सत्यं शिवं सुन्दरम्

Chapter-2

Literature Review

2.1 *FERONIA LIMONIA* LINN

Feronia limonia (family Rutaceae, subfamily Aurantioideae), is commonly known as kaitha or wood apple and is widely distributed in deciduous and arid landscapes of several countries in South Asia (Chopra, 2003). In India, it is found throughout the planes, particularly in dry situations of Aravallis in south-east Rajasthan & also up to an elevation of 1500 feet in western Himalayas (Anonymous, 1995). It is also frequently grown throughout the dry warm regions of Bangladesh, Barma, Ceylon, Java, Srilanka, northern Malaysia and Penang Island (Hooker, 1875; Allen, 1967). Taxonomical classification and vernacular names of *Feronia limonia* are given in Figure 2.1 and Figure 2.2.

Figure 2.1 Taxonomical classification and photograph of *Feronia limonia*

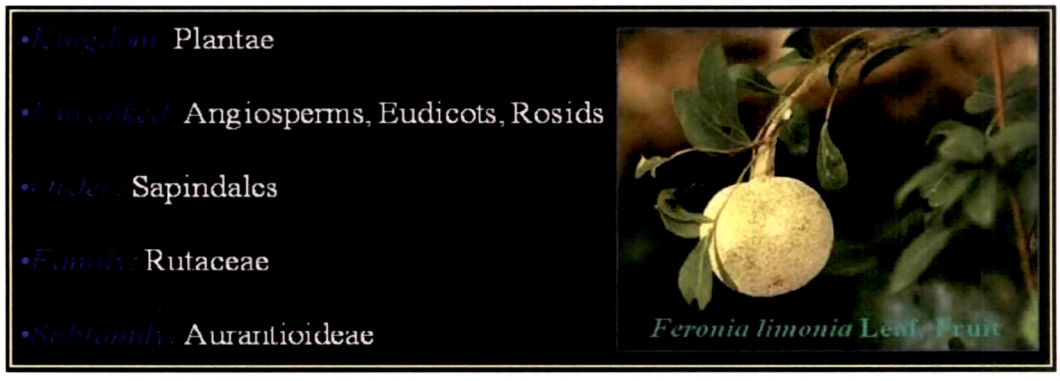
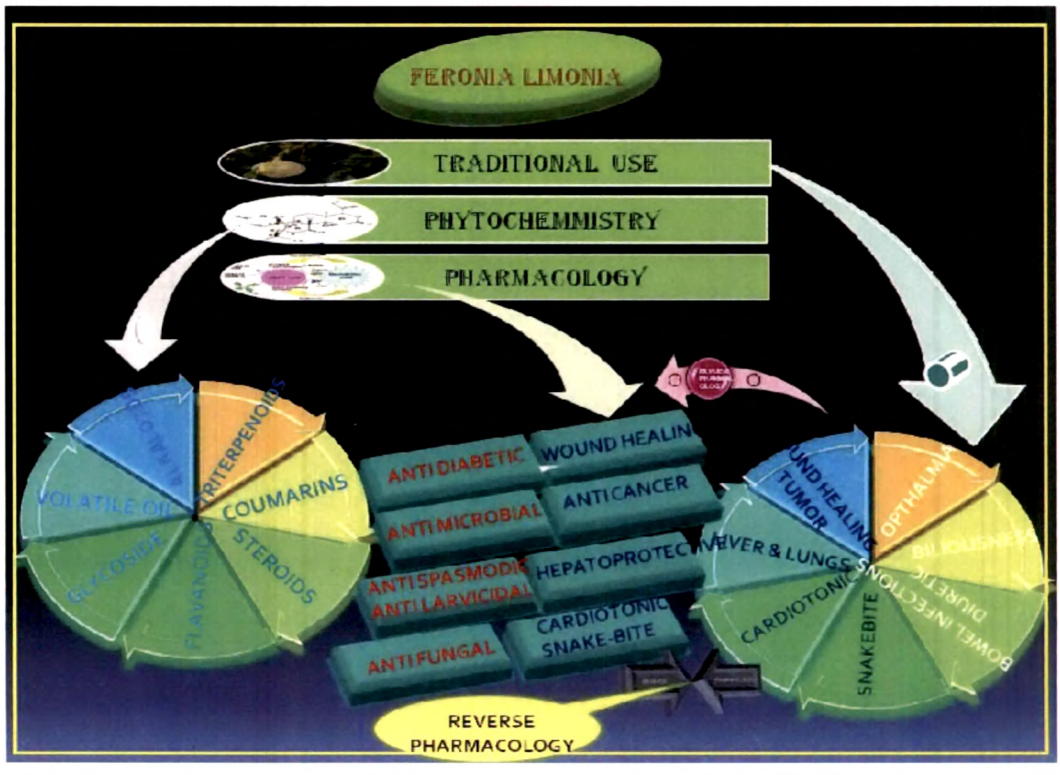


