosettes, 32-85μ</td>
<td>Absent</td>
</tr>
<tr>
<td>11. Tannin cells</td>
<td>Form a tube</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>12. Pollen grains</td>
<td>20-25μ, rounded, spiny</td>
<td>16-25μ, rounded, spiny</td>
<td>27-30μ, rounded, spiny</td>
<td>16-20μ, hexagonal to rounded, smooth</td>
<td></td>
</tr>
</tbody>
</table>

The microscopical characters of *E alba* as described in the literature are mentioned below:

**Leaf:** 290-296

Transverse section of the leaf passing through the midrib shows single layered upper and lower epidermis covered with thin cuticle. It contains anomocytic and anisocytic stomata and simple covering multicellular trichomes- more in number on the lower surface. Mesophyll tissue is composed of both palisade cells- 2 layered lying below the upper epidermis and spongy parenchyma traversed with small collateral vascular bundles. Midrib contains five vascular bundles, the central one being the largest in size.

**Stem:** 290-296

The transverse section of stem shows single layer of epidermis covered with thin cuticle and few unicellular hairs. Hypodermis is made up of collenchymatous cells. There are many air cavities in the inner cortical parenchyma. Vascular bundles are open, collateral, arranged in the form of a ring, and are covered externally with sclerenchymatous caps. Vessels are pitted and spiral. Fibres are thick-walled, lignified and with bifurcate tips. Pith is large, parenchymatous and contains acicular crystals of calcium oxalate.

**Root:** 290, 292B, 297

Except three references 290, 292B, 297, which mention few salient microscopical features as mentioned below, no one has studied the detail microscopical characters of the root.

The root has a diarch structure with normal secondary growth. The endodermis is indistinct. Few layers of cork cells are present. Secondary cortex cells are elongated or rounded in shape with air cavities. Stone cells are scattered in the secondary cortex. Medullary rays are multiseriate, rarely uni to biseriate.
The diagnostic characters of the powders are as follows:

**Leaf**: Multicellular hairs with calcium oxalate prisms, parenchymatous cells with secretory cells, anomocytic and anisocytic stomata and annular to spirally thickened xylem vessels.

**Stem**: Unicellular trichomes with bulbous base, reticulate to pitted thickened xylem vessels, and thick walled group of fibres.

**Root**: Cork cells; pitted, thin-walled stone cells and xylem vessels and starch grains.

Subba Rao et al. (1983) have studied the effect of heavy oil effluent on morphological characters and pigment concentration of *E. alba*. Decrease in pigment concentration and increase in the number of branches, leaves, flower buds, fruits and leaf area of *E. alba* growing in polluted places have been reported.

Shrotiya & Singh (1986) have reported growth performance study of *E. alba* in relation to Cadmium treatment. Cadmium, a heavy metal was found to be toxic to the growth of the plant at all the concentrations tested. Tolerance of the plant to some degree was also noticed.

Siddiqui et al. (1987) have studied the salinity effect of sodium sulphate (S.S.) on seed germination of *E. alba*. Seeds of two population (saline & non saline) of *E. alba* were germinated in various concentrations of sodium sulphate containing ethrel. Exogenous application of ethrel inhibited the seed germination in both population and germination continued up to 6 atm. only in saline population. Increasing concentration of S.S. also lowered % germination of seeds in both the populations. Higher concentration of ethrel reduced % of germination of seed in non saline population.

Saeed et al. (1996) have carried out comparative cytological studies of T.S of fresh herb and dried powder of various parts of *Conyza ambigua, Eclipta alba, Sonchus asper*. Chloroform & methanol extracts of the leaves, roots and flowers of all the herbs exhibited prominent erythema on mice’s skin. Extracts of *Sonchus asper* flowers were more potent than the other extracts of the same species and also the other herbs. The possible relationship of anatomical structures with phytochemistry and irritancy of these species has been discussed.

### 5.1.4. CHEMICAL CONSTITUENTS:

Different types of chemical constituents like alkaloids, alkenynes, cardiac glycosides, coumarins, flavonoids, lipids, polyacetylene compounds, triterpenes, steroids, saponins, steroidal alkaloids etc. have been reported by various workers in different parts of *E. prostrata* from different countries. Genetic, environmental or other factors like geographical source, season and time of collection may also cause the variability in these constituents where by contradictory reports regarding presence or absence of certain constituents are found to exist. The reported compounds are mentioned below. The place from where these reports are published and parts of plant are mentioned in the bracket.
Entire plant (India) is reported to contain an alkaloid nicotine301 but other reports 296,302-306 have shown its absence in the different parts of the plant. It is also reported to contain Alkanes- henriciacontan-1-ol307 (Rt- India), heptacosan-14-ol307 (Rt- India) and heptacosane-n308 (Ar- India); Alkyne- tetradeca-4-6-diene-8-10-12 triyne (Rt), trideca-1-ene-3-5-7-9-11-pentayne (Rt), trideca-cis-1-7-diene-3-5-9-11-tetrayne-8-methyl sulfonate (Rt), trideca-trans-1-7-diene-3-5-9-11-tetrayne-5-methyl sulfonate309 (Rt) and cardiac glycosides302 (Ent- Nepal). Coumarins - wedelolactone66,310-318 and demethylwedelolactone66,311,314,319 are hepatoprotective66 constituents of the plant. It also contains demethylwedelolactone-7-0-β-D-glucoside311. Flavonoids - cynaroside318 (Ar- Egypt), apigenin66 (Ar- India) and unspecified304 type (Ar- Iran); Lipids - heptacosan-5-one-1-ol-myristate308 (Ar- India) and pentadeca-1-ol-palmitate-11-hydroxy308 (Ar- India) are also reported to be present. Roots and aerial parts are reported to contain various types of number of (more than 26 number) polycyclic sulfur compounds like - bithienyl derivatives320-322, terthienyl derivatives309,323, thiophene derivatives309,320,323. Triterpenes - β- amyrin318 (Ar- Egypt), enchinocystic acid324 (Ent- China), Eclabasaponins325,326 1 to X, triterpene acid glucoside318, Ecliptasaponin A324, Ecliptasaponin B324 and Ecliptasaponin D327; oleanolic acid324, Steroids - β-sitosterol316 (Ar- Brazil) and stigmasterol312 (Ar- India320, Ar- Brazil315, Ent- China317, Rt- India307); haemolytic saponins302,304-306, steroidal alkaloids - Ecliptalbine328 (Lf-Suriname), Verazine and its derivatives328 (Lf-Suriname) are reported to be present in E. prostrata.

N.B.: Abbreviations . Ar=Aerial parts, Ent=Entire herb, Lf=Leaf, Rt=Root

5.1.5. PHARMACOLOGICAL REVIEW:

Eclipta prostrata is reported to have different types of biological activities on different animals and tissues at different dose levels. These activities are produced by various types of extracts obtained from different parts of the plant and are reported by different countries. It has antibacterial, antifungal, antiviral, antyeast, mollucidial, nematocidal, ovicidal, anti diarrhoeal, antihepatotoxic, antihepatitis B, analgesic(weak), anti convulsant(weak), anti-inflammatory, antipyretic, antispasmodic, antihistaminic, antivenin, hypolipidemic, hypotensive, cytotoxic, weak uterus stimulant(pregnant rat) etc. activities. Clinical trials have proved its antihepatotoxic activity. All these activities have been elaborated below. The name of the country where these activities have been reported and part of plant used are mentioned in the bracket.

(1) Antibacterial:
- 80% alcohol extract (Ar- Iraq) is active against Mycobacterium smegmatis at 1 mg/ml303.
- Chloroform and methanolic extracts of the plant(Sudan) are active against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus at 1.0 g/ml362.
- The ethanol extract(Hb- Suriname) is inactive at 50 mg/ml, versus B. subtilis, E coli, P aeruginosa and S aureus349.
• Ethanol extract (Hb- India) is active against *Staphylococcus albus* (100 mcg/disc), *E. coli* (250 mcg/disc), *Shigella flexeri* (250 mcg/disc), *Staphylococcus aureus* (30 mcg/disc) but it is inactive against *Proteus vulgaris* (250 mcg/disc), and other bacteria, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi A, B, C, S. typhi*, *Staphylococcus citreus* at 500 mcg/disc concentration. It has weak activity on *Shigella boydii, Sh. dysenterica, Sh. schmitzii and Sh. sonnei* at 500 mcg/disc concentration.

• Ethanol extract (Lf- India) is inactive at 10 mg/ml against *Corynобacterium diptheriae, Diplococcus pneumoniae, S. aureus, Streptococcus pyogens, Streptococcus viridans*.

• Seeds (India) of the herb when placed on nutrient media have activity against *B. subtilis, E. coli, Pseudomonas cichorri*, but inactive against *Salmonella typhimurium*.

(2) Antifungal activity :
• Ethanol extract (Ar- India) is active against *Helminthosporium turcicum*.
• Ethanol extract (Hb- Suriname) is inactive against *Aspergillus niger* at 50 mg/ml conc.
• Ethanol extract (Hb- India) is inactive against *Microsporum canis, Phylophora jeanselmei, Piedaria hortae, Trichophyton mentagrophytes* and *Microsporus gypseum* at 10 mg/ml conc.

(3) Antiviral activity :
• Ethanol:water (1:1) extract (Hb- India) has weak antiviral activity against Ranikhet virus at 50 mcg/ml concentration.
• Juice (undiluted) of the leaf (India) has weak activity against Bean mosaic virus.
• Decoction (Hb- China) is active against *Herpes simplex* type 1 at conc. of 100 mg/ml by cell culture method.
• Alcohol extract (Hb- China) has weak activity against *Herpes simplex* at 10 mg/ml concentration.
• Ethyl acetate extract of the plant is inactive at 100 mcg/ml while water extract has weak activity against *Herpes simplex* virus-1 at the same concentration.
• Water extract of the plant is inactive against HIV type 1 (Protease inhibition) at 200 mcg/ml concentration.

(4) Antiyeast activity :
• 80% ethanol extract (Ar- Iraq) is inactive against *Candida albicans* at 1 mg/ml.
• Ethanol extract (Hb-Surinam) is also inactive even at higher concentration (50 mg/ml) against *Candida albicans*.
• Ethanol, water and hexane extracts (Hb- India) are inactive against *Candida albicans* and *Candida tropicalis* at 10 mg/ml conc.
• Methanol extract (Hb-Surinam) is active against *Saccharomyces cervisiae* strain 1138, *S. cervisiae* strain 1140 and *S. cervisiae* strain 1353 at the conc. 16 mcg, 26 mcg and 9 mcg per ml respectively.
(5) Mollucidal activity:
- Ethanol extracts (Lf & St, Brazil) are active against *Biomphalaria glabrata* at 100 ppm conc. but the hexane extract is inactive at 100 ppm concentration. 

(6) Anthelmintic activity:
- Ethanol, water and n-hexane extracts (Lf- India) have no anthelmintic activity at 10 mg/kg. 

(7) Antinematodal (Nematocidal) activity:
- Water extract (Hb- India) has strong activity against *Meloidogyne incognita*. 

(8) Ovicidal activity:
- Water extract (St & Rt- India) is active against ova of *Sitostroga Cerealella*, ED50 is 25% extract. 

(9) Antidiarrhoeal activity:
- Hot and cold water extracts (Hb- India) are active against *E. coli* enterotoxin induced secretion at 300 mg dose, on rabbit and guinea pig ileal loop. 

(10) Antihepatotoxic activity:
- Leaf juice (Burma) is hepatoprotective to female guinea pig by oral route if given four days prior to CCl4 treatment. 
- Chloroform and water extracts (Hb- India) are active against Hepatitis B surface antigen at 2% concentration. 
- Ethyl acetate fraction of the methanol extract (Hb- India) is active at 0.1 mg/ml conc against rat hepatocytes intoxicated with CCl4 and galactosamine; P<0.01 and P<0.001 respectively. 
- Aerial powder of the herb 500 mg/kg for 9 days, by gastric intubation has hepatoprotective activity in male rats intoxicated with CCl4, inhibit AAT, GTP, AP. Ethanol extract 1 mg/ml stabilized human RBC membrane. 
- Ethanol extract (Hb- India) is active versus CCl4 toxicity in the rats. 
- Ethanol extract (Hb- India) 50 mg/kg oral, increases the bile flow and liver weight in the rats. 
- Extracts of aerial parts and roots are reported to have antihepatitis B surface antigen activity in *in vitro* studies. Red pigment was the active constituent. 
- Red pigment of the herb (0.85 Rf) inactivates hepatitis B surface antigen in vitro. 
- Total aqueous and successive aqueous extracts (Hb- India) have hepatoprotective activity (20% and 12% activity respectively) versus CCl4 toxicity in rats at 500 mg/kg/i.p.; chloroform, methanol and petrol extracts are inactive. Ethanol extract (Ar- India) 62.5 mg/kg/intragastric to mouse inhibit zoxazolamine induced paralysis. ED50, 175.9 mg/kg/intragastric to mouse decreases bromosulphalein clearance time, ED50, 156.7 mg/kg/intragastric to mouse reduces barbiturate sleeping time in CCl4 treated animals.
• Ethanol extract (Hb- India) is hepatoprotective to rat and mouse 381.
• Ethanol extract (Hb- India) is active against CCl₄ toxicity in rabbit at 100 mg/kg/intragastric dose 386.
• 60% ethanol extract is active against Hepatitis B-virus (DNA polymerase inhibitor) at 10 mg/ml in the cell culture 366.

**Polyherbal formulations:**
• Eclinol- a polyherbal formulation (PHF- India) reduces liver and serum lipids in rats by oral route, which are induced by CCl₄ 367.
  • PHF (four herbs- India) is active versus CCl₄ toxicity in rats 374
  • A herbomineral preparation (India) is also active in rats versus hepatocellular jaundice 378.
• Hepatogard, a PHF (India) is active versus CCl₄ toxicity in rats 379.
• Hepatomed, a PHF (India) 3ml/100gm for 15 days is active versus cumene hydroperoxide toxicity 28.

(11) Analgesic activity:
• Hot water extract (Hb- Nepal) at 500 mg/kg/intragastrically has a weak analgesic activity in mouse versus acetic acid injury but was inactive against hot plate 348.

(12) Anticonvulsant activity:
• Hot water extract (Hb- Nepal) at 500 mg/kg/intragastric has a weak anticonvulsant activity versus superamxial electroshock induced convulsions in male mouse but inactive versus strychnine induced convulsions 348.

(13) Anti-inflammatory activity:
• Powder of aerial parts, 1.5 g/kg/intragastric, is active against carrageenin induced pedal oedema in male rats 151.
• Decoction (Hb- India), 1.0 g/kg/intragastric is active versus chronic inflammation in rats but weak activity versus carrageenin induced pedal oedema and andjuvant induced arthrities 357.

(14) Antimalarial activity:
• Ethanol:water (1:1) extract (Hb-India) is inactive against *Plasmodium berghei* at 100 mcg/ml concentration *in vitro* and 1 g/kg/intragastric in mouse 334.

(15) Antioxidant activity:
• Herb (India), (EC₅₀ 2.28 mg/ml) inhibits lipid peroxide formation and scavenges hydroxyl radical 385.

(16) Antipyretic activity:
• Hot water extract (Hb- Nepal) has antipyretic activity at 500 mg/kg/ gastric intubation in male mouse 348.
(17) **Antispasmodic activity:**
- Alcohol : water(1:1) extract (Hb- India) has antispasmodic activity versus acetyl choline and histamine induced spasms on guinea pig ileum 336.
- Hot water extract (Hb- Nepal) has no anticholinergic activity in male mouse at 500 mg/kg/gastric intubation 348.
- Decoction (Hb- India) has antihistaminic activity at 1.0 g/kg/gastric intubation in rats 357.

(18) **Antiulcerogenic activity:**
- Cauvery 100, a PHF (India) has antiulcerogenic activity in rats through oral route versus indomethacin induced ulcer 382.

(19) **Antivenin (Antisnake venom) activity:**
- Ether, ethanol(40%) and ethanol(95%) extracts (Brazil) have antivenin activity at 0.5 mg, 2.5mg and 1.8 mg per animal respectively. Hexane extract is inactive (2-3mg/animal)316.
- Water extract has kinase inhibition (skeletal muscle of mouse) and creatinine kinase inhibition (in mouse) activity at 8.5 mcg/ml and 250 mcg/kg respectively 316.
- The plant (Brazil) extract through i.v. route to mouse, protects against snake venom injection 312.
- Herb is having antivenin activity. It is antimyotoxic and antihaemorrhagic also 383.

(20) **Activity on blood:**
- Water extract of the plant, 1.0 g/kg/i.p. has a haemostatic activity in mouse 340.
- Methanol extract (IC50 2.0 mcg/ml) and wedelolactone (IC50 2.5μmol) have lipoxygenase-5-inhibitory activity on leucocytes of pig 347.
- Hexane extracts (Lf & St- Thailand) has no coagulant, fibrinolytic or platelet aggregation stimulation activity364.
- PHF, Abana (India), 50 mg/kg/intragastric has hypolipidemic activity in rat 368.

(21) **Blood pressure:**
- Ethanol:water(1:1) extract(Hb- India) has hypotensive activity on dog at 50 mg/kg/i.v. 336.
- Ethanol extract and columbin (the active compound) have hypotensive activity on anaesthetized cat 380.

(22) **Cytotoxic activity:**
- Methanol extract of the herb is active against CA-Ehrlich Ascites cell culture at 1.25 mg/ml. 337.
- Water extract (Ar- China) is inactive at 500 mcg/ml versus CA-mammary microalveolar cell culture 330.
- Ethanol:water(1:1) extract (Hb- India) is inactive at ED50 >20.0 mcg/ml versus CA-9KB cell culture 336.
- Water and ethyl acetate extracts of the herb are inactive at 100 mcg/ml in cellvero 361.

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(23) **Tranquilizing activity:**
- Hot water extract (Hb- Nepal) is neither having tranquilizing activity nor spontaneous activity at 500 mg/kg/gastric intubation to male mouse.  

(24) **Uterine activity:**
- Water extract (Lf- India) has no stimulant activity on the uterus of non pregnant guinea pig.  
- Water extract (Rt- India) is having no stimulant activity on the uterus of rat.  
- Hot water extract of the herb has no stimulant activity on the uterus of non-pregnant rat but has a weak activity on the uterus of pregnant rat.  
- Ethanol:water(1:1) extract (Ar), 100 mg/kg/oral has no anti-implantation activity on the female rat.  

**Clinical Trials - *E. prostrata* alone:**

**Antihepatotoxic activity:**
- Powder (Hb- India), 50 mg/kg/oral has antihpatoxicity activity, 40 children recovered from jaundice out of 50.  
- Powder (Hb- India), 500 mg/person, three times a day for 3-4 weeks cured 55% of hepatic patients and for 8 weeks treatment cured 75% patients.  

**Dyspepsia:**
- 20 g powder (Hb- India) in a syrup base in three divided doses (oral) for six weeks, cured 90% of patients (out of 30) from nonulcerative and peptic ulcer dyspepsia by reducing the gastric acidity.  

**Gastritis:**
- 12 gm of the herb powder (India) in three divided doses (oral) for 45 days has excellent response in 52% patients (out of 25) of gastritis by reducing the gastric acidity.  

**Clinical Trials with Polyherbal Formulations (PHF):**
- Shen Mari Yin (China) has good anti-aids activity if taken for 8 months orally.  
- Tefrolti (India) is active against hepatitis virus, orally.  
- Geriforte and Rumalaya (India) are active against inflammation, orally.  
- Pilex tablet and ointment (India) orally and externally respectively have anti-inflammatory activity.  
- A polyherbal capsule(1 cap. twice a day for 3, 6, 9, 12 months, orally) and the cream (externally, twice a day) cured 40 to 66% patients of leucoderma(out of 40 patients).  
- A polyherbal (six herbs) capsule (450 mg/oral), for 15 days was active against renal calculi.  
- A polyherbal (extracts of 10 herbs) tablet, 1 tablet thrice a day for 21 days/oral has excellent result in 25/50 patients in dyspepsia(Amlapitta).  
- A polyherbal preparation(including fruit of *E. alba*) containing vitamins is active externally in controlling grey hair (China).
Toxicity:
- Alcohol extract (India), 2g/kg/mouse/intragastric has no toxicity in mouse.
- 0.2 mg ext./day/oral route in mouse for 90 days has no mortality or weight loss.
- Ethanol:water (1:1) extract (India) 1 mg/kg/i.p. in mouse is the maximum tolerated dose.

Abbreviations: Ar = Aerial parts; Hb = Herb; Lf = Leaf; Rt = Root; St = Stem

5.1.6. TISSUE CULTURE:
- Das et al. (1991) have developed callus culture from the young stem of E. prostrata on revised M. S. medium supplemented with 2,4-D, N.A.A. and kinetin. It was subcultured on M. S. media supplemented with N.A.A. and kinetin as well as I A.A. and kinetin. Wedelolactone and demethyl wedelolactone were present in the callus of all the three media.
- Franca et al. (1995) have established a protocol for in vitro micropropogation of E. prostrata from the nodal segment explants. The maximum number of shoots were obtained after 60 days of culture in Murashige and Skoog (MS) medium supplemented with 4.4 μm benzyladenine. Multiple rooting was achieved using MS medium supplemented with 2.4 μm 2-isopentyladenine. Wedelolactone was present in shoots cultured in media containing cytokinins.

