Chapter II

Research Envisaged and Literature
Review of Selected Plant
2. RESEARCH ENVISAGED AND LITERATURE REVIEW OF SELECTED PLANT

2.1 Research envisaged and plant selection

Plant drugs play a vital role in the treatment of various ailments based on the information available in the ancient literature. Despite their sound traditional background, it is essential to investigate the rationality of their use in modern scientific terms.

The potential use of plants as a source of new drugs is still poorly explored, of the estimated 2,50,000-5,00,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties. In most cases, only pharmacological screening or preliminary studies have been carried out. It is estimated that only 5000 species have been studied for medicinal use (Mabberley, 1997).

As natural product research continues to be an important part of the drug discovery, the author was interested in taking up the phytochemical investigation of selected plant to isolate active principles. The main advantage of natural products as a source of lead compounds is the tremendous molecular diversity found in nature. Advances in the methodologies used to evaluate extracts and pure compounds for biological activity has enabled the possibility to isolate and identify interesting biologically active compounds on sub milligram scale (Robert, 1996).

Due to high cost of this type of research selection of the specimen to be analyzed is one of the critical points. According to Elisabetsky and Moraes (Elisabetsky and Moraes, 1988) there are three different ways of approach for the selection of medicinal plants:

1. Randomized, which does not use any criteria, i.e., and the investigation takes an arbitrary course, every time a specimen is available.
2. Chemotaxonomical or phylogenetical, where the species are selected according to a given chemical category of substances in a genus or family.
3. Ethnopharmacological, in which selection of plants is based on their therapeutic use by an ethnic group.
There is another aspect with which everyone agrees. If the selection of plants is made on the grounds of their traditional use, the chance of research success is greater (Trotter et al., 1982; Elisabetsky and Wannmacher., 1993).

*Phyllanthus amarus* Schum. & Thonn. is an important plant of Indian Ayurvedic system of medicine. There was strong evidence among the tribals of Paderu, Visakhapatnam forest region in which they are using *Phyllanthus amarus* Schum & Thonn for the treatment of wound healing and joint pains. Traditionally this plant is used in the problems of wounds, ulcers, scabies, stomach, genitourinary system, liver, kidney, gonorrhea, menorrhagia, other genital infections, gastropathy, diarrhoea, dysentery, intermittent fevers. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. Along with these traditional uses the *P.amarus* extracts and its lignans also showed *in vitro* cytotoxic activity on cancer cell lines (Dixit et al., 2011).

After analyzing the above aspects, the author has considered both chemotaxonomy as well as ethnopharmacology before the selection of *Phyllanthus amarus* Schum. & Thonn. for this research. To prove the Folkloric claims and make *P.amarus* useful to the human community by contributing to herbal drug development as there was lacking in the treatment of many diseases by allopathic medicines, the author felt the plant *Phyllanthus amarus* is worthy to consider for this research.

### 2.2 Plan of work

The plan of research work was as follows:

1. Introduction
2. Literature review on the selected plant i.e., *Phyllanthus amarus* Schum. & Thonn.
3. Qualitative phytochemical evaluation of the plant extracts.
4. Isolation of bioactive lignans that were reported during the previous investigations (Kassuya et al., 2006; Singh et al., 2009 and Pramyothin et al., 2010) which were not pursued due to rigidity of the isolation methods and poor yields of pure lignan molecules.
5. Acute oral toxicity studies of the hexane, ethyl acetate, methanol extracts of *P.amarus* aerial parts.
6. Evaluation of Anti-oxidant activity of the hexane, ethyl acetate, methanol extracts and isolated lignans of \emph{P.amarus}.

7. Evaluation of Anti-inflammatory activity of the hexane, ethyl acetate, methanol extracts and isolated lignans of \emph{P.amarus}.

8. Evaluation of Anticancer activity of the hexane, ethyl acetate, methanol extracts and isolated lignans of \emph{P.amarus} by \emph{in vitro} and \emph{in vivo} methods.

9. Formulation of conventional and pegylated liposomes for the isolated bioactive lignans and their pharmacokinetic studies.

The results obtained are presented and discussed in the subsequent chapters.