Figure 2.1 Vernacular names and photograph of *Feronia limonia*

2.1.1. Description of Feronia limonia

The slow-growing tree is erect, with a few upward-reaching branches bending outward near the summit where they are subdivided into slender branchlets drooping at the tips. The bark is ridged, fissured and scaly and there are sharp spines: 3/4 to 2 in (2-5 cm) long on some of the zigzag twigs. The deciduous, alternate leaves, 3 to 5 in (7.5-12.5 cm) long, dark-green, leathery, often minutely toothed, blunt or notched at the apex, are dotted with oil glands and slightly lemon-scented when crushed. Dull-red or greenish flowers to 1/2 in (1.25 cm) wide are borne in small, loose, terminal or lateral panicles. They are usually bisexual. The fruit is round to oval, 2 to 5 in (5-12.5 cm) wide, with a hard, woody, grayish-white, scurfy rind about 1/4 in (6 mm) thick. The pulp is brown, mealy, odorous, resinous, astringent, acid or sweetish, with numerous small, white seeds scattered through it (Morton and Miami., 1987). The graphical outline containing traditional uses, phytochemistry and pharmacology of *Feronia limonia* is given in Figure 2.3

Figure2.3 Graphical outline containing traditional uses, phytochemistry and pharmacology of *Feronia limonia*



2.1.2 Ethnomeditional and folk meditional uses of Feronia limonia

Feronia limonia occupied a reputed position of having valuable medicinal properties in both folk and classical streams of indigenous medicinal systems. Literature on ethnobotanical research work undertaken on various forests, tribal/rural populations scattered in India shows that it occupied widely by them for the management of various ailments. Medicinal usage on various plant parts of *Feronia limonia* in indigenous system of medicines in India and other countries is outlined below:

Unripe Fruits: The unripe fruits which are sour, aromatic, astringent, constipating and alexipharmic by nature are used in the treatment of diarrhoea, pharyngodynia, pruritus in Ayurvedic medicinal system (Bakshi et al., 2001; Warrier et al., 1994; Orwa et al., 2009). Generally it is employed alone or in combination with *Aegle marmelose* and other medicine in the treatment of diarrhoea and dysentery (Panda, 2000).

Ripe Fruits: Pulp of the ripe fruit, tastes like coagulated milk and is eaten with sugar; it is useful in salivation; sore throat and other infection of gums and throat. It quenches thirst & cures biliousness, stops bad breath, bleeding from gums, supplies sufficient vitamin C and increases body resistance against infections. Pulp of the fruit mixed with cardamom, honey and cumin seeds are given as a medicine to cure indigestion, diarrhea, piles, cirrhosis of liver in children due to malnutrition etc. Its regular use early in the morning acts as a medicine to tone up the sagging breast and uterus to cures sterility due to deficiency of progesterone hormones. Ripe pulp is rubbed to alleviate pain caused by venomous strings (Pullaiah, 2006; Aman et al., 1969). It is also reported to be a curative agent, for various ailments such as pruritis, impotency, heart disease, vomiting, and anorexia, in the treatment of asthma and tumours, and as a 'liver tonic' in the traditional systems of medicine (Pandey and Dravyaguna, 2001).