5.1.7. ANALYSIS OF ACTIVE CONSTITUENTS:
Only two methods have been described for the estimation of the active constituents, wedelolactone and demethyl wedelolactone in E. prostrata herb.
1. Wagner et al. (1986) have described a HPLC method using C_{18} column, U.V. detector (254nm) and gradient mixture of (A) water and (B) acetonitrile as a mobile phase. Both A and B contain 10 ml of 0.1 M phosphoric acid per litre, and concentration of B was increased from 15% to 25% in 25 minutes.
2. Das et al. (1990) have described a spectrophotometric method using 351 nm for the estimation of wedelolactone and demethylwedelolactone.

5.1.8. ANOTHER RELATED INDIAN HERB:
*Wedelia chinensis* (Osbeck.)Merr. (a substitute of *E. prostrata*):

SYNONYM: *Wedelia calendulacea* (Linn.) Less.
DISTRIBUTION: Bengal, Assam, Kerala, Madras, Thailand, Srilanka, Burma, China, Japan.

*W. chinensis* morphologically resembles in all the characters with *E alba*, except the colour of its flower which is yellow and hence is known as “Peeta Bhangra”. Ayurvedic physicians believe that this plant is superior than that of *E alba* and recommend its use in hepatoprotective formulations but the recent reports say that they are less active.

A perennial herb 0.3-0.9m high; stem procumbent at the base and rooting at the lower nodes, terete, more or less appressedly hairy. Leaves opposite, subsessile, 2.5-7.5
cm by 1-2.8 cm, lanceolate-oblong, entire or irregularly crenate-serrate, scabrous with short white hairs or at length more or less glabrate, base tapering. Heads 2-3.2 cm diameter, solitary, peduncle 2.5-15 cm long, erect, slender, slightly thickened beneath the heads. Involutrals bracts herbaceous, oblong or slightly obovate, hairy, subobtuse, much longer than the disk flowers. Disc florets bisexual, corolla tubular, 5-lobed. Ray-flower ligulate, ligule yellow, 2-3 toothed. Style-arms of female flowers long, acute, recurved. Pappus a toothed membranous cup. Achenes of the ray tapering, slightly pubescent; disc achenes compressed and tuberculate.

Summarising the overall review of the literature of the herb, *E prostrata*, the following investigations were thought worth to carry out. To study in detail microscopy of leaf, stem and root.

1. Sufficient details have not been found to be reported for the histological characters of these parts of the plant. Hence they also need further study.

2. Some controversy exists regarding the cell contents of the stem, e.g. Patel mentions the presence of prisms while Gopalkrishnan et al. mention the presence of Ca-oxalate raphides in the parenchymatous cells of the pith. This needs further clarification.

Except Patel none has mentioned the presence of Ca-oxalate prisms in the trichomes of the leaf and rosette in the parenchymatous cells of the mesophyll. This also needs further clarification.

(II) Evaluation of the quality of the polyherbal formulation by estimating the active principles of the incorporated herbs is very essential. But as yet none of such formulations have been found evaluated for this. Hence it was thought worth to estimate wedelolactone- the active constituent of *E prostrata* in polyherbal hepatoprotective formulations.

5.2. *Andrographis Wall.*

A genus of erect or procumbent annual herbs or small shrubs, distributed in tropical Asia. About 21 species are reported to be present in India, but only 4 are available. They are *A paniculata, A. echiodes, A. serpyllifolia* and *A wightiana*

*Andrographis paniculata* (Burm. f.) Wall. ex Nees. : 432-436

5.2.1 INTRODUCTION :

SYNONYM: 434

*Justicia paniculata* Burm. f.

*Andrographis subspathulata* C.B.Clarke.

PARTS USED: In Ayurvedic system of medicine, whole herb is used.

FAMILY: Acanthaceae
Andrographis paniculata (Burm.f.) Wallich ex Nees. 1, Twig; 2, Flower bud; 3-4, Flowers.
COMMON NAMES:
Bengal: Kabnegh, Mahatita
English: The creat, King of bitter
Gujarati: Kariyatu, Kiryata
Hindi: Charayetah, Kiryat, Mahatita
Malayalam: Kiriyattu, Nalaveppu
Marathi: Olikiryata
Sanskrit: Bhunimba
Tamil: Nilavembu
Telugu: Nelaveemu

CONTROVERSY REGARDING COMMON NAMES:
Ayurvedic system of medicine prescribes the use of the entire herb, but the aerial parts of the plant are found to be used often in medicine.
Common names many times create confusions in the correct identification of the plant, e.g. under its common names, Kanyatu (in Gujarati) other drugs like Picrorhiza kurroa and Swertia chirata are also available in the market. Similarly its other common name Bhunimba (in Sanskrit) is also used for naming other drug, S. chirata. Hence to overcome this confusion its Ayurvedic name ‘Kalmegh’ should be used for this plant.

DISTRIBUTION:
The plant is found wild throughout tropical India and Srilanka.
It is common in uncultivated ground and also as an undergrowth in forests.
Chiefly found from Himachal Pradesh to Assam and Mizoram and all over South India.

DESCRIPTION:
Much branched, erect, annual herb. 0.3 to 1m in height, stem dark green 2-6mm in diameter and quadrangular with longitudinal furrows and wings on the angles of the younger parts, slightly enlarged at nodes; leaves simple opposite, short petioled (0.6-0.8cm) up to 8.0 cm long and 2.5cm broad, lanceolate, pinnate. Flowers small, white with purplish blotches in terminal and axillary panicles. Calyx 5-partite, glandular-pubescent; corolla bilabiate, hairy outside, the lower lip deeply 3-lobed, deflexed; upper oblong, slightly 2-fid; stamens 2, filaments hairy, anthers 2-celled, connate, deep purple; fruit a linear-oblong compressed capsule, longitudinally furrowed on broad faces, 20mm x 3mm; Seed numerous, sub quadrate, yellowish brown.

Medicinal uses:
Survey of the Ayurvedic literature revealed the following medicinal uses of the plant:
- **Tonic and Antipyretic**: The drug is bitter and is reputed as a bitter tonic. The decoction or infusion of the leaves is used in general debility and dyspepsia. The tincture of the root is stimulant and apparant. The drug is recommended in cases of various types of fevers especially intermittent fevers, “sannipat” fever, influenza etc.
• **Blood purifier**: It is used in various types of skin diseases like itching, swelling, ulcers. It is one of the major ingredients in Ayurvedic formulation “SG-1 Swattendilepa” which is effective in treating vitiligo- a dermatological disease.

• **Antidisenteric and Anthelmintic**: It is astringent and laxative and is used in cases of dysentry. It is used for removing worms. The macerated leaves and juice together with certain spices such as cardamom, clove and cinnamon, called as ‘Alui’ in Bengal, is dried in sun and small pills are prepared out of this. These pills are prescribed for infants to relieve griping, irregular stools and loss of appetite. It is given in cases of oedema to reduce swellings.

• **Its other therapeutic uses** are: It is anodyne and possesses alexipharmic, antidiabetic, cooling properties. It overcomes difficulty in breathing, hemopathy, morbidity of kapha and pitta, burning sensation, thirst, acidity and liver complaints.

5.2.2 ETHNOMEDICAL USES:

The drug is highly valued amongst the tribal people residing in various parts of the country. The survey of the literature regarding these uses are found to be much more similar with that which have been mentioned above. These uses have been discussed below:

**Anaemia**: People of Kani tribe (Kerala) use decoction of *A. paniculata* in anaemic condition.533

**Apettizer**: People of Kani tribe (Kerala) and Chenchu tribe (A.P.) use decoction of the plant as a good appetizer.533,534

**Anthelmintic**: Two teaspoonful of decoction of the leaf is drunk as an anthelmintic three times a day by the people of Kondh tribe (Dhenkanal district, Orissa).535

**Scabies**: Above decoction is also used in similar way by same tribe for scabies.535

**Antimalarial**: People of Kanitribe (Kerala) use decoction of mixture of *A. paniculata*, *Ocimum sanctum* and dried rhizomes of *Gingiber officinalis* for malaria.533 People of Chenchu tribe (A.P.) drink decoction of *A. paniculata* with jaggary for malaria.534

**Cholera**: People of Chenchu tribe(A.P.) mix leaf juice of *A. paniculata* with decoction of bark of *Azadirachta indica* and leaf juice of *Tinospora cordifolia*, it is drunk with sugar in cholera.534

**Cough and Cold**: The tribal people of Chittoor district use leaf powder internally for cough, cold and fever due to cold.536

**Jaundice**: The traditional healers of Chittoor district(A.P.) take shade dried mature plant of *A. paniculata* and powder it with a few pieces of garlic; 3g of this is given orally with buttermilk two times a day for four days in acute jaundice.537

**Snake bite**: The tribal people of Chittoor district use leaf powder internally for snake bite.536

5.2.3: PHARMACOGNOSTICAL REVIEW:

Prasad et al. (1957)438 have described complete morphology and microscopy of all the aerial parts of the plant viz. leaf, stem, flower and fruit. The salient microscopical features of their observations are as follows:

**Stem**: Stem is quadrangular particularly in the upper regions with four buldges arising at the four corners. Epidermis is characterised by the presence of glandular (unicellular stalk
and 8 celled head) and simple covering (mostly unicellular) trichomes, caryophyllaceous stomata and cystolith. Endodermis is distinct. Cortex and pith contain acicular crystals of calcium oxalate.

**Leaf**: It is attached at the base with small winged petiole. Glandular and simple (1-3 celled) trichomes are similar to that of stem. Stomata are present on the lower surface only. Cystoliths are present in the epidermis of the lamina and midrib cells.

**Floral axis**: Floral axis has a structure essentially similar to that of the stem.

I.P. '55\(^{437}\) has also described the morphology and microscopy of leaf and stem. Singh et al. (1972)\(^{439}\) have carried out pharmacobotanic studies on the plant. Their morphological and microscopic studies tally with that carried out by Prasad et al. They have grown the plants by seed propagation.

Washi (1980)\(^{440}\) has studied the patterns of energy activation in *A. paniculata* as influenced by various levels of light intensity. Plants grown under shade give maximum dry matter.

Chen and Jiang (1980)\(^{441}\) have reported the anatomical features of leaves and stems of *A. paniculata*, *Strobilanthes cusia* (*Baphicacanthus cusia*), *Didiptera crinata* and *S. japonicus*.

Roy et al., (1985)\(^{442}\) have reported complete pharmacognosy of Kalmegh.

Edwin and Chungath (1987)\(^{443}\), have described the distinguishing microscopic and fluorescence characters of *A. paniculata* and *Swertia chirata*. In *S. chirata* the stomata are anisocytic type and trichomes are of covering type while in *A. paniculata* stomata are dacytic and trichomes are covering as well as of glandular type.

Indian Herbal Pharmacopoeia (1998)\(^{436}\) has mentioned the microscopic characters of the leaf and stem and their powders.

Banerjee and Datta (1991)\(^{444}\) have studied the effect of season, age, phase and hormones on accumulation of medicinal compound in *A. paniculata*.

Girach et al., (1994)\(^{445}\) have given enough reasons on the basis of similar vernacular names and therapeutic action to accept *A. paniculata* as a possible substitute for *Swertia chirata*.

Gupta and Srivastava (1995)\(^{446}\) have studied 10 wildly growing populations of *A. paniculata*. The highest biological activity was recorded in IC-111287 and maximum andrographolide yield was found in IC-111288.

Kapoor (1997)\(^{447}\) has studied the total nitrogen content of the plant and the factors affecting its percentage. Light was found to favour the accumulation of the nitrogen while advanced growth stage has adverse effect, causing decrease in its nitrogen content.

Munirampara and Farroqui (1997)\(^{448}\) have studied the effect of certain elements like nitrogen (0,50,75 and 100 kg/ha), phosphorous (0,25,50 and 75 kg/ha) and potassium (50 kg/ha) on the growth of the plant. Nitrogen (100 kg/ha) was most effective for inducing the maximum height of the plant and for yielding maximum fresh and dry weight of the plant.

Padmesh and Seeeni (1998)\(^{449}\) used Random Amplified Polymeric DNA (RAPD), for studying the molecular biology and found the existence of moderately high genetic variability in this species.
Sabu and Seeni (1998)\textsuperscript{450} have studied the patterns on allozyme diversity in natural population of \textit{A. paniculata}.

Kapur (1999)\textsuperscript{451} has studied the effect of light on andrographide content of the plants, the fully exposed plants showed maximum andrographide content.

5.2.4 : CHEMICAL CONSTITUENTS :

The major chemical investigation has been carried out on \textit{A. paniculata} amongst the four available species of \textit{Andrographus} in India. Various chemical constituents which include numerous diterpenoids, flavonoids and their glycosides etc. have been isolated from the different parts of the plant. They have been described by Bhat and Nanavati (1978)\textsuperscript{452} and Saxena et al. (1998)\textsuperscript{453} in their review articles as follows.

Diterpenoids :- Andrographide, a main, bitter, colorless, crystalline diterpene lactone (M.P. 231°C) was first isolated by Boorsma (1896)\textsuperscript{454} and its structure was proved by Gorter (1911)\textsuperscript{455} and Cava et al. (1962). The second diterpene lactone isolated was a non bitter glucoside, neoandrographide (M.P. 167-168°C) by Kleipool (1952)\textsuperscript{457}. Its structure was proved by Cava et al. (1965)\textsuperscript{458}. Besides these two major compounds, several other minor diterpenoids and few of their glycosides were reported from the plant. They are,

- 14-Deoxy-11-12-didehydroandrographide\textsuperscript{459}, 14-deoxy-11-oxoandrographide\textsuperscript{459}, 14-deoxoandrographide\textsuperscript{459}, 3-14-dideoxy andrographide (Androgranin)\textsuperscript{460,461}, Andrographoside, 14-deoxyandrographoside (Andropanoside), 14-deoxy-12-methoxyandrographide\textsuperscript{460-461}, Procumbide\textsuperscript{462}, Ent-14B(1H)-8(17), 12-labddenien-16,15-olde-3B, 19-oxide\textsuperscript{463}, a major compound in Malaysian \textit{A. paniculata}, 14-Epiandrographide\textsuperscript{464}, isoandrographide12-epi-14-deoxy12-methoxy andrographide, 14-Deoxy-12-hydroxyandrographide, 14-Deoxy-11-hydroxyandrographide, 14-deoxy-11,12-didehydroandrographide, 6'-acetylneoandrographide, Bisandrographide A, Bisandrographide B, Bisandrographide C, Bisandrographide D\textsuperscript{464}.

Flavonoids : Two flavone derivatives, oroxyllin and wagnon were isolated from the leaves\textsuperscript{467} and the following flavonoid compounds were reported from the root:

- 5-hydroxy-7,8,2',3'-tetramethoxy flavone\textsuperscript{468}, Apigenin-7,4'-di-O-methylether\textsuperscript{468-470}, Panicolin(2',5-dihydroxy-7,8, dimethoxy flavone)\textsuperscript{469-470}, Andrographin(5-hydroxy-2,7,8 trimethoxyflavone), 5-hydroxy-7,8-dimethoxy flavone (7-O-methyl wognin), 5-hydroxy-3,7,7,2'-tetramethoxyflavone\textsuperscript{471}, 5-hydroxy-7,8-dimethoxyflavonone, Andrographidine A\textsuperscript{472}, Andrographidine B, Andrographidine C, Andrographidine D, Andrographidine E, Andrographidine F\textsuperscript{472}.

Miscellaneous : The polyphenols- Caffeic acid, chlorogenic acid and mixture of dicaffeoylquinic acid\textsuperscript{475}, Carvacrol, eugenol, myristic acid, hentriacontane and tritriacontane\textsuperscript{476} were reported from the leaves of \textit{A. paniculata}. In addition, two acidic polysaccharides PA and PB were isolated from the pectin of the plant. The acidic polysaccharide PA contains galactose, arabinose and rhamnose in a ratio of 5:2.8:1 and 25.6% of galactouronic acid. The polysaccharide PB contains galactose, arabinose and rhamnose in a ratio of 3.4 : 1.7 : 1.\textsuperscript{477}
A. paniculata, because of its importance in indigenous system of medicine, is one of the most extensively investigated medicinal plants. A broad spectrum of biological activities have been reported with different parts and different preparation of the plant parts. In addition, its major phytochemical constituent, andrographolide and related diterpenoids have also been extensively studied. The reported biological activities have been enumerated below.

1. **Immunomodulation**:
   - The water soluble fraction of the extract of herb and water insoluble fraction was given through oral route to mice to assess the effect on cell involved phagocytosis through carbon clearance test on humoral antibody response and on cell mediated immune response by delayed hypersensitivity test. The test drug stimulated humoral response. The water insoluble portion stimulated immunity. In contrast to this the water soluble portion showed suppression.
   - The ethanol extract of *A. paniculata* and the purified diterpene andrographolide induced significant stimulation of antibody and delayed type hypersensitivity response to sheep red blood cells in mice. The extract also stimulated nonspecific immune response of the animal and proliferation of splenic lymphocytes. The stimulation of both antigen specific and nonspecific immune response was, however of lower order with andrographolide than with ethanol extract, suggesting thereby that substance(s) other than andrographolide present in the extract may also be contributing to immunostimulation.

2. **Antiallergic**: Andrographolide and neoandrographolide are reported to produce antiallergic activity in a dose dependent manner, (dose of 10, 50 and 100 mg/kg, p.o.); when tested against passive cutaneous anaphylaxis (PCA) in rats. They also inhibited induced mast cell membrane stabilizing effect. These findings are corroborated by Madhav et al. They have reported that andrographolide produced significant decrease in mast cell degranulation and histamine release from them at the dose level of 30, 100 and 300 μg/ml. However, it prolonged delayed hypersensitive reaction.

3. **Antimalarial**: *A. paniculata* was studied in a four day suppressive test against *Plasmodium berghei* NK 65 in mastomys. The crude ethanol extract and its fractions reduced the level of parasitaemia in a dose dependent manner. The n-butanol fraction was more effective than hexane, chloroform and aqueous fractions. Among the four diterpenes isolated from the n-butanol fraction, neoandrographolide and deoxyandrographolide were more effective than andrographolide and andrographoside in the inhibition of parasitaemia.

4. **Antifertility**:
   - Six week old male and female mice were put on diets supplemented to the extent of 0.75% with powdered stem of *A. paniculata* for one, two, three and four weeks and effect on fertility and gestational period observed. Significant reduction in fertility after three and four weeks of feeding in the group containing the treated males and
untreated females was observed; gestation period was also prolonged after four weeks of feeding. There was virtually no change in fertility and gestational period in the group having treated females and untreated males.

- Akbarsha et al. (1990) gave 20mg powder of *A. paniculata* to albino rats orally per day for 60 days, it resulted in cessation of spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells and degenerative changes in the epididymis, seminal vesicles, ventral prostate and coagulating gland. The treatment also resulted in accumulation of glycogen and cholesterol in testis and increased activities of lactate dehydrogenase in testis and alkaline phosphatase in testis and ventral prostate. The results suggest antispermatic and/or antiandrogenic effect of the plant.

- Muragaian and co-workers evaluated the effect of administration of andrographolide suspension in gum acacia in a dose of 10 mg/day for 45 days on male rats. Histological analysis and observations made during the study indicated that andrographolide has the potential to be developed as an antifertility agent.

- However, Burgos and co-workers have reported that administration of a standardized dried 70% alcoholic extract containing 5.6% andrographolide did not produce any toxic effect on testis when administered to male rats for 60 days in the dose of 20, 200 and 1000 mg/kg through oral route. The parameter assessed were ponderable changes in the reproductive organ weight, testicular histology, ultrastructural analysis of Leydig cells and testosterone level. According to these workers the difference between the results obtained in their study and that of Akbarsha and co-workers may be due to the difference in the test material used. While the Burgos team used ethanolic extract of the plant material, Akbarsha and co-workers had used dried leaf powder.

5. *Analgesic, antipyretic and antiulcerogenic activities* :-

- Apigenin-7,4'-di-O-methyl ether, a flavone isolated from *A. paniculata* was tested for its antigastric ulcer property in various animal models. The flavone produced a significant dose dependent antulcer activity in Shay rats, histamine induced ulcer in guineapig and aspirin induced ulcers in rats. It is suggested that antisecretory activity and a protective effect on the gastric mucosa may be responsible for the antiulcer action. *A. paniculata* has antipyretic effect in experimental rats. Oral administration of andrographolide was studied for analgesic, antipyretic and antiulcerogenic activities(30,100 and 300mg/kg, oral). Andrographolide did not show any analgesic activity in hot plate test in mice while it showed significant (P < 0.05) analgesic activity in acetic acid induced writhing in mice and Randall selitto’s test in rats at 300mg/kg dose. Andrographolide(100 and 300 mg/kg, oral) produced significant (P<0.05) antipyretic effect after 3h of administration in Brewer’s yeast induced pyrexia in rats. It also possessed significant (P< 0.05) antiulcerogenic activity at 100 and 300 mg/kg dose in aspirin induced ulceration in rats.