\subsection*{2.3 Literature review of \textit{Phyllanthus amarus} Schum. & Thonn.}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig201.png}
\caption{\textit{Phyllanthus amarus} Schum. & Thonn.}
\end{figure}
2.4. Taxonomical classification

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Euphorbiales
Family : Euphorbiaceae
Genus : Phyllanthus
Species : amarus

2.5. Botanical Name : Phyllanthus amarus Schum. & Thonn.

2.6. Vernacular names

Tamil : Keelanelli (Keezhanelli)
Hindi : Bhuyiavla, Jangli amla
Bengali : Bhuiamala, Sadahazurmani
Telugu : Nela uirika, Nelavusari
Kanada : Nela – nelli, Kirunelli

2.7. Geographical distribution

The genus Phyllanthus belonging to family Euphorbiaceae consists of about 6500 species in 300 genera, of which 200 are American, 100 African, 70 from Madagascar and the remaining Asian and Australasian (Webster, 1994). It is indigenous to the rainforests of the Amazon and other tropical countries like India, China and Bahamas, (Morton, 1981; Tirimana, 1987), Philippines (Chevallier, 2000). *P. amarus* is a common tropical weed that grows well in moist, shady and sunny places (Cabieses, 1993; Nanden, 1998). *P. amarus* is distributed all over India and is considered as the most widely occurring Phyllanthus species in India.

2.8. Botanical description

Erect herb grows up to 10-20cm height. Leaves disposed alternately on branchlets, about 2-3 cm long, aborate, rounded at base stipulate; flowers are greenish white, in axillary or lateral pairs, cymes bisexual terminal, 1 or 2 male, the other axils
with 1 male and 1 female flowers, stamens 3, ovary glabrous styles 3, bifid at tip; capsule globose; seeds -6, wedge shaped, ribbed (Dixit et al., 2011).

2.9. Flowering and Fruiting: Throughout the year.

2.10. Parts used: Root, bark, fruits, leaves, seeds.

2.11. Traditional Uses

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Country</th>
<th>Traditional Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amazonia</td>
<td>Gallstones, Kidney Diseases, Kidney Stones</td>
</tr>
<tr>
<td>2</td>
<td>Bahamas</td>
<td>Aperitif, Cold, Constipation, Fever, Flu, Laxative, Stomach Ache, Typhoid</td>
</tr>
<tr>
<td>3</td>
<td>Brazil</td>
<td>Joint Ache, Antispasmodic, Bladder Diseases, Cystitis, Diabetes, Diuretic, Fever, Gallbladder Diseases, Gallstone, Hepatitis, Hydropsy, Kidney Trouble, Kidney Stones, Liver, Prostate and Urinary Diseases</td>
</tr>
<tr>
<td>4</td>
<td>Haiti</td>
<td>Stomach Ache., Carminative, Colic, Digestive, Diuretic, Fever, Malaria, Stomachic, Tenesmus</td>
</tr>
<tr>
<td>5</td>
<td>India</td>
<td>Anemia, Asthma, Bronchitis, Cough, Diuretic, Dysentery, Gonorrhea, Hepatitis, Jaundice, Thirst, Tuberculosis, Abdomen Tumor</td>
</tr>
<tr>
<td>6</td>
<td>Java</td>
<td>Cough, Gonorrhea, Stomachache</td>
</tr>
<tr>
<td>7</td>
<td>Malaya</td>
<td>Caterpillarsting, Dermatosis, Diarrhoea, Diuretic, Itch, Miscarriage, Pesticide, Renosis, Syphilis, Vertigo</td>
</tr>
<tr>
<td>8</td>
<td>Marianas</td>
<td>Dysentery, Itch, Rectitis, Vaginitis</td>
</tr>
<tr>
<td>9</td>
<td>Peru</td>
<td>Diuretic, Hepatitis, Gallstone, Kidney stones</td>
</tr>
<tr>
<td>10</td>
<td>Elsewhere</td>
<td>Blennorrhagia, Diabetes, Diarrhoea, Diuretic, Dropsy, Dysentery, Dyspepsia, Emmenagogue, Fever, Gallstone, Gonorrhea, Kidney Stones, Malaria, Tonic</td>
</tr>
</tbody>
</table>

2.12. Phytochemical review

The Phyllanthus genus has been the subject of much phytochemical studies since the mid 1960’s. The various types of secondary metabolites previously reported
in *P. amarus* are alkaloids, flavonoids, ellagitannins, lignans, polyphenols, triterpenes, sterols and volatile oils (Dixit *et al*., 2011).

### 2.12 Lignans

Lignans isolated from Phyllanthus species mostly belongs to two groups, the 1,4-diarylbutane and 1-aryltetralin though neolignans and lignans with other skeleton were also reported from this plant. The following lignans have been reported. (Morton, 1981; Chevallier, 2000; Srivastava *et al*., 2008; Kassuya *et al*., 2006; Huang *et al*., 2003; Maciel *et al*., 2007; Singh *et al*., 2009).

