CHAPTER - 2
REVIEW OF LITERATURE

2.1 Clerodendrum phlomidis

2.1.1 Genus Clerodendrum

The genus *Clerodendrum* of the family Lamiaceae is a very large and diverse genus of about 560 (Moldenke, 1971) and 580 (Munir, 1989) species of small trees, shrubs, lianas, or occasionally perennial herbs, mostly in the tropics and subtropics of the old world (Verdcourt, 1992). This genus was first described by Linnaeus in 1753 based on the type species *Clerodendrum infortunatum* from India, later Adanson changed the Latinized form "*Clerodendrum*" to its Greek form "*Clerodendron*" in 1763. After almost two centuries Moldenke readopted the Latinized word "*Clerodendrum*" in 1942, which is now commonly used by taxonomists for classification and description of the genus (Hsiao and Lin, 1995). *Clerodendrum* displays a high degree of morphological and cytological variations, many species have been described by more than one author (Steane et al., 1997). Throughout its taxonomic history *Clerodendrum* has been delimited in many ways, some delimitations being more inclusive than others. *Clerodendrum* has been divided between as many as a dozen different genera; sometimes these smaller genera were divided among different families (Westman, 1744; De Necker, 1790). 19th and 20th century taxonomic and phylogenetic studies did much to rectify this, but even now, especially with the development of molecular systematic methods, the delimitation of *Clerodendrum* continues to be modified (Steane et al., 2004). Phenetic and cladistic studies have led to the suggestion that *Clerodendrum* is paraphyletic (Stenzel et al., 1988) or polyphyletic (Cantino, 1992; Rimpler et al., 1992). Parsimony analysis of 456 potentially informative characters identifies four large discrete clades (Clades I-IV) within *Clerodendrum* (Steane et al., 1997). The sequence analysis of internal transcribed spacers of the nuclear ribosomal DNA concluded the genus to be polyphyletic (Steane et al., 1999).

Clerodendrum is a chemically diversified genus, terpenoids are the major secondary metabolite, chiefly steroids (Subramanian et al., 1973; Akihisa et al., 1989; Yang et al., 2000b), neo-clerodane diterpenes (Kumari et al., 2003; Pandey et

al., 2005b), triterpenes (Rangaswami and Sarangan, 1969; Ganapaty and Rao, 1985), and iridoids (Jacke and Rimpler, 1983; Wei et al., 2000). Phenolic compounds have frequently been reported among which phenyl propanoids (Yang et al., 2000a; Kim et al., 2001) and flavonoids predominate (Vendantham et al., 1977; Sinha et al., 1981). Few species have been reported for macrocyclic alkaloids (Bashwira and Hootele, 1988; Lumbu and Hootele, 1993) and cyanogenetic glycosides (Adsersen et al., 1988; Miller et al., 2006). *Clerodendrum* have been studied for a number of biological activity mainly anti-inflammatory (Panthong et al., 2003; Park and Kim, 2007), hepatoprotective (Vidya et al., 2007; Gopal and Sengottuvelu, 2008), anti-hypertension (Hsu and Hsing, 1962; Wang and Liao, 1990), antioxidant (Rajlakshmi et al., 2003; Chae et al., 2006), cytotoxicity (Cheng et al., 2001), antitumour (Shi et al., 1993), antifeedant (Kumari et al., 2003) and CNS activity (Zhu et al., 1996).

2.1.2 Taxonomical hierarchy

Domain : Eukaryota
Kingdom : Plantae
Subkingdom : Viridaeplantae
Phylum : Tracheophyta
Subphylum : Euphyllophytina
Infraphylum : Radiatopses
Class : Magnoliopsida
Subclass : Lamiidae
Superorder : Lamianae
Order : Lamiales
Family : Lamiaceae
Subfamily : Ajugoideae
Genus : *Clerodendrum*
Species : *phlomidis*

2.1.3 Botanical and geographical source

Clerodendrum phlomidis Linn. f. (syn.: *Clerodendrum multiflorum* (Burm.f) O. Kuntze, *Volkameria multiflorum* Burm. f.) (Figure 2.1) belongs to the family Lamiaceae. It is commonly known as Clerodendrum or Wind-killer in English and has different vernacular names in India (Table 2.1) (Nadkarni et al., 1954; Singh, 1955; Kirtikar and Basu, 1975; Anonymous, 2001; Shafi et al., 2001; Pandey et al., 2008).

Figure 2.1: *Clerodendrum phlomidis*

C. phlomidis is a common shrub of arid plains, low hills and tropical deserts. They are distributed throughout the drier parts of India (Andhra Pradesh, Uttar Pradesh, Diu Island, Delhi, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Bihar, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal); Pakistan (Sindh, Baluchistan and north western provinces); Sri Lanka; Myanmar and south-eastern Asia (Watt, 1889; Moldenke, 1948; Chopra et al., 1956; Kirtikar and Basu, 1975; Anonymous, 2001; Pandey et al., 2008).

Table 2.1: Different vernacular names of C. *phlomidis* used in India

Language	Vernacular name
Sanskrit	*Agnimantha, Agnimanthini, Arani, Gandhapatra, Gandhapushpa, Ganikarika, Jatha, Jaya, Jayanti, Jayarini, Krishanuga, Kshudra-Agnimantha, Laghumantha, Nadeyi, Parnaka, Prasarini, Tanutvacha, Tapana, Tarkari, Tejovriksha, Vaijayanti, Vataghni, Vijaya*
Tamil	*Takkari, Thalanji, Thalludhalai, Tirugudalai, Sayandi, Vadamadakki*
Marathi	*Airanamula, Arani, Arni, Iran, Takalimula, Tekar*
Hindi	*Arni, Piran, Pirun, Urni*
Telugu	*Nelli, Taluki, Takkolamu, Tekkali*
Malayalam	*Munja, Peruvelum, Tirutalai*
Bengali	*Arani, Ganiyari, Goniari*
Gujarati	*Aranimula, Arni, Irun*
Kannada	*Taggi, Taggi-Beru*
Oriya	*Hontari, Ganiary*

2.1.4 Controversy regarding its synonym in Ayurveda

Agnimantha and/or Arani is an important drug of Ayurvedic system of medicine. In some Nighantus (Materia Medica) of Ayurveda, only one type of Agnimantha is described but in some, two types of Agnimantha have been mentioned i.e. Laghu

or Kshudra (Small) and Brihad (Large) (Purandare, 1896; Madhavakara et al., 1974; Chaturvedi et al., 1983) having minor differences in their medicinal properties. Some texts refer the source of Kshudra Agnimantha or Laghu Agnimantha as dried mature roots of *C. phlomidis* and dried mature roots of *Premna integrifolia* as Brihad Agnimantha (Singh, 1955; Madhavakara et al., 1974; Nair, 2004). Some consider the smaller type as *P. integrifolia* and larger ones as *C. phlomidis* (Acharya, 1950).

Nair (2004) quotes *P. integrifolia* is used as Agnimantha in Kerala, India and both *C. phlomidis* and *P. integrifolia* may be used as Agnimantha according to availability. Some refer Agnimantha to *P. integrifolia* and Arani to *C. phlomidis* (Jain, 1986) but others quote both as Agnimantha and/or Arni (Vaidya 1965; Sharma, 1996; Bishnupriya et al., 2003; Nair, 2004). Nadkarni et al., 1954 clearly mentions *C. phlomidis* as Agnimantha and also quotes Haines in addition who recognized two varieties, the white (Safed Tekar) and the black (Kala Tekar), the former alone being useful. In "Ayurvedic Formulary of India" the Latin name for Agnimantha is *C. phlomidis* and states that *Premna* sp. can be used as a substitute (Warrier, 1996).

Though Tarkari is regarded as a synonym of Agnimantha, Susruta has enumerated both Agnimantha and Tarkari side by side in one group (Susruta Sutr, XXXVIII), while dravyagunavigyan (Science of treatment) considers Agnimantha to be Valiya munna (*P. mucronata*) and Tarkari to be Ceriya munna (*C. phlomidis).* However through out the state of Kerala, India *Premna* sp. is used for both Agnimantha and Tarkari (Warrier et al., 1996), but Takari also refers to *P. integrifolia, Sesbania aegyptiaca* Pers. and *Cassia tora* Linn. (Meulenbeld and Wujastyk, 2001).

A comparative study of Chavanprasha (a polyherbal formulation containing Agnimantha as ingredient) mentioned in Charak Samhita, Ayurvedic Pharmacopoeia and a commercial house (Baidyanath) was carried out. The results

showed *P. integrifolia* as Agnimantha in Charak Samhita and *C. phlomidis* in Ayurvedic Pharmacopoeia, while Baidyanath showed absence of Agnimantha (Puri, 2002). Moreover, the study of market samples has also indicated that in most cases stem portions are used in place of roots. In Pondicherry, India a stem drug is sold under the name Agnimantha. A pharmacognostical study of the commercial samples supplied as Agnimantha neither, resembled each other nor *C. phlomidis* morphologically or anatomically (Krishnamurthy et al., 1972). A comparative morphological, microscopical and HPTLC studies (using clerodendrin-A as chemical marker) have been carried out for the roots of *Clerodendrum phlomidis* and *Premna integrifolia* (Gokani et al., 2008). Although morphologically both roots resemble each other except for their color and size, they have been differentiated microscopically. The HPTLC studies showed high concentration of clerodendrin-A in *C. phlomidis* (0.073 %w/w) than *P. integrifolia* (0.04 %w/w). In spite of all this studies it is still difficult to decide pharmacolinguistically between the two botanical claimants viz., *C. phlomidis* and *P. integrifolia*, and in addition both of these plants are used as Agnimantha and/or Arani even now by Ayurvedic Vaidyas (Krishnamurthy, 1971).