The Ayurveda also mentions their use in the treatment of various ear ailments like *ear ache, puti karna,*and *karn sarva.* Freshly collected luke warm juice of *kapittha* (*Feronia limonia*), *matulunga, srngvera* is used for the treatment of ear ache (Dash and Kashyap, 1984). It is much used as a liver and cardiac tonic and is also found to

be effective for the treatment of scurvy, sore throat, hiccough and other diseases of the gums (Orwa et al., 2009). Also, a useful remedy for the treatment of piles and hiccough is given in *Charaka samhita* (An Indian Medicinal Treatise) which recommends the soup of *kapittha* and *bilva* (Aegle marmelos) (60-120 ml) to treat piles and the juice mixed with *Piplli* (Piper Longum) and honey, in hiccough (Khare, 2004).

Leaves: Ayurveda mentions the use of Feronia leaves in treating the vitiated conditions of vatta and vayu (wind). Being aromatic, astringent, carminative, purgative, sudorific in nature they are useful in the treatment of gastropathy, anorexia, diarrhoea, indigestion, flatulence, vomiting, hiccough and other troubled conditions of vatta and cardiotonic and in management of other cardiac debilities (Bakshi et al., 2001; Warrier et al., 1994; Parajapati et al., 2003). *Charaka* and *Sushruta* included the leaves and fruits of *Kapittha* in prescriptions for diarrhoea, urinary disorders, ringworm and other chronic skin infections (Khare, 2004). Tribals of Saurashtra region, Gujarat (Western India) they apply the paste of Feronia leaves topically for the treatment of piles or haemorrhoids (Jadeja et al., 2006). Tribal population of Maharastra, (Western India) they use 1 tsp leaf powder/ extract in one cup of water once or twice a day to treat acidity/ ulcers (Kamble et al., 2010; Kamble et al., 2008). Some ethnic communities also use powder of the leaves dried in shade with equal quantity of sugar candy, one teaspoon full thrice daily with cold water is given to cure spermatorrhea, pre-mature ejaculation & functional impotency with very gratifying results. An ounce of fresh leaf juice mixed with cumin taken twice daily cures urticaria (Pullaiah, 2006).

Bark and Trunk Gum: The bark possesses aromatic and cooling effect, and is useful in the vitiated conditions of *Pitta* (fire & water – "bile") (Warrier et al., 1994). The *Santal* community use bark in treating asthma, bronchitis, phthisis. They also use bark to treat rinderpest of cattle. *Ethenic Communities of Dhasan valley use bark in* alleviating itching (Bakshi et al., 2001). Bark is prescribed in the form of powder or decoctions for biliousness and the juice is externally applied to the skin eruptions caused by biliousness. The medicine (powder or decoctions) is prescribed under the name of *panch kapittha* meaning 'five parts of *kapittha*' and contains the five parts of

Feronia limonia i.e, flowers, roots, leaves, bark and fruits (Panda, 2000). The bark paste is applied on venomous wounds and is chewed with that of *Barringtonia* (Orwa, 2009).

Transparent gummy substance exuding from the stem when cut and broken, resembles gum arabic, may be used in bowel affections and to relieve tenesmus; reduced to powder and mixed with honey to cure dysentery and diarrhoea (Panda, 2000; Vayaskara, 1953). Gum 20 gms dissolved in sugared cow's milk is given thrice daily as a valuable medicine for spermatorrhea, pre-mature ejaculation, scanty urination, high acidity of urine etc (Aman et al., 1969). Gum secreted from tree trunk is constipating, demulcent, useful in diabetes, diarrhea, dysentery, gastropathy and haemorrhoids (Bakshi et al., 2001; Warrier et al., 1994).

Roots: *Ethinic communities of Orissa* (North- India) like *Kondhs, Gadabas, Koyas, Oraon, Santals and Juangs* use the roots in relieving body pain (Bakshi et al., 2001).

Ayurvedic Formulations

Kapitthaashtaka churna is recommended in '*Sarangadhara*' which is used in dose of 1 drachm in chronia, diarrhoea, dysentery, loss of appetite and in affections of throat. Ghee medicated with the paste prepared from bark, root, leaves, flower and fruits of *Feronia elephantum* are prescribed in rat-bite poisoning and it's after effects (Vayaskara., 1953) 29.

