

2.1. PLANT SELECTED FOR THIS STUDY



Plate.2.1. *Calotropis gigantea* (L.)R.Br.

[1. Flowering branch; 2. gynostegium in longitudinal branch; 3. pollinium; 4. follicle.]

From pre-historic times to the modern era in many parts of the world and India, plants, animals and other natural objects have profound influence on culture and civilization of man. Since the beginning of civilization, human beings have worshiped plants and such plants are conserved as a genetic resource and used as food, fodder, fibre, fertilizer, fuel, febrifuge and in every other way. *Calotropis gigantea* is one such plant (Royal Botanic Gardens, Kew, 1900). In ancient ayurvedic medicine the plant *Calotropis gigantea* is known as “sweta Arka” and *Caotropis procera* as “Raktha Arka”. Both of them are often similar in their botanical aspects and also have similar pharmacological effects (Mueen Ahamed *et al.*, 2005). The systematic position, vernacular names, vegetative characters of the plant are given in the following Tables (2.1 -3). In the present study henceforth this plant is referred to as *Calotropis*.

Table 2.1. Systematic position of the selected plant

Kingdom	Plantae
Order:	Gentianales
Family:	<i>Asclepiadaceae</i>
Subfamily:	<i>Asclepiadoideae</i>
Genus:	<i>Calotropis</i>
Species:	<i>Gigantea</i>

Table 2.2. Vernacular names

India	(Sanskrit) Arka, Ganarupa, Mandara, Vasuka, Svetapushpa, Sadapushpa, Alarka, Pratapass, (Hindi) Aak, Madar, (Kannada) Ekka, (Tamil and Malayalam) Erukku, (Telugu) Jilledu Puvvu
Malaysia	Remiga, rembega, kemengu.
English	Crown flower, giant Indian milkweed.
Indonesia	Bidhuri (Sundanese, Madurese), sidaguri (Javanese), rubik (Aceh)
Philippines	Kapal-kapal (Tagalog).
Laos	Kok may, dok kap, dok hak.
Thailand	Po thuean, paan thuean (northern), rak (central).
Vietnam	B[oot]ng b[oot]ng, l[as] hen, nam t[it] b[at].
French	Faux arbre de soie, mercure vegetal.

Table 2.3. Vegetative characters

Habit:	Shrub or a small tree up to 2.5 m (max.6m) height.
Root:	Simple, branched, woody at base and covered with a fissured; corky bark; branches somewhat succulent and densely white tomentose; early glabrescent. All parts of the plant exude white latex when cut or broken.
Leaves:	Opposite-decussate, simple, sub sessile, extipulate; blade-oblong obovate to broadly obovate, 5-30X2.5-15.5 cm, apex abruptly and shortly acuminate to apiculate, base cordate, margins entire, succulent, white tomentose when young, later glabrescent and glaucous.
Flowers:	Bracteate, complete, bisexual, actinomorphic, pentamerous, hypogynous, pedicellate, pedicel 1-3 cm long.
Floral Characteristics:	Inflorescence: A dense, multiflowered, umbellate, peduncled cymes, arising from the nodes and appearing axillary or terminal
Calyx:	Sepal 5, Polysepalous, 5 lobed, shortly united at the base, glabrescent, quincuncial aestivation.
Corolla:	Petals five, gamopetalous, five lobed, twisted aestivation.
Androecium :	Stamens five, gynandrous, anther dithecous, coherent.
Gynoecium:	Bicarpellary, apocarpus, styles are united at their apex, peltate stigma with five lateral stigmatic surfaces. Anthers adnate to the stigma forming a gynostegium.
Fruit:	A simple, fleshy, inflated, subglobose to obliquely ovoid follicle up to 10 cm or more in diameter.
Seeds:	Many, small, flat, obovate, 6x5 mm, compressed with silky white pappus, 3 cm or more long.

(Gamble, 1923 and 1935; Lindley, 1985; Mueen Ahamed *et al.*, 2005)

2.1.1. Ecology and Distribution

2.1.1.1. Natural habitat

Calotropis is drought resistant, salt tolerant to a relatively high degree, grows wild up to 900 meters (msl) throughout the country (Sastry and Kavathekar, 1990) and prefers disturbed sandy soils with mean annual rainfall: 300-400 mm. Through its wind and animal dispersed seeds, it quickly becomes established as a weed along degraded roadsides, lagoon edges and in overgrazed native pastures. It has a preference for and is often dominant in areas of abandoned cultivation especially disturbed sandy soils and low rainfall. It is assumed to be an indicator of over cultivation (Mueen Ahamed *et al.*, 2005).