2.1.5 Commerce and trade

C. phlomidis leaves and roots are one of the highly traded medicinal plants from tropical forests. The estimated consumption/trade of *C. phlomidis* was 306 metric tonnes for the year 2005-06 and estimated annual trade is 200-500 MT. It is one of the highly traded medicinal plants from tropical forests, as they are used in folklore, Ayurveda, Siddha and Unnani medicines. Its sold under the trade name of "Arnimul" (leaf and root), the price range being 15-20 rupees per kg (Ved and Goraya, 2008).

2.1.6 Morphology, anatomy, histology, powder-microscopy and proximate analysis

Anatomical, histological and powder characteristics of different parts of C. phlomidis are shown in Table 2.2 (Haines, 1961; Chunekar and Raghunathan,

1967; Krishnamurthy et al., 1972; Matthew, 1995; Anonymous, 2001). The leaf constants like stomatal number, stomatal index, vein islet number and vein termination number varies from those reported by Chunekar and Raghunathan (1967) and Krishnamurthy et al. (1972). Ayurvedic pharmacopoeia of India has specified the TLC pattern and some parameters viz., foreign matter (NMT 2 %), total ash (NMT 6 %), acid insoluble ash (NMT 1 %), alcohol soluble extractive (NLT 2 %) and water soluble extractive values (NLT 5 %) for identity, purity and strength of roots of *C. phlomidis* (Anonymous, 2001).

Table 2.2: Morphological, histological and powder characteristics of different parts of *C. phlomidis*

Part	Morphological characteristics	Histological characteristics	Powder characteristics
Root	7-15 X 0.2-3.0 cm; occasionally branched; cylindrical; tough; yellowish brown externally; bark thin; outer surface rough; light yellow wood; hard fracture.	Exfoliating cork; rhomboidal crystal of calcium oxalate packed in xylem parenchyma and xylem rays; usual elements all being lignified; abundant round starch grains measuring 6-17 μ in diameter.	Dull yellow, slightly astringent in taste; small, pointed, aseptate, lignified fibres; lignified cells packed with rhomboidal crystals of calcium oxalate; numerous simple, round to oval starch grains.
Leaf	Simple; opposite; exstipulate; deltoid ovate to rhomboid ovate; 1.5-5 X 1-4 cm; entire to sinuate-crenate; sub-acute to obtuse; petiole 3.5 cm long; both surfaces of leaf are puberulous; reticulate; unicostate; 4-7 pairs of secondary nerves.	Lamina is dorsiventral; non-glandular trichomes, slightly warty; glandular trichomes with one celled stalk and 4-8 celled head; cruciferous type of stomata; open collateral vascular bundles; few pericyclic fibres.	Pale green, taste is bitter and astringent; cruciferous type of stomata; amoeboid intercostals cells in the upper epidermis; sunken glandular trichomes.
Stem	Straight; unbranched; cylindrical; 9x2.5 cm; surface uneven; made up of irregularly interconnected axially elongated light coloured ridges alternating with similarly oriented but darker depressions; no lenticels markings.	Tracheidal fibres, end walls sometime present, oblique, the number of tracheidal fibres intervening two adjacent medullary structures is 3-4; pitting usually not seen; medullary structures less elongated mostly 2 regions, 3 celled.	Ash grey, indistinct odour, insipid in taste; tracheidal fibres; medullary structures less elongated mostly 2 regions, 3 celled.

2.1.7 Ethnomedical uses

Indian system of medicines particularly Ayurveda and Siddha uses *C. phlomidis* as a single drug or in combination with other drugs. It was found out that, a village nearby Pondicherry, India is called after the Tamil name, *Thalludhalai* (Krishnamurthy, K.H., 1971). The Ayurvedic properties of *C. phlomidis* are; *Rasa* (taste) – Tikta (bitter), Katu (pungent/acrid), Kashaaya (astringent), Madhura (sweet); *Guna* (quality) – Rooksha (non-unctuous), Laghu (light); *Veerya* (potency) – Ushna (heat); *Vipaka* (transformation with digestion) - Katu (pungent) (Anonymous, 2001). Due to its bitter and pungent nature *C. phlomidis* is considered to normalize the vitiated Kapha and Vata dosa (Chaturvedi et al., 1983). It is an ingredient of number of *Ayurvedic* formulations indicated for digestive disorders, acidity, gas, diarrhoea, laxative, liver tonic and general health tonic (Tyagi, 2005).

The roots are used in different Ayurvedic formulations like Ayushyavardhaak tel, Bhratpanchamula, Chandraprabha vati, Lavanbhasker churna, Abhayarisht, Chavanprasha, Dasamularista, Ashwagandharishta, Mritasanjivani, Dasamula Kvatha Churna, Haritakiavleh, Indukanta Ghrta, Dhanvantara Ghrta, Gorocanadi Vati, Narayana Taila, Ras pitari, Vrahat Panchamuli (Nadkarni et al., 1954; Chunekar, 1960; Chaturvedi et al., 1983; Anonymous, 2001; Puri, 2002, Tyagi, 2005) and Muthu Marunthu (a Siddha polyherbal formulation). *C. phlomidis* is an ingredient of many stress/pain relief massage oil blends and many polyherbal formulations that are used as rejuvenation tonic. Though root is considered to be the authentic drug it is the leaf that finds application in folklore medicines (Krishnamurthy et al., 1972). The ethnomedical uses of different parts of *C. phlomidis* are given in Table 2.3.

Table 2.3: Ethnomedical uses of different parts of *C. phlomidis*

Part	Ethnomedical uses	Reference
Root	12-24 g as decoction are used in Sotha (inflammation, swelling), Pandu (jaundice), Arsa (haemorrhoids, piles), Vibandha (constipation), Agnimandya (slowness of digestion, dyspepsia), Adhmana (swelling of the body), Gulma (a chronic enlargement of the spleen or any glandular enlargement in the abdomen), Mutrakrcchra (painful discharge of urine, a class of urinary affections) and Mutraghata (urinary disease)	Anonymous, 2001
	Used as bitter tonic, antidote, analgesic, asthma, inflammatory diseases and in rheumatism	Kirtikar and Basu, 1975; Katewa et al., 2004
	Used as bitter tonic, nervous disorder and in debility	Khare, 2007
Root bark	Used in cough, asthma, cold, anaemia, oedema and nervous disorders	Singh et al., 1980
Root and root bark	Used as alterative, bitter tonic, and is given in the convalescence of measles by natives of Western India	Watt, 1889 ; Nadkarni et al., 1954 ; Chopra et al., 1958
Root decoction	Used as aromatic, astringent and as demulcent in gonorrhea	Nadkarni et al., 1954; Katewa et al., 2004
Root juice	Used to reduce over-corpulence	Manohar, 2005
Whole plant	Used as hypoglycemic	Krishnamurthy, 1971; Marles and Farnsworth, 1995
	Used for ailments involving swellings, joint pains and inflammation The properties are quoted same as those of *P. integrifolia* but *C. phlomidis* is considered better in inflammation	Krishnamurthy et al., 1972 Puri, 1970 ; Nair, 2004
	The tribes "Santals" rub the plant over their bodies in dropsy	Watt, 1889; Kirtikar and Basu, 1975; Anjaria et. al., 2002
	The tribals "Sahariya" use in fever, postnatal complaints, dyspepsia, colic and anthrax	Anis et al., 2000

	Used in colics, body-ache, diarrhoea, cholera, dysentery, dyspepsia, fever, head-ache, post-natal fever, stomach-ache, during convalescence from measles and specially used for mental diseases	Johnson, 1999; Mitra and Rangesh, 2003; Tyagi, 2005; Patil and Patil, 2006; Khare, 2007
Whole plant decoction	Used to treat diabetes	Mishra, 2003
Whole plant and root	Used as bitter tonic and for neglected syphilitic complaints	Shafi et al., 2001
Leaf	Used as a remedy to treat diabetes in southern parts of India especially tribals of Nilgiris	Dhanabal et al., 2008
	Used in fever due to sunstroke and malaria and as febrifuge	Pandey et al., 2005a
	Ground leaves are given in stomach pain, dyspepsia, digestive disorders, eye complaints, lung diseases, rheumatism, asthma, inflammatory diseases, swellings	Anonymous, 1992
	Locally tied for the treatment of guinea worms	Katewa et al., 2004
Leaf juice	Used to treat mental tension and mental disturbance in Tamilnadu	Murugesan et al., 2001
Leaf and leaf juice	Used as bitter tonic, alterative and prescribed in neglected syphilitic complaints in doses of half an ounce or more twice daily in Southern India	Watt, 1889; Nadkarni et al., 1954; Kirtikar and Basu, 1975; Shafi et al., 2001; Anjaria et. al., 2002
Leaf decoction	Used for inflammation, and is effective in treating bronchitis, headache, weakness, drowsiness and digestive problems	Nadkarni et al., 1954
Leaf and root	Used for body-ache, head-ache and unconsciousness	Patil and Patil, 2006.
Aerial parts	The tribals "Sahariya" apply the paste on body joints for about a month to reduce pain or stiffness of joints	Anis et al., 2000

2.1.8 Veterinary Uses

C. phlomidis finds lot of application in Indian traditional veterinary practices (Srivastava et al., 2000). The tribals, Santhals feed *C. phlomidis* to their cattle for diarrhea and worms or when the stomach swells (Watt, 1889; Kirtikar and Basu, 1975; Anjaria et al., 2002). Extract of leaves is applied on body of domestic animals to kill lices. Leaves are good fodder especially for goats (Pawar and Patil, 2008). Leaf paste is applied on infested hooves to give relief for the animal and reportedly cures foot and mouth disease and or secondary infections (Bhagore, 1991). Fresh leaf extract is pasted on animals having skin problems (Keshabhai, 1992) and used for hypothermia or shivering in cattle (Vaidyar, 1997). In Chittoor and Ananthapur districts of Andhra Pradesh, in Southern India *C. phlomidis* is used for alleviating diseases of livestock by the local traditional herbal practitioners. Leaves are given orally twice daily to cure convulsive seizures and trypanosomosis infection until cured (Rao et al., 2008).