2.1.3 Phytochemical aspects

The phytochemical study of *Feronia limonia* can be dated back to 1905 when the rubber of *Feronia limonia* was examined for its constituents, with enzymes & sugars being the compounds, first isolated from it (Lemeland, 1905). The genus is enriched with glycosides, coumarins, flavanoids, & essential oils, where essential oils & coumarins constituted the majority of compounds. Tabular representation of some important compounds isolated from various parts of *Feronia limonia* is given in Table 2.1.

Table 2.1 Some important chemical constituents isolated from various parts of *F. limonia*

Plant Parts	Components	Molecular formula	Melting Point	References
Fruit	**Esters**			
	methyl hexanoate,	$C_7H_{14}O_2$	-71.00	[Macleod and Pieris, 1981]
	ethyl -3-			[Macleod and Pieris, 1981]
	hydroxyhexanoate	C_3H_7COOH	−7.9 °C	[Macleod and Pieris, 1981]
	butanoic acid			
	Amino Acids			
	Phenylalanine	$C_9H_{11}NO_2$	173	[Barnabas and Badve, 1954]
	Tyrosine	$C_9H_{11}NO_3$	295	[Barnabas and Badve, 1954]
	Arginine	$C_6H_{14}N_4O_2$	221	[Barnabas and Badve, 1954]
	Lysine	$C_6H_{14}N_2O_2$	196	[Barnabas and Badve, 1954]
	Leucine	$C_6H_{13}NO_2$	286-288	[Barnabas and Badve, 1954]
	Alanine	$C_3H_7NO_2$	314	[Barnabas and Badve, 1954]
	Histidine	$C_6H_9N_3O_2$	282	[Barnabas and Badve, 1954]
	Cystine	$C_3H_7NO_2$	258-261	[Barnabas and Badve, 1954]
	Tyramine Derivatives			
	Acidissimin	$C_{26}H_{28}O_8$	284	[Ghosh et al., 1989]
	Acidissiminol	$C_{25}H_{32}N_1O_3$	85-87	[Ghosh et al., 1991]
	Acidissimin epoxide	$C_{43}H_{66}N_1O_5$	105-106	[Ghosh et al., 1991]
	N-benzoyltyramine	$C_{28}H_{51}O_3$	159-160	[Ghosh et al., 1991]
Fruit Shell	2,6- dimethoxy- Benzoquinone	$C_8H_8O_4$	242-245	[Bandara et al., 1988]
	Xanthotoxin	$C_{12}H_8O_4$	146-148	[Reisch et al., 1985]
Leaf:	**Steroids**			
	Stigmasterol acetate	$C_{31}H_{50}O_2$	142-146	[Gupta et al., 1979]
	Cholesterol	$C_{26}H_{46}O$	148-150	[El-Fishawy, 1994]
	Compesterol	$C_{28}H_{48}O$	157-158	[El-Fishawy, 1994]
	Stigmasterol	$C_{29}H_{48}O$	160-164	[El- Fishawy & El-Khrisy,1994]
	β-Sitosterol	$C_{29}H_{50}O$	130-145	[El- Fishawy & El-Khrisy,1994]
	Glycosides			
	Psoralen	$C_{11}H_6O_3$	163-164	[Gupta et al., 1979]
	Bergapten	$C_{12}H_8O_4$	190-193	[Gupta et al., 1979]
	β-amyrin,	$C_{30}H_{50}O$	77	[El- Fishawy, 1994]
	lupeol	$C_{30}H_{50}O$	204-206	[El- Fishawy, 1994]
	Xanthotoxin,	$C_{12}H_8O_4$	146-148	[El- Fishawy & El-Khrisy,1994]
	Umbelliferone	$C_9H_6O_3$	230	[El- Fishawy & El-Khrisy,1994]
	Marmesin	$C_{14}H_{14}O_4$	185-186	[El- Fishawy & El-Khrisy,1994]
	Isopimpinellin	$C_{13}H_{10}O_5$	150-151	[El-Khrisy,1994]
	Imperatorin	$C_{16}H_{14}O_4$	102	[El-Khrisy,1994]
	Flavanoids			
	Orientin	$C_{21}H_{20}O_{11}$	265-267	[Gupta et al., 1979]
	Vitexin	$C_{21}H_{20}O_{10}$	203-204	[Gupta et al., 1979]
	Saponarin	$C_{27}H_{30}O_{15}$	228-236	[Gupta et al., 1979]
	Fatty acids			
	Palmitic acid	$C_5H_{11}COOH$	63	[El- Fishawy, 1994]