2.1.1.2. The chief features of the plant are:-

- The plant grows very well in a variety of soils and different environmental conditions
- It does not require cultivation practices
- It is one of the few plants not consumed by grazing animals (Sharma, 2003).
- It thrives on poor soils particularly where overgrazing has removed competition from native grasses (Smith, 2002)
- Some times this plant is the only survivor in some areas, where nothing else grows (Sastry and Kavathekar, 1990; Oudhia, 2001).
- It is drought tolerant and the pioneer vegetation in desert soil (Oudhia, 1997).
- Presence of latex, extensively branched root system and thick leaves with waxy coverage are the xerophytic adaptations (Mueen Ahamed, 2005).
- Hence, it is distributed in tropical and subtropical area of the world and throughout India. (Sastry and Kavathekar, 1990).

2.1.1.3. Geographic distribution

It is a native of India, China and Malaysia and distributed in the following countries: Afghanistan, Algeria, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Egypt, Eritrea, Ethiopia, Gambia, Ghana, guinea-Bissau, India, Iran. Iraq, Israel, Kenya, Kuwait, Lebanon, Libyan, Arab Jamahiriya, Mali, Mauritania, morocco, Mozambique, Myanmar, Nepal, Niger, Nigeria, Oman, Pakistan, Saudi Arabia, Senegal, sierra Leone, Somalia, Sudan, Syrian Arab Republic, Tanzania, Thailand, Uganda, United Arab emirates, Vietnam, Yemen, Republic of

Zimbabwe, Exotic: Antigua and Barbuda, Argentina, Australia, Bahamas, Barbados, Bolivia, Brazil, Chile, Colombia, Cuba, Dominica, Dominican Republic, Ecuador, French Guiana, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Jamaica, Martinique, Mexico, Montserrat, Netherlands Antilles, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, St Kitts and Nevis, St Lucia, St Vincent, and the Grenadines, Surinam, Trinidad and Tobago, Uruguay, Venezuela and Virgin Islands (US) (Mueen Ahmed *et al.*, 2005).

2.1.1.4. Propagation and management

The seeds freely float in the air and natural regeneration is very common. Vegetative propagation through stem and root cuttings is very useful in large scale multiplication of the superior genotypes.

Calotropis has been cultivated in South America and on the Caribbean Islands for the production of fibres at a spacing of 1-1.5m. When cultivated annually yields of up to 500kg/ha are expected. A single harvest per season is preferable to a double or triple harvest; a single harvest would result in a net saving of energy input both on the farm and in the processing plant. It is well suited for intensive energy farming in arid or semi-arid regions where frost is not a limiting factor (Mueen Ahamed *et al.*, 2005).

2.1.2. Phytochemistry of *Calotropis*

The previous workers have reported many phytochemical constituents in the various parts of *Calotropis gigantea* especially in the leaves. Usharin, gigantol, calcium oxalate, alpha and beta-calotropol, beta-amyrin, fatty acids (both saturated and unsaturated), hydrocarbons, acetates and the benzoates, a mixture of tetracyclic triterpene compounds, terols, giganteol and gigantol are also found to be present (Bhaskara Rama Murti and Seshadri, 1943, 1944, 1945a, b). Cardenolide calotropin (Kupchan *et al.*, 1964), α -amyrin, β -amyrin, taraxasterol, β -sitosterol, α -amyrin methylbutazone, β -amyrin methylbutazone, α -amyrin acetate, β -amyrin acetate, taraxasteryl acetate, lupeol acetate B, gigantursenyl acetate A, gigantursenyl acetate B (Sen *et al.*, 1992; Habib *et al.*, 2007), flavonol glycoside, akundarol, uscharidin, calotropin, frugoside, calotroposides A to G (Kshirsagar, *et al.*, 2010) are responsible for many of its activities. The following cardenolides are also described in the literature: calactin, calotoxin, calotropagenin, proceroside, syriogenine, uscharidin, uscharin, uzarigenin and voruscharin (Crout *et al.*, 1963 a,b; Brischweiler *et al.*, 1969a,

b; Lardon *et al.*, 1970; Singh and Rastogi, 1972; Seiber *et al.*, 1982). Other compounds found are benzoylisolineolon and benzoyllineolone (Chandler *et al.*, 1968).