2.1.9 Utilization in Agriculture

In *Surapala's Vrkshayurveda* (a 1,000 AD text) root of *C. phlomidis* is mentioned for various tree disorders (Nene, 2006). C. *multiflorum* is used as an herbal pesticide particularly for insect pest like aphids and red hairy caterpillar (Upadhyay et al., 2002; Bharvad, 2005; Singh and Saratchandra, 2005; Belsare, 2007). Leaf extract is also used for preserving grains (Charpot, 1998), and to control green worms (*Heliothis sp.*) (Ghanch, 1998). Jowar (Sorghum) seeds are treated at the spike forming stage with leaf juice of *C. multiflorum* to protect from fungal infection (*Sporisorium* sp.) (Parmar, 1997).

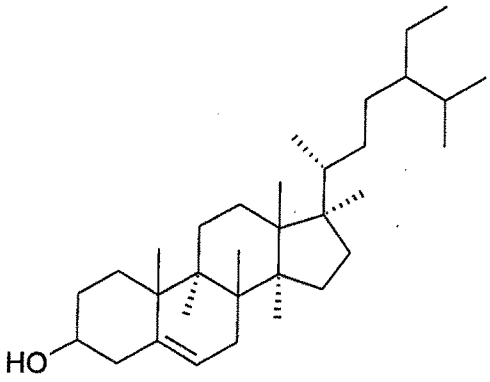
2.1.10 Chemistry

A crystalline non-glucosidal bitter principle ($C_{17}H_{16}O_6$, m.p. 213 °C), ceryl alcohol, β-sitosterol, γ-sitosterol, palmitic acid, cerotic acid and an unidentified sterol ($C_{28}H_{48}O$, m.p. 155 °C) were isolated from leaves. The water extract was found to contain glucose, arabinose and potassium nitrate. Two compounds ($C_{14}H_{28}O_2$, m.p.

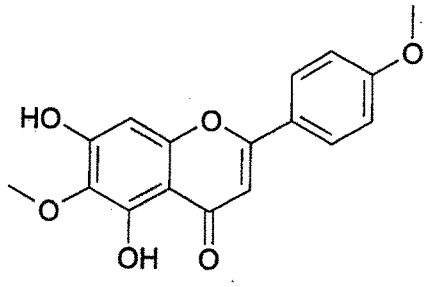
67 °C and $C_{31}H_{64}O_2$, m.p. 93 °C) and a bitter resinous substance reported (Bhakuni et al., 1962) are yet to be characterized. D-mannitol, β-D glucoside of β-sitosterol, β-sitosterol and ceryl alcohol were also isolated from stem (Gupta et al., 1967). The stem, leaf and flower parts were reported positive for alkaloids and negative for saponins and tannins (Hungund and Pathak, 1971).

Scutellarein (5,6,7,4'-tetrahydroxy flavones), pectolinaringenin (6,4'-dimethoxy scutellarein) and a flavanone have been isolated from leaves (Subramanian and Nair, 1972). The crystalline non-glucosidal bitter principle earlier reported by Bhakuni et al. (1962) was identified as pectolinaringenin by Subramanian and Nair (1972).

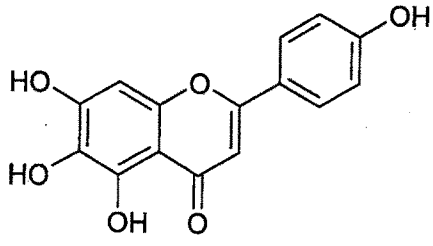
A chemotaxonomic marker of the genus, (24S)-ethylcholesta-5,22,25-triene-3β-ol (C_{29} H_{46} O, m.p. 151-153 °C) was isolated from leaves (Subramanian et al., 1973). β-sitosterol, γ-sitosterol, ceryl alcohol, clerodin ($C_{24}H_{34}O_7$), clerosterol ($C_{29}H_{48}O$) and clerodendrin-A ($C_{27}H_{26}O_{17}$) were also isolated from roots (Joshi et al., 1979). Seth et al., 1982 reported 6,4'-dimethyl-7-acetoxyscutellarein, pectolinarigenin, hispidulin, apigenin and luteolin from flowers. A chalcone glycoside (4, 2', 4' – trihydroxy-6'-methoxychalcone-4,4'α-D-diglucoside, mp. 186-188 °C, $C_{28}H_{34}O_{15}$), pectolinarigenin, 7-hydroxy flavone and 7-hydroxy flavanone 7-*O*-glucoside were isolated reported from flowers and leaves (Roy and Pandey, 1994; Anam, 1999). α-L-Rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-7-O-naringin-4'-O-α-D-glucopyranoside-5-methyl ether ($C_{34}H_{44}O_{19}$) was isolated from root by Anam (1999). Lup-20(29)-en-3-triacontanoate ($C_{60}H_{108}O_2$), tetratriacontanol and 24β-ethylcholesta-5,22*E*,25-triene-3β-ol were isolated from aerial parts (Pandey et al., 2008). Shanker et al. (2008) reported a new validated TLC method for the quantification of marker sterol, 24β-Ethylcholesta-5,22*E*,25-triene-3β-ol with chloroform-methanol (98.5: 1.5) (R_f 0.54 ± 0.05) and densitometric evaluation at 650 nm after anisaldehyde derivatization.



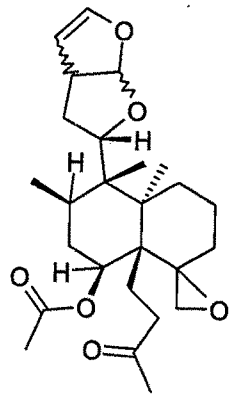
β-sitosterol



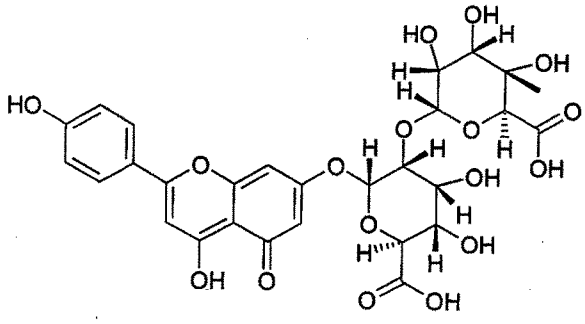
Pectolinaringenin



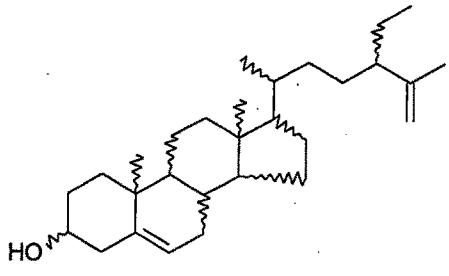
Scutellarein



Clerodin

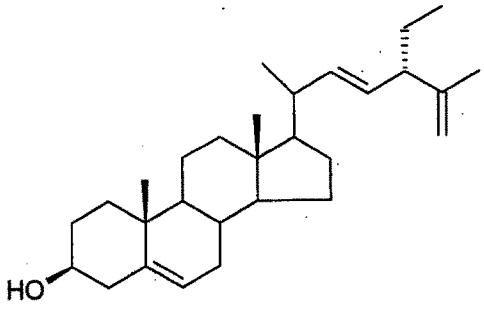


Clerodendrin

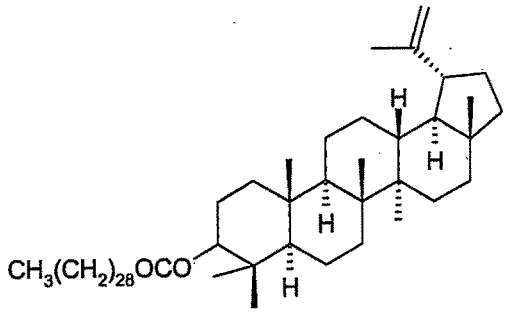


Clerosterol

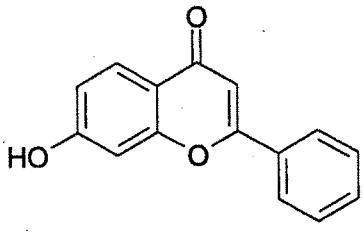
Figure 2.2. Chemical structures of some isolated compounds from *C. phlomidis* (Cont.)