6. *Antithrombotic effect*:

- The flavone fraction obtained from the roots of *A. paniculata*(TFAP) was given to dogs through i.v. administration. The results indicated that TFAP might promote the
synthesis of PG-I and inhibit the production of TxA₂, stimulate the synthesis of cAMP in platelets, impede aggregation of platelets and thereby prevent the formation of thrombi as well as the development of myocardial infarction.⁴⁹¹

- The crude extract of *A paniculata* has been reported to significantly inhibit the one phase and two phase platelet aggregation. The potency of *A paniculata* appears to be stronger than ligustrazine and persanthin injection in vivo. The rapid effect of these drugs on platelet aggregation in vivo suggested that *Andrographis* can be absorbed quickly. Influence on coagulation and thromboelastrogram was determined. The study suggested that *A paniculata* is a promising antithrombogenic agent. This drug could be beneficial in preventing and treating arterial thrombotic disease.⁴⁹²

- *A.paniculata* was found to alleviate significantly atherosclerotic iliac artery stenosis induced by deendothelialization and high cholesterol diet and re-stenosis following angioplasty in rabbits.⁴⁹³

7. **Antidiarrhoeal activity** : The alcoholic extract of *A paniculata* exhibited significant antidiarrhoeal activity against *E.coli* enterotoxins in animal models. The activity was further located in n-butanol fraction, which led to the isolation of four diterpenes. Among the four diterpenes, andrographolide and neoandrographolide showed similar activity to loperamide against (*E.coli*) LT and LT/ST enterotoxins. Andrographolide was found to be superior against ST enterotoxin, the most common cause of epidemics of neonatal diarrhoea.⁴⁹⁴ (LT= Thermolabile, ST= Thermostable).

8. **Antidiabetic activity** : The extract of leaves and stem of *A paniculata* and andrographolide were found to be without effect on blood sugar levels in normal or diabetic rats when administered either by oral or subcutaneous route.⁴⁹⁵ However, in another study, aqueous extract (2g/kg, p.o., rats) has been found to possess antihyperglycaemic effect in glucose tolerance test. This indicates that extract inhibits absorption of glucose from intestine.

9. **Anti-inflammatory activity** :

- Andrographolide and the related compounds, deoxyandrographolide, deoxydidehydroandrographolide and neoandrographolide were tested for anti-inflammatory activity in animals with inflammation caused by croton oil. The anti-inflammatory effects of andrographolide and related compounds were lower than those of corticosteroids and of conventionally used nonsteroidal drugs. The pharmacological effect was highest with deoxydidehydroandrographolide followed by deoxyandrographolides, neoandrographolide and andrographolide. The minimum lethal dose of these compounds by oral administration was greater than 20g/kg. The anti-inflammatory effects of all four compounds was absent in adrenalectomized animals, indicating a possible involvement of the pituitary and adrenal systems. These four compounds did not significantly affect inflammatory hyperplasia and migration of leucocytes into the inflammatory focus. The anti-inflammatory mechanism was speculated to be different from that of conventional drugs.⁴⁹⁷

- An aqueous extract of *A.paniculata*, 20mg/kg body wt. orally showed 65.35% inhibition of oedema induced by carrageenin in rat in comparison to control group.⁴⁹⁸
10. Cell differentiation inducing activity: The methanolic extract of the aerial parts was partitioned between ethyl acetate and water. The ethylacetate fraction and some of the compounds isolated from it showed significant differentiation inducing activity towards MI cells.

11. Hepatoprotective activity:

- Andrographolide has been shown to be hepatoprotective in a number of experimental models including carbon tetrachloride, D-galactosamine, paracetamol, and ethanol induced hepatotoxicity. The aqueous extract of leaf is reported to increases bile flow, lower weight and shorten hexabarital sleeping time, suggesting induction of hepatic drug metabolizing enzymes, similar effects are also reported for andrographolide. An aqueous extract of A. paniculata also decreases CCl₄ induced hepatic microsomal lipid peroxidation. Aqueous extract of A. paniculata has significant liver protective activity in albino rats. Successive methalonic extract has better hepatoprotective activity than successive aqueous and total aqueous extract against CCl₄ induced toxicity in rats.

- Andrographolide was found to be more potent than silymarin, a known hepatoprotective drug against CCl₄, paracetamol and galactosamine toxicity. Andrographolide given by oral route, exhibited dose dependent (3-12 mg/kg) protection against galactosamine induced hepatic damage. Alcoholic extract of the leaves, 300mg/kg prevented CCl₄ induced liver damage.

- Aqueous extract of A. paniculata (12mg/kg) protected liver of mice against tumors induced by hexachlorocyclohexane (BHC).

- Administration of a single dose of leaf extract (0.5g/kg and 1g/kg) and andrographolide (5.0mg/kg and 10mg/kg) to male albino rats, both at higher and lower doses characteristically inhibit hepatic microsomal aniline hydroxylase, N-demethylase and O-demethylase after varying time (4-12 h) of treatment and kinetic study indicates the nature of inhibition to be noncompetitive.

- Crude extracts of A. paniculata, Eclipta alba, Phyllanthus niruri, Ricinus communis, Tinospora cordifolia and Wedelia calendulacea showed in vitro inactivation of hepatitis-B surface antigen.

12. Hypotensive activity:

- Ethylether extract of A. paniculata leaves is reported to possess hypotensive activity. K-21 isolated from A. paniculata produced a positive inotropic effect on frog’s heart.

- The cardiovascular activities of crude water extract (WE) of A. paniculata, its three semipurified fractions ethylacetate (FA), n-butanol (FB) and aqueous (FC) as well as andrographolide have been studied in anaesthetized rats. FA and andrographolide did not affect mean arterial blood pressure (MAP) while WE, FB and FC produced significant fall in MAP in a dose dependent manner without significant decrease in
13. **Anti HIV activity**: Water extract of the leaf of *A paniculata* was tested for inhibitory effect on human immunodeficiency virus type-1 (HIV-1). It was found to be active, ED₅₀ was 70µg/ml for HIV-1 infected MT-4 cells.\(^{516}\)

14. **Antifilarial activity**: Water decoction of *A paniculata* leaves *in vitro* killed microfilarial larvae within 40 minutes. Three subcutaneous injections of the extract injected into infected dogs at 0.06ml/kg body weight reduced the number of microfilaria in body by more than 85% \(^{517}\).

15. **Antimicrobial and Antiprotozoal activity**: Alcoholic (50%) extract of *A paniculata* has no antibacterial activity on *E coli, Streptococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Protease vulgaris*.\(^{518}\) However 10 and 90% methanolic extract of the plant was active against *Salmonella typhosa*\(^{519}\) but inactive against gram positive bacteria *Micrococcus pyrogens var aureus, Streptococcus pyogens, Diplococcus pneumoniae* and *Bacillus subtilis* and gram negative bacteria, *Vibrio comma* and *Shigella dysenteriae*\(^{519}\) and 90% methanolic extracts of the plant have no antifungal activity against *Candida albicans, Microsporum gypseum, Trichophyton mentagrophytes* except *Helminthosporium sativum*\(^{519}\). Alcoholic (50%) extract of the plant also has no antifungal activity against *Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes, Aspergillus fumigatus, Sporotrichum schenckii*\(^{518}\). This extract is devoid of antiprotozoal activity against *Entamoeba histolytica*; and antiviral activity against Ranikhet disease and *Vaccinia virus*\(^{518}\).

16. **Miscellaneous activities**: *A. paniculata* is reported to have good antisnakevenom activity.\(^{520}\)

**Clinical Trials on *A. paniculata***:
- Decoction of *A paniculata* was given to 20 patients of infective hepatitis. Complete remission was observed in 16 patients and good relief in 4 patients at the end of 24 days. Serum transaminase level, bilirubin level and thymol turbidity also dropped to normal level by the end of the study.\(^{521}\).
- In China *A paniculata* is used both in injection and tablet form. The effective dose for adults is 100-150mg of andrographolide. Tablets are used for dysentry, upper respiratory tract infections, tonsillitis and other infectious diseases. In one of the study all the patients suffering from upper respiratory tract infections and 93% of tonsillitis patients recovered after receiving andrographolide tablets. An injection prepared from *A. paniculata* was 92% effective against upper respiratory tract infections when administered at doses of 100-200 mg i.v.\(^{522}\)
- A double blind clinical study was carried out on 53 patients of common cold. *A. paniculata* dried extract at the dose of 1200mg/day for four days decreased symptoms and showed improvement in the recovery from common cold.\(^{523}\)
Polyherbal formulations containing *A. paniculata*:

- **Livereen syrup** and aqueous ext. of *A. paniculata* (3.75 ml/kg in mice Po) increased bile flow and decreased hexabarbital sleeping time significantly\(^5\)
- **Tefroli** when studied on 31 patients of viral hepatitis, showed effectiveness in its management and was safe\(^5\),\(^2\). When male patients (32-55yrs) of cirrhosis were treated with Tefroli for 3 weeks, there was reduction in half life of antipyrine from 29.3hrs to 17.9hrs (Control group 28 hrs to 20 hrs). The formulation also protected rats against CCl\(_4\) treatment\(^5\).
- **PHP-A**, a polyherbal formulation 250mg/kg (p.o.) was given to rats for 12 days, it protected against CCl\(_4\) toxicity\(^5\).
- **Livomyn**, protected rats intoxicated with CCl\(_4\)\(^2\). Its syrup 1ml/100g per day for 2.4 or 6 weeks also protected livers of rats against toxicity of Ketoconazole (an antifungal agent) 20mg/100g for 2.4 or 6 weeks\(^5\).
- Pretreatment with **HD-03** 750mg/kg for 15 days has anticholestatic activity against thioacetamide (100 mg/kg) induced cholestasis in anaesthetized guinea pigs\(^5\).
- **Adliv-75, Biligen, Kalmegh compound, Livarin, Liverin, Livol, Livosin, Livotone, Livotrit, M-Liv, Stimuliv** have also liver protective activity in rats against CCl\(_4\) induced toxicity\(^2\). Uptake of bromosulphalein by liver slices of rats treated with CCl\(_4\) and LIVP-7 was increased\(^1\).
- **Ayush-57** showed good activity in the treatment of Vitiligo patients\(^5\).
- A trial was carried out with **AV/LTP115** on 28 dogs of different breeds and sex for management of anorexia syndrome. It showed 82.1% response\(^5\).

5.2.6. : TISSUE CULTURE STUDIES :

Tissue culture derived from hypocotyl and stem of *A. paniculata* has been shown to produce sesquiterpene lactones and apparently lack the ability to synthesize diterpenes. The sesquiterpene lactones based on bisabolene were paniculides A, B and C. Andrographolide or related substances could not be detected in the tissue culture\(^5\).

Using callus culture of *A. paniculata* Overton and Picken have collected evidence that biosynthesis of \(\gamma\)-bisabolene has the Z-configuration and its biosynthetic precursor is 2-cis, 6-trans and not 2-cis, 6-cis farnesol pyrophosphate.\(^5\)

5.2.7. ANALYTICAL REVIEW :

IP'55 has described a gravimetric method for estimation of andrographolide in the herb as well as the extract. 20 g herb powder is extracted with chloroform in a Soxhlet extractor. Chloroform is evaporated and the residue is washed repeatedly with benzene till colouring matter is removed. The residue is extracted with ethyl acetate till it gives no bitter taste. Combined ethyl acetate extract is evaporated and the residue is weighed. The herb should not contain less than 1% andrographolide\(^5\).

Srivastava et al. (1959) have modified above gravimetric method because it is difficult to wash the dry chloroform extract with benzene and extract with ethyl acetate. They have extracted the drug with alcohol. Alcohol is concentrated, diluted with water and washed with benzene. The aqueous phase is extracted with ethyl acetate. It is dried and evaporated.\(^5\).
Maiti et al. (1959) have described a very sensitive colorimetric method for the estimation of andrographolide (0.6mg to 7.2mg) by developing colour of the extract with 50% KOH (methanolic). But colour is very unstable.

Rao (1962) has described another sensitive method, called as lactone titration method.

Gaind et al. (1963) have described a very sensitive spectrophotometric method for andrographolide (5.0μg to 45μg/ml), measuring absorbance of the extract at 266nm.

Talukdar et al. (1968) have given correction factors for nullification of the presence of impurities for titrimetric as well as spectrophotometric methods.

Talukdar and Dutta (1969) have described a TLC method for the estimation of andrographolide.

Burgos et al. (1997) have described a HPLC method for estimation of andrographolide using a column of licrospher RP-18, 5μ(125 × 4mm); mobile phase-25% acetonitrile and 6% acetic acid, 1.5ml/min, detector at 228nm, Rt = 5.68 to 5.7 minutes.

Indian Herbal Pharmacopoeia (1998) has also described HPLC method using C_{18} ODS column (3μ, 25cm×4mm); mobile phase, methanol : water (65:35) at the rate of 1.0ml/min, detection at 223nm, retention time 4.5min.

Handa et al. (1990) have described HPLC method using Si column (5μ, 3.9 × 150nm); Chloroform : methanol (9:1) as mobile phase, flow rate 0.7ml/min, detector at 254nm, Rt 2.9 min.

Chauhan et al. (1999) have described HPTLC method using prepared Si plate, ethylacetate as mobile phase, detector at 260nm; linear range of 0.1 to 1.0μg

5.2.8. OTHER INDIAN SPECIES OF ANDROGRAPHIS:

To investigate a suitable substitute of *A. paniculata*, its other species have also been studied as follows.

*A echoides* Nees:

(Guj-Kalukariya, Hindi-Birkabat, Mal & Tam.-Gopuram thangi, Mar. - Banchimani.)

It is an erect, hairy, annual herb, up to 60 cm in height, with oblong or subelliptic, obtuse leaves, numerous flowers, having pink or white corolla with dark purple blotches on the lower lip, in axillary racemos and many seeded, hairy capsules, found in drier parts of tropical India.

The juice of herb is given in fever. The plant is stated to have properties similar to those of *A. paniculata*. The whole plant collected from Madras gave echioindinin (0.017%) and echinodin. Andrographolide is absent.

*A serpyllifolia* Wight:

It is a trailing and rooting, procumbent herb, arising from a stout roots stock, with densely hairy stems, orbicular-reniform leaves, pale or white flowers with purple blotches on the lower lip and elliptic capsule, found in Tamil Nadu, Andhra Pradesh up to 900m. Tribal people of Chittoor district (A.P.) take orally, paste prepared from the leaves of *A serpyllifolia* along with pepper and garlic for chronic epilepsy. The leaves from
Salem(TN) on analysis gave apigenin-7-4'-di-O-methylether. The dried stems and roots were found to contain tectochrysin, apigenin-7-4'-di-O methylether and a new flavone, serphyllin (M.P. 170°C).

*A wightiana* Arn.ex. Nees : 435

It is a herb, widely distributed in the hills of Kerala, Tamil Nadu, Karnataka, up to 750m. The dried stems and roots collected from Peringalkuthu(Kerala) have been found to contain wightin. The dried leaves gave echinoidinin and a bitter principle wightionolide.

The overall review of the literature reveals that hepatoprotective herbal market formulations have not been evaluated for the active constituents of *A paniculata*, hence the following work was thought worth to carry out:

1. TLC studies of the various market formulations containing *A paniculata* as one of the ingredients.
2. Estimation of andrographolide, the active constituent, by HPLC and HPTLC methods.

### 5.3. *Phyllanthus* Linn.

A genus of herbs or undershrubs chiefly distributed in tropical and subtropical regions of the world. About 24 species occur wild in India but very few have been used in medicine.

*Phyllanthus amarus* 548-551 Schum & Thonn.

#### 5.3.1. INTRODUCTION:

**SYNONYM:** *Phyllanthus niruri* avet non L.

**FAMILY:** Euphorbiaceae

**PARTS USED:** Entire plant of *P. amarus* is used in medicine.

**COMMON NAMES:**

- Bengali: Bhui amla
- Gujarati: Bhony aanmali
- Hindi: Bhuinavalah, Jaramla, Jangli amli
- Kan. : Nela nelli, Kiranelli gida
- Mal. : Kizha nelli
- Marathi: Bhuivavali
- Sanskrit: Bhumyamalaki, Bahupatra, Bahuphala, Bahupushpi
- Tamil : Kilaneli
- Telugu : Nelausirika
- Oriya : Bhui aola, Badianla

**CONTROVERSIAL ASPECTS OF THE SPECIES:**

The herb commonly named as “Bhumyamalaki” and “Bhuinavalah” in Sanskrit and Hindi respectively was wrongly described as *Phyllanthus niruri* L by “Flora of British India” (Hooker, 1877) and by various other taxonomists of India. True *Phyllanthus niruri* is endemic to the West Indies and is not found in India. Webster (1957)
Phyllanthus amarus Schum. & Thonn. 1, Habit; 2, short-Shoot from the axil of a cataphyll; 3, Female Flower; 4, Male flower 5, Female flower L.S.; 6, stamens in male flower; 7, Fruit; 8, Seed
and Mitra and Jain (1985) have shown that *P. niruri* L. of Hooker is actually represented by three different species, viz. *P. amarus* Schum & Thonn, *P. fraternus* Webster and *P. debilis* Klein ex Wild. The later is restricted to the coastal regions of the country. Much of the botanical, phytochemical and pharmacological work reported by earlier scientists in India was in fact on these three *Phyllanthus* species which are wrongly designated as *P. niruri*.

**DISTRIBUTION**: Probably native to America, but found throughout India and almost all tropical countries.

**DESCRIPTION**

Erect annual herb, 10-60 cm tall, main stem simple or branched, terete, smooth or scabridulous in younger parts. Cataphylls, stipules 1.5-1.9 mm long, deltoid-acuminate. Deciduous branchlets 1.5-14 cm long, subterete, smooth or a few lower nodes sometimes scabridulous with 13-30 distichous leaves. Leaves, 3-11 x 1.5-6 mm, elliptic-oblong, obovate-oblong or even obovate, obtuse or minutely apiculate at apex, obtuse or slightly inequilateral at base; petioles 0.3-0.5 mm long; stipules 0.8-1.1 mm long, triangular-acuminate. Flowers in axillary unisexual and bisexual cymes on deciduous branchlets; proximal 2-3 axils with unisexual cymes of 1-3 male flowers; all succeeding axils with bisexual cymes, each consisting of 1 male and 1 female or 2 male and 1 female or 1 male and 2 female flowers or combinations thereof. Male flowers, pedicel 1 mm long, calyx lobes 5 (rarely 6), subequal, 0.7 x 0.3 mm, elliptic or oblong elliptic and abruptly acute at apex, stamens 3, filaments connate, 0.2-0.3 mm high. Female flowers; pedicils 0.8-1 mm long; calyx lobes 5 (rarely 6), subequal, 0.6 x 0.25 mm, ovate-oblong or oblong, acute at apex, midsepaline band green, disc flat, deeply 5-lobed; styles 3, free, shallowly bifid at apex. Capsule; 1.8 mm across; oblate, rounded; pedicel 1.5 mm long; calyx 1.0 x 0.3 mm. seeds 0.9 mm long, triangular, with 6-7 longitudinal ribs and numerous minute transverse striae on back.

**Medicinal Uses**: Whole plant is bitter, stomachic and medicinal. The drug is highly reputed as a single drug remedy in the treatment of jaundice in traditional medicine. It is carminative, styptic, astringent, cooling and used in cough and indigestion. It is also used in diabetes. It is also much used as a diuretic in dropsical affections, gonorrhoea and other troubles of the genito-urinary tract. An infusion of the young shoot is given in dysentery. The powdered leaves and roots are made into poultice with rice-water and used to lessen the oedematous swellings and ulcers.

The fresh root is said to be an excellent remedy for jaundice. In the Konkan, the root rubbed down with rice water is given as a remedy for menorrhagia.

Leaves are stomachic, juice is a good application to offensive sores. A poultice of the leaves with salt cures scabby affections (antifungal action) and without salt may be applied to bruises (antibacterial action) etc.

The fruit is bitter, useful for tubercular ulcers, wounds, sores, bruises, scabies, ringworm etc. (antifungal, antibacterial activity).
In the Gold Coast, the leaves are pounded and used to cure gonorrhoea. Parts of the plant are used to cure constipation. The leaves are boiled and the liquor is drunk to stop acute pains in the stomach. The chief use of the plant is to allay griping in cases of dysentery.

In La Reunion, the plant is very much used in blennorrhagia, dropsy and diarrhoea.

The decoction of the root and leaves is very bitter and is a favourite remedy among the natives of Port Rico for the cure of intermittent fevers.

Kirtikar (1933) has also proved the efficacy of the plant in the cases of paroxysm, where tincture was made up from whole plant and two drachms was given in the morning. Sometimes the dose was repeated, which acted upon the bowels as a slight purgative and was very useful in inveterate intermittents with infarcts of the spleen and liver.

The infusion of the root and leaves is a good tonic, and a diuretic when taken cold in repeated doses.

5.3.2. ETHNOMEDICAL USES:

Different parts of the plant are used by the traditional people in different countries for various ailments. In India, it is mainly used in jaundice, pyrexia, veneral diseases, eye diseases, skin diseases, diabetes etc. The plant has more or less similar uses in other parts of the world which are mentioned below; name of place and parts of plants are mentioned in the bracket.

1) **Antiasthmatic**: Decoction (Hb- India) is used orally.

2) **Antidiabetic**: Decoction (Sd and Ft-Brazil or Hb-India, East Indies and West Indies or Lf-India) are taken orally.

3) **Antidiarrhoeal and antidysentric**: Powder of young shoot (Orissa, India or decoction of the fresh shoot are used orally in the treatment of dysentry (India). Decoctions (Ar-India or Lf-Papau New Guinea) are used orally in the treatment of diarrhoea. Infusion of dried leaves is administered orally for the treatment of diarrhoea and dysentry (Fiji).

4) **Anti-inflammatory**: Decoction (Hb- Thailand) is administered orally as an antiinflammatory.

5) **Antimalarial**: Decoctions (Ar-Thailand and Hb-West Indies) are used orally.