Flavonoids (Chopra *et al.*, 1956; Singh and Rastogi, 1972), triterpenoids (Pal and Sinha, 1980), alkaloids, steroids, glycosides, saponins, terpenes, enzymes, alcohol, resin, fatty acids and esters of calotropeols (Seiber *et al.*, 1982), volatile long chain fatty acids (Sen, *et al.*, 1992), glycosides and proteases (Kitagawa *et al.*, 1992) have been isolated from the various parts of the plant *Calotropis gigantea*.

Cleverson *et al.*, (1996) worked out the laticifer fluid of *Calotropis*, and found to have strong proteolytic activity, having the enzyme cysteine proteinase and aspartic proteinase. Due to the presence of these components, the plants are resistant to phytopathogens and insects mainly in leaves where the latex circulates abundantly. The milky latex of the plant is rich in lupeol, calotropin, calotoxin, and uscharidin, the latex protein. Sharma and Sharma (1999) screened the major phytochemicals viz. alkaloids, carbohydrates, glycosides, phenolic compounds/tannins, proteins and amino acids, flavonoids, saponins, sterols, acid compounds, resins in flower, bud, root of *Calotropis* (Table 2.4).

Table 2.4. Phytochemical components in *Calotropis*

S.No.	Class of Compounds	Plant Part			Tests performed
		Flower	Bud	Root	
1.	Alkaloids	+	+	+	Dragendorff's test, Mayers test
2.	Carbohydrates	+	+	+	Molish test, Fehling test
3.	Glycosides	+	+	+	Keller killiani test
4.	Phenolic compounds/tannins	+	+	+	Ferric chloride test
5.	Proteins and amino acids	+	+	+	Xantho protein test
6.	Flavonoids	+	+	+	Ammonia test
7.	Saponins	+	+	+	With water With sodium bicarbonate
8.	Sterols	+	+	+	Liebermann-Burchard test Salkowski reaction Hesse's reaction
9.	Acid compounds	+	+	+	With sodium bicarbonate With litmus paper
10.	Resins	+	+	+	With double distilled water With acetone and

					conc.Hydrochloric acid
11.	Peroxides	-	-	-	Potassium Iodide test
12.	Polyuronoids	-	-	-	Haemotoxylin test

(Sharma and Sharma, 1999).

2.1.3. Economic values of *Calotropis*

2.1.3.1. Medicinal properties

Different parts of the plant have immense potential to cure various diseases and disorders (Table 2.5). It is used in various polyherbal preparations (The Wealth of India, 1992; Tenpe, *et al.*, 2007). There are more than hundred activities described in detail by Duke (1992). *Calotropis* is used alone and sometimes with other plants to cure variety of human and animals ailments.

Table 2.5. Medicinal properties

S.No.	Medicinal properties	References
1.	Asthma	Kirtikar and Basu, 1935; Shah and Joshi, 1971; Jain <i>et al.</i> , 1973; Chaudhuri <i>et al.</i> , 1975; Bhalla <i>et al.</i> , 1982; Saxena, 1986; Caius, 1986; Das, 1996; Snigdha Roy 2008
2.	Abortifacient	Saha <i>et al.</i> , 1961; Patel and Patel, 2004
3.	Analgesic, anticonvulsant, anxiolytic and sedative	Nadkarni, 1976; Allen, 1994; Aminuddin Girach, 2001; Argal and Pathak, 2006; Pathak and Argal, 2007; Argal and Diwivedi, 2010
4.	Antifertility and emmenagogue	Patel and Patel, 2004
5.	Anti-inflammatory activity	Pardesi <i>et al.</i> , 2008; Das <i>et al.</i> , 2009
6.	Antinociceptive activity	Soares <i>et al.</i> , 2005
7.	Anthelmintic activity	Zafar Iqbal <i>et al.</i> , 2005
8.	Anti cancer activity	Choedon <i>et al.</i> , 2006
9.	Anti dote for Scorpion stings and insect bites	Hutt and Houghtom, 1998; Narumon., 2005; Kadhivel <i>et al.</i> , 2010
10.	Anti tumor activity	Dash, 1991; Jayaweera, 1980–1982; Dassanayake, 1980–2000; Pal and Jain, 1998; Taylor <i>et al.</i> , 1996
11.	Anti-diarrheal and anti dysentery activities	Satyavati <i>et al.</i> , 1976; Dash, 1991; Jayaweera, 1980–1982; Dassanayake, 1980–2000; The Wealth of India, 1992; Pal and Jain, 1998; Taylor <i>et al.</i> , 1996; Caius, 1986; Das, 1996; Havagiray <i>et al.</i> , 2004; Chitme <i>et al.</i> , 2004; Chitme <i>et al.</i> , 2005
12.	Antimicrobial activity	Valsaraj <i>et al.</i> , 1997; Samy & Ignacimuthu, 2000; Rao, 2000; Ashraful <i>et al.</i> , 2008