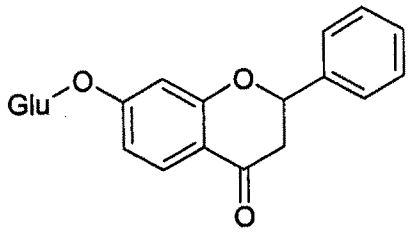
24β-Ethylcholesta-5,22*E*,25-triene-3β-ol



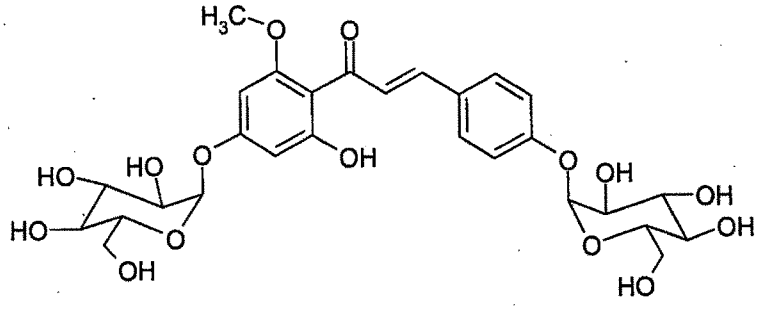
Lup-20(29)-en-3-triacontanoate



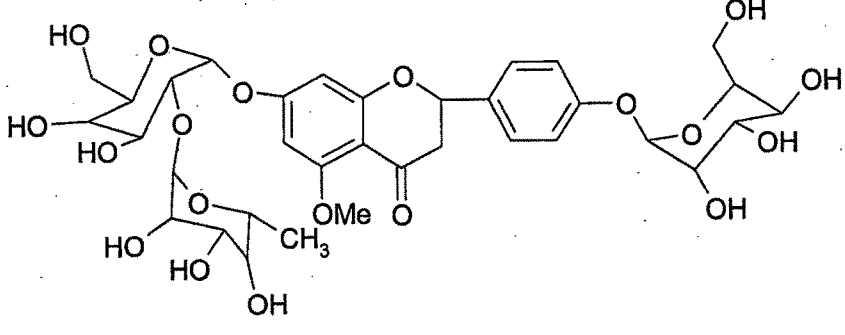
7-hydroxy flavone



7-hydroxyflavanone-7-O-glucoside



4, 2', 4' – trihydroxy-6'-methoxychalcone-4,4'α-D-diglucoside



α-L-Rhamnopyranosyl-(1→2)α-D-glucopyranosyl-7-O-naringin-4'-O-α-D-glucopyranoside-5-methyl ether

Figure 2.2. Chemical structures of some isolated compounds from *C. phlomidis*

The amount quantified in different solvent extracts were hexane 0.0552, chloroform 0.0547, ethanol (95 %) 0.0338, methanol 0.0927 and ethyl acetate 0.1047 %w/w. Figure 2.2 shows the chemical structures of some isolated compounds from *Clerodendrum phlomidis*.

2.1.11 Non-clinical investigations

Many studies have reported the diversified biological activity exhibited by *C. phlomidis*, these have been described in greater detail in the following section.

Analgesic activity

The ethanolic extract of leaves (150 and 300 mg/kg, i.p.) was evaluated for analgesic activity in Albino mice (either sex, 20-25 g) by Eddys hot plate method. Extract at 300 mg/kg showed significant activity and supports the folklore claim as analgesic (Srinivasa et al., 2007).

Antiamnesic activity

The aqueous extract of bark (yield 2 %w/w yield) at 100 and 200 mg/kg, p.o. were evaluated for antiamnesic activity in younger Swiss mice (8 weeks, either sex) and older Swiss mice (28 weeks, either sex). Acute toxicity studies showed hypersensitivity, grooming, convulsions, sedation, hypothermia, ptosis and mortality at dose above 1000 mg/kg. 200 mg/kg dose more significantly enhanced the learning and memory of aged animals rather than the young ones. The extract profoundly increased SDL (step down latency) indicating improvement in the memory of younger mice and significantly inhibited the AchE activity indicating its potential in the attenuation of learning and memory deficits especially in aged mice. The study concluded *C. phlomidis* as a potential nootropic and anti-cholinesterase agent (Joshi and Megeri, 2008).

Anti-asthmatic activity

The aqueous extract (yield 7.9 %w/w) of leaf was studied for anti-asthmatic activity in male Albino mice (Swiss strain, 22-25 g). The effect of extract (2, 4, 10

mg/ml) on goat tracheal chain were also studied and significant activity was observed at 4 and 10 mg/ml indicating relaxant effect (depression of histamine H_1 receptor). The extract at dose levels of 25, 50 and 100 mg/kg, i.p. in milk induced eosinophilia showed significance at 100 mg/kg suggesting the antagonizing effect. In three days treatment of the extract (25, 50 and 100 mg/kg, i.p.), the 100 mg/kg dose showed 73.25 % protection of mast cell degranulation. The extract (25, 50 and 100 mg/kg, i.p.) when studied for capillary permeability, the 100 mg/kg dose level significantly decreased % transmittance, an evident of its effect on optical density of the eye. The overall study lends credence to the beneficial use of aqueous extract in the treatment of asthma and related conditions (Vadnere et al., 2007). The authors have quoted a back reference regarding the antiulcerogenic activity of *C. phlomidis*, which is misleading. In fact, *C. splendens* was screened for antiulcerogenic activity in the mentioned reference.

Anti-diarrhoeal activity

The successive methanolic extract (yield 7.5 %w/w) of leaves showed no mortality till an oral dose of 1 g/kg. The extract at doses of 200, 400, 600 and 800 mg/kg were evaluated for castor-oil induced diarrhea, gastrointestinal motility and PGE_2-induced interpooling in Albino rats (Wistar strain, 180-200g, either sex). The extracts, 600 and 800 mg/kg showed significant inhibition of defecation frequency and decrease in propulsion of the charcoal meal through gastrointestinal tract. The extract also significantly inhibited PGE_2-induced interpooling in almost all the dose levels. The mechanism appears to be spasmolytic and anti-enteropooling (Rani et al., 1999). The extract has shown the presence of steroid, alkaloid and flavonoid only but the author's concluded that the exhibited activity of the extract might be due to tannins, which is controversial.

Anti-inflammatory activity

Screening was done in four sets Albino rats in different period of time with slight variations in the interval between two consecutive measurements. In first three

sets the leaf aqueous extract was administrated intramuscularly at plantar region in both hind limbs, while in fourth set the alcoholic extract was administered by intubation and also applied externally at the site of inflammation. The leaf treated group showed general decrease in the size of the swelling following certain initial fluctuations and reduction in suppuration especially in its frequency in general drying up of the pus (Krishnamurthy et al., 1972). No details were found regarding the dose of the extract administered.

Antimicrobial studies

20 µl of defatted methanolic (yield 4.4 %w/w) and acetone (yield 1.7 %w/w) extract of stems and leaves (combined) were screened for five Gram-positive bacteria, seven Gram-negative bacteria and three fungi species by agar diffusion method. Acetone extract was not active while the methanolic extract showed inhibition against *Citrobacter freundii* and *Staphylococcus epidermidis* (Vaghasiya and Chanda, 2007). The preliminary phytochemical analysis of *C. phlomidis* extracts mentioned were not clear whether it is for methanolic or acetone extract. Further it was astonishing to note that the data indicates absence of alkaloids, tannins, cardiac glycosides, steroids, flavonoids and saponins while the authors concluded that the antimicrobial activity exhibited may be attributed to various active constituents present in them either individually or in combination. The ethyl acetate and hexane extract of leaf (yielded 8.4 % and 1.1 %w/w) and stem (yielded 3.21 % and 0.52 %w/w) at concentration of 1 mg/ml were screened for human pathogens and plant pathogens by poison plate technique. The leaf extracts (particularly hexane extract) was more active than stem extracts on both pathogens, however the stem extracts were only inhibitory to plant pathogens. The study revealed that both extracts are more effective in controlling plant pathogens than human pathogens and could be utilized in pesticide formulations (Rajasekaran and Kannan, 2006). Antifungal activity of two flavones, flavonone glucoside and one chalcone glucoside isolated from *C. phlomidis* were studied.

Chalcone glucoside was highly promising followed by pectolinaringenin, flavonone glucoside and flavones (Roy et al., 1995).

Antiplasmodial activity

Ethanolic leaf extract showed 96% inhibition at 100 µg/ml and an IC_{50} value of 25 µg/ml against *Plasmodium falciparum*. Study concluded that the activity may be due to the presence of iridoids (Simonsen et al., 2001) but no iridoids have been reported yet from *C. phlomidis*.

Antiviral studies

The ethanolic extract of leaves was evaluated for antiviral activity against sunnhemp rosette virus (SRV) on *Cyamopsis tetragonoloba*. The virus inhibitory activity percent was 29 and no significant antiviral response (Khan et al., 1991).

Brine shrimp lethality study

The biological activity and toxicity of aqueous extract of root using *Artemia salina* (Brine shrimp test) was studied. The extract with an LC_{50} value of 3,750 µg/ml, showed no brine shrimp lethality (Krishnaraju et al., 2006).

Hypoglycemic activity

Chaturvedi et al. (1983) studied the effect of decoction and alcoholic extract of Arani plant on adrenaline induced hyperglycemia and alloxan induced diabetics in rats. Arani exhibited significant inhibitory effect particularly alcoholic extract on adrenaline induced hyperglycemia. Both decoction and alcoholic extract brought down the blood sugar levels effectively and exhibited same degree of action in alloxan induced diabetic rats. In a comparative study between the immediate effect (hourly basis) and on long term effect (30 days) of decoction in normal rats, Arani produced comparable fall in blood sugar both on immediate as well as on long term use. The defatted ethanol extract of leaves was screened for hypoglycemic activity in alloxan-induced diabetic rats at two dose levels, viz., 100 and 200 mg/kg. The extract at 200 mg/kg dose level exhibited significant

hypoglycemic activity and also correction of altered biochemical parameters viz., cholesterol and triglycerides. In the histopathological studies more prominent islet cells were seen in the positive control and ethanol extract of *C. phlomoidis* (200 mg/kg) treated groups, while the diabetic control showed no endocrine glands (Dhanabal et al., 2008).