	Linoleic acid	$C_{17}H_{31}COOH$	-5	[El- Fishawy, 1994]
	Oleic acid	$C_{17}H_{33}COOH$	13	[El- Fishawy, 1994]
	Linolenic acid	$C_{17}H_{29}COOH$	-11.3	[El- Fishawy, 1994]
	Stearic acid .	$C_{17}H_{35}COOH$	69	[El- Fishawy, 1994]
	Alkaloids			
	Integriquinolone	$C_{33}H_{38}N_2O_9$	257-260	[Wijeratne et al., 1992]
Stem Bark	**Flavanoids**			
	Glucopyranoside	$C_{28}H_{34}O_{14}$	130	[Shukla and Tiwari, 1971]
	Urosolic Acid	$C_{30}H_{45}O_3$	286	[Shukla and Tiwari, 1971]
	Dihydroxy-4'-methoxy-6",6"-dimethylchromeno-flavanone.	$C_{21}H_{20}O_6$	44-41	[Mukhlesur and Gray, 2002]
	Tetranortriterpene			
	Acidissimin (Limonoid)	$C_{26}H_{28}O_8$	284	[Macleod and Moeller, 1989]
	Coumarins :			
	Bergapten	$C_{12}H_8O_4$	185-186	[Talapatra et al., 1973] [Wijeratne et al., 1992]
	Marmesin	$C_{14}H_{14}O_4$	185-186	[Talapatra et al., 1973]
	Psoralen	$C_{11}H_6O_3$	162-163	[Wijeratne et al., 1992] [Bandara et al., 1988]
	Demethylsuberosin	$C_{14}H_{14}O_3$	-	[Rahman and Gray, 2002]
Root Bark	**Coumarins:**			
	Xanthotoxin	$C_{12}H_8O_4$	144-146	[Bandara et al., 1988]
	Osthenol	$C_{14}H_{14}O_3$	122-124	[Bandara et al., 1988]
	Osthol	$C_{15}H_{16}O_3$	83-84	[Talapatra et al.,1973]
	Auraptan	$C_{19}H_{22}O_3$	66-67	[Talapatra et al., 1973]
	Isopimpinellin	$C_{13}H_{10}O_5$	150-151	[Talapatra et al., 1973]
	6-methoxy7-geranyloxy	-	87-88	[Talapatra et al., 1973]
	Tetranortriterpene			
	Obacunone	$C_{26}H_{30}O_7$	227-229	[Macleod and Moeller, 1989]
	Acidissimin (Limonoid)	$C_{26}H_{28}O_8$	284	[Macleod and Moeller, 1989]
	Steroids:			
	Stigmasterol	$C_{29}H_{48}O$	160-164	[Bandara et al., 1988]
	Alkaloids :			
	Integriquinolone	$C_{33}H_{38}N_2O_9$	255-258	[Talapatra et al., 1973]
Root	**Coumarins:**			
	Marmesin	$C_{14}H_{14}O_4$	185-186	[Agrawal et al., 1989]
	Fernolin	$C_{22}H_{20}O_7$	262	[Agrawal et al., 1989]
	Aurapten	$C_{19}H_{22}O_3$	66-67	[Agrawal et al., 1989]
	Bergapten	$C_{12}H_8O_4$	185-186	[Agrawal et al., 1989]
	Xanthotoxin	$C_{12}H_8O_4$	144-148	[Agrawal et al., 1989]
	Marmin	$C_{10}H_{24}O_5$	110-120	[Wijeratne et al., 1992]
	Glycoside			
	Methoxyflavone			[Inthekhab et al., 2008]