13.	Antiviral activity	Locher <i>et al.</i> , 1995
14.	Anxiety and pain	Boericke, 2001; Sharma, 2001
15.	CNS activity	Argal and Pathak, 2006
16.	Cold	Caius, 1986; Das, 1996
17.	Expectorant	Kirtikar and Basu, 1975; Shiddamallayya. <i>et al.</i> , 2010
18.	Cytostatic activity	Smit <i>et al.</i> , 1995
19.	Cytotoxic activity	Ayoub and Kingston, 1981; Smit <i>et al.</i> , 1995; Locher <i>et al.</i> , 1995; Kupchan <i>et al.</i> , 1964; Oliveira <i>et al.</i> , 2007
20.	Dyspepsia	Blair, (1907).; Ghosh 1988
21.	Eczema	Caius, 1986; Das. 1996 ; Kirtikar KR and Basu, 1998; Chitme <i>et al.</i> , 2004; Chitme <i>et al.</i> , 2005
22.	Elephantiasis	Caius, 1986; Das, 1996
23.	Epilepsy	Jain <i>et al.</i> , 2001; Pathak and Argal, 2006
24.	Elephantiasis of the legs and scrotum	Kirtikar and Basu, 1975
25.	Expectorant	Kirtikar and Basu, 1935
26.	Fever	Caius, 1986; Das. 1996
27.	Fibrinolytic activities	Rajesh <i>et al.</i> , 2005
28.	Free radical Scavenging activity	Mueen Ahmed <i>et al.</i> , 2003
29.	Healing the ulcers and blotches	Blair, 1907; Ghosh 1988; Ferrington 1990
30.	(Goat) Motility of mature <i>Haemonchus contortus</i> of goat origin	Sharma <i>et al.</i> , 1971
31.	Indigestion	Kirtikar and Basu, 1975
32.	Kesarayer disease	Kumar and Vallikannan, 2009
33.	Leprosy	Shah and Joshi, 1971; Jain <i>et al.</i> , 1973; Chaudhuri <i>et al.</i> , 1975; Jayaweera, 1980–1982; Bhalla <i>et al.</i> , 1982; Saxena, 1986; Dash, 1991; Dassanayake , 1980–2000; Taylor <i>et al.</i> , 1996; Pal and Jain, 1998; Kirtikar and Basu, 1998; Chitme <i>et al.</i> , 2004
34.	Liver injuries as well as on oxidative stress, Hepatoprotective	Jayaweera, 1980–1982; Dash, 1991; Dassanayake , 1980–2000; Pal and Jain, 1998; Taylor <i>et al.</i> , 1996; Lodhi <i>et al.</i> , 2009
35.	Mental disorders	Upadhyaya <i>et al.</i> , 1994; Sivastava <i>et al.</i> , 2007
36.	Migrine	Prusti and Behera, 2007
37.	Nasal ulcer, laxative, rheumatoid arthritis, bronchial asthma, diabetes mellitus, nervous disorders	Narumon, 2005
38.	Piles	Shiddamallaya <i>et al.</i> , 2010
39.	Pregnancy interceptive activity	Srivastava <i>et al.</i> , 2007