Immunomodulatory activity

Methanolic extract of root was evaluated for specific immune response (HA titre, PFC assay and DTH response) and non-specific immune response (carbon clearance and *E. coli* induced abdominal sepsis). The specific immune response was studied in BLAB/c Albino mice either sex (22-25 g) for 7 days. The extract at 300 mg/kg showed significance in HA titre, PFC assay and DTH. In carbon clearance test (5 days treatment) and *E. coli* induced abdominal sepsis (15 days treatment) the extract showed increased phagocytic index, significance clearance of carbon particles and only 20 % mortality in 24 h particularly without any symptoms of peritonitis in surviving animals. The study concluded that the methanolic extract exhibit higher response in most of the studied models and the phytoconstituents diterpenoids and flavonoids might be contributing towards the immunomodulatory activity (Gokani et al., 2007).

Nematicidal activity

Aqueous leaf extracts showed moderate nematicidal property against larvae of root-knot nematode *Meloidogyne incognita* and antifungal effect (43.58 % inhibition) against *Fusarium oxysporum* f. sp. cumini (Sharma and Trivedi, 2002).

Psychopharmacological activity

The successive methanolic leaf extract (yield, 7.5 %w/w) was studied in Swiss Albino mice either sex (20-25 g) for phenobarbitone sodium induced sleep, general behaviour test, muscle relaxant activity and exploratory behavior (rat model) at doses of 200, 400 and 600 mg/kg. The 400 and 600 mg/kg, i.p. dose significantly

lengthened the phenobarbitone sodium induced sleep time in mice. The same dose levels produced slight/moderate spontaneous activity, sound, pain and touch responses. All the studied dose levels produced significant loss of motor co-ordination and tone of muscles. Significant decrease was shown by all three dose levels in the extraploratory behaviour of rats in Y-maze test and in head dips responses in mice. The study concluded that the extract possess most of the pharmacological activities characteristic of minor tranquilizers (Murugesan et al., 2001). The author has quoted that the extract is non-toxic and caused no death even up to an oral dose of 3.2 mg/kg while the doses selected for the study was 200, 400 and 600 mg/kg, which is contradictory.

Pharmacological effects of pure compounds

The isolated compound 7-hydroxy flavone acts on targets like aromatase, alcohol dehydrogenase, 17β hydroxyl steroid oxydoreductase, multidrug resistance transporter (MDR-TR)- P-glycoprotein transporter (PGP-TR) and 3,5- cyclic nucleotide phosphodiesterase. This flavone also exerts *in vivo* antinociceptive activity (Polya, 2003) and was also active towards FAAH (fatty acid amide hydrolase) with an IC_{50} value of 0.5-1 muM. Further it also reduced the FAAH-dependent uptake of anandamide and its metabolism in intact RBL2H3 basophilic leukaemia cells (Thors et al., 2008).

Studies on formulation containing C. phlomidis

The 50 % ethanolic extract of Chyavanprasha showed nitric oxide scavenging activity (Jagetia et al., 2004). Defatted 50 % ethanolic extract of Chyavanaprasha showed radioprotective effect in mice exposed to lethal dose of γ-radiation (Jagetia and Baliga, 2004). Muthu Marunthu showed very good controlling capacity on the biochemical events during tumor progression, without producing any toxic effects (Palani et al., 1999). The aqueous and alcoholic axtract of Amrit nectar tablets and the herbal Ayurvedic food supplement, M-4 (Maharishi-4) reduced the toxicities induced by Adriamycin and Cisplatin (Dwivedi et al., 2005) and showed

antineoplastic properties (Sharma et al., 1990; Engineer et al., 1992; Prasad et al., 1992; Prasad et al., 1993; Mazzoleni et al., 2002). The studies conducted on formulation containing *C. phlomidis* are not emphasized much as they are polyherbal formulation and each herb comprising numerous chemical constituents.

2.1.12 Clinical investigations

In a clinical study, 22 % reduction of blood sugar was observed when 8 pills of alcoholic extract were administered to 10 normal and 33 maturity onset diabetic patients (Bhattacharya and Bajpai, 1975). The manuscript reads as "each pill weighing 0.5 g containing 4 g of crude drug" which is practically impossible to formulate, again it reads as "8 pills of Arani containing 32 g of crude drug" leading to a conclusion that each pill may be weighing more than 4 g. Effects of age, sex and complications on the results were not considered in this study.

A case report states that a 70 yr male patient with complaints of polyuria, polydypsia, constipation, loss of vision, tingling, numbness in extremities for the duration of 10 years and complicated with pulmonary tuberculosis when administered with Arani decoction daily in 4 divided doses for three weeks along with anti-tubercular treatment simultaneously responded well without any adverse effect. Significant lowering of fasting blood sugar and improvement of polydypsia and polyuria were reported. In a clinical study reported of 23 patients, 46% treated with *C. phlomidis* (a 1:4 decoction prepared from 15-30 g in daily divided doses for 5 weeks) showed 7.1 % mean fall of blood sugar and 18.2 % fall in urine sugar with no side effects (Chaturvedi et al., 1983). But the study does not report sufficient data for re-analysis and lacks specific data (no means, no standard deviations), the small number of patients in the multiple arms of the study make generalization difficult. However the authors conclude *C. phlomidis* as a good oral hypoglycemic drug.

Namboodari et al., 1984 have reported the combination of *Dasmularista*, *Pippalyasava* and *Vettumaran Gutika* were effective in the 90 days treatment of acute rheumatoid arthritis. Park and Ernst (2005) have evaluated the methodological quality of all randomized controlled trials (RCTs) on the effectiveness of Ayurvedic medicine for rheumatoid arthritis (RA). The Jadad score assigned by Park and Ernst (2005) for the study by Namboodari et al. (1984) was 2 due to poor study design and the inability to draw any meaningful conclusions.

2.2 Nymphaea stellata

2.2.1 Nymphaeaceae and nymphaea

Palaeobotanical studies (Soltis et al., 1999; Friis et al., 2001; Buzgo et al., 2005; Yoo et al., 2006) support the view that the so-called ANITA clads (Amborellaceae, Nymphaeales, Illiciales, Trimeniaceae, Austrobaileyaceae) were the first line to diverge from the main branch of the angiosperm phylogenetic tree. Nymphaeaceae is classified under the order Nymphaeales in the group of the "basal families" in the recent molecular based angiosperm phylogeny (Anonymous, 2003). Nymphaeaceae is a primitive family, fossil record goes back to the early cretaceous period (Friis et al., 2001). Nymphaeaceae Salisb. is cosmopolitan with about 6 genera and 75 species (Mabberley, 1997). The genus *Nymphaea* includes approximately 40 species found in tropical and temperate climates on both hemispheres. *Nymphaea* is divided into two main groups, which in turn is divided into five subgenera. Group Apocarpiae includes the subgenera *Anecphya*, *Brachyceras* and group Syncarpiae consists of subgenera *Hydrocallis*, *lotos* and *Nymphaea*.

2.2.2 *Nymphaea stellata*

Nymphaea stellata Willdenow (syn. *Nymphaea nouchali* Burman f.) belongs to the family Nymphaeaceae (Figure 2.3). In Greek nymphala refers to water nymph and stellata in Latin means star-shaped (Wiart, 2006). For a variety of reasons much synonymy occurs for *N. stellata* (Danin, 2000; Merlin, 2003). The synonymy are; *Nymphaea cyanea* Roxb., *Nymphaea malabarica* Poir., *Nymphaea minima* F. M. Bailey, *Nymphaea punctata* Edgew, *Nymphaea versicolor* Sims. (Stephens and Dowling, 2002). *N. caerula* is also considered as synonymy by some taxonomists, while some include as a variety, *Nymphaea nouchali* Burm.f. var. caerulea (Savigny) Verdc. (Viljoen and Notten, 2002). Other varieties recorded are, *N. nouchali* var. cyanea (Hooker F. & Thomson) Almeida (syn. *N. stellata* var. cyanea) and *N. nouchali* var. versicolor (Roxburgh) Hooker f. & Thomson (syn. *N. stellata* var. vesicolor) (Slocum, 2005). Verdcourt has quoted that *N. nouchali* should be

synonymy to *N. stellata* and not to *N. pubescens* as some have done (Verdcourt, 1989a ; Simmonds and Howes, 2006). But in some literatures and books *N. stellata* and *N nouchali* have been differentiated as two species (Singh and Sandhu, 2003). Till now there exists a controversy among botanists regarding the synonymy and the varieties. But this review is constructed considering *N. nouchali* as a synonymy of *N. stellata.*