#### 2.12.1 Diarylbutane lignans

The following diarylbutanes were previously reported. Phyllanthin (1), hydroxyniranthin (2), 2,3-desmethoxy seco-isolintetralin (3), niranthin (4), demethylenedioxyxyniranthin, seco-isolariciresinol trimethyl ether (5), 2,3-desmethoxy seco-isolintetralin diacetate (6), linanthin (7), demethylenedioxy niranthin (8) and nirphyllin (9) (Morton, 1981; Anjaneyulu *et al*., 1973). A detailed extraction, isolation and characterization method was also optimized for phyllanthin (Hamrapurkar *et al*., 2009).

![Phyllanthin -R1 = R2 = CH3 R3 = R4 = H R5 = R6 = CH3 (1)](image)

![Hydroxyniranthin-R1 + R2 = CH2 R3 = CH3 R4 = OH R5 = R6= CH3 (2)](image)

![2,3-desmethoxyseco-isolintetralin-R1 + R2 = CH2 R3 = R4 = R5 = R6 = H (3)](image)

![Niranthin-R1 + R2 = CH2 R3 = CH3 R4 = H R5 = R6= CH3 (4)](image)

![Seco-isolariciresinol trimethyl ether-R1 = R2 = CH3 R3 = R4 = R5 = H R6 = CH3 (5)](image)

![2,3-desmethoxyseco-isolintetralin diacetate-R1 + R2 = CH2R3 = R4 = H R5 =R6 = COCH3 (6)](image)
2.12.1.2 Aryltetralin lignans: Hypophyllanthin (10), phyltetralin (11), lintetralin (12), nirtetralin (13), isolintetralin (14), neonirtetralin (15) (Anjaneyulu et al., 1973; Huang et al., 2003)

2.12.1.3 Neolignan: Phyllnirurin (16) (Singh et al., 2009).

2.12.1.4 Other lignans: Two new lignans, namely cubebin dimethyl ether (17) and urinatetralin (18) are isolated recently (Elfahmi et al., 2006). Seco-4-hydroxylintetralin (19), 3,4-methylenedioxybenzyl-3′,4′-dimethoxybenzylbutyro lactone (20) (McDoniel and Cole, 1972).
Lintetralin (12)  
Nirtetralin (13)  
Isolintetralin (14)  
Phyllnirurin (16)  
Cubebin dimethyl ether (17)
Urinatetralin (18)

Seco-4-hydroxylintetralin (19)

3,4-Methylenedioxybenzyl-3’,4’-dimethoxybenzylbutyrolactone (20)
2.12.2 Flavonoids

The following flavonols and flavanone subclasses and their respective glycosides have been reported for this plant i.e., astragalin(21), kaempferol(22), rutin(23), quercetin(24), quercitrin(25), isoquercitrin(26), kaempferol-4’‐rhamno pyranoside(27), eridictyol-7-rhamno pyranoside(28), Prenylated flavanone glycoside(29), Gallocatechin(30), nirurin(31), niruriflavone(32), quercetol(33) and Quercetin 3-O-β-glucopyranosyl (2-1) O-β-D-xylopyranoside(34) (Ishimaru et al., 1992; Morton, 1981; Foo and Wong, 1992; Foo, 1993a & 1995).
2.12.3 Terpenes

Monoterpenoids like Limonene (35), p-Cymene (36) has been previously isolated from this plant. Lupeol (37), phyllanthenol (38), phyllanthenone (39), phyllantheol (40), Oleanolic acid (41), ursolic acid (42). 2Z, 6Z, 10Z, 14E, 18E, 22E-farnesylfarnesol (43) (Maciel et al., 2007; Foo and Wong, 1992).
2.12.4 Tanins

Tannin precursors like ellagic acid(44), gallic acid(45), simple tannins like 1,6-digalloylglucopyranose(46), 4-O-galloylquinic Acid(47) and complex tannins like Ellagitannin(48), amaritin(49), furosin (50), geraniin acid B (51), amariinic acid(52), amarulone(53), repandusinic acid A(54), Geranin(55), isocorilagin(56), elaeocarpusin(57), phyllanthusiin(58), melatonin(59) and corilagin(60) have been previously isolated (Foo and Wong, 1992; Foo, 1993a, 1995; Houghton et al., 1996; Kassuya et al., 2006).
2.12.5 Sterols
Amarosterol A (60) and amarosterol B (61) are isolated previously along with β-sitosterol (63) (Ahmad and Alam, 2003).