40.	Purgative	Baldwin, 1979
41.	Removing anemia	Blair, 1907; Ghosh 1988; Ferrington 1990
42.	Rheumatism	Srivastava <i>et al.</i> , 2007
43.	Ringworm of the scalp	Kirtikar and Basu, 1975
44.	secondary syphilis, gonorrhoea, ascites, helminthiasis, and jaundice	Kirtikar and Basu, 1998; Chitme <i>et al.</i> , 2004
45.	Skin diseases	Dash, 1991; Jayaweera, 1980–1982; Dassanayake, 1980–2000; Taylor <i>et al.</i> , 1996; Pal and Jain, 1998
46.	Spleen disorder	Shiddamallayya, <i>et al.</i> , 2010
47.	Swelling and inflammation in sprain	Manandhar, 1990
48.	TB and leprosy	Kirtikar and Basu, 1935; Grange and Davey, 1990
49.	Uterus stimulant	Saha <i>et al.</i> , 1961; Chopra <i>et al.</i> , 1965
50.	Vermicidal activity	Garg and Atal, 1963
51.	(Vertenery) Camel diseases treatment	Sharma <i>et al.</i> , 1971; Antoine-Moussiaux <i>et al.</i> , 2007
52.	Worms	Dash, 1991; Jayaweera, 1980 – 1982; Dassanayake, 1980–2000; Taylor <i>et al.</i> , 1996; Pal and Jain, 1998
53.	Wounds and ulcers	Jayaweera, 1980–1982; Dassanayake, 1980–2000; Caius, 1986; Dash, 1991; Das, 1996; Taylor <i>et al.</i> , 1996; Pal and Jain, 1998
54.	Wound healing activity	Biswas and Mukherjee, 2003; Havagiray <i>et al.</i> , 2004; Chitme <i>et al.</i> , 2004; Rajesh <i>et al.</i> , 2005; Snigdha Roy, 2008; Pradeep <i>et al.</i> , 2009; Nalwaya <i>et al.</i> , 2009

2.1.3.2. Various other uses

Calotropis is a highly potential plant resource. The various uses of this plant are given in the Table (2.6).

Table 2.6. Other uses of *Calotropis gigantea*

S.No.	Activities	Parts Used	References
1.	Arrow poison	Latex	Ashok and Vaidya, 1998, sharma, 2003
2.	Biocidal activity	Latex	Jain <i>et al.</i> , 1989
3.	Biogas and substitute for petroleum products	Whole plant	Shilpker <i>et al.</i> , 2007
4.	Brewing and to curdle milk	The bark and latex	Pereira and Seabrook, 1996
5.	Cleansing water	Leaves and its Saps	Pereira and Seabrook, 1996
6.	Energy plantation	Whole Plant	Pereira and Seabrook, 1996
7.	Fibers	Bark, and the silky hairs from its seeds	Nart <i>et al.</i> , 1984

8.	Fodder	Young pods, Senescing leaves and flowers	Orwa <i>et al.</i> , 2009
9.	Fungicidal, insecticidal properties	Whole Plant	Ganapathy and Narayanasamy., 1993; Haque <i>et al.</i> , 2000; Ashraful <i>et al.</i> ,2009; Usha <i>et al.</i> , 2009
10.	Isomers Accumulation	Whole Plant	Abhilash and Singh (2008)
11.	Larvicide	Whole Plant	Girdhar <i>et al.</i> , 1984
12.	Latex or rubber	Latex	Mueen Ahamed, 2005
13.	Leather tanning	Whole Plant	Singh <i>et. al.</i> 1996, Mueen Ahamed, 2005
14.	Manna like sugar and liquor (bar)	Sap	Pereira and Seabrook, 1996
15.	Manure, Pest repellent	Twigs and Leaves	Pereira and Seabrook, 1996; Rathood, 1998; TNAU, 2008
16.	Molluscicidal activity	Whole plant	Hussein and El- Wakil, 1996; Bakry, 2009
17.	Indicators of Heavy Metals	Leaf and Stem	Samantaray <i>et al.</i> , 1997
18.	Mosquitocidal potential	Whole plant Petroleum ether– acetone extract,	Neraliya and Srivastava, 1996
19.	Poly aromatic hydrocarbon contamination	Leaves	Sharma & Tripathi, 2009
20.	Pollution Monitoring	Whole Plant	Singh <i>et al.</i> , 1995
21.	Reclaiming salt lands	Whole plant	Pereira and Seabrook, 1996
22.	To cool the air around homes	Plantation of Calotropis	Pereira and Seabrook, 1996
23.	Substitute for paper	Leaves	Pereira and Seabrook, 1996

2.2. STUDY AREA

Seasonal and locational influences on the phytochemistry, the anatomy and the anticancer potential of *Calotropis gigantea* are studied during Sep 2007 - Aug 2008 in Tamil Nadu, India.