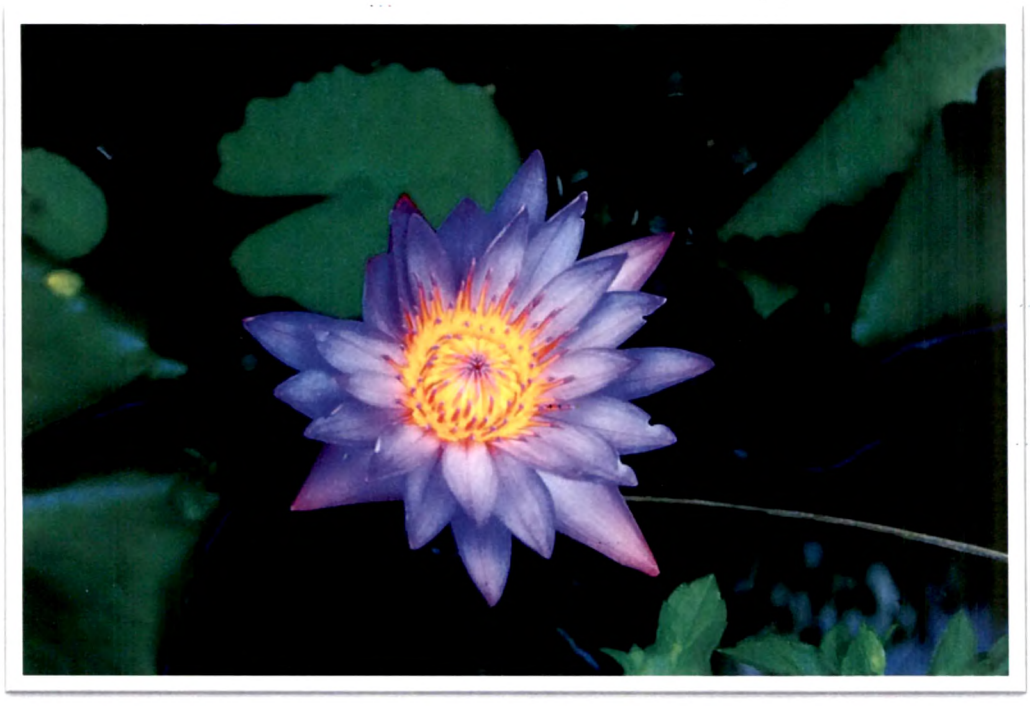


Figure 2.3: *Nymphaea stellata*

N. stellata is commonly known as Indian blue water lily/Indian water lily in English and has different vernacular names in India (Table 2.4) (Anonymous, 2001). Sometimes this water lily is often referred as "blue lotus of India", but it is not a lotus (Slocum, 2005). Many reports specify that "blue lotus of the nile" and the "blue lotus of India" are *N. caerulea* and *N. nouchali* respectively, while others report "sacred blue lily" as *Nymphaea nouchali* var. caerulea. In India the vernacular names used for *N. nouchali*, besides the correct local name "Nilotpalam", include "Allithamarai" and "Vellambal" (Tamil), which are in fact applicable to *N. pubescens* (Sanjappa et al., 2005). Another name "Nilotpala" refers

to three plants – *N. stellata*, *N. rubra* and *Monochoria hastate*. *N. stellata* itself alone has 17 names including Indivar, Nilakamala, Nilotpala and Utpala (Nair, 2004). Lotus has no blue coloured flowers in India, the name "Neelathamara" is applicable only to water lily with bluish flowers which is *N. stellata*. These vernacular names referred for *N. stellata* in India are sometimes conclusive but mostly diverges dramatically, making identification of the plant complicated.

Table 2.4: Different vernacular names of *N. stellata* used in India.

Languages	Vernacular names
Sanskrit	*Kumuda, Indivar, Nilakamala, Nilotpala, Utpala, Padma, Kamala, Indeevararn*
Tamil	*Alli, Ambal, Allithamarai, Vellambal, Nilotpalam*
Marathi	*Kamoda, Neel Kamal*
Hindi	*Neel Kamal, Kumudinee*
Telugu	*Allitamara, Kaluvapoovu, Kaluva, Neelattamara*
Malayalam	*Ambal Poovu*
Bengali	*Kumud, Sundi*
Gujarati	*Poyanu*
Kannada	*Neeltare*
Punjabi	*Neel Kamal, Kamalini*
Assamese	*Boga bhet, Seluk*
Urdu	*Neelofar*

Karyotype analysis on *N. stellata* showed 2n=28 (Ping-he and Wei-pei, 1994), another study on the chromosome number of *N. nouchali* showed euploidy in nature. Three types have been identified, *N. nouchali* (Type-1) 2n=56, *N. nouchali* (Type-2) 2n=84, and *N. nouchali* (Type-3) 2n= 70 have been identified. *N. nouchali* (Type-1) 2n=56 (4x) chromosomes may have evolved by the doubling of chromosome (2n=28) from ancestral species. Similarly *N. nouchali* (Type-2) 2n=84 (6x) may have evolved by the chromosome doubling of *N. daubeniana* 2n=42 (3x).

N. nouchali (Type-3) 2n=70 (5x) might have originated by the crossing of *N. pubescens* 2n=84 (6x) and *N. nouchali* (Type-1) 2n=56 (4x). Most of the species in Nymphaea hybridize freely among themselves naturally and thereby generating uncertainty regarding their identity (Hossain et al., 2007). Much work remains to be done to improve the understanding of this wide-ranging and highly variable taxon and its relationship to related taxa. Genotypic studies reveal that carnivory is polyphyletic (Albert et al., 1992). Phylogenetic trees prepared on the basis of taxonomy suggest a strong evolutionary linkage between some carnivorous families such as Nepanthaceae and Sarraceniaceae to Nymphaeaceae, which is conclusive from *N. stellata* as it indulges in a primitive form of insectivory. No insectivorous flowering plant has been reported and *N. nouchali* may be the missing link in the evolutionary history of other highly evolved carnivorous plant families (Tetali et al., 2008). *N. stellata* is also reported for its allelopathic potential and being more toxic to the growth of hyacinth (Gupta and Saxena, 2004).

2.2.3 Geographical source

N. stellata is a perennial aquatic rooting herb, wild/cultivated, generally found in tanks and ponds throughout the warmer parts of India particularly, Eastern Ghats. For centuries it has been cultivated in Southeast Asia, especially around temples (Slocum, 2005; Anonymous, 2004). Native to Borneo, Philippines, Srilanka, Myanmar, Afganistan, Pakistan, Bangladesh, Nepal, Cambodia, Malaysia, Laos, Thailand, Vietnam, New Guinea, Indochina to China, Taiwan and Indonesia, distribution also reported in Africa and Australia (Kirtikar and Basu, 1975; Satyavati, 1987; Shui et al., 2001; Anonymous, 2001; Pullaiah and Rao 2002; Anonymous, 2003a; Wiart, 2006).

2.2.4 Cultivation

N. stellata is cultivated in portions of paddy fields left uncultivated during the Southwest monsoon. Seed/roots are first selected, then air dried and stored, finally propagated in shallow mud and later transplanted to positions in rows 3 feet apart

by 2-3 feet apart in the rows. The mud is first prepared and manured with old weeds or stubble, or special compost (Soyza, 1936; Irvine and Trickett, 1953)

2.2.5 Morphology, anatomy, histology, powder-microscopy and proximate analysis

N. stellata is rather weak or stout herb from cone-like tuberous rhizome bearing small white pithy roots; stems often reddish violet. Rhizomes are short, erect, stout, fleshy, unbranched, pyriform and about size of an egg. The rhizomes are enclosed in a thin covering which turns horny on drying. The covering is itself covered with a cottony substance, especially at the apex. The rhizome is full of starch and is very palatable when boiled or prepared with curry, being more palatable than the Yam (*Dioscorea*) and Cocoyam (*Colocasia*) (Irvine and Trickett, 1953). The fruit is about 2.5-3 cm in diameter, globose, 6.5 cm, glabrous and contains round, flask-shaped seeds less than 1 mm in diameter. Seeds many, berries, ellipsoid-globose, black with a white aril, 0.5-1.3 mm, with longitudinal rows of hairs (Stephens and Dowling, 2002; Butchart, 2000; Pullaiah et al., 1998). Table 2.5 shows the anatomy, histology and powder microscopy of flower and leaf of *N. stellata* (Farooqui, 1980; Gupta et al., 1980; Satyavati, 1987; Verdcourt, 1989; Dassanyake, 1996; Pullaiah and Mohammed, 2000; Anonymous, 2001; Slocum, 2005; Dhanabal et al., 2006; Yakandawala and Paebotuwage, 2007; Mohanmarugaraja et al., 2008).

The Ayurvedic pharmacopoeia of India has specified the proximate analytical parameters viz., foreign matter (Not More Than 2 %), total ash (NMT 8 %), acid insoluble ash (NMT 0.5%), alcohol soluble extractive (Not Less Than 5 %) and water soluble extractive values (NLT 22 %) for identity, purity and strength of flowers of *N. stellata*. TLC pattern of alcoholic flower extract in chloroform : ethylacetate : formic acid (5 : 4 : 1) showed three visible spots at R_f 0.59, 0.68 and 0.81 (all bluish grey) and on spraying with 10 % aqueous ferric chloride solution two spots appeared at R_f 0.68 and 0.81 (both blue colour) (Anonymous, 2001).

Table 2.5: Morphological, histological and powder characteristics of different parts of *N. stellata*.