6) **Antipyretic**: Decoctions (Hb-Thailand, Haiti, Bohmian Island and Fiji; Ar-Puerto Rico and Lf-Dominican Republic) are used orally; the leaf decoction is taken orally and is also used for bathing as an antipyretic(Haiti, French Guiana). Decoction (Lf-India) is administered orally in intermittent fever.

7) **Antiseptic**: Fresh juice of leaf is applied externally on cuts and wounds. Decoction of dried entire plant is used to bath newborns. It removes disease causing elements from the skin (Philippines).

8) **Cholagogue**: Decoction (Lf-French Guiana) is administered orally.

9) **Cough Treatment**: Decoction (Hb- Philippines) is used orally for cough in infants.
10) **Diuretic**: Decoction (Ar-India\textsuperscript{620} and Hb-E. Africa \textsuperscript{621}, Thailand \textsuperscript{612}, Peru \textsuperscript{622} and West Indies\textsuperscript{615}) are used orally.

11) **Emetic**: Decoction (Lf-Mexico\textsuperscript{623}) is taken as a strong tea.

12) **Eye diseases**: People (Kondh tribe, Orissa\textsuperscript{624}) use drops of plant juice to treat conjunctivitis. Plant juice mixed with castor oil is applied to the eyes(Fiji\textsuperscript{610}).

13) **Gall stone**: Decoction (Hb- Peru\textsuperscript{612}) is administered orally.

14) **Genitourinary disorders**: Fresh plant juice is taken orally for genito-urinary troubles (India\textsuperscript{626}).

15) **Jaundice**:
   - People (Kani tribe, Kerala\textsuperscript{627}) take paste of the plant with cow’s milk for 3 days to cure jaundice.
   - Traditional healers (Chittoor district, A. P.\textsuperscript{628,629}) prepare a paste of leaves with few pieces of *Allium sativum*, *Piper nigrum* fruits and butter milk. The paste is given orally for seven days for the treatment of jaundice.
   - Traditional people use decoction (Rt-Brazil\textsuperscript{602} or fresh Ar-India\textsuperscript{606}) orally for the treatment of jaundice. Dried entire plant is churned with buttermilk and is used orally (Fiji\textsuperscript{610}) for the same purpose.

16) **Viral Hepatitis**: Herbalists (Anantapur district, A.P.\textsuperscript{607}) make small pills (Size of *Ziziphus mauritiana* seeds) from powder of *P. amarus*, cardamom and pepper with the help of tamarind juice. One pill per day is given orally to treat viral hepatitis.

17) **Laxative**: Traditional people use orally decoction (Hb-Bahamian Island\textsuperscript{615}).

18) **Menstrual regulation**:
   - People use decoction (Hb-Argentina\textsuperscript{630}, Philippines\textsuperscript{631}) as an emmenogogue.
   - Decoction (Hb-East Indies\textsuperscript{604}) is administered orally for menstrual troubles.
   - Infusion of fresh roots is taken orally to treat heavy menstrual periods (Fiji\textsuperscript{610}).
   - Decoction of the leaves is given orally after a miscarriage, it is also used as an emmenogogue\textsuperscript{632}.

19) **Ringworm**: Fruits are used externally (India\textsuperscript{580}).

20) **Scabies**: Fruits are used externally (India\textsuperscript{580}).

21) **Sores**: The herbalists (Anantapur district, A.P.\textsuperscript{607}) apply juice of the plant externally for offensive sores.

22) **Spasmolytic**: Hot water extract of the entire plant is administered orally (Haiti, French Guiana\textsuperscript{614}).

23) **Stomachic**: Decoctions of leaves(India\textsuperscript{580}) and herb(Virgin Island\textsuperscript{633}) are used orally.
   - Decoction of roots of *P. nururi* and *Citrus aurantifolia* are mixed and taken orally to increase the appetite (West Indies\textsuperscript{605}).

24) **Tonic**: Decoction (Hb-East Indies\textsuperscript{604} and Fiji\textsuperscript{610}) is taken orally.

25) **Urinary calculi**:
   - Infusion of dried leaves and stem is taken orally to treat kidney and bladder calculi(Brazil\textsuperscript{605}).
Decoction (Hb-Peru) is administered orally for renal calculi.

26) Veneral diseases:
- People (New Ireland of Papua New Guinea) use orally the juice squeezed from the leaves or decoction of whole plant daily as per requirement to treat veneral diseases. They also chew the roots for the same purpose. In Manns Island decoction of the bark and leaf is used to combat gonorrhoea. People of Admiralty Island also use hot water extract of dried bark and leaves twice a day orally (500 ml) up to six months to treat acute conditions of veneral diseases.
- Decoction (Hb-India) is administered orally for leucorrhoea, gonorrhoea and urogenital tract infections.
- Decoction (Hb-Tanzania) is used orally for gonorrhoea.

N.B.: Abbreviations: Ar = Aerial parts; Ft = Fruit; Hb = Herb; Lf = Leaf; Rt = Root; Sd = Seed.

5.3.3. PHARMACOGNOSTICAL REVIEW:
- Saha and Krishnamurthy (1959) have studied the pharmacognosy of *P. niruri*.
- Webster (1957) has reported a revision of the taxonomy of the genus *Phyllanthus*. He has shown that *P. niruri* is an entirely American species, found in West Indies. The other three closely related species are reported from India viz. *P. amarus*, *P. debilis* and *P. fraternus*.
- Mitra and Jain (1985) have described the concept of *P. niruri*. Their studies have also revealed that *P. niruri* in "Flora of British India" is a mixture of three distinct species viz. *P. amarus* Schum & Thonn, *P. fraternus* Webster and *P. debilis* Klein ex. Willd. They have given a key to identify these species and also given their detailed description.
- De and Datta (1990) have reported detailed microscopy of leaf, stem and root of *P. amarus*, the salient features of these are mentioned below.

Leaf: It is dorsiventral, palisade cells are present below upper epidermis. Epidermal cells are polygonal, wavy walled, with paracytic stomata, sometimes anomocytic stomata also seen. Spongy parenchyma show two types of Ca-oxalate crystals, rosette (abundant) and prismatic.

Stem: Parenchymatous cells of cortex and pith also show two types of calcium oxalate crystals, rosettes and prisms. Patches of fibres are seen in the inner cortex.

Root: Inner cortex shows patches of macro-sclereids. Xylem shows fibres, xylem vessels and parenchyma.

Histochemical tests, ash and extractive values, fluorescence behaviour of leaf, stem and root are also reported.

Bagchi et al. (1992) have reported morphological and microscopical characters of four species of *Phyllanthus* viz. *P. amarus*, *P. fraternus*, *P. urinaria* and *P. virgatus*. They have compared the microscopical characters of leaf, branch, stem and root. A key is
also given to identify these four species\textsuperscript{553}. These characters are mentioned in the following table.

**TABLE - VIII**

**COMPARATIVE MORPHOLOGICAL AND MICROSCOPICAL STUDY ON FOUR SPECIES OF *PHYLLANTHUS*\textsuperscript{553,556}**

<table>
<thead>
<tr>
<th>1.</th>
<th>Herb</th>
<th><em>P. amarus</em></th>
<th><em>P. fraternus</em></th>
<th><em>P. urinaria</em></th>
<th><em>P. virgatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Stem</td>
<td>Erect</td>
<td>Erect</td>
<td>Erect</td>
<td>Prostrate</td>
</tr>
<tr>
<td>3.</td>
<td>Sepals</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>4.</td>
<td>Fruit</td>
<td>Smooth</td>
<td>Smooth</td>
<td>warty</td>
<td>Smooth</td>
</tr>
<tr>
<td>5.</td>
<td>Stomata</td>
<td>Anisocytic (mostly Paracytic, sometimes Anomocytic\textsuperscript{556})</td>
<td>Anisocytic</td>
<td>Paracytic</td>
<td>Paracytic</td>
</tr>
<tr>
<td>6.</td>
<td>Stomatal index</td>
<td>3-8\textsuperscript{92}</td>
<td>19-23</td>
<td>4-10 \textsuperscript{92}</td>
<td>11-16 \textsuperscript{92}</td>
</tr>
<tr>
<td></td>
<td>upper</td>
<td>23-27</td>
<td>22-26</td>
<td>16-22</td>
<td>21-26</td>
</tr>
<tr>
<td></td>
<td>lower</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Epidermal cells</td>
<td>Wavy wall</td>
<td>Wavy wall</td>
<td>Wavy wall</td>
<td>Straight wall</td>
</tr>
<tr>
<td>8.</td>
<td>Ca-oxalate</td>
<td>Druse (Rosettes and prisms\textsuperscript{556})</td>
<td>Absent</td>
<td>Absent (Prisms and clusters\textsuperscript{556})</td>
<td>Absent</td>
</tr>
<tr>
<td>9.</td>
<td>Branchlet T. C. outline</td>
<td>Round</td>
<td>2\textsuperscript{+}4 ridges</td>
<td>Planoconvex, 2 ridges</td>
<td>Round, 2 ridges</td>
</tr>
<tr>
<td>10.</td>
<td>Branchlet pericyclic fibres</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>11.</td>
<td>Ca-oxalate in branchlet</td>
<td>Druse type</td>
<td>Absent</td>
<td>Absent</td>
<td>Sandy crystals</td>
</tr>
<tr>
<td>12.</td>
<td>Starch in pith of branchlet</td>
<td>Absent</td>
<td>Absent</td>
<td>Less</td>
<td>Abundant</td>
</tr>
<tr>
<td>13.</td>
<td>Hypodermis of branchlet</td>
<td>No tannin cell</td>
<td>No tannin cell</td>
<td>No tannin cell</td>
<td>Tannin present in all cells</td>
</tr>
<tr>
<td>14.</td>
<td>Stem epidermis</td>
<td>Single layer</td>
<td>Single layer</td>
<td>Single layer with 5 grooves and ridges</td>
<td>2-5 layers of cork</td>
</tr>
</tbody>
</table>

\textsuperscript{92}
15. **Stem cortex**  
Ca-oxalate  
Pruse type  
(prisms and rosettes\(^{556}\))  
Absent  
Absent  
Prisms  

<table>
<thead>
<tr>
<th>16. <strong>Stem pericyclic fibres</strong></th>
<th>Present</th>
<th>Present</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>17. <strong>Pith starch</strong></td>
<td>Absent</td>
<td>Absent</td>
<td>Less</td>
<td>Abundant</td>
</tr>
<tr>
<td>18. <strong>Root starch</strong></td>
<td>In endodermis</td>
<td>In endodermis</td>
<td>In endodermis</td>
<td>Abundant in parenchyma</td>
</tr>
<tr>
<td>19. <strong>Root, sclereids in cortex</strong></td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
</tr>
</tbody>
</table>

**N.B.** De and Datta’s\(^{556}\) observations which differ from those of Bagchi et al.\(^{553}\) are mentioned in the brackets.

- Blumeberg et al.(1991,1993) have reported that these plants can be cultivated without loss in the activity.
- Joshi (1996) has mentioned the characters to distinguish *P. fraternus* from *P. amarus*, they have 6 and 5 sepals respectively\(^{558}\).
- Choudhary et al.(1998) have reported that seeds sown in April, plantlets transplanted in May and herb harvested in September gives maximum yield of leaves as compared to harvesting in October, November and December. Sowing the seeds in May or June, transplanting the plantlets in June and July and harvesting from Sept. to Dec. also gives low yield of leaves. However, yield of leaves in September is maximum. Leaves are rich in the active constituents (Phyllanthin and Hypophyllanthin), so they should be more when the herb is harvested\(^{588}\).
- Indian Herbal Pharmacopoeia, vol. II (1999) has described morphology of the herb; microscopical characters of powdered leaf, stem and fruit; extractive and ash values of the aerial parts\(^{560}\).

5.3.4. CHEMICAL CONSTITUENTS:

Various types of chemical constituents have been isolated from *P. amarus*, either under the name of *P. niruri* or *P. amarus* in India and outside India. Lignans, phyllanthin and hypophyllanthin and a triterpene, triacontanal are reported to be hepatoprotective constituents of the plant\(^{597}\). Besides lignans, it is also reported to contain various flavonoids, alkaloids, tannins, sterols, triterpenoids, aliphatic diterpene and triterpene derivatives etc.

Phyllanthin (a bitter principle) and hypophyllanthin(a non-bitter principle) were isolated by Krishnamurthy and Seshadri(1946), their structures were not assigned\(^{564}\). Row et al.(1964,1966,1967) have established the structures of phyllanthin and hypophyllanthin\(^{565-567}\). Structure of hypophyllanthin was proposed and revised several times\(^{565,566,568-570}\) and was established finally by Somanbandhu\(^{571}\) et al.(1993). Following constituents have been reported from the plant.
Lignans:
Phyllanthin^{564,567,571,576,579,597,600}, hypophyllanthin^{564,572,576,596,600}, nirtetralin^{570,573,578}, lintetralin^{573,576,578}, phylltetralin^{570,573,578}, niranthis^{576,578}, seco-4-hydroxy lintetralin, seco-isolariciresinol trimethyl ether, hydroxy niranthis, dibenzyl butyrolactone^{574}, nirphyllin, phyllnirurin^{575}, isolintetralin^{576}, niruriside^{577} etc.

Flavonoids:
Quercetin, quercetrin, isoquercetrin, astragalin^{579}, rutin^{579,581,584}, quercetin-3-O-glucopyranoside^{581}, kaempferol-4'-rhamnopyranoside, eriodictyol-7-rhamnopyranoside^{580}, nirurin^{582} (a prenylated flavonoid), fisetin-4'-O-glucoside^{583} and 3,5,7,4'-tetrahydroxy flavone^{584}.

Alkaloids:
Ent-nor securinine^{585,587}, 4-methoxy securinine(phyllanthin), 4-methoxy nor securinine^{586}, 4-methoxy dihydrosecurinine, 4-methoxy tetrahydrosecurinine, 4-methoxy securinine, securinol A, securinol B, securinine, dihydrosecurinine, tetrahydro securinine, allosecurinine, nor securinine^{588}, isobubbialine and epibubbialine^{589}.

Tannins:
Geraniin^{581,590}, gallic acid^{590}, ellagic acid^{590}, phyllanthusiin-D^{591}, amariin, corilagin, 1,6-digalloyl glucopyranoside^{581} and amirinic acid^{592}.

Triterpenes:
Lupeol, lupeol-3-acetate^{593}, 7-lupeol, α-amyrin^{584}.

Euphane Triterpenoids:
Phyllanthanol, phyllanthenone and phyllanthanol^{594}.

Sterols:
β-sitosterol and isopropyl cholesterol^{578}.

Steroidal hormone:
Estradiol^{595}.

Acyclic diterpene: Trans-phytol^{596}.

Acyclic triterpenes:
Triacantanal, triacantanol^{597}, 32-methyl-1-triacontanol^{584}, dotriacontanoic acid^{578}.

Phthalic acid bis-ester: Phyllester^{578}.

Miscellaneous:
Vitamin C^{598}, ricinoleic acid, linoleic acid, linolenic acid^{599}.

5.3.5. PHARMACOLOGICAL REVIEW:
Phyllanthus amarus is reported to have varieties of activities from various types of extracts prepared from its different parts using different solvents. It is reported to have hepatoprotective, antihepatitis B, anticancer, antihypertensive, antinociceptive, antifungal, contraceptive, diuretic, hypoglycaemic, hypotensive etc. activities. The literature survey of all these activities are separately mentioned below:

1. Anticancer activity:
   - Dhar et al. (1968) have reported that ethanol: water(1:1) extract was active when administered (i.p.) to mice on LEUK(Friend Virus solid). However, the extract was inactive on CA-9KB at ED_{50}>20.0 mcg/ml^{590}. 

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• Macrae et al. (1988) have reported the activity of the ethyl acetate and aqueous fraction of methanolic extract of aerial part (Amazon). The EC$_{50}$ value is 0.05 and 1.31 µg/ml against Agrobacterium induced tumour.

• Srinivasulu (1992) has reported that phyllanthin and hypophyllanthin, lignan constituents of P. niruri when tested on various cancer cell lines, did not show significant cytotoxic effect on any cell line. Phyllanthin showed modest inhibition on small cell lung line and hypophyllanthin on CNS cancer cell line. However, dibenzyl-butyrolactone which is also present in P niruri is reported to exhibit antitumor activity.

• Somanabandhu et al. (1993) have reported that neither phyllanthin nor hypophyllanthin demonstrated significant cytotoxic activity when cultured with battery of cultured mammalian cells, but both were found to enhance the cytotoxic response mediated by vinblastine with multidrug-resistant KB cells. In addition, phyllanthin was found to displace the binding of vinblastine with membrane vesicles derived from this cell line, suggesting an interaction with the P-glycoprotein.

2. Anticlastogenic activity:
Dhir et al. (1990) have found that oral administration of aqueous extract of P. niruri leaves to mice for a week, significantly reduced the cytotoxic action of lead nitrate and aluminium sulphate. The frequency of chromosomal breakage, gaps and rearrangements induced by three concentrations of these salts was decreased when compared to control animals which had received the salts alone. The extract was given at the dose of 685 mg of leaf per kg body weight. It was effective in modifying the clastogenic effects of both the salts. Same group of workers have also reported similar results for the protection afforded by the aqueous extract of P. niruri at the same dose level against toxicity produced by three different doses of nickel chloride on mice.

3. Antihepatotoxic activity:
• Syamsunder et al. (1985) have shown the significant antihepatotoxic activity of the hexane extract of the plant in rat hepatocyte intoxicated with CCl$_4$. The individual isolates of the extract, phyllanthin, hypophyllanthin, triacontanal and triacontanol were also tested separately in rat hepatocytes intoxicated with CCl$_4$ and galactosamine. The former two compounds showed significant antihepatotoxic activity in these two models while triacontanal showed activity against later intoxicant only and triacontanol did not show significant activity in any.

• Naduveettil (1985) has also reported the activity of phyllanthin and hypophyllanthin, isolated from P. niruri and its extract (alcohol : water) through oral route on mice intoxicated with CCl$_4$. Phyllanthin (100 mg/kg) normalised the sleeping time of hexobarbitone, hypophyllanthin (20 mg/kg) was inactive and the extract (600 mg/kg) did not show significant activity.

• Rao (1985) has shown that pretreatment with water extract of P. niruri 2 ml/kg for one month protected the livers of albino rats against CCl$_4$ toxicity.

• Urmani et al. (1985) administered the powder of P. niruri orally for 45 days (200 mg/kg) to ethanol induced fatty liver rats and studied its effect on the increased
deposition of triglycerides, cholesterol and phospholipids of liver, heart and kidney. All of them were brought down to the normal value.  

- Murthy et al. (1993) have studied the effect of alcoholic extract of *P. niruri* on rats intoxicated with CCl₄. It reduced SGPT, SGOT, serum bilirubin etc. indicating its significant antihepatotoxic activity.  

- Prakash et al. (1995) have reported the effects of alcohol extracts of three species of *Phyllanthus* against carbon tetrachloride induced (chronic) hepatic damage on rats. The extracts of *P. niruri*, *P. urinaria* and *P. simplex* were administered orally at three different dose levels 10, 40 and 400 mg/kg to the rats. Statistically significant reversal of the elevated serum levels were observed in the animals in *P. niruri* and *P. urinaria* extracts. Hepatoprotective activity of *P. urinaria* was about 60% in comparison with *P. niruri*, it may be considered as a substitute of *P. niruri*, but *P. simplex* lacked in significant activity.  

- Sane et al. (1995) have compared the hepatoprotective activity of *P. amarus* and *P. debilis* on the rats intoxicated with CCl₄. Slurry prepared from each of the whole plant powder was fed 0.66 g/kg for 3 days, CCl₄ was given on the first day. Study of serum enzyme levels and hepatocellular damage revealed that both the plants are effective in protecting the liver damage. *P. debilis* has more activity than *P. amarus*.  

- Subramanian (1995) has criticized the hepatoprotective studies of Sane et al. and pointed out some of the mistakes in his calculations. He has suggested the following formula for finding out the hepatoprotective activity of the drug:

\[
\text{% of protection} = 100 - \frac{100 \times (\text{Difference in the values between CCl}_4 \text{ control) - Difference in the values between CCl}_4 \text{ control and normal control})}{\text{CCI}_4 \text{ control and normal control}}
\]

**Antihepatotoxic polyherbal formulations:**

- The pharmacological activity of antihepatotoxic polyherbal formulations have not been found to be much investigated. Reports of some of the formulations mentioned below are available.  

- Sharma et al. (1991) have reported hepatoprotective activity of M-Liv against CCl₄ induced toxicity in rats.  

- Bhaumik and Sharma (1993) have studied the antihepatotoxic activities of a formulation consisting of mixture of equal proportion of *P. niruri*, *Andrographis paniculata* and *Solanum nigrum*. Sheep were intoxicated with paracetamol and subsequently administered 1.0 g/kg drug by gastric intubation daily for 10 days. The drug protected the animals from jaundice and increased AST, ALT etc.  

- Kapur et al. (1994) have reported hepatoprotective activity of Jigrine, a polyherbal formulation containing 14 medicinal plants, some amongst them being *Phyllanthus niruri*, *Solanum nigrum*, *Cichorium intybus* and *Foemculum vulgare*. The effects of oral pretreatment with Jigrine (0.5 ml & 1.0 ml/kg for 7 days) were studied on hepatic
damage induced by alcohol- carbon tetrachloride (40% alcohol 2.0 ml/100g, p.o. for 21 days and CCl₄ 1.4 in groundnut oil, 0.1 ml/kg, s.c. on 20th day) and paracetamol (750 mg/kg, i.p.) in rats. The study of biochemical parameters and histopathology confirmed its hepatoprotective activity³⁰,⁶⁵¹.