2.2.1. General status of Tamil Nadu

2.2.1.1 Climate

The climate of Tamil Nadu is tropical in nature with little variation in summer and winter temperatures. While April - June is the hottest summer period with the temperature rising up to the 40 °C mark, November - February is the coolest winter period with temperature hovering around 20 °C, making the climate quite pleasant. Surprisingly, Tamil Nadu gets all its rains from the northeast monsoon between

October and December, when the rest of Tamil Nadu remains dry. The average of annual rainfalls in Tamil Nadu ranges between 635 and 1,905 mm a year. During summer (April - June), the coastal regions of Tamil Nadu become uncomfortably warm and humid, but the cool sea breezes in the afternoon make the nights cool and pleasant. In this period the enchanting hill stations of the state provide much needed respite from heat and humidity of the plains. -

2.2.1.2 Geography

India, which lies between 804' N and 3706' N latitude and 6807' E and 97025' E longitude, has a total geographic area of 32, 87,782 km². This is only 2.42 % of the total geographic area of the world. The Tamil Nadu is the southern most state of India, surrounded by Andhra Pradesh in the North, Karnataka and Kerala in the west, Indian Ocean in the South and Bay of Bengal in the East. Cape Comorin or Kanyakumari, the southern most point of India lies in the state of Tamil Nadu. The Eastern and Western Ghats (mountain ranges) run along the eastern and western borders of the state and meet at Sittlingi in Dharmapuri district, Tamil Nadu. The Western Ghats, bordering Tamil Nadu, breaks only at two points - Palakkad (25 km wide gap) and Shencottah, which connect the state with Karnataka and Kerala. The state of Tamil Nadu roughly extends between the 8° 04' N latitude (Cape Comorin) to 13.35" and the 78° 0' E to 80.20" longitude (Plate 2.2).



Plate 2.2. Location of Tamil Nadu in India Map (Govt. India, 2003)

2.3. STUDY DESIGN

2.3.1. Seasonal study

Table 2.7. Seasons and months

Season Code	Seasons	Months
S1.	Northeast monsoon	September 2007 - November 2007
S2.	Pre-summer season	December 2007 - February 2008
S3.	Summer season	March 2008 - May 2008
S4.	Southwest monsoon	June 2008 - August 2008

The seasons chosen for the study are found in Table (2.7). The location, selected for the seasonal study, Thirumalai samudram village is situated on the northern side of Tiruchirappalli -Thanjavur highways (NH-47) between Sengipatti and

Vallam village. It was ensured that the sampling land was not under cultivation for the past five years.

2.3.2. Locational study

The samples for the locational studies were collected during August 2008 from five different places in Tamil Nadu, India (Table.2.8. and Plate 2.3 and 2.4).

Table 2.8. Details of the study area

Station Code	Nature Of the Location	Name of the Station	Geographical extension	Altitude [Meters (msl)]
L1	Coastal tract (Plate 2.4.L1)	Sothavilai beach, Kanyakumari	8°07'28. 88" N, 77°29'38. 96"E	6
L2	Hilly terrain (Pate 2.4 L2)	Anaimalayan patti Hills, Theni	9°45'25. 71" N, 77 °21'49. 67"E	600
L3	Riverine zone (Plate 2.4. L3)	Cauveri River bed - Tiruchirappalli	10°50'07.19" N, 78°42'51.18" E	70
L4	Terrestrial -rural stretch (Plate 2.4.L4)	Thirumalai Samudram, Near Tiruchirappalli	10°43'42. 43"N, 79°00'44. 60"E	78
L5	Terrestrial -urban area (Plate 2.4.L5)	Ponmalai locoshed, Tiruchirappalli.	10°47'34.46"N, 78°42'37.00"E	85