Part	Morphological characteristics	Histological characteristics	Powder characteristics
Flower	Flowers solitary, fragrant, open all day. Sepals usually 4, green but sometimes marked with dark purple or crimson lines or dots and sometimes with reddish purple or crimson margins, oblong-ovate to oblong-lanceolate. Petals 12-27, usually blue but sometimes pink, mauve or white, lanceolate to oblong-lanceolate. Stamens 30-250, pale to dark blue. Carpels 14-47; stigmatic appendages densely papillate.	Sepal - unicellular hairs; tanniniferous content in collenchymatous cells. Stamen - stellate air canals; anther shows 4 splitting pollen chambers attached with parenchymatous connective tissues; vascular tissues and stellate idioblasts; endothecium consisting of single layered columnar cells; stromium in both the chambers.	Powder - Brown; shows groups of parenchymatous cells, stellate air canals, uniseriate hairs, yellowish-brown rounded pollen grains, measuring 22 - 27 μ in diameter, having thick, smooth, exine and thin intine.
Leaf	Leaves round to elliptic, 5-(8)-35(-45) cm long, 5-30(-40) cm wide, rounded or retuse at the apex, incised or cordate to hastate at the base, the margins entire. The upper surface green and smooth, the lower green, red or purple or green and purple-spotted and/or with purple margins; primary lateral nerves 5-8 on each side and 4-5 additional pairs from the midrib, the nervation flat or raised beneath.	Leaf is hydromorphic, lamina is 400μm thick; stellately branched, long armed sclerenchyma cells called 'trichosclereids'; minute prismatic crystals are densely deposited on arms of trichosclereids; collateral vascular bundles differ in size from different zones of the lamina; ranunculaceous or anomocytic stomata.	Abundant trichosclereids of either entire or broken pieces, the surface of the sclereid is warty due to deposition of minute prismatic crystals, large masses calcium oxalate crystals.

2.2.6 Ethnomedical uses

Indian system of medicines particularly Ayurveda and Siddha uses *N. stellata* as a single drug or in combination with other drugs. It is also known as *Utpala* in Sanskrit but this name refers only to the dried flowers. *N. stellata* are ingredients of many ayurvedic formulations like, Asokarista, Arvindasava, Usirasava, Candanasava, Kalyanaka Ghrta, Samangadi Curna, Kanaka Taila, Jatyadi Taila, Tungadrumadi Taila, Manjesthadi Taila, Candanadi Lauha, and Triphala Ghrta (Anonymous, 2001). It is also an ingredient of many poly herbal formulations for anti-aging, rejuvenation, and menstrual irregularities. The traditional uses of different parts of *N. stellata* are given in Table 2.6.

The rhizome, fruit, leaf petiole, roots, flowers, tubers and seed are used as edible parts in different ways by people (Ngugi, 1999; Venu, 1999; Sharma, 2001; Kumar and Bohra, 2005; Patiri and Borah, 2007; Sarma et al., 2008). It has also been cultivated for food in Srilanka as the rhizomes are full of starch and reputedly quite tasty when boiled (Slocum, 2005). The roots and rhizomes are considered to be nutritious when eaten either raw or roasted (Michler, 2004). Flowers are used in temples, rhizomes in medicine, flower and flower stalks as vegetables, green manure and fodder. *N. stellata* is considered as one of the ten most common noxious aquatic weeds in India (Varshney and Rzoska, 1976).

Table 2.6: Ethnomedical uses of different parts of *N. stellata*.

Part	Ethnomedical uses	Reference
Whole plant	Used for the treatment of liver disorders in Ayurveda. Leaves, roots and flowers are used for diabetes, blood disorders, antifertility, heart troubles, dysentery, eruptive fevers, indigestion and as cardiotonic, emollient, diuretic, narcotic, stimulant and aphrodisiac.	Kirtikar and Basu, 1975; Nadkarni, 1982; Sharma, 1998; Cridland and Koonin, 2001; Tirkey et al., 2001; Deutschlander et al., 2009.
	The flowers and roots are having mild sedative properties; used for mind-altering purposes. The whole plant is used as anti-periodic and cardiac stimulant in Kashmir.	Lawrence, 1991; Kaul, 1997; Merlin, 2003; Tyagi, 2005.
Flower	3-6 g of the drug is used in Pipasa daha (burning thirst), Raktapitta (bile-blood), Chardi (vomiting), Murccha (fainting), Hrdraoga (heart disease), Mutra Kecchra (painful discharge of urine, a class of urinary affections), Jvaratisara (diarrhoea with fever).	Anonymous, 2001.
	The flowers are used in the treatment of diabetes mellitus (Madhumeha) and liver disorders in the Ayurveda and siddha system of medicine. The flower has an acrid, bitter-sweet taste, removes impurities from blood, cools, and alleviates cough, is used for biliousness, as an aphrodisiac, for vomiting, giddiness, worm infestation, and burning of the skin. The decoction of the flower is used in palpitation of the heart and as a narcotic; syrup of the flower is used in high fever, apoplexy, inflammatory diseases of the brain, and also in dysuria. The filaments of the plants are used as an astringent and a cooling agent in burning sensation of the body, bleeding piles, and menorrhagia.	Watt and Brandwijk, 1962; Kirtikar and Basu, 1975; Nadkarni, 1982; Satyavati, 1987; Manjunatha et al., 2004.
Rootstock	Powder is used to treat dyspepsia, diarrhoea and piles	Kirtikar and Basu, 1975; Satyavati, 1987.
Root	The roots are as emollient, diuretic and used to treat diabetes, blenorrhagia, infections of the urinary passages, infertility.	Watt and Brandwijk, 1962; Arnold and Gulumian, 1984; Simmonds and Howes, 2006;

Leaf and flower	The tender leaves and flower peduncles of used as curries in Ceylon	Soyza, 1936.
Rhizome and stem	An infusion is considered to be an emollient, diuretic, and used for treatment of blennorrhagia and diseases of the urinary tract.	Kirtikar and Basu, 1975; Satyavati, 1987.
Flower and rhizome	Flowers and rhizomes are astringent, demulcent, mild sedative, spasmolytic, antiseptic, used in infusion internally for chronic diarrhea, as a douche for leucorrhoea and vaginitis, as a gargle for sore throat; also given internally in prostates.	Sarma, 2008.
	Rhizomes and flowers used as a remedy for kidney problems.	Ngugi, 1999.
Leaf	Leaves are applied topically in erysipelas whereas the macerated leaves are used as a lotion in eruptive fevers.	Kirtikar and Basu, 1975; Satyavati, 1987; Wiart, 2006.
Seed	The seeds are said to be stomachic and restorative.	Kirtikar and Basu, 1975; Satyavati, 1987, Singh et al., 2007.
	Seeds are prescribed as diet in diabetes mellitus in the Aurvedic system of medicine.	Watt and Brandwijk, 1962; Achariya, 1996; Subbulakshmi and Naik, 2001.
Rhizome	It is often eaten in India and Ceylon mainly as a famine food.	Irvine and Trickett, 1953.
	The rhizomes are eaten after roasting in hot embers. Rhizome paste is used to treat menstruation problem.	Crevost et al., 1917; Partha and Hossain, 2007.
	Rhizomes are used to treat gastrointestinal disturbances.	Watt and Brandwijk, 1962.
Petiole	Petiole paste along with little common salt, seed powder of *Cuminum cyminum*, butter and few drops of honey is taken against excessive menstrual discharge. Stripes along with roots of *Pinus longifolia* is taken against fever, dysentery, nausea, cough, vertigo, pain and bleeding during pregnancy.	Singh and Sandhu, 2003.

2.2.7 Chemistry

Different solvent extracts of the entire plant have shown the presence of sterols, alkaloids, saponins, tannins and flavonoids. Nymphayol (25,26-dinorcholest-5-en-3b-ol) (Figure 2.4), a new sterol have been isolated from the successive chloroform extract of flower (Subashbabu et al., 2009). Protein, pentosan, mucilage and tannins are reported in seeds (Gujral et al., 1955; Kapoor et al., 1975). Astragalin, corilagin, gallic acid, gallic acid methyl ester, isokaempferide, kaempferol, quercetin-3-methyl ether, quercetin, 2,3,4,6-tetra-o-galloyl dextroglucose; 3-o-methylquercetin-3'-o-beta dextroxylopyranoside have been identified from flowers (Mukherjee et al., 1986; Kizu and Tamimori, 2003). Gallic acid, corilagin and 1,2,3,4,6-penta-O-galloyl-β-d-glucose has been identified from 50 % methanol flower extract (Huang et al., 2010). HPTLC method for quantitative determination of gallic acid from hydroalcoholic dried flower extract has been reported (Rakesh et al., 2009). The leaves and shoots of *N. stellata* (Blue water lily) and *N. nouchali* (Red water lily) as different species have been studied for their chemical composition.

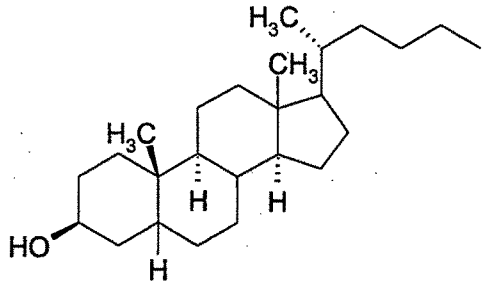


Figure 2.4: Chemical structure of nymphayol

The proximate analysis showed dry matter-8.4 %, crude protein-16.8, ash-18.7, crude fat-2.8, crude fibre-26.3, nitrogen free extract-35.4 for *N. nouchali* and dry matter-7, crude protein-16.7, Ash-14.1, crude fat-2.6, crude fibre-24, nitrogen free extract-42.6 for *N. stellata* respectively. Mineral content showed sodium-1.19, potassium-2.23, calcium-0.52, phosphorus-0.32, calcium/phosphorus ration 1.63

for *N. nouchali* and sodium-0.93, potassium-1.30, calcium-0.95, phosphorus-0.21, ca/p ratio-4.52 for *N. stellata*. Alkaloids have been detected in fraction A (extracted with chloroform from an ammonical solution) for both, while *N. nouchali* and *N. stellata* differed in their nitrates content with 2 % and 0.9% respectively. *N. nouchali* showed polyphenols total-8.7 %; free-5.9 %, bound-2.8 % and *N. stellata* polyphenols total-10.2 %; free-9.3 %, bound-0.9 % (Banerjee and Matai, 1990). The nitrogen and protein content (Table 2.7) of *N. stellata* and *N. nouchali* have also been reported as two different species (Dewanji et al., 1997). There is not much difference quantitatively between the two and trivial difference in plant content is familiar. *N. stellata* (Blue water lily) and *N. nouchali* (Red water lily) may be still same for the reason that blue, pink, mauve or white blue flower colours are also recorded in *N. stellata*. It is also possible that they may two varieties. Apomorphine, nuciferine and nornuciferine have been reported (Perry et al., 2002) from *N. stellata* in fact they have been isolated from *N. caerulea*.