**Hepatoprotection - Clinical trials:**

- Dixit and Achar(1983) have studied the results of clinical trials by administrating the powder of *P. niruri* to 160 children (1 to 12 yrs. old) suffering from jaundice. Majority of them (90%) were admitted after 5 days of jaundice attack while the remaining were still more severe cases (6 to 10 to 15 or more days attack). They were given 50 mg *P. niruri* powder per kg body weight in three divided doses for the period of 6 weeks. They were examined twice a week for 6 weeks clinically and by laboratory reports and followed up for 3 months after the recovery. There was restoration of appetite within one week (77% cases) to two weeks (20% cases); disappearance of jaundice, liver tenderness and bile pigment salts from urine within 1 to 3 weeks in about 95% cases. There was complete recovery within six weeks in 101 patients out of 160 patients who continued the treatment. There was a drop out of 59 patients within 1 or 2 weeks. The follow up study for 3 months indicated there was no reappearance of jaundice. These studies indicated the remedial cure of jaundice by *P. niruri*⁶⁵².

4. **Antihepatitis B Surface Antigen activity:**

- Mehrotra et al. (Thyagarajan et al.(1982) have reported *in vitro* inactivation of HBsAg by *P. niruri* extract. Four extracts were prepared separately from aerial parts and roots of the plants, by macerating at room temperature as well as by Soxhlet extraction. 0.2 ml solution of all the four extracts (2% solution of dried extracts) incubated with 0.2 ml of HBsAg positive sera (1:64 CEP titre), brought about *in vitro* inactivation of HBsAg within 24 hours at 37°C and also at room temperature. All these extracts contain a red pigment, which is active⁶⁵³.

- Venkateswaran et al.(1987) have reported *in vitro* and *in vivo* studies of an aqueous extract of *P. niruri* on hepatitis B and Woodchuck hepatitis viruses. The extract inhibits endogenous DNA polymerase of Hepatitis B virus and binds to the surface antigen of hepatitis B virus *in vitro*. The extract also inhibits Woodchuck hepatitis virus (WHV) DNA polymerase and binds to the surface antigen of WHV *in vitro*. In a trial using six long term WHV-carrier Woodchucks, five treated animals showed a faster decrease in Woodchuck hepatitis virus surface antigen titre compared to untreated control. In animals recently infected with WHV, the extract was effective when administered i.p. in three out of four animals eliminating both the surface antigen titre and DNA polymerase activity in serum. The treatment was discontinued after 10 weeks, and the treated animals have remained free detectable markers of WHV for more than 45 weeks. In contrast, three untreated controls remained positive for both markers for WHV for more than 45 weeks. One of the control died after 8 weeks. In a third trial with long term carriers, test animals treated subcutaneously with the extract for 12 weeks did not respond; but on switching the mode of administration to i.p., two out of the five animals showed a significant decrease in Woodchuck hepatitis virus surface antigen titre compared to controls⁶⁵⁴.
Venkateswaran et al. (1987) have also reported that both water and methanol extracts of the dried entire plant were active against Woodchuck hepatitis virus at variable concentrations. They inhibited DNA polymerase of Woodchuck hepatitis virus, water extract afforded 25% inhibition at 50 mg/ml concentration. Jayaram et al. (1989) have reported in vitro inactivation of the Hepatitis B surface antigen by the crude extract of *P. niruri* in 48-72 hours. A red pigment was found to be active.

Mehrotra et al. (1990) have reported that alcoholic extract of *P. niruri* has in vitro anti-HBsAg activity (68%) at a concentration ranging from 1.6 to 2.6 mg/ml against 0.039 µg/ml HBsAg at 37 °C after 24 hours incubation. HBsAg were collected from different types of patients, like patients with acute HBV infection, chronic carriers of HBV or having liver cirrhosis along with HBV.

Mehrotra et al. (1991) have also further studied in vitro effects of *P. amarus* on hepatitis B virus. Ethanolic extract of whole plant and subsequent fractions prepared from it, viz. hexane, chloroform, butanol and finally water were tested for in vitro effects on HBsAg, HBeAg and HBV-DNA in serum samples positive for HBV antigens. The extracts were effective against HBV antigens, the n-butanol extract being the most potent; its further fractionation showed enhanced activity. The active fractions inhibited the interaction between HBsAg/HbeAg and their corresponding antibodies suggesting anti-HBs, anti-HBe like activity and also an effect on HBV-DNA.

Unander et al. (1991) have studied in vitro activity of different species of *Phyllanthus* against DNA polymerase of hepatitis viruses, effects of growing environment and inter and intraspecific differences. (i) Aqueous extracts of several herbaceous species of *Phyllanthus* inhibited the endogenous DNA of hepadnaviruses in vitro. This inhibitory activity varied among the species viz. *P. urinaria*, *P. amarus*, *P. debilis*, *P. fraternus*, *P. mimics*, *P. odontadenius*, *P. carolmensis*, *P. niruri* etc. (ii) Within *P. urinaria*, there were highly significant differences among plants grown from seeds of diverse origin. Within *P. amarus*, plants from seeds of diverse origin did not differ significantly. (iii) One seed lot which was tested for intra-accession variability showed highly significant plant to plant differences in viral DNA polymerase inhibition, but these differences were not highly heritable. (iv) Differences in general fertility, soil moisture, pH or calcium generally did not significantly affected the in vitro inhibition of DNAp. (v) Plant grown at low temperature (winter) showed decreased DNAp activity as compared to high temperature (summer) grown plants. (vi) Plants of *P. amarus* were successfully grown as a raw crop with fertilizers and irrigation without loss of activity relative to samples from the wild. (vii) Roots of *P. amarus* were more active than the herb.

Pousset et al. (1993) have reported in vitro inactivation of HBsAg by water and methanol extract of the dried leaves; IC₅₀ for water extract was 3.3 mcg/ml and for later extract 1.2 mcg/ml.

**Ant-hepatitis B virus activity - Clinical Trials:**

Thyagarajan et al. (1988) have reported the effect of *P. amarus* on chronic carriers of hepatitis B virus. 200 mg of dried and sterilised powder prepared from whole plant...
was given in a capsule form; 3 capsules per day for 30 days to the chronic carriers of hepatitis B virus. 22 of 37 (59%) treated patients lost hepatitis B surface antigen (HBsAg) when tested 15-20 days after the end of the treatment; compared with placebo treated control, 1 of 23 (4%) lost HBsAg. Some subject followed for 9 months, in no case the surface antigen has returned. Clinical observation revealed no toxic effects.

- Thumlikitul et al. (1991) have studied the efficacy of *P. amarus* for eradication of hepatitis B virus in chronic carriers in Thailand. The aerial parts of *P. amarus* (grown in Central part of Thailand, at a dose of 200 mg TDS for 60 days or 400 mg TDS for 30 days through oral route has very minimal effect on eradication of HBsAg from Thai adult asymptomatic chronic carriers. These results are contradictory to that reported by Thyagarajan et al. (1988) from India probably because of the different environmental condition or the age of the plant or the subjects in India were younger than those in the present study.

- Doshi et al. (1994) have studied the role of *P. amarus* in eradication of Hepatitis B virus in carriers. They have shown that *P. amarus* is not effective in clearing HBsAg in asymptomatic carriers at the doses of 250 mg and 500 mg TDS for 4 to 8 weeks in 30 subjects.

5. Anti HIV activity:
- Ogata et al. (1992) have shown that water extract of dried entire plant is active on HIV-1 virus; ID$_{50}$ is 50.0 mcg/l.

- Hussain et al. (1995) have shown that phyllanthin, hypophyllanthin and nirtetralin inhibit labelled ET-1 binding receptor expressed in Chinese hamster ovary cells (CHO-ETA), but were inactive against the recombinant ETB receptors.

- Cutrone et al. (1996) have isolated niruriside from the methanol extract of the plant. It inhibited HIV REV/RRE binding, the IC$_{50}$ value was 3.3 μm.

6. Antihypercholesterolemic activity (Antihyperlipemic activity):
- Urmani et al. have reported that dried entire plant was active in the rats in which fatty liver was induced with alcohol. The drug powder was given orally, 200 mg/kg for 45 days. It reduced the increased deposition of triglycerides, cholesterol and phospholipids in the liver, heart and kidney that resulted from alcohol treatment.

7. Antinociceptive activity:
- Antinociceptive activity of *P. niruri* has been compared with number of other species of *Phyllanthus* found in China and America. These activities are mentioned below:

- Analgesic effects of hydroalcoholic extracts (HEs, 50% alcohol-water) of *P. urinaria*, *P. tenellus*, *P. niruri* and *P. sellowiarius* have been investigated. The extracts of four species of *Phyllanthus* (1-90 mg/kg, i.p.) caused a dose related inhibition of acetic acid induced abdominal constriction in mice with ID$_{50}$ values of 5.4, 8.5, 18.2 and 53 mg/kg and maximal inhibition (%) of 80, 67, 63 and 50 for *P. urinaria*, *P. niruri*, *P. tenellus* and *P. sellowiarius*. 99
In the formalin tests, the HEs of all *Phyllanthus* species (0.3-60 mg/kgm i.p.) caused graded inhibition of both the phases of formalin induced pain, but they were, however, more potent in relation to the second phase of the pain. The ID$_{50}$ values (mg/kg) for the first phase were 20, 23, >60 and >60 for *P. urinaria*, *P. tenellus*, *P. niruri* and *P. sellowiarius* respectively and percentage of maximal inhibition were 63, 70, 41 and 46 respectively. The ID$_{50}$ values (mg/kg) for the second phase were 0.71, 4.87, 7.7 and 33 with maximal inhibition (%) of 91, 97, 97 and 92 respectively. Given orally, the extracts caused significant antinociceptive profile, but they were about one-tenth to one-twentieth as potent when given intraperitoneally. However, the HEs of *Phyllanthus* failed to affect formalin induced rat paw oedema and did not interfere with the performance of animals in the rota-rod test. Naloxone (5mg/kg) completely reversed the analgesic effect caused by morphine (5mg/kg), but had no effect against the analgesic effect of the HEs of *Phyllanthus*. Furthermore, the HEs of *Phyllanthus* in contrast to morphine had no analgesic effect in either tail-flick or hot-plate tests.

The antinociceptive activities are in following order

\[ urinaria > P. corcovadensis > P. tenellus > P. niruri > P. sellowiarius \]

- The methanolic extracts of callus cultures (grown on media containing 2,4-D; IAA and IBA) of *P. tenellus*, *P. corcovadensis* and *P. niruri* have antinociceptive activity (3-90 mg/kg, i.p.). The extracts caused graded inhibition of abdominal constrictions induced by acetic acid (0.6). ID$_{50}$ values are 30, 19 and >30 mg/kg for *P. corcovadensis*, *P. niruri* and *P. tenellus* respectively.

8. **Antipyretic activity**:
- Mokkhamit et al. (1972) have reported that ethanol water (1:1) extract of commercial sample of entire plant, when administered at variable dosage levels by gastric intubation to rabbits was inactive against yeast induced pyrexia.

9. **Antispasmodic activity**:
- Patel (1965) has reported that phyllanthin, alcohol extract and aqueous extracts have antispasmodic action on rat’s duodenum against Ba induced contraction. Activity of Phyllanthin was more than alcohol and water extracts.
- Dhar et al. (1968) have reported that ethanol : water (1:1) extract of the entire plant was active on guinea pig ileum against acetylcholine and histamine induced spasms.
- Santos et al. (1994) have reported that methanol extract of dried callus tissue at a concentration of 320.0 mcg/ml was inactive on guinea pig ileum against acetylcholine induced contractions.

10. **Contraceptive effects**:
- Feeding an alcoholic extract of *P. amarus* to male mice at a dose of 500 mg/kg for 45 days, induced gradual inhibition of fertility potential. There was 72% reduction in the fertility, it reduced sperm count, sperm motility, sperm viability, weight of testis etc. It has no adverse effect on blood biochemical profile and cell counts. Upon withdrawal of feeding the extract, the antifertility effects were reversed gradually.
11. Diuretic activity:
- Woerd (1941) has reported diuretic activity of the plant.
- Patel (1965) has also reported that aqueous extract of *P. niruri* is having significant diuretic activity on rats at the dose of 0.05 ml/kg (1 ml extract = 1 g drug) through oral route. Alcohol extract has less activity and phyllanthin has feeble activity.

12. Hypoglycaemic activity:
- Jain and Sharma (1967) have reported that alcohol extract of entire plant is inactive in reducing blood sugar level at 10 mg/kg dose level through oral route in normal rabbits.
- Ramkrishnan et al. (1982) have reported that an aqueous extract of the leaves of *P. niruri* at a dose of 5 ml (representing 5 g leaf) per kg body weight, through oral route, lowered blood sugar level in normal and alloxan diabetic rabbits and the effect is more than that of tolbutamide (250 mg/kg, p.o.). The extract also lowers blood sugar level even after the administration of glucose.
- Hukeri et al. (1988) have reported that water extract of dried entire plant when administered by gastric intubation to rats, was active against alloxan induced hyperglycaemia.
- Moshi et al. (1997) have reported that aqueous extract of aerial parts of *P. amarus* (in Tanzania), 0.1 and 1 g/kg body weight, significantly enhanced clearance of glucose from the blood as compared to control during an oral glucose tolerance test, using normal fasted albino rabbits. Both doses had no effect on blood glucose in the unfed rabbits. Chlorpropamide, 0.1 g/kg body weight, showed a greater effect than both doses, on glucose clearance in the fed state and on blood glucose in fasted rabbits. A methanol extract of the aerial parts, 1 g/kg body weight, worsened glucose tolerance causing a significant increase in glucose in the blood, use of an aqueous extract as suggested by traditional healers appears to be the correct remedy.

Hypoglycaemic activity - Clinical Trials:
- Sivaprakash et al. (1995) have carried out clinical studies of *P. amarus* on 25 diabetic patients in the age group of 35-55 years with moderate to severe diabetic blood sugar level (250-400 mg/100ml). The drug brought down statistically significant lowering of blood sugar levels at a dose of 1 g thrice a day for the period of 3 months.

13. Hypotensive, chronotropic and cardiotoxic activities:
- Patel (1965) has reported that aqueous extract, alcohol extract and phyllanthin have hypotensive activity on dog. Phyllanthin is having maximum activity followed by alcohol and water extracts.
- Mokhamit et al. (1971) have reported that ethanol : water (1:1) extract of a commercial sample of the entire plant was devoid of hypotensive, chronotropic or cardiotoxic activities at variable concentration through i.v. route.
Diuretic, Hypoglycaemic and Hypotensive activity - Clinical trials:

- Srividya and Periwal (1995) have carried out a clinical trial on nine mild hypertensive patients (four of them also suffering from diabetes mellitus). They were treated with 5 g pellets (prepared from whole plant powder and honey) per day (orally) in three divided doses for 10 days. After the treatment there was a significant increase in 24 hour urine volume, urine and serum sodium levels. There was also a significant reduction in systolic blood pressure in non-diabetic hypertensives and female subjects. There was also reduction in blood glucose levels (5 to 50 mg/100ml) in both diabetic and non-diabetic subjects.

14. Antibacterial activity:

- Collier and Van (1949) have reported that saline extract of leaves was active on Pasteurella pestis and Staphylococcus aureus and inactive on Escherichia coli at a 10% concentration using agar plate method.
- Khan et al. (1978) have reported that water extract of fresh entire plant of P. niruri was inactive on Neisseria gonorrhoea at 1.0% concentration on agar plate.
- Khan et al. (1980) have reported antibacterial activity of alcoholic extract of P. niruri against Staphylococcus aureus and E. coli using filter paper disc assay method with 1% solution of the extract for the assay.
- Farouk et al. (1983) have reported that chloroform extract at a concentration of 1.0 g/ml on agar plate was inactive on Bacillus subtilis, E. coli, Pseudomonas aeruginosa and Staphylococcus aureus. Methanol extract was active on Staphylococcus aureus but inactive on B subtilis, E. coli and P. aeruginosa.
- Macrae et al. (1988) have reported the activity of ethyl acetate and water fractions of methanol extract of aerial part (Amazon). The ethyl acetate fraction is inactive against E. coli and S. aureus while aqueous fraction is active against S. aureus but inactive against E. coli.
- Macrae et al. (1988) have reported the activity of ethyl acetate and water fraction of the methanol extract of the aerial part (Amazon). Ethyl acetate fraction and aqueous fraction are active against Microsporum canis, M. gypseum and Trichophyton gallinae. Aqueous fraction is also active against M. fulvum.

15. Antifungal activity:

- Bhatnagar et al. (1961) have shown that petroleum ether extract of whole plant has antifungal activity against Helminthosporium sativa.

16. Antiviral activity:

- Khan et al. (1991) have reported that ethanol extract of fresh entire plant when tested on Tobacco Mosaic Virus in cell culture was equivocal. The viral inhibitory activity was 7%.
- Saigopal et al. (1986) have reported that the fresh leaf and fresh root extract (in 0.05 M phosphate buffer, pH 7.5) of the plant have antiviral activity on pea nut mosaic virus, Tobacco Mosaic Virus and Tobacco Ring Spot Virus at 4% concentration.
- Macrae et al. (1988) have reported the activity of ethyl acetate and aqueous fractions of the methanol extract of the aerial parts (Amazon) against Sindbis virus and Murine 102 virus.
Ethyl acetate fraction is active (1 to 100 μg/ml) against pre-infection by both the viruses treatment (100% activity) but inactive against post-infection treatment (1 to 100 μg/ml concentration level); while ethyl acetate fraction is active against both the viruses by pretreatment at 10 to 100 μg/ml concentration (100% activity). Aqueous fraction is having moderate activity when used for post-infection treatment against Sindbis virus and pre-infection treatment against Murine cytomegalovirus.

**17. Anti-yeast activity**: Macrae et al. (1988) have reported the activity of ethyl acetate and aqueous fractions of the methanolic extract of aerial parts (Amazon). Both the extracts are inactive against *Saccharomyces cerevisiae* and *Candida albicans*.

**18. Brine Shrimp mortality**: Macrae et al. (1988) have reported the activity of ethyl acetate and aqueous fractions of methanolic extract of the aerial part (Amazon). Ethyl acetate fraction has LC50 value 114 μg/ml, aqueous fraction has no activity.

**19. Molliscidal activity**:
- Ahmed et al. (1984) have reported molluscidal activity of certain Sudanese plants. Successive petroleum ether extract and successive alcohol extract of *P. niruri* have molluscidal activity at concentration of 25 ppm and 250 ppm respectively. These extracts produced 100% mortality in *Bulinus truncatus* and *Biomphakria pfeifferi*.

**20. Nematocidal activity**:
- Kiuchi et al. (1989) have shown that decoction of commercial sample of bark, at a concentration of 1.0 mg/ml was active on *Toxacara canis*.

**Biochemical Effects**:
John and Krishnamurthy (1993) have studied some biochemical effects of *P. niruri* after oral administration to rats. *In vitro* tissue respiration and hepatic K+, Mg++ and inorganic phosphorous content were not significantly altered by the drug for a period of two weeks, however, the concentration of Na+ in the liver was elevated by 4 doses of the drug. Aspartate and alanine transaminases and alkaline phosphatase of serum and liver as well as liver microsomal glucose-6-beta phosphatase, ali-esterase and glucopyronyl transferase were unaffected by feeding the aqueous extract of the drug. Erythrocytes from rats receiving the drug were more resistant to chronic hemolysis. The drug however, showed antidiuretic and antioxidant activity.

**Toxicity assessment**:
Dhar et al. (1968) have reported that ethanol : water (1:1) extract of the entire plant administered orally to mice, tolerated a maximum dose of 1 g/kg.

Naduveettil (1985) has reported LD50 for phyllanthin 800 mg/kg and hypophyllanthin 20 mg/kg in mice.

Venkateswaran et al. (1987) have reported that water extract of dried entire plant at a dose of 0.1 mcg/animal was inactive. No weight loss was found seven days after the treatment.
Jayaram et al. (1987) have reported the safety study of *P. niruri* *in vitro* and *in vivo* while using it for anti-HBV properties, using mice as the model and Vero cell-line as the tissue culture system. Acute and chronic toxicity was carried out. There was no mortality, weight loss, behavioural changes, changes in histopathological pictures of liver, spleen and kidney or biochemical profile after 90 days treatment. Vero cell-line also indicated no cytotoxicity\(^{685}\).

### 5.3.6. ANALYTICAL REVIEW:

Phyllanthin and hypophyllanthin are reported to be the hepatoprotective compounds of *P. amarus*\(^{597}\). They are also major ingredients of the herb. So analytical methods which are available help to estimate these active constituents in the herbs as well as formulations. These methods are based on sophisticated instruments like HPTLC and HPLC, they are as follows:

- **Handa et al. (1993)** have described the HPLC method for estimation of phyllanthin and hypophyllanthin in the different parts of the *P. amarus* using reverse phase HPLC system, methanol : water (66:34) as mobile phase, detector at 230 nm., Rt 9.6 min and 8.6 min(for phyllanthin and hypophyllanthin respectively). Leaves contain maximum amount of phyllanthin (0.7% w/w) and hypophyllanthin (0.3% w/w) as compared to stem, fruit, root etc.\(^{686}\). Same method is also described in Indian Herbal Pharmacopoeia, vol. II(1999)\(^{560}\).