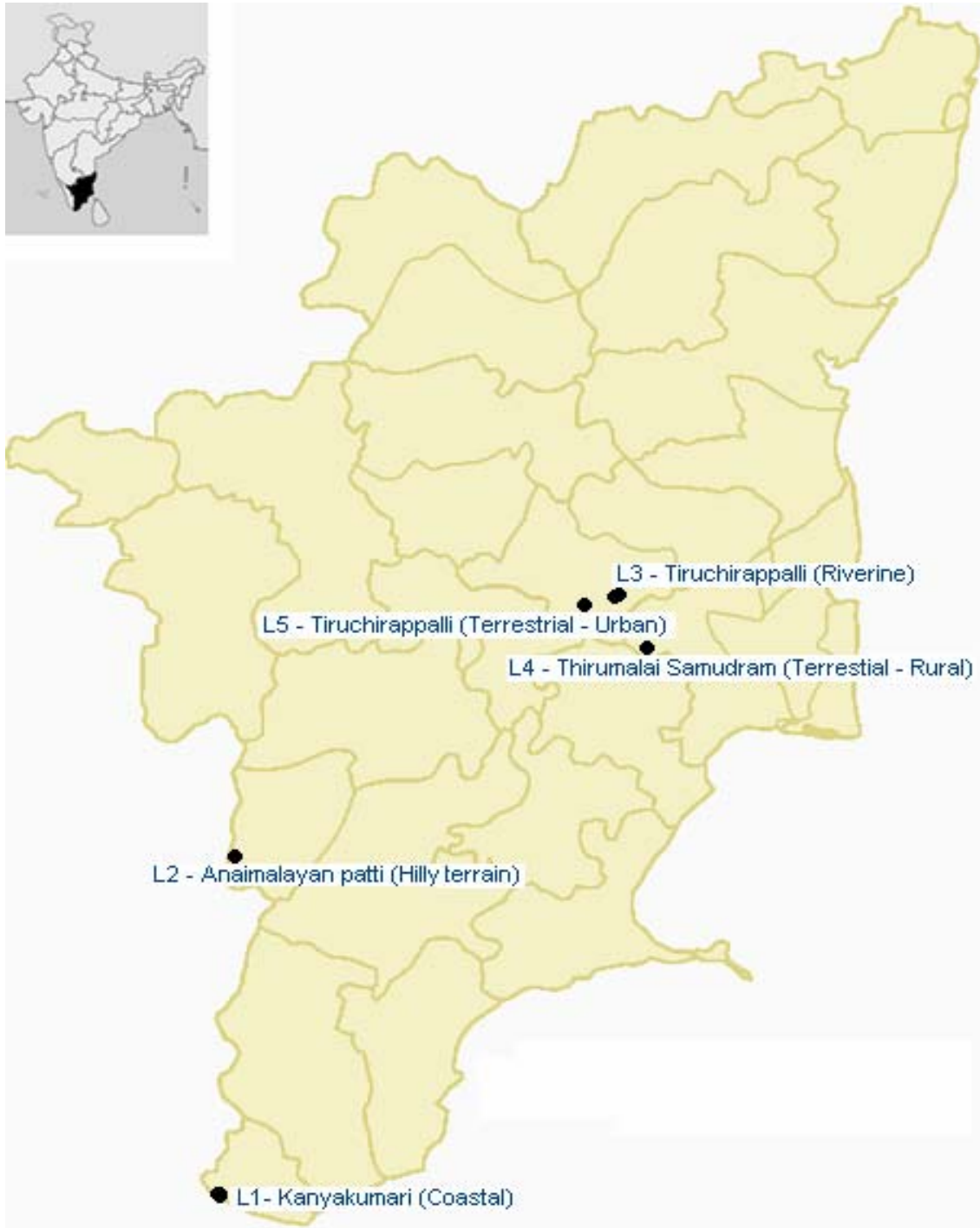


Plate 2.3. Study locations



L1 – Kanyakumari (Coastal tract)



L2 Anaimalayan Patti (Hilly terrain)

Plate 2.4 (L1 & L2) Study locations



L3- Tiruchirappalli (Riverine zone)



L4 – Thirumalaisamudram (Terrestrial – rural stretch)

L5 – Tiruchirappalli (Terrestrial –urban area)

Plate 2.4 (L3, L4 & L5) Study locations

2.4. COLLECTION OF SOIL SAMPLES AND PLANT MATERIALS

The soil samples were collected to analyse the soil nutrients and heavy metal status; the aerial parts of the plant parts were collected to study the phytochemistry and anticancer potential. The stem, the root and the leaves from the third nodes are collected to study the anatomy in the four different seasons and five different locations. The sampling was performed between 3 pm to 4 pm on the respective days of sampling. The plant was vouched by the Department of Botany, Bishop Heber College, Tiruchirappalli. Tamil Nadu, India and a type specimen was submitted to the herbarium.

The specific data sources, methodology, and instruments for climatology, edaphic elements, phytochemistry, plant anatomy and anticancer potential are enumerated in the respective chapters.

2.5. STATISTICAL ANALYSIS

One sample 't' test is performed for the seasonal, soil, phytochemical, anatomical and *invitro* cytotoxicity data from the pooled sample. The Karl Pearson correlation analysis is performed where ever necessary to know the relationship among these parameters. All statistical analysis is performed by using SPSS (version15) package.