2.12.6 Volatile oil
Volatile oils like Linalool (64) and Phytol (65) have been reported (Moronkola et al., 2009).

2.12.7 Alkaloids
Alkaloids like securinine (66), dihydrosecurinine (67), tetrahydrosecurinine (68), securinol (69), phyllanthine (70), allo-securnine (71), norsecurinine (72), epibubbialine (73), isobubbialine (74), 4-methoxy-nor-securnine (75), 4-methoxy dihydrosecurinine (76), 4-methoxytetrahydrosecurinine (77) and 4-Hydrosecurinine (78), Nirurine (79) and Phyllochrysine (80) have been isolated. Securinine is a selective antagonist of GABA recognition sites on mammalian central neurons (Foo and Wong, 1992; Houghton et al., 1996; Kassuya et al., 2006).

R = H Securinine (66)
R = OCH3 Phyllanthine (70)
2.13. Pharmacological review

2.13.1 Anticancer activity

Administration of the aqueous extract of *P. amarus* to cancer bearing mice, lowered the tumor incidents, level of carcinogen-metabolizing enzymes levels of liver cancer markers dose dependently (Kumar and Kuttan, 2005). Cytotoxicity of the crude extracts (aqueous and methanolic) and their fractions of *P. amarus* inhibited A549 (lung carcinoma) cells growth in *in vitro* experiments. It was previously reported that antimetastatic activity of this plant is mostly associated to the presence of polyphenol compounds in its extracts (Lee et al., 2011).

Significant increase in the survival of hepatocellular carcinoma harboring animals was observed upon administration of aqueous extract of *P. amarus* (Rajeshkumar and Kuttan, 2000). A mixture of phyllanthin and hypophyllanthin (1:1), isolated from *P. amarus* exhibited antitumor activities against EAC in Swiss albino mice. (Islam et al., 2008). The hexane extract, the lignans rich fraction and the lignans nirtetralin, niranthin and phyllanthin exerted cytotoxic effects on K-562 cells with of cell death, respectively (Leite et al., 2006).

2.13.2 Antiulcer activity

The pretreatment of *P. amarus* leaf aqueous extract (500 mg/kg) significantly inhibited ulceration induced by oral administration of absolute ethanol (Shokunbi and Odetola, 2008).

2.13.3 Antiamnesic activity

The aqueous extract of leaves and stems of *P. amarus* showed dose-dependent improvement in memory of young and older mice by reversing the amnesia induced by scopolamine and diazepam by reducing the brain cholinesterase activity (Joshi and Parle, 2007).

2.13.4 Antibacterial activity

Hexane, methanol and water extracts of aerial parts of *P. amarus* exhibited remarkable antimicrobial activities against *M. lylae, S. haemolyticus, B. lentus, B. firmus, P. stutzeri, P. aeruginosa* and *S. aureus* (Koday et al., 2009).
2.13.5 Antifungal activity

The chloroform fraction of the aerial part of the *P. amarus* showed significant inhibitory effect against dermatophytic fungi *M. gypseum* (Agrawal *et al.*, 2004). Norsecurinine (alkaloid) was effective against most of the fungi (Sahni *et al.*, 2005).

2.13.6 Anti-inflammatory, Analgesic and anti-oedematogenic activity

It was previously reported that the aqueous leaf extract of *P. amarus* showed significant dose related increased inhibition of the carrageenan-induced paw oedema in the rats. It was reported that extract produced a marked analgesic activity by inhibiting both early and late phases of pain stimulus in formalin induced paw licking rats (Iranloye *et al.*, 2011). The methanolic extract of *P. amarus* inhibited induction of interleukin (IL)-1β, IL-10, and interferon in human blood and reduced the TNF-γ production *in vivo*. The anti-allodynic and anti-oedematogenic effects of the hexane extract, lignan-rich fraction and purified lignans from aerial parts of *P. amarus* was reported in the inflammatory and neuropathic models of nociception (Mahat and Patil, 2007).

2.13.7 Antinociceptive activity

The extract of the Phyllanthus species when given intraperitoneally, produced dose related and pronounced antinociception when assessed against chemical models of nociception, including acetic acid, formalin and capsaicin-induced pain (Santos *et al.*, 2000).

2.13.8 Antioxidant activity

The aqueous extract of whole plant of *P. amarus* found to have significant potential in scavenging free radicals, in inhibiting lipid peroxidation and cytoprotective efficiency against Cr(VI)-induced oxidative cellular damage (Guha *et al.*, 2010). Amariin, repandusinic acid and phyllanthusiin D showed higher antioxidant activity among the ellagitannins and were comparable to the flavonoids, rutin and quercetin 3-Oglucoside (Londhe *et al.*, 2008).
2.13.9 Antiplasmodial activity

The ethanolic, methanolic and methylene chloride extracts of entire plant of *P. amarus* showed significant activity against the chloroquine sensitive strain of *P. falciparum* 3D7 (Adjobimey *et al.*, 2004). The extracts of the whole plant of *P. amarus* possess repository and chemotherapeutic effects against resistant strains of *P. yoelii* (Ajala *et al.*, 2011) and *P. berghei* (Dapper *et al.*, 2007) parasites in Swiss albino mice.