- **Deb and Mandal (1996)** have described TLC-Densitometric method for estimation of phyllanthin and hypophyllanthin in *P. amarus* and polyherbal formulations, using 4 µg to 25 µg standard per spot, mobile phase hexane : ethyl acetate (2:1), silica gel plates, detection at 268 nm. But they have not mentioned percentage of above constituents in the herb or in the formulations\(^{686}\).

- **Sane et al. (1997)** have studied the HPTLC chromatograms of chloroform extracts of *P. amarus* and 3 other species of Phyllanthus to identify them. Phyllanthin and hypophyllanthin were noticed in *P. amarus* only\(^{557}\).

- **Sane et al. (1997)** have described the HPTLC method for estimation of phyllanthin and hypophyllanthin in the herb of *P. amarus* collected from different regions and in different seasons of India. Sample from Bombay, Allahabad and Trichur. None of them showed any significant variation in phyllanthin content but did show variation in hypophyllanthin content which was 0.855%, 0.825% and 0.660% respectively. Phyllanthin content was maximum in monsoon (0.709%), followed by winter (0.595%) and summer (0.505%). HPTLC was carried out on silica gel prepared plates, using Toluene : ethyl acetate (3:1) as mobile phase, detector at 205 nm. and linearity in the range of 100 to 700ng\(^{687}\).

- **Chaudhary et al. (1998)** have described the HPLC method using normal phase of column, micro porasil (39 mm. x 300 mm.); Ethyl acetate : Hexane (1:1) as a mobile phase, flow rate 1 ml/min, detector at 280 nm. and Rt 27.93 min for phyllanthin.
5.3.7. OTHER INDIAN SPECIES OF PHYLLANTHUS INVESTIGATED:

To investigate the good hepatoprotective herbal drug besides *P. amarus* number of other species of *Phyllanthus* have been studied as follows:

**P. urinaria L.**: Common name- Lal Bhuinavalah
- De and Datta (1990)\(^5\) have compared the morphology, microscopy, histochemical characters and extractive values of *P. amarus* and *P. urinaria*
- Bagchi et al.(1992)\(^5\) have described the morphological and microscopical characters of the plant as- Plant erect, stem red and hairy, capsule verucose, calyx lobes 6, fibres absent in the branchlets; parenchyma of stem and root contain few or no starch.
- Sane et al (1997) did not notice the presence of phyllanthin and hypophyllanthin in this plant, when it was analysed with HPTLC method\(^5\).

**P. debilis Klein ex Willd**: Syn.- *P. airy-shawii* Burnal and Roux
- Sivarajan and Balachandran (1984,1994) have described the characters to distinguish this species from *P. amarus*. It states ‘the plant as an erect, delicate herb, young stems angular, sepals 5 to 6, fruiting perianth as long as capsule, capsule smooth.
- Sane et al. (1995) have compared the hepatoprotective activity of *P. debilis* with *P. amarus* (0.66 g powder/kg, orally) in rats against CCl\(_4\) induced toxicity. *P. debilis* showed better activity than the later herb\(^6\).
- Subramanian (1995) has criticized the above results saying “Both *P. debilis* and *P. amarus* have less than 50% hepatoprotective activity, so they are not good hepatoprotective drugs.” He has carried out studies on rat intoxicated with paracetamol administering the herb powders orally in the form of slurry, 1 g powder/kg body weight\(^6\).
- Sane et al.(1997) did not notice the presence of phyllanthin and hypophyllanthin in this herb, which was analysed by HPTLC method\(^5\). The hepatoprotective activity\(^6\) may be due to compounds other than phyllanthin and hypophyllanthin.

**P. virgatus Frost**: Syn.- *P. simplex* Retz.
- Bagchi et al. (1992)\(^5\) have described the morphological and microscopical characters of the plant, as-plant, prostrate, female flowers with pedicels upto 6 mm long, calyx lobes 6, and parenchymatous cells of stem and root full of starch grains.
- Prakash et al. (1995) have studied hepatoprotective activity in rats intoxicated with CCl\(_4\) and showed its insignificant hepatoprotective activity\(^5\).

**P. fraternus Webster**:
- Bagchi et al. (1992) have described the morphological and microscopical characters of leaf, rachis, stem, root etc. to distinguish it from *P. amarus*\(^5\).
- Sane and Kuber(1993) have reported hepatoprotective activity against CCl\(_4\) induced toxicity in rats\(^5\). Sane et al. did not find the presence of phyllanthin and hypophyllanthin when the herb was analysed by HPTLC method\(^5\).
**P. maderaspatensis:**
- Kirtikar and Basu (1933) and Wealth of India (1969) describe the plant as "Annual erect herb, sometimes undershrub, leaves scattered, sepals six.
- Asha and Pushpangadan (1998) have shown it to possess hepatoprotective activity in rats intoxicated with paracetamol and mice intoxicated with CCl₄.

**P. kozhikodianus:**
- Asha and Pushpangadan (1998) have shown it to possess hepatoprotective activity in rats intoxicated with paracetamol and mice intoxicated with CCl₄.

Steroids isolated from *P. corcovadensis* produced partial but significant antinociceptive effects against acetic acid and formalin induced algesic responses in mice at the doses level 10 to 100 mg/kg, i.p. The steroids isolated are β-sitosterol, stigmasterol and stigmasterol acetate. They are also present in other species of *Phyllanthus*.

Two ellagitannins, furosin and geraniin, isolated from *P. sellowiiarius* have significant antinociceptive activity (3 to 30 mg/kg, i.p.) when given 30 min. before testing, against acetic acid induced abdominal constrictions in mice. Both these tannins have dose related activities. Geraniin is also isolated from *P. amarus*, *P. niruri* and *P. tenellus*. It may be present in other species of *Phyllanthus* also. Geraniin is also having ACE inhibitory activity.

After summarizing the review of the literature it was noticed that eventhough much of the pharmacognostical, pharmacological and chemical evaluation of *P. amarus* is reported the polyherbal formulations were not chemically evaluated so far for their quality. Hence phyllanthin, the active chemical constituent of *P. amarus* was decided to be estimated from the polyherbal formulations by the HPLC and HPTLC methods.

**5.4. Solanum:**
A large genus of herbs, shrubs and rarely trees, found throughout the temperate and tropical parts of the world. Over 50 species have been recorded in India and a few ornamental exotics have been introduced into the garden.

**5.4.1. INTRODUCTION:**
*Solanum nigrum* L.
SYNONYM: *S. rubrum* Mill.
FAMILY: Solanaceae
Polyploidy is very common in this family and three chromosomal races, diploids, tetraploids and hexaploids are reported in *Solanum* species. Morphologically these three races can be differentiated with the help of their colour and size of the fruit. Diploid races (2n=12; *S. americanum*) are with shiny bluish black coloured fruits, tetraploids (4n=24, very much morphologically similar to *S. luteum*) with orange red coloured fruits while that of hexaploids(6n=36, *Solanum nigrum*) with bigger sized and purplish black coloured fruits.

PARTS USED: Whole plant and fruits.
*Solanum nigrum* L. 1, Flowering branch; 2, Inflorescence; 3, Fruits.
COMMON NAMES:
Assam: Pichkati
Bengal: Gurkamani, kakmachi
English: Black Nightshade
Gujarati: Piludi
Hindi: Makoi
Marathi: Kakmachi, Meko
Punjabi: Kachmach, Mako
Sanskrit: Kakamachi, Bahuphala, Katuphala.
Tamil: Munatalakal
Telugu : Kachchipundu, Kachi, Kamachi

DISTRIBUTION:
The plant is found throughout India as a weed in open waste lands, gardens, cultivated land and roadsides up to an elevation of 2,100 m. It is also found in all temperate and tropical regions of the world like C. and W. Asia, Europe, Africa etc.

DESCRIPTION:
An annual herb, 30-45 cm high, stem erect, glabrous or more or less pubescent, much divaricately branched. Leaves numerous, 2.5-9 by 2-5 cm, ovate-lanceolate, subacute or acuminate, glabrous, thin, entire minute toothed, tapering into petiole; petioles 2 cm long. Flowers small, in extra-axillary subumbellate 3-8 flowered cymes; peduncles 6-20 mm long, slender; pedicels 6-10 mm long, very slender. Calyx 3 mm long, glabrous or nearly so; lobes 5, oblong, obtuse, 1.25 mm long, not enlarged in fruit. Corolla 4-8 mm long, divided more than half-way down into 5 oblong subacute lobes. Filaments short, flattened, hairy at the base; anthers 2.5 mm long, yellow, oblong, obtuse, notched at the apex. Ovary globose, glabrous; style cylindric, hairy. Berry 6 mm diameter, globose, usually purplish black, but sometimes red or yellow, smooth, shining. Seeds discoid, 1.5 mm diameter, minutely pitted, yellow.

Medicinal uses:
The entire herb and its individual parts are separately used in various types of diseases as mentioned below:

Entire plant:
- Cardiotonic and antidiabetic,
- Used internally for cardialgia and gripping; externally in nephritic colic, corroding ulcers, suppurring chancre, severe burning and herpes (Guiana and Madagascar).
- It is used internally for convulsions and externally for anthrax pustules and to disinfect the meat from anthrax. An infusion is used as an enema to infants with abdominal upsets (S. Africa).
- Juice of plant is given in chronic enlargement of liver, in blood-spitting, piles, dysentery etc.
- Young shoots are given in chronic skin diseases including psoriasis (Konkan-Karnataka).
- Internally used for malaria, blackwater fever, dysenteries etc.; Ointment prepared from decoction is used for foul ulcers (Rhodesia).

**Berries:**

The berries are laxative, alternative, aphrodisiac, tonic, diuretic, improve appetite and taste; useful in diseases of the heart and the eye, in pains, piles, inflammation, "tridosh", leucoderma, itch, worms in the ear, dysentery, hiccup, vomiting, asthma, bronchitis, fever, urinary discharges; improve voice; favour conception and facilitate delivery; useful in erysipelas and rat bite. It is also useful in thirst due to fever. Employed in fever, diarrhoea, eye diseases, hydrophobia etc. (Bengal). Paste of green berries is applied to ring worm (S. Africa). The seeds are laxative; useful in giddiness, gonorrhoea, thirst and inflammation.

**Root Bark:**
- The root bark is laxative; useful in diseases of the ear, eye and nose; good for ulcers of the neck; burning of the throat, inflammation of the liver, chronic fever, griping; but not recommended to the pregnant women.
- Roots and leaves are used in cases of arthritis, dropsy and skin diseases in the form of decoction.

**Leaves:**
- Used for headache and diseases of the nose.
- Decoction is used, one drachm three times a day, for dropsical affections; diuretic and laxative.
- Juice alleviates pain and inflammation of the kidneys and bladder and virulent gonorrhoea (China).
- Decoction is used as diuretic and depurative (Guinea).

### 5.4.2. ETHNOMEDICAL USES:

Besides above medicinal uses, number of traditional uses of the entire plant and its various parts have also been reported which are mentioned below. The part of the plant and the country in which it is used are mentioned in the bracket.

1) **Abortifacient:** Decoction (Hb- Korea; Ft- S. korea; Rt bark- India) are used orally.

2) **Analgesic:** Decoction (Hb- India) is used orally or poultice (Lf- Israel and Italy) is applied externally as local analgesic.

3) **Antianaemic:** Decoction (Ar- Mauritius) is used orally.

4) **Antiangiastic:** Decoction (Hb- India) or juice (Ar- Fiji) are used orally.

5) **Antibacterial:** Decoction (Ar- Saudi Arabia) is used orally.

6) **Anticancer:**
   - Decoction of herb of *S. nigrum*, *S. lyratum* and *Duchesnea indica* is used orally (China).
   - Decoction (Hb- Korea) is used orally for uterine cancer.

7) **Antiacaterrhal:** Decoction (Hb- Korea) is taken orally.

8) **Anticonvulsant:** Decoction (Fresh Lf- Nigeria) is used orally.

9) **Antidiabetic:** Decoction (Hb- Korea) is administered orally.

10) **Antidiarrhoeal:** Fruits (India) are used orally.

11) **Antidysenteric:** Fresh ripe fruits are used orally (Fiji).
12) **Antidote for opium**: Decoction (Lf- India\textsuperscript{706}) used orally to remove the effects of opium.

13) **Antihaemorrhoidal**:
- Decoction (Ar- Italy\textsuperscript{707}; Ft- Peru\textsuperscript{708} and Hb- India\textsuperscript{709}) or paste (Lf- Iran\textsuperscript{711} and Israel\textsuperscript{712}) is applied externally in rectum.
- Decoction (Hb- India; Lf\textsuperscript{697}, Canary Island\textsuperscript{710}) or ripe fruits (Fiji\textsuperscript{701}) are taken orally.

14) **Antihypertensive**: Decoction (Hb- Mauritius\textsuperscript{700}) is used as antihypertensive. The leaves are cooked as a vegetable and used to ease hypertension (Rodrigues, Mauritius\textsuperscript{713}).

15) **Anti-inflammatory**:
- Decoction (Lf- Hawai\textsuperscript{714}; Hb- India\textsuperscript{709} and Nepal\textsuperscript{715}) or poultice (Hb- India\textsuperscript{716}) or fresh leaves cooked in buttermilk are applied externally (Israel\textsuperscript{698}). Hot leaves are applied externally (India) to treat inflamed scrotum and testicles\textsuperscript{705}. Paste of the leaves is applied externally in animals in case of body swelling (M.P., India\textsuperscript{717}).
- Decoction (Ar- Saudi Arabia\textsuperscript{702} and Korea\textsuperscript{694}; Lf- Canary Island\textsuperscript{710}) are used orally.
- Juice (Lf- India\textsuperscript{705}) is used orally in Kidney and bladder inflammation.
- Decoction (Lf- Iran\textsuperscript{711}) is used as an enema to reduce inflammation of caecum, genitourinary tract and bladder.

16) **Antimalarial**: Decoction (Lf- Nigeria\textsuperscript{704}) is used orally.

17) **Antipyretic**:
- Fresh fruits (Fiji\textsuperscript{701}) or decoction (Ft- Peru\textsuperscript{708}) are taken orally or the decoction is applied externally (India\textsuperscript{718}).
- Decoction (Lf) is used orally in scarlet fever (Nigeria\textsuperscript{704}).
- Decoction of *S. nigrum* (part is not mentioned) with *Cyperus rotundus* is used orally in S. India\textsuperscript{720} in recurring fever.
- Paste (Fresh Rt- India\textsuperscript{721}) is applied on forehead or taken orally.

18) **Antirheumatic**: Decoction (Fresh Lf- Nigeria\textsuperscript{704}; Fr- Peru\textsuperscript{708}) are used orally or leaf decoction (Iran\textsuperscript{711}) is applied externally in rheumatoid arthritis.

19) **Antiseptic**: Juice (Hb- Seychelles\textsuperscript{722}) is taken orally in infections of the tongue. Decoction (Hb- Korea\textsuperscript{694}) is used externally for sores in mouth.

20) **Antispasmodic**: Decoction (Fresh Lf- Nigeria\textsuperscript{704}) is used orally.

21) **Aphrodisiac**: Decoction (Hb- India\textsuperscript{697} and Nepal\textsuperscript{715}) is used orally.

22) **Apoplexy**: Decoction (Hb- Korea\textsuperscript{694}) is used orally.

23) **Beri-beri**: Decoction (Hb- Korea\textsuperscript{694}) is used orally.

24) **Bronchitis**: Decoction (Lf- Canary Island\textsuperscript{710} and Spain) is used orally.

25) **Burns**:
- Extract (Fr- Tunisia\textsuperscript{723}) or decoction (Lf- Iran\textsuperscript{711}) or juice (Hb- Minnesota in U.S.A.\textsuperscript{724}) are applied externally.
- Leaf juice with olive oil is applied externally (Israel\textsuperscript{712}).
- Decoction (Hb- Korea\textsuperscript{694}) is taken orally.

26) **Calculi**: Juice (Fresh Hb- India\textsuperscript{725}) is used orally for kidney stones.
27) **Chestpain**: Juice (Ar- Fiji\(^{701}\)) used orally.

28) **Convulsions**: Decoction (Hb- Korea\(^{694}\)) is used orally for convulsions in infants.

29) **Cough**:
- Juice (Ar- Iran\(^{711}\)) or decoction (Fr- Peru\(^{708}\)) is used orally in whooping cough.
- Decoction of the herb with seeds of *Glycosmis mauritania* and/or wood chips of *Santalum album* is used orally (India\(^{721}\)).

30) **Diaphoretic**: Decoction (Ar- Saudi Arabia\(^{702}\); 60 to 180 mg Lf- USA\(^{726}\); Lf- Nigeria\(^{704}\)) is used orally.

31) **Diuretic**: Decoction (Hb- India\(^{697}\) and Nepal\(^{715}\); Fr- West Indies\(^{727}\)) or juice (Fresh Hb- India\(^{725}\)) is used orally.

32) **Earache**: Juice (Fresh Lf- India\(^{716}\) and Fiji\(^{701}\)) is used as eardrops.

33) **Erysipelas**: Decoction (Hb- Korea\(^{694}\) and Fr- Peru\(^{708}\)) is used externally.

34) **Galactogogue**: Decoction of aerial parts of the plant with *Moringa Pterygosperma* and beach peebles is used orally (Philippines\(^{728}\)).

35) **Gonorrhoea**: Decoction (Hb- India\(^{705}\)) or juice (Hb- India\(^{729}\); Ar- Fiji\(^{701}\)) 5 ml juice, 5 times a day is taken orally.

36) **Headaches**: Fresh leaves (N. India) are cooked and eaten\(^{720}\) or juice (Fresh Hb-Fiji\(^{701}\)) is taken orally and applied externally on forehead.

37) **Heart diseases**: Fresh ripe fruits (India\(^{705}\) and Fiji\(^{701}\)) or decoction (Hb- India\(^{697}\)) or infusion (Lf- Egypt\(^{731}\)) are taken orally.

38) **Hemoptysis**: Decoction of the herb with seeds of *Glycosmis mauritania* and wood chips of *Santalum album* is taken orally (India\(^{721}\)).

39) **Hemostatic**: Crushed fresh leaves are applied externally (Ivory coast\(^{732}\)).

40) **Jaundice**:
- Leaf powder (about 6 g) is taken orally with a glass of buttermilk early in the morning for six consecutive days (Chittoor, A.P., India\(^{733}\)).
- 10 ml juice of the aerial parts (obtained by crushing) is given twice a day for 30-40 days (India\(^{734}\)).
- A paste prepared from 10 ml juice of the leaves, 10 g powder of *Picrorrhiza kurroa* and 5 mg “loh-bhasma” is divided into 14 equal doses. The preparation is given twice a day for seven days. With this, 1 ml fresh cow urine is also given to the patient in the early morning (India\(^{734}\)).
- A crude drug combination of leaves, fruits and seeds of *Solanum nigrum, Eclipta alba, Althea rosea, Cassia tora* and *Ocimum sanctum* is used in jaundice (India\(^{735}\)).
- Leaf extract of *S. nigrum* is used in liver diseases (Jaunsari tribe, Dehra Dun, India\(^{736}\)).
- Paste is prepared from equal quantities of leaves of *S. nigrum, Ricinus communis* and *Boerhaavia diffusa*, 10 g is taken once a day orally for 7 days (India\(^{737}\)).
- Infusion (Dry Lf- India\(^{738}\) and Egypt\(^{731}\)) is used orally.
- Juice (Hb- India\(^{705}\)) is used in case of liver enlargement.

41) **Laxative**: Herb (Turkey\(^{739}\)) or dried ripe fruits (Egypt\(^{731}\), India\(^{705}\), Nepal\(^{715}\), Pakistan\(^{740}\)) or infusion (60 to 180 mg Lf- USA\(^{726}\)) are used orally as a laxative.
42) **Leucoderma**: Decoction (Hb- India[^697]) is used externally.

43) **Menstrual disorder**: Decoction (Ar- Philippines[^741]) is used orally.

44) **Narcotic**: Decoction (Teaspoonful Lf- USA[^726]) or juice (Ar- Iran[^711]) of the plant (part not mentioned, Turkey[^739]) is taken orally.

45) **Neuralgia**: Decoction (Fr- Peru[^708]) is taken orally.

46) **Ophthalmic**: Juice (Fresh Lf- India[^742]) is used as eyedrops for eye pain and other eye ailments.

47) **Palsy**: Decoction (Hb- Korea[^694]) is used orally.

48) **Pruritis**: Decoction (Ar- Iran[^711]) is used as a douche to relieve vaginal pruritis

49) **Respiratory tract disorders**: Decoction (Dry Lf- Hawai[^714]) is used orally.

50) **Rickets**: Leaves of *S nigrum* along with *Erythnia variegata* and *Datura metel* are ground with egg white and warmed with sesame oil, two spoonfuls are given in a day to the children for rickets(India[^743]).

51) **Sedative**: Decoction (Hb- Turkey[^739]; Ar- Saudi Arabia[^702]; Lf- Nigeria[^704]; and teaspoonful Lf- USA[^726]) is used orally.

52) **Skin diseases**:

- **Boils**: Fresh leaves are heated and applied externally (California in USA[^744]). Powdered leaves with or without coconut oil are applied externally around the boils(Cook Island[^745]).
- **Cracks**: Decoction(Ar or Lf- Iran[^711]) or juice (Fresh Lf- Iran[^711]) is applied externally on cracks of the nipples in the females.
- **Dermatitis**: Decoction (Ar-Korea[^694]) is used externally.
- **Skin eruption**: Decoction (Lf- Hawai[^714]) is used orally.
- **Eczema and Erythema**: Extract (Fr- Tunisia[^723]) is used externally.
- **Ring worm**: Paste of green leaves is applied externally(Jaunsai Tribe, Dehra Dun in India[^736]).
- **Scabies**: Decoction (Fr- Israel[^712]) is applied externally.
- **Sore and ulcers**: Decoction (Ar or Lf- Iran[^711]) or juice(Fresh Lf- Iran[^711]) is applied externally on sores and traumatized parts of the body to relieve soreness and painful ulcers.
- **Extract**: Lf- India[^716] is used externally in boils, sores and chronic skin diseases.
- **Decoction**: (Ar- India[^718]) is used externally in skin diseases.
- **Decoction**: (Ar- Saudi Arabia[^702]) is used orally in skin diseases.