Table 2.7: Nitrogen and protein content of *N. stellata* and *N. nouchali*

Plant	Plant nitrogen %	Total protein		Leaf protein
		% of pulp nitrogen extracted	% of pulp nitrogen extracted as protein	
N. stellata	2.70	26.64	11.53	5.37
N. nouchali	2.72	21.80	12.61	5.56

2.2.8 Non-clinical investigations

Antidiabetic activity

The defatted ethanolic leaf extract (14.26 %w/w) at a dose of 100 and 200 mg/kg were studied for hypoglycemic activity in alloxan induced diabetic rats (Wistar, 150–220 g). Oral treatment significantly and dose-dependently reduced the hyperglycemia. Moreover, it decreased the levels of cholesterol (CHL) and triglyceride (TGL) increased by alloxan treatment. On the contrary, no effect was seen in normal rats both in glucose and lipids plasma level. The

hypocholesterolaemic effect of the ethanolic extract of leaves of *N. stellata* could possibly be related to its amino acid and saponin composition (Dhanabal et al., 2007). Hydroalcoholic extract (yield: 6.8 %w/w) of flowers at 200, 300, and 400 mg/kg (oral) were studied in normoglycemic and alloxan-induced diabetic rats (Male, Wistar strain, 150–200 g). Showed no hypoglycemic effect in normoglycemic animals, but showed statistically significant antihyperglycemic activity by improvement of OGTT. The flower extract caused significant reduction in blood glucose level of diabetic rats. The dose of 300 mg/kg showed significant blood glucose level reduction (45 %) at 4 h after administration of the flower extract. The hydroalcoholic extract also showed a dose dependent response (Rajagopal et al., 2008).

The antidiabetic effect of *N. stellata* hydroalcoholic extract of flower could be linked to more than one mechanism. The possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors. The antihyperglycemic action may be due to potentiation of pancreatic secretion of insulin, which is clearly evident by the increased level of insulin in treated rats. *N. stellata* also acts as a hepatoprotective agent (Bhandarkar and Khan, 2004) so it could have improved the function of liver and maintained glucose uptake, enhanced transport of blood glucose to peripheral tissue and utilization, which may be another mechanism of action. The extract might have also stimulated glycogenesis and/or inhibited glycogennolysis in the diabetic rat liver. Administration of extract reduced TC, TG, LDL, VLDL and also improved HDL level. Serum phospholipid was elevated whereas the phospholipids in the liver and kidney were decreased. Treatment could have restored the normal metabolism by shifting the balance from lipids metabolism to carbohydrate metabolism (Rajagopal and Sasikala, 2008).

Oral administration of *N. stellata* flower extract for 30 consecutive days to diabetic rats also decreased their food consumption and improved body weight. This could

be due to a better control of the hyperglycaemic state in the diabetic rats. Administration of flower extract to diabetic rats significantly increased the level of total haemoglobin and this might be due to the decreased level of blood glucose. Oral administration of flower extract improved total protein concentration in the serum. Administration of *N. stellata* flower extract to diabetic rats reversed the changes and improved the HDL levels. These results unmistakably indicate that the flower extract could effectively manage the diabetic complications such as body weight maintenance, hyperlipidaemia, cardiovascular complications in diabetes mellitus and progression of atherosclerosis (Rajagopal and Sasikala, 2008a). Nymphayol (25,26-dinorcholest-5-en-3b-ol),a new sterol isolated from the bioactive successive chloroform flower extract have been reported for its antidiabetic activity at 20 mg/kg bw in streptozotocin induced diabetic rats. Oral administration of Nymphayol for 45 days significantly restored the plasma glucose levels and increased the plasma insulin levels to near normal in STZ-diabetic rats. Light microscopy and immunocytochemical staining of Nymphayol treated diabetic pancreas revealed increased number of insulin positive β-cells. The mode of action of Nymphayol may be due to the reversal of the damaged endocrine tissue and thereby stimulating the secretion of insulin in β-cells as revealed by insulin assay. The active principle Nymphayol enhances the antioxidant defense against reactive oxygen species produced under hyperglycemic condition and thus protects the pancreatic β-cells against loss (Subashbabu et al., 2009). 50 % methanolic flower extract and 1,2,3,4,6-penta-O-galloyl-β-d-glucose inhibited maltase activity in a dose-dependent manner, and the EC_{50} values were 0.1 0mg/ml and 0.17 mg/ml respectively. 1,2,3,4,6-penta-O-galloyl-β-d-glucose, corilagin and gallic acid showed 76 %, 32 % and 26 % inhibitions, respectively in α-glucosidase activity in Caco-2cells (Huang et al., 2010).

Tumor inhibition studies

Methanolic extract of *Nymphaea nouchali* roots at 200 μg/ml were screened for their inhibitory activity toward tumor promoter 12-*O*-hexadecanoylphorbol-13-

acetate induced Epstein-Barr virus activation in Raji cells. The extract was inactive with zero inhibition rate (Murakami et al., 2000).

Antihepatotoxic effect

The alcoholic extract (yield, 9 %w/w) of *N. stellata* flowers was evaluated against carbon tetrachloride-induced hepatic damage in albino Wistar rats (8-10 weeks, 100-120 g) at 250, 500 and 750 mg/kg (orally) in the form of aqueous suspension once a day for 10 days. The hepatoprotective activity exerted by the extract may be due to the cell membrane stabilization, hepatic cell regeneration and activation of antioxidative enzymes such as glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase (Bhandarkar and Khan, 2004). The petroleum ether extracts of *N. stellata* seeds tested against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats and mice at a dose of 300 mg/kg i.p. The extract markedly reduced the prolongation of sleeping time and significantly prevented the CCl_4-induced increase in weight and volume of the liver, and mortality. The extract also prevented necrosis of the liver and promoted to some extent liver generation (Singh et al., 1978).

Cholinergic activity

The alcohol extract of defatted fruits of *N. stellata* produced mild sedation and ataxia, potentiated hexobarbitone-induced hypnosis in mice, and also produced a sharp and transient hypotension blocked by pretreatment with atropine. In large doses were administered after atropinization, a rise in blood pressure and also a stimulant effect on guinea pig ileum was observed indicating the presence of some unstable cholinergic principle (Satyavati, 1987).

Analgesic and anti-inflammatory activity

The extract had a significant analgesic activity as revealed by aconitine-induced writhing in mice and antipyretic activity against carrageenin-induced rat paw

edema. The anti-inflammatory activity exhibited was comparable to that of hydrocortisone (Singh et al., 1977).

Antimicrobial activity

Flowers of *N. nouchali* were effective against *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* (Vasu and Singaracharya, 2008). *N. stellata* also demonstrated a broad spectrum of activity against phytopathogenic bacteria. The ethanolic extract of *Nymphaea stellata* leaves has shown considerable antibacterial activity against *E. coli* (Mohanmarugaraja et al., 2008).

Acute oral toxicity study

Mice after administration at doses up to 10,000 mg/kg of 50 % methanolic extract of flowers did not show any mortality. And there were no clinical signs of toxicity until the 14 days of observation period (Huang et al., 2010).

Chromosomal aberration assay

There were no significant increases in structural and numerical chromosomal aberrations at any dose of 50 % methanolic extract treated for 6h, 24h and 48h (Huang et al., 2010).

In vivo mouse micronucleus assay

50 % methanolic extract of flowers did not induce any statistically significant increases in MNPCE satany dose treated compared to then egative control (Huang et al., 2010).

Other activities

The LD_{50} of the 50 % ethanol extract of *N. stellata* was found to be 681 mg/kg in albino mice. *N. stellata* was found to be inactive as an antibacterial, antifungal, antiprotozoal, antiviral, diuretic, and with no effect on cardio vascular system and central nervous system (Aswal et al., 1984).

2.2.9 Conclusion

Detailed literature on ethnomedicinal information, phytochemical studies and biological activities showed that both the selected plants are widely used as herbal medicines for various ailments but lack systematic scientific studies. The selected bitter plants are traditionally claimed for antidiabetic effect but are unscientifically exploited and/or improperly used. These plants deserve detailed studies in the light of modern medicine. Investigations on the chemical composition of the bioactive extracts/fractions of these bitter plants may lead to new potent chemical entities.

2.3 Research envisaged

The present study was envisaged for systematic validation of two selected medicinal plants designated as bitters in relation to their traditional anti-diabetic claims. Plan of work is;

- To perform qualitative evaluation to confirm identification for the selected morphological part and compiling data as per WHO guidelines.
- Selection of extract/fraction for isolation of chemical entities.
- Isolation, separation and characterization of isolated compounds.
- Development of analytical method for isolated compounds.
- Anti-diabetic evaluation of extracts/fractions/isolated compounds.