2.13.10 Antiviral activity

The methanolic extracts of root and leaves of *P. amarus* inhibited the NS3 and NS5B enzymes of hepatitis-C virus were screened by *in vitro* enzyme assays. The aqueous extract of *P. amarus* showed antiviral activity against white spot syndrome virus in shrimp (Balasubramanian *et al.*, 2007). *P. amarus* derived preparations (water–alcohol extract, geranin) blocked the interaction of HIV-1 gp120 with its primary cellular receptor CD4. Inhibition was also evident for the HIV-1 enzymes integrase, reverse transcriptase and protease (Notka *et al.*, 2004).

2.13.11 Aphrodisiac activity

The methanolic extract of *P. amarus* leaves (50–800 mg/kg) caused a statistically significant increase in the level of testosterone of the male guinea pigs. It also caused an insignificant change in the level of leutenizing (LH) and follicle stimulating (FSH) hormones (Obianime and Uche, 2009).

2.13.12 Hepatoprotective activity

*P. amarus* extract and phyllanthin were found to have protective effect on CCl4-induced toxicity in human hepatoma HepG2 cell line (Krithika *et al.*, 2009). The hepatoprotective mechanism of *P. amarus* was neither related to inhibition on cytochrome P450, nor induction on sulfate and glucuronide conjugation pathways of paracetamol, but partly due to the antioxidant activity and the protective effect on the decrease of hepatic reduced glutathione (Wongnawa *et al.*, 2005). *P. amarus* effectively modified alcohol and thermally oxidized polyunsaturated fatty acid-induced fibrosis by modulating the expression of matrix metalloproteinases (Surya Narayanan *et al.*, 2011).
2.13.13 Hypoglycemic and hypocholesterolemic activities

The hypoglycemic activity was observed for the aqueous extract of whole plant of *P. amarus* (Mbagwu et al., 2011). The significant decrease in the body weight, hyperglycemia and hyperlipidemia was observed in STZ-induced diabetic rats with the treatment of aqueous extract of *P. amarus* in diabetic treated group (Karuna et al., 2011). The ethanol and hexane extracts of *P. amarus* exhibited appreciable α-amylase inhibitory activity (Tamil et al., 2010). Hydro-alcoholic extract of leaves of *P. amarus* showed significant hypolipidemic activity (Umbare et al., 2009).

2.13.14 Immunomodulatory activity


2.13.15 Nephroprotective activity

It was found that the aqueous extracts of leaf and seeds of *P. amarus* showed significant protective effects against acetaminophen and gentamicin-induced nephrotoxicity (Adeneye and Adokiye, 2008).

2.13.16 Radioprotective effect

Kumar and Kuttan (2004) reported that the *P. amarus* extracts and its compounds (amariin, 1-galloyl-2, 3-dehydrohexahydroxydiphenyl (DHHDP)-glucose, repandusinic acid, geraniin, corilagin, phyllanthusiin D, rutin, and quercetin 3-O-glucoside) showed radioprotective activity by reducing the lipid peroxidation levels, which were increased after radiation, both in serum and liver.

2.13.17 Spasmolytic activity

Potential spasmolytic activity was exhibited by *P. amarus* extracts on the smooth muscle contraction of a 2 cm long piece of guinea pig ileum induced by acetylcholine (Mans et al., 2004).
2.13.18 Effect on reproductive organs

Phyllanthin and hypophyllanthin treatment recovered the damage caused by a carbamate insecticide, carbofuran on estrous cycle and follicular growth in virgin female wistar rats (Islam et al., 2008a).

2.13.19 Toxicological assessment and contraindications

Previous reports on acute oral administration of *P. amarus* extract showed that the plant extract was non-toxic to the rat liver, even at a dose of 5 g/kg body weight. The chronic toxicity studies of *P. amarus* extracts administration (of 100–800 mg/kg body weight) showed the absence of cumulative toxicity as reflected by the non-significant change in the parameters studied as well as from the results of the histological studies (Sirajudeen et al., 2006). It has been considered in herbal medicine to be abortive (at high dosages) as well as an emmenagogue. Although it is not studied in humans but animal studies do indicate that it has uterine relaxant effects. It is therefore contraindicated during pregnancy. It has been documented with female antifertility effects in mice (the effect was reversed in 45 days after cessation of dosing) (Rao and Alice, 2001).