53) **Stomachaches**: Green flowers (India[^727]) and leaves(India[^727,746]) decoction (Lf- Canary Island[^710]; Hb- Korea[^694]) are taken orally in stomachaches.

54) **Tranquilizer**: Infusion (Lf- Canary Island[^710]) is used orally.

55) **Toothache**: Cut or cooked fruits(Israe[^698,712]) are applied externally or its steam is inhaled (Israel[^698]) or leaves are smoked (USA[^719]) or decoction (Lf- Peru[^708]) is taken orally.

56) **Tonic**: Decoction (Hb- India[^697,709] and Nepal[^715]) or fruit(West Indies[^727]) or leaf juice (India[^743]) is used orally.

57) **Tonsilitis**: Decoction (Hb- Korea[^694]) is used orally for tonsilitis and inflammation of lymphatic gland in groin.
58) **Ulcers**: Extract (Lf- Puerto Rico747) is used orally.

59) **Wounds**: Poultice of fresh or green leaves or ash of burnt leaves (Israel698) or crushed leaves (India742, Hawai745) or decoction (Dry Lf- Hawai714, Fresh Fr- Israel698) are applied externally.

60) **Toxicity**: Dried fruits (USA726) are considered as toxic.

Abbreviations used: Ar=Aerial parts; Fr=Fruit; Hb=Herb; Lf=Leaf; Rt=Root

5.4.3. **PHARMACOGNOSTICAL REVIEW**:

- Gupta and Kundu et al.(1965) have determined average vein islet termination and stomatal numbers of the leaf *S. nigrum*748.
- Wallis(1967) has described the types of trichomes of the leaf and its palisade ratio, 2 to 4829.
- Brinda et al. (1982) have reported detailed pharmacognosy of the plant749.
- Sonare (1994) has described the morphology of the herb750.
- Rai et al. (1995) have described morphology and microscopy of leaf, stem and fruit751.
- Nayar et al. (1991) have reported chemico-botanical standardisation of *S. nigrum* fruits. They have described the detailed morphology, microscopy and powder characters of the fruit. Besides this, the fluorescence analysis, physical constants, qualitative inorganic and organic analysis, T.L.C etc of the fruit was also carried out for the correct identification of the drug. Some of the salient features of these are mentioned below752.

**Microscopical characters**:

**Fruit** :-

*Epicarp*: A single layered epidermis composed of rectangular to polygonal shaped cells, covered with cuticle.

*Mesocarp*: It is a fleshy mass, made up of few layered collenchymatous tissue underneath the epidermis and many layered parenchymatous tissue filled with simple and compound starch grains752.

*Seeds*: Seeds are embedded in the parenchymatous tissue of the fleshy mesocarp. In T.S. it shows a single layer of stony, light brown to yellow epidermis. Endosperm contains fixed oil and aleurone grains. Embryo is cylindrical with hypocotyl and radicle752.

*Peduncle*: The epidermis of peduncle is single layered, covered with thick cuticle, the cortex consists of thin-walled large parenchymatous cells with intercellular spaces. The vascular bundles are bicollateral arranged around the central parenchymatous pith752.

**Powder study (Fruit)**:

The powder is light brown to light yellow in colour. The diagnostic microscopical characters of the fruit powder are: lignified thick-walled stone cells of the seed epidermis, parenchymatous cells of the mesocarp filled with starch grains and endosperm cells with oil globules and aleurone grains752.

**Leaf** :

Jadhav (1996) has reported the microscopical characters of the leaf. It is dorsiventral with single layer of palisade, lying below the upper epidermis. It is
discontinuous over the midrib where lies the collenchymatous tissues. Both the epidermis have smooth cuticle, multicellular (3-4 cell long) simple, covering trichomes and anisocytic stomata. The vascular bundle of the midrib is bicolateral. Calcium oxalate crystals of prisms and clusters and few microsphenoidal are present throughout the parenchymatous cells of the section.

Root:
Iyer and Kolammal (1960) have described detailed microscopy of the root. Its transverse section is circular in outline with central wood and narrower outer bark which is about one-third the thickness of the wood.

Cork: It is very thin consists of two to four rows of cells with brownish wall. The cells are mostly narrow thin-walled, nearly rectangular and tangentially elongated, not uniform in shape. Phellogen is not distinct.

Cortex: Outer cortex consists of large slightly elongated thin walled cells. Groups of sclerechyma, each group of about 8 to 20 cells are seen in the inner cortex. Most of cortical cells are filled with the black contents. Starch is seen in fewer cells but it is seen in most of the cells in young root.

Phloem(bast): The cells are small, thin-walled and polygonal in shape. Most of them contain the black contents. Sieve tubes and companion cells are small.

Cambium: It consists of a row of narrow thin-walled rectangular cells.

Wood: Vessels are arranged in groups of two or three in radial rows. The ray cells are radially rectangular and somewhat thick-walled.

5.4.4. CHEMICAL CONSTITUENTS:
The plant *Solanum nigrum* and its different parts are reported to contain an alkaloid solasodine and its glycoside solamargine. Besides these, certain other constituents like steroidal alkaloids, steroids, flavonoids, vitamins, mineral elements etc. have also been reported. Literature survey reports the following constituents in the various parts of the plant.

**Plant:**
Alkaloids, flavonoids, saponins and tannins; steroidal alkaloids, solasodine (0.1-0.7%) and its glycosides, β-solamargine (trace), solasonine (0.2%) and solamargine (0.25%). It also contains other steroidal alkaloids like N-methyl solasodine, 12-β-hydroxy solasodine, tomatidinol and solanocapsine. Steroids, diosgenin (0.09-1.2%) and titogenin etc. Large number of mineral elements like Ca(0.288%), Cu(0.0013%), Fe(0.0545%), Mg(0.1668%), Zn(0.0026%), Ba(0.000163%), K(0.45%), Ua (0.124%), Al(0.1442%), Mn(0.0006%), Ce(0.003%), Sr(0.004%), V(0.00265%), Hg(0.0244%), Co(0.00045%) etc.

**Stem:**
Solasodine (0.02%), α-solasonine, α-solamargine, uttronin-β, titogenin etc.
Leaves:
Steroidal alkaloids, solasodine (0.03%), solasonine and solamargine and flavonoid glycosides of quercetin like quercetin-3-glucoside, quercetin-3-diglucoside, quercetin-3-β-glucosyl(1-6)-β-galactoside, quercetin-3-galactoside and quercetin-3-O-(2-α-rhamnosyl-β-glucosyl-(1-6)-β-galactoside. Presence of steroids like β-sitosterol, stigmasterol, cholesterol and campesterol; vitamin A (9666 IU/100g), riboflavin (0.011%), Ca (0.410%), P (0.070%), Fe (0.0205%) have also been reported.

Fruits:
Steroidal alkaloids solasodine (about 0.7%), α-solasonine, α-solamargine, β-solamargine, solanigrin etc. They also contain 26-O-(β-D-glucopyranosyl)-22-methoxy-25-D-5-α-furost-3β-26-diol-3-O-β-glycotetroside, titogenin, diosgenin; α-carotene. Green unripe fruits contain 4.2% glycoalkaloids, α-solamargin and α-solasonine, solamargine and solasonine.

Roots:
Solasodine (0.02%), titogenin, uttronin-β.

Seeds: Protein (17.5%) and fixed oil (21.5%); the fatty acids reported in the fixed oil are stearic, oleic and linoleic acid.

• Ontogenic variations in sterols in leaf and tissue cultures derived from leaf:
  Bhatt and Bhatt (1984) have studied the composition of β-C-3 sterols of S. nigrum during aging and in vitro morphogenesis. Cholesterol, campesterol, stigmasterol and sitosterol were detected in the leaves and in the differentiated and undifferentiated tissue cultures. The content of cholesterol declined during leaf aging. The ratio of stigmasterol to sitosterol was 0.1 in the young leaves but increased to 0.16 in the mature leaves. Campesterol content does not vary much.

• Total sterol content in the in vitro differentiated shoot was about 1.5 times higher than that of the initial leaf explant. Among the tissue cultures, sterols were about 3 times higher in the differentiated cultures (roots and shoots) than in the undifferentiated cultures (callus). Stigmasterol to sitosterol ratio was about three times higher in the callus grown on benzyladenine containing medium than that grown on indole acetic acid containing medium.

• Ontogenic variations in solasodine content: Eltayeb et al. (1997) have reported various changes in the percentage of solasodine during the development of the plant in its different parts. There is variation in percentage of solasodine content and absolute amount of solasodine in the leaf, stem, root and fruit at different time interval in the plant grown from the seed. The percentage of solasodine is maximum in small and young leaves as compared to the mature leaves, more in root as compared to stem; more in small fruits as compared to young, mature and ripe fruits. Total amount of solasodine is more in mature leaves as compared to young and small leaves; root as compared to stem; small fruits as compared to young, mature and ripe fruits. The percentage solasodine is maximum in the leaves and fruits of 16 weeks old plants, when all types of fruits are seen on them but it is maximum in the stem and root in 24 weeks old plants.
• **Biochemical prediction**: Khan et al. (1997) have studied the effect of supply of extra urea to the plant at the time of seed sowing; there is accumulation of more amount of nitrogen in the leaves and solasodine in the fruits. Further they have correlated the amount of solasodine content of the fruits, which is found to proportional to that of the nitrogen content of the leaf. For this they have estimated the nitrogen content of the leaves from 85 days old plant and solasodine content of the fruits from 175 days old plant. For calculating the % of solasodine in 175 days old fruits on the basis of nitrogen content of 85 days old leaves, they have given the equation. If nitrogen content of the 85 days old leaves is found low, they have recommended supply of nitrogen through spray of urea(source of nitrogen) to increase solasodine content of the mature fruits\(^781\)(175 days old fruits).

5.4.5. **PHARMACOLOGICAL REVIEW**:

Survey of the literature indicated that the herb and its various parts possess different types of activities like antibacterial, antifungal, anticancer, anti-inflammatory, antioxidant, antispasmodic, gastric protective, hepatoprotective etc.. These activities, reported by different scientists of the world, are discussed below:

1) **Abortifacient activity**: Prakash and Mathur (1976) have reported that ethanol : water (1:1) extract of the seeds is inactive in female rat at 150 mg/kg dose level, through oral route\(^782\).

2) **Analgesic activity**: Mohsin et al. (1989, Saudi Arabia) have reported that ethanol extract of the aerial parts is inactive against hot plate method on mouse, at the dose of 500 mg/kg, through intragastric route\(^783\).

3) **Analgesic and antispasmodic**: Henry (1938) has mentioned the use of dry extract of the plant in glycerin suppository as analgesic and antispasmodic. It is useful in neuralgia, painful spasms of viscera, trembling in multiple sclerosis and parkinsonism. It is also of value in anxieties and insomnia caused by neuro-vegetative system\(^845\).

4) **Antibacterial activity**:
   - Taniguchi et al. (1978) have reported that ethanol (70%) extract of fresh leaf is inactive against *Bacillus subtilis* and *E. coli* at 100 mcg/ml concentration by broth culture method\(^784\).
   - Ayneci et al. (1982, Iran) have reported that the mixture of ethanol (80%) extracts of fruit, leaf and stem at a concentration of 100 mcg/ml is active against *Bacillus anthracis* and *Vibrio cholera* but inactive against *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Shigella sonnei* and *Staphylococcus aureus*\(^785\).
   - Al-Mashal et al. (1982) have reported that methanol extract of the entire plant is active against *Proteus vulgaris* and *Staphylococcus aureus* but inactive against *E. coli*, *Pseudomonas aeruginosa* and *Salmonella* species by agar plate method\(^786\) (concentration not given).
Jawad and Jaffer (1988, Iraq) have reported the activity of 80% alcohol extract of the aerial parts and their alkaloidal and lactone fractions against \( B. \ subtilis \), \( S. \ aureus \), \( E \ coli \) and \( P \ aeruginosa \) by agar plate method. The extract was inactive against all four organisms, while the fractions, at 0.2 ml/well concentration were active against the former two but were inactive against the later two organisms\(^{787} \).

Naovi et al.(1991) have studied the activity of the water extract of the plant at a concentration of 10 mg/ml by agar plate method. It is inactive against \( Corynebacterium \ diptheriae \), \( Diplococcus \ pneumoniae \), \( Staphylococcus \ aureus \) and \( Streptococcus \ viridens \) organisms but weak activity was noticed against \( Streptococcus \ pyogenes \)\(^{788} \).

Taniguchi and Kubo (1993, E. Africa) have reported the activity of the methanol (80%) extract of the leaf at 500 mcg/ml concentration using agar plate method and have stated it to be inactive against \( B. \ subtilis \) and \( E \ coli \)\(^{789} \).

Awachie and Ugwu (1997) have reported the activity of alcoholic extract of the herb against \( B. \ subtilis \) and \( P. \ aeruginosa \). It is active (minimum inhibitory concentration 1250 mcg/ml) against the former one but not so against the later one\(^{790} \).

Sushil kumar et al. (1997) have reported the activity of the seeds. When the sterile seeds were placed directly on agar media inoculated with the bacteria (\( Bacillus \ subtilis \), \( Escherichia \ coli \), \( Pseudomonas \ cichorii \) and \( Salmonella \ typhimurium \)), they formed zones of inhibition surrounding the seeds\(^{386} \).

### Anticancer activity:

Dhar et al.(1968) have reported that alcohol : water (1:1) extract of the entire plant is inactive in CA-9KB cell culture\(^{336} \) (ED\(_{50} \) >20.0 mcg/ml).

Wang et al. (1982) have reported that hot water extract of aerial parts is active against CA-Ehrlich-Ascites at variable dose levels through IP route in mouse\(^{791} \).

Sauter and Wolfensberger (1989) have reported that water extract of the fruit is inactive against CA-Mammary-BT-20 cell culture at 2.0 ml concentration\(^{792} \).

Lin et al. (1990) have reported that solasodine and solamargin have significant cytotoxic effects against human PLC/PRF/5 cells in vitro; ED\(_{50} \) values are 1.53 and 3.75 µg/ml respectively. Solasodine is also cytotoxic to KB cells, ED\(_{50} \) value is 3.74 µg/ml\(^{793} \). (PLC/PRF/5 cells are established from human hepatoma and are known to produce hepatitis B surface antigen continuously in culture fluids).

Aruna and Sivaramakrishnan (1990) have reported that leaves have protective activity against carcinogenesis. Mice fed with leaf, 600 mg/g diet for 14 days. It increased GST (Glutathione-S-transferase) activity by more than 78% in the stomach, liver and oesophagus, high enough to be considered as protective agents against carcinogenesis. This plant also suppressed significantly chromosome aberrations caused by benzo(A) pyrene in mouse bone marrow cells. It can act as protective agent in cancer\(^{794} \).

Park and Kim (1992) have reported that aqueous, chloroform and methanol extracts of the plant (part not specified) are inactive against CA-A 549 cell culture at 100 mcg/ml concentration\(^{795} \).
Arana and Sivaramakrishnan (1992) have reported that aqueous extract of the herb is active against benzo(A) pyrene induced carcinogenesis in mouse at a dose of 600 mg/g diet.

Park et al. (1993) have reported that methanol extract of aerial parts is inactive against leuk (Shay) (IC_{50} 183.2 mcg/ml), Human-Snu-1 cells (IC_{50} 222.2 mcg/ml) and Human-SNU-C4 cells.

Arisawa (1994, Japan) has reported that methanol extract of the fruit has cytotoxic activity against CA-KB-Human culture at 50.0 mcg/ml, 22% inhibition.

Nikawa et al. (1995) have reported that ethanol (60%) and water extracts of stem are inactive on Salmonell typhimurium TA 98 against 3-amino-1,4-dimethyl-5H-pyrid [4,3-β] indole (Trp-p-1) induced mutagenesis.

Locher et al. (1996) have reported that methanol extract of leaf is inactive against MT-4 cell culture at ED_{50} 124.3 mcg/ml.

6) **Anticoagulant activity**: Kone-Bamba et al. (1987) have reported that fresh leaf extract is active against whole human blood at 50% concentration.

7) **Anticonvulsant**: Adesina (1982) have reported that ethanol (70%) extract of fresh leaf is active against metrazole and strychnine induced convulsions in mouse at variable dose level through i.p. route.

8) **Antifilarial activity**: Ghosh et al. (1994) have reported antifilarial activity of glycoalkaloid solamargine. It killed in vitro 100% adults and microfilariae (mf) of Setaria cervi at a 4 mg/ml concentration in 60 and 88 min. respectively. When the drug was administered orally at 100 mg/kg to rats, in which S. cervi adults were implanted intraperitoneally it reduced blood mf count by more than 30% after the first phase of treatment for 10 days. The mf count showed an increase of 72% after the second phase of treatment. Following the third and the fourth phases of treatments, the mf density was reduced by more than 90%. 100% reduction of mf count was obtained 15 days after the fourth and final phase of treatment which was 5 days duration. At the evaluated dose (100mg/kg x 4 phases), solamargine killed 100% of adult worms without incident toxicity.

9) **Antifertility activity**: Matusi et al. (1967, China) have reported that water extract of entire plant is inactive in female mouse through s.c. route (dose not stated).

10) **Antifungal activity**:

- Neme et al. (1968) have mentioned that aqueous extract of fresh shoot is inactive against plant pathogen, Helminthosporium turcicum, at undiluted concentration by agar plate method.

- Taniguchi et al. (1978) have reported that ethanol (70%) extract of fresh leaf is inactive against Penicillium crustosum at 100 mcg/ml concentration by broth culture method.
Masood and Rajan (1991) have reported 26% of inhibition of aflatoxin produced by *Aspergillus flavus*, with 1 ml conc. of aqueous extract of the leaf. But contradictory observations were later on reported by Mishra et al. (1993) who did not observe any such activity (conc. not mentioned).

Taniguchi and Kubo (1993, E. Africa) have reported that methanol (80%) extract of the leaf is inactive against *Penicillium crustosum* at conc. 500 mcg/ml, using agar plate method.

Singh et al. (1994) have reported that aqueous extract of fresh leaf has weak antifungal activity against *Fusarium oxysporum f. sp. lentis* at a concentration 1:1, using agar plate method.

Awachie and Ugwu (1997, Nigeria) have reported that alcoholic extract of entire plant is active against fungi *Curvularia clavata* and *Candida albicans*, minimum inhibitory concentration is 625 and 1250 mcg/ml respectively.

11) **Antihemolytic / Haemolytic activity:**

- Basu and Lahiri (1977) have examined haemolytic activity of solasonine using human R.B.C. The concentration of solasonine which produced 50% haemolysis is likely to be nearly $10^{-4}$, taking distilled water as 100%. No haemolysis was found with $10^{-6}$ and $10^{-5}$ concentration of solasonine.

- Sulochana et al. (1984) have shown that 0.2 ml extract of the herb inhibited chloropromazine induced haemolysis of human normal erythrocytes in vitro.

12) **Antihistaminic activity:** Reddy et al. (1990) have reported that decoction of the herb has antihistaminic activity in rats at a dose of 1.0 g/kg, through intragastric route.

13) **Anti-HIV activity:**

- Pacheco et al. (1993, Chile) have reported that the ethanol (70%) extract and hydro alcoholic extract of the entire plant have no antiviral activity against Herpes simplex 1 virus or simplex 2 virus or HIV at a conc. 100 mcg/ml by cell culture method.

- El-Mekkaway et al. (1995, Egypt) have reported that water and methanol extracts of the fruits are inactive in inhibition of reverse transcriptase of virus-HIV-1 and virus-HIV-2 at IC$_{50}$ 1.0mg/ml.

- Locher et al. (1996) have shown that methanol extract of leaf has weak anti virus-HIV-1 activity at IC$_{50}$ 124.3 mcg/ml.

14) **Anti-implantation activity:**

- Matusi et al. (1971) have reported that the extract is inactive in mouse at a dose of 0.2 ml/animal through s.c. route (plant part and type of extract not specified).

- Kamboj (1988) has reported that the extract (type not stated) is inactive in female rat (dose not stated) through i.p. route.
15) **Anti-inflammatory**:
- Basu and Lahiri (1977) have examined solasonine for anti-inflammatory activity with cotton pellet implantation granuloma test and carrageenin paw oedema test in rats at the dose of 20 mg/kg (s.c. route). It showed significant anti-inflammatory activity comparable with 0.5 mg/kg betamethasone.805.
- Reddy (1989) has reported anti-inflammatory activity of decoction of herb using the two experimental models viz. the acute inflammatory model, inflammation induced by carrageenin and chronic inflammatory model, inflammation induced by Freund's adjuvant in albino rats. In both the models it is active at a dose level 1.0 g/kg (intragastric route). It also inhibited higher levels of histamine up to 48.69% in the blood due to chronic inflammation.357.
- Nadeem and Hussain (1996) have reported that successive petroleum ether and successive 50% alcohol extracts of the fruits have significant anti-inflammatory activity in rats. The rats were treated with the petroleum ether extracts (0.4, 0.8 and 1.2 mg/kg) and 50% alcohol extracts (100, 200 and 400 mg/kg) 10 days prior to induction of inflammation by carrageenin. Petroleum ether extract at the dose level of 1.2 mg/kg and 50% alcohol extract at 200 and 400 mg/kg have significant anti-inflammatory activity as compared to acetylsalicylic acid (100 mg/kg).810.

16) **Antimalarial activity**: Misra et al. (1991) have reported that alcohol-water (50%) extract of aerial parts is inactive at a dose of 1.0 g/kg for 4 days, against *Plasmodium berghei* in mouse. The *in vitro* studies have shown 52% inhibition of *P. berghei* at a concentration of 100 mcg/ml of the above extract.819.

17) **Antioxidant**: Sultana et al. (1995) have reported that the crude extract of the leaves inhibits (29.5%) free radical mediated DNA damage at the dose of 25 mcg/ml. The leaf powder was extracted with alcohol; concentrated and defatted with petroleum ether and then extracted with diethyl ether. The diethyl ether soluble portion was passed from the column (silica gel) and eluted with petroleum ether, benzene and ethyl acetate. The benzene + ethyl acetate fraction protected calf thymus DNA against oxidative damage to its deoxyribose sugar moiety. The effect was dependent on the concentration of the plant extract (5 to 25 mcg/ml). The hepatoprotective effect of this plant extract may be due to their ability to suppress the oxidative degradation of DNA in the tissue debris.811.

18) **Antipyretic activity**:
- Dhar et al. (1968) have reported that 50% alcohol extract of the plant is active in mouse at a dose of 500 mg/kg through i.p. route.812.
- Mohsin et al. (1989) have reported that alcoholic extract of the aerial parts is inactive against yeast induced pyrexia in mouse at the dose of 500 mg/kg through intragastric route.783.
19) **Antispasmodic activity** : Dhar et al. (1968) have reported that ethanol-water (1:1) extract of the entire plant is active against acetylcholine induced spasms in guinea pig ileum\(^{336}\).

20) **Antispermatogenic activity** : Dey et al. (1965) have reported that ethanol extract of entire plant 100 mg/kg (orally) was inactive\(^{813}\).

21) **Antiviral activity** :
   - Roychoudhury and Basu (1983) have studied the activity of the aqueous extract of unripe fruit and leaf on Tobacco Mosaic virus and Sunnhemp rosette virus. The extracts were inactive against both the viruses but the polyphenolic fraction and flavonoid fraction of it were inactive against tobacco mosaic virus\(^{841}\).
   - Sauter and Wolfensberger (1989, Switzerland) have reported that water extract of the fruit is active against influenza virus at 2.0 ml concentration\(^{792}\).

22) **Antiyeast activity** :
   - Taniguchi et al. (1978) have reported that ethanol (70%) extract of fresh leaf is inactive against *Saccharomyces cerevisiae* at 100 mcg/ml concentration\(^{784}\).
   - Al Mashal et al. (1982, Saudi Arabia) have reported that methanol extract of entire plant is inactive against *Candida albicans* by agar plate method (conc. not mentioned)\(^{786}\).
   - Jawad et al. (1988, Iraq) have studied the activity of 80% alcohol extract of aerial parts of the herb and its alkaloidal and sesquiterpene fractions at a conc. of 0.2 ml/well, against *Candida albicans* and *Candida pseudotropicalis* by agar plate method. The extract was active against *Candida albicans* but the fractions did not show any activity against any of the two organisms\(^{787}\).
   - Taniguchi and Kubo (1993) have reported that methanol (80%) extract of leaf is active against *Saccharomyces cerevisiae*, at a conc. 500 mcg/ml using agar plate method\(^{789}\).

23) **Brine Shrimp lethality** : Awachie and Ugwu (1997) have reported that alcoholic extract of entire plant is inactive against brine shrimp, *Artemia salina* at a concentration between 10 to 1000 ppm\(^{790}\).

24) **Cardiovascular system** : Basu and Lahiri (1977) have reported following activities of solasonine on cardiovascular system using various animal models\(^{805}\):
   - **Anaesthetised cat** : Solasonine has hypotensive activity in anaesthetised cats at a dose level of 1-2 mg/kg through i.v. route. The fall in B.P. was moderately marked but temporary. It did not affect significantly the hypotensive response to acetylcholine, histamine and 5-hydroxy tryptamine or the pressor response to adrenaline and noradrenaline. The hypotensive response was not blocked by atropine, mepyramine, cyproheptadine or propanol\(^{805}\).
   - **Cat's heart in situ** : Solasonine (1 mg/kg, i.v.) reduced amplitude of contraction, diastolic relaxation was incomplete\(^{805}\). 

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Isolated guinea pig heart: 5 mcg or more of solasonine produced an increase followed by a sustained decrease of rate and amplitude. Coronary outflow was markedly reduced.

Rabbit ear: Solasonine (2.5μg) produced a marked vasoconstriction on perfused isolated rabbit ear, roughly of the same order as induced by 10 ng adrenaline on the same preparation.

25) CNS-stimulant activity: Basu and Lahiri (1977) have studied the effect of solasonine (20 mg/kg, i.p.) on the behavioural activity in mice. It did not affect significantly pentobarbitone sleeping time or rectal temperature. Analgesic, anticonvulsant or amphetamine toxicity protective activity was also not noticed.

26) CNS depressant activity: Perez et al.(1998) have reported that alcoholic extract of fresh ripe fruits of *S. nigrum* has CNS depressant activities on rats and mice at the dose levels of 51, 127.5 and 225 mg/kg through i.p. route (LD₅₀ value is 510 mg/kg, through i.p. route in mice). It significantly increased pentobarbitone induced sleep time, produced alteration in general behaviour pattern, suppressed aggressive behaviour, affected locomotor activity, reduced spontaneous motility etc. in different experimental models.

27) Embryotoxic effect: Prakash and Mathur (1978) have reported that ethanol-water (1:1) extract of seed is inactive in female rats at a dose of 150 mg/kg through oral route.

28) Gastric protective activity: Basu and Lahiri (1977) have reported that solasonine (20mg/kg, s.c.) was found to reduce significantly the free acid level but not the total acid level or gastric content volume in Shay rats. An examination of the gastric mucosa showed that as compared to control rats, mucosal erosion and small bleeding points were fewer in solasonine treated rats.

29) Gastric antiulcerogenic activity: Akhtar and Munir (1989) have evaluated the powder of aerial parts and its aqueous and methanolic extracts, through intragastric route in rats for the various activities like (i) Acid neutralising: 400 mg concentration showed strong to water extract, none to methanol extract and weak to powder activity. (ii) Antiulcer activity: Aspirin induced ulcerogenic rats at a dose of 4 g/kg showed activity to methanol extract and powder but not to water. (iii) Gastric secretion inhibitory activity and pepsin binding activity were carried under same condition as mentioned in (ii). All were inactive in inhibiting the gastric hexosamine and pepsin secretion, except methanol extract which showed strong activity for pepsin. Powder showed activity for pepsin binding but the other two showed very weak activities.

• Urinogastric effect- Clinical trials: Pevchikh et al. (1966) have studied the changes of gastric functions under the influence of solasodine, at the doses of 0.024 g (total dose 0.5 g), in 40 patients with rheumatism and rheumatic arthritis. It increased pepsin in gastric juice and uropsin in the urine. K⁺ content in the
gastric juice decreased from 43 to 33 mg\% and Na\(^+\) increased slightly from 153 to 161 mg\%\(^8\)16.

30) **Hepatoprotective activity:**
- Basu and Lahiri (1977) estimated liver glycogen in bilaterally adrenalectomised mice after glucose administration. Solasonine (20 mg/kg, i.v.) induced significant increase in liver glycogen deposition\(^8\)05.
- Saxena and Garg (1979) have reported that when aqueous extract of the leaves 0.125 ml/kg was given orally to rats for 11 weeks, it reduced hexobarbitone sleeping time. It also stimulated hepatic mitochondrial enzymes, but has no effect on the hepatic lysosomal or microsomal enzymes\(^8\)21.
- Pandey (1980) has reported hepatoprotective activity of alcoholic extract of the herb on chlorpromazine induced liver injury in albino rats. Extract was dissolved in propylene glycol and a dose of 25 mg/kg(i.p.) was given. The drug reduced serum alkaline phosphatase, SGPT, and BSP clearance test values to the highly significant levels. The decrease in SGOT and thymol turbidity was also to significant level. There was increase in body weight and in RNA and DNA concentration of the liver. It dilated portal tract with mild periportal necrosis\(^8\)20.
- Lin et al. (1988) have reported hepatoprotective activity of solasodine, solamargine, solasonine, ursolic acid and carpesterol, the constituents isolated from the fruits of *S. incanum*. First three constituents are also present in *S. nigrum*. These constituents are reported to protect mice against CCl\(_4\) induced toxicity at the dose levels of 3, 1 and 0.1 mg/kg, orally respectively\(^8\)22.
- Moundipa and Domngang (1991) have studied the effect of fresh leaf at variable dose levels against aflatoxin B\(_1\) induced toxicity on some liver drug metabolising enzymes in female rats. It was active in stimulation of alkaline phosphatase (50\% activity increased) and aminopyrine-N-demethylase induction (75\% activity) but inactive in aspartate aminotransferase induction and \(\beta\)-gluco-uronidase stimulation. It was active in induction of Gamma glutaryl transferase (200\% activity), glutathione -S- transferase (65\% activity) and uridine diphosphate glucuronyl transferase (99\% activity)\(^8\)23.
- Nadeem et al. (1991 and 1997) have reported hepatoprotective activity of the fruits. The slurry of the powdered fruits at dose of 5, 10 and 20 g/kg has significant hepatoprotective activity against CCl\(_4\) and paracetamol induced toxicity. It reduced significantly serum enzymes aspartate transferase(\(\text{AST}\)), alanine transferase(\(\text{ALT}\)), alkaline phosphatase(\(\text{SACP}\)) and acid phosphatases(\(\text{SACP}\)). It also restored hepatic glutathione (GSH) level. Histopathological studies confirmed these findings against CCl\(_4\) induced damage but not against paracetamol toxicity at the same dose levels. Petroleum ether extract(at a dose level of 2.5, 5 and 10 mg/kg) and 50\% ethanolic extracts(at a dose level of 1.19, 2.39 and 4.78 g/kg) also have slight to moderate hepatoprotective activity\(^8\)24,\(^8\)25.

- **Hepatoprotective activity - Clinical trials**: Pevchikh et al. (1966) have studied the changes of hepatic functions under the influence of solasodine at the dose of 123
0.024 g (total dose 0.5 g) in 40 patients with rheumatism and rheumatic arthritis. It normalised some disturbed indices of hepatic function i.e. levels of bilirubin, prothrombin and total protein.

- **Hepatoprotective activity of polyherbal formulations:**
Liv 52 is the most popular hepatoprotective polyherbal formulation amongst all the market herbal formulations. Hence its pharmacological reports are many in comparison to the others. It contains 7 herbal drugs (mentioned in Table I) one of them being *Solanum nigrum*. The investigated reports on this are as follows:

i. Jogelkar et al. (1963) have administered Liv 52 to male mouse, orally, and studied its activity against CCl₄ induced toxicity. 70% animals survived.

ii. Sethi and Sharma (1978) administered hot water extract of Liv 52 to adult patients suffering from jaundice and proved its hepatoprotective efficacy.

iii. Saxena and Garg (1979) have reported that when 0.125 ml/kg Liv-52 is given to the rats (orally) for 11 weeks, it stimulated hepatic mitochondrial enzymes.

iv. Sharma et al. (1991) have reported hepatoprotective activity of Liv 52 against CCl₄ induced toxicity in rat.

The reports for the other formulations are as mentioned below:

v. Bardhan et al. (1985) have reported that butanol, petroleum ether and water extracts of a polyherbal formulation are inactive while chloroform extract is active against CCl₄ induced damages in liver homogenate at a dose of 500 mcg/ml. It inhibited acid phosphatase and acid ribonuclease.

vi. Bhaumik and Sharma (1993) have reported that intragastric administration of the formulation, 1 g/kg, for 10 days after paracetamol intoxication ameliorated the changes induced by the toxin in the sheep (the formulation consists of *A. paniculata, P. niruri* and *S. nigrum*).

vii. Sharma et al. (1991) have reported hepatoprotective activity of Hepa-10, Livarin, Livokin, Livomyn and M-Liv in rat against CCl₄ induced toxicity.

viii. Sharma et al. (1993) have reported hepatoprotective activity of a polyherbal formulation consisting of equal parts of *Solanum nigrum, Berberis aristata* and *Achyranthus aspera* against paracetamol induced hepatotoxicity in the goats. The drug was given for 10 days orally at a dose of 1 g/kg body weight.

Besides antihepatotoxic activity, polyherbal formulations were also studied for:

31) **Anti-viral hepatitis activity - Clinical trial:** Sama et al. (1978) have shown that Liv-52 is active against acute viral hepatitis in human adults through oral route.

32) **Hypocholesterolaemic activity:** Dixit et al. (1992) have reported that solasodine at the dose of 50 mg/kg (through oral route) has hypocholesterolaemic and antiatherosclerotic activity in cholesterol fed rabbits. To the cholesterol fed rabbits (for 60 days), solasodine was given from 61 to 120 days. It reduced serum cholesterol and LDL cholesterol by 73.3% and 73.5% respectively and prevented atherosclerosis. The cholesterol phospholipid ratio was decreased by 42.02% while the HDL ratio was raised significantly. It prevented accumulation of cholesterol in the liver and aorta and regressed plaque size in the thoracic and
abdominal aorta. Faecal excretion of cholesterol and phospholipids was significantly (P >0.001) increased suggesting that modulation of absorption was affected.

33) **Hypoglycaemic activity**: Basu and Lahiri (1977) have reported that solasonine (20 mg/kg, i.p.) enhanced blood sugar levels in fasting rabbits which returned to normal by about 4 hours.

34) **Hypotensive activity**:
- Karaev et al. (1955) have reported that galenical preparation of the plant caused an appreciable reduction in blood pressure in acute experiments. In isolated rabbit ears vasodilation was observed.
- Dhar et al. (1968) have reported that 50% alcoholic extract of the entire plant has hypotensive activity in dog at a dose of 50 mg/kg through i.v. route.
- Basu and Lahiri (1977) have reported the activity of solasonine in anaesthetised cat at a dose level of 1-2 mg/kg through i.v. route. The fall in B.P. was moderately marked but temporary. It did not affect significantly the hypotensive response to acetylcholine, histamine and 5-hydroxy tryptamine or the pressor response to adrenaline and noradrenaline. The hypotensive response was not blocked by atropine, mepyramine, cyproheptadine or propanol.
- Kloos et al. (1987) have reported that ethanol (70%) extract of fresh leaf is active in rat at variable dose levels through i.v. route.

35) **Immunomodulating activity**: Bahr and Hansel (1982) have shown that solasodine isolated from *S. nigrum* has immunosuppressive actions *in vitro* model. Solasodine inhibited proliferation of murine spleen cell cultures.

36) **Insecticidal activity**: Heal et al. (1950) have reported that water extract of the leaf is active against *Blatella germanica* at variable concentration and *Pteriplaneta americana* at 40 ml/kg, through i.v. route (strong activity).

37) **Miracidal activity**: Broberg and Suomen (1980) have reported that aqueous extract of dried leaf is active against *Fasciola gigantica* at 0.1% conc., resulting death in 5 minutes.

38) **Mollucidal activity**:
- Mendes et al. (1984) have reported the activity of the ethanol and hexane extracts of the dried leaf are inactive against *Biophalaria glabrata* at a conc. 100 ppm.
- Kloos et al. (1987) have reported that aqueous extracts of dried stem, root and fruit are active against *Biophalaria pfeifferi* (conc. used is not stated).
- Mkoji et al. (1989) have reported that water extract of the fruits is inactive against *Lymnaea natalensis* at conc. 100 mg/L.
39) **Respiratory activity**: Basu and Lahiri (1977) have reported that solasodine at the dose of 2 mg/kg (i.v.) increases the rate of respiration but not the amplitude, in guinea pigs; respiration in the rat was not significantly affected.

40) **Smooth muscular activity**: Basu and Lahiri (1977) have reported the effect of solasonine on the smooth muscles of different organs of various animals as follows:

- **Guinea pig ileum**: It did not produce any contraction up to concentration of $1 \times 10^{-5}$. It inhibited contractile response of acetylcholine at concentration of $5 \times 10^{-7}$ but not that of histamine or barium and at still higher concentration (above $10^{-5}$) small contraction was noticed.

- **Rabbit intestine**: It contracts rabbits intestine at $10^{-4}$ concentration.

- **Isolated rat uterus**: It produces contractions of rat uterus at $10^{-7}$ to $10^{-6}$ concentration.

- **Isolated cat tracheal ring**: It did not produce a contractile response at $4 \times 10^{-5}$ concentration but if pretreated for 2 min. reduced acetylcholine response.

41) **Uterine activity**: Dhar et al. (1968) have reported that alcohol: water (1:1) extract of the entire plant has no uterine stimulant (estrogenic) activity in female rat.

42) **Toxicity studies**:

- Dhar et al. (1968) have reported maximum tolerated dose of alcohol: water (1:1) extract of the entire plant as 1 g/kg in mouse through i.p. route.

- Mohsin et al. (1989) have reported that alcohol extract of aerial parts 3.0 g/kg, through intragastric route produces no death in mouse.

- Akhtar and Munir (1989, Pakistan) have reported that powder of aerial parts at a dose of 6 g/kg through intragastric route is inactive, and with no general toxicity.

- Dugan and Gumbmann (1990) have shown that when 8% seeds were fed to rats along with the diet for 1-9 days, it reduced glutamate oxaloacetate, cholesterol and sugar level in serum but when 16% seeds were fed, it stimulated glutamate pyruvate transaminase and with 32% seeds, no general toxic effect was noticed.

- Nadeem and Hussain (1996) have reported the maximum tolerated doses of the petroleum ether and 50% alcoholic extract of fruit; they are 10 mg/kg and 1500 mg/kg body weight of the rats respectively. No mortality was observed at these two dose levels up to 10 days.

- Perez et al. (1998) have reported LD$_{50}$ of alcohol extract of fresh ripe fruits. It is 510 mg/kg, i.p. in mice. There is no acute (during two hours) or delayed (3 days
after administration) toxicity in the mice at 51, 127.5 and 255 mg/kg, through i.p. route in mice.

- Indian Herbal Pharmacopoeia vol. II (1999) mentions that higher doses of fruit powder may cause lethargy, diarrhoea and pyloric obstruction. Children who have eaten berries from the plant have complained headache, vertigo, nausea, vomiting and tenesmus.

- Basu and Lahiri (1977) have reported LD$_{50}$ (mouse, i.p.) of solasonine to be 77.3 mg/kg. Solasonine administration (10 mg/kg per day for 20 days) produced no significant changes either in body weight, haemoglobin, R.B.C. or W.B.C. count as compared with saline treated control.

5.4.6. ANALYTICAL REVIEW:

All the parts of the plant are reported to contain solasodine and its glycosides. They are also reported to have various biological activities including hepatoprotective activities. Number of methods are available to determine the quantity of solasodine in the herb and its various parts.

e.g. gravimetric, titrimetric, colorimetric, TLC, GLC, HPLC etc. Some of the drawbacks of these methods are as follows:

- Larger quantity of sample is required in the first three methods, and they are not specific. Moreover, TLC method is semi-quantitative. GLC method requires purification and derivatization prior to analysis. Detectors for HPLC method are not sensitive.

- Birner (1969) has described a colorimetric method for estimation of solasodine in the leaf or fruit drugs. Colour is developed with methyl orange and estimated at 420 nm, it obeys Beer's law in concentration from 10-120 mcg in 5 ml. Colour can also be developed with antimony chloride or bromothymol blue.

- Weiler et al.(1980) have described radio-immunoassay method for estimation of solasodine and its glycosides in the plant and herbarium specimens. It is rapid, specific and sensitive method, and can detect as little as 0.7 ng of solasodine glycosides(solasonine, solamargine) in crude plant extract.

- Indian Herbal Pharmacopoeia (1998) has described HPLC method for estimation of solasodine in Solanum xanthocarpum, which can also be applied in cases of other plant containing solasodine. In this method, standard solution of solasodine is prepared having concentration of 0.2-1.6 mg/ml; column-μ Bondapack C$_{18}$ (30x3.9 mm); mobile phase- TRIS buffer (10 mm, pH 7.5) : Acetonitrile (1:9); flow rate- 2 ml/min; detector - U V., 200 nm; Retention time - 10.0 min.
Literature survey reveals that no one has tried to identify the hepatoprotective compound/s of the herb or its parts by structure activity guided fractionation method. However, Lin et al. have reported that solasodine, solasonine and solamargine are the hepatoprotective compounds of *Solanum incanum*. These constituents are also present in all parts of *S. nigrum*. Flavonoids are also reported to have antioxidant and hepatoprotective activities. Flavonoid, quercetin glycosides are reported in the leaves of the plant. Sultana et al. have also reported antioxidant activity of the purified fraction of leaf containing phenolic compounds. Other workers have also reported hepatoprotective activities of the leaf, fruit and the herb. But no one has done estimation of solasodine and its glycosides (the hepatoprotective compounds) in the polyherbal hepatoprotective formulations containing the herb or its parts. We have planned to do estimation of solasodine in the herb, its different parts and the available market formulations. Immature fruits are reported to have toxic effect.