	Glucopyranoside			
Whole Plant	**Coumarins:**			
	Luvagentin	$C_{15}H_{14}0_4$	104	[Patra et al., 1988]
	Xanthotoxin	$C_{12}H_8O_4$	144-148	[Patra et al., 1988]
	Marmesin	$C_{14}H_{14}O_4$	185-186	[Patra et al., 1988]
	Triterpinoids:			
	Lupeol	$C_{30}H_{15}0$	213-215	[Patra et al., 1988]
	Limonin	$C_{26}H_{30}0_8$	298	[Patra et al., 1988]
	Steroids :			
	Sitosterol	$C_{29}H5_00$	136-137	[Patra et al., 1988]
	Sitosterol-O-β-D-Glucoside	$C_{43}H_{68}0_8$	148-149	[Patra et al., 1988]

Fruit & Fruit Shell: Results of study done by some researchers on the fruit of *Feronia limonia*for its characteristic aroma revealed that 3 components are majorly responsible for characteristic wood apple aroma- methyl hexanoate, ethyl 3-hydroxyhexanoate, and butanoic acid. Most aroma components were esters (-45% of the total sample) and included β-hydroxy esters which have previously only been located in tropical fruits (MacLeod and Pieris, 1981).

In yet another phytochemical study performed on ripe & unripe fruits, for the amino acid content revealed that phenylalanine, tyrosine, arginine, lysine, leucine, alanine, histidine, and cystine were all found in substantial amounts in ripe and unripe fruits but unripe fruits contained less of the last 4 amino acids than ripe fruits. Also when ripe and unripe fruits are estimated in terms of nitrogen content results shows that they contained 0.88% N and 0.64% N, resp. When fed to rats as N source, fruit pulp was found to be nearly equal to skimmed-milk powder in biological value (Barnabas and Badve, 1954).

Tyramin derivatives acidissimin, acidissiminol, acidissiminin epoxide, and N-benzoyltyramine were also isolated from the fruits of *Limonia acidissim.* (Ghosh et al., 1989; Ghosh et al., 1991). Studies on fruit pericarp extracts of *Feronia limonia* shows that alkaloids, coumarins, fatty acids, and sterols were present in pericarp extracts, namely it contains umbelliferone, dictamnine, xanthotoxol, scoparone, xanthotoxin, isopimpinellin, isoimperatorin, and marmin (Reisch et al., 1985).

A benzaquinone derivative - 2, 6- dimethoxy-benzoquinone has also been isolated from the chloroform extract of fruit shell which possess antifungal activity (Bandara

et al., 1988).

Leaves: A wide variety of work had been done on the leaves of *F. limonia*. One of the earlier phytochemical reports on its volatile oil composition shows that estragole was the first compound isolated from the leaves of *F.limonia* (Bhati and Deshapande, 1949). Furthur, 32 compounds were isolated and identified in oil out of which its major components were methyl chavicol (27.2%), thymol (24.4%), t-anethol (10.94%), p- cymen-7-ol (7.3%) and 1,4-dimethoxy-2-allylbenzene (Ahmad et al., 1989; Nigam and Nigam, 1979).

More than 50 years of intensive research on leaves resulted in the isolation and identification of some new components of oil like Linalool, caryophyllene, cis-anethole, p-methoxy phenyl-2-propanone, elemicine, 3,4-dimethoxy benzaldehyde, 3,4-dimethoxy cinnamic aldehyde and p-methoxy cinnamic alcohol (Garg, 2003).

Apart from volatile oils, C- flavanoid glycosides have also been isolated from the leaves including orientin, Vitexin , isovitexin, and saponarin being the major components. Gas chromatographical analysis of saponificable and unsaponificable matter revealed the presence of a series of C_{16}- C_{32} compounds like cholesterol, campesterol, stigmasterol and β-sitosterol compounds in unsaponifiable fraction and 12 fatty acids were identified in the saponifiable fraction respectively. Palmitic, linoleic, oleic, linolenic and stearic acids were the major components (84%). Leaves also contains coumarins among them furanocoumarins- bergapten (0.10 %) and , marmesin, and psoralen are the important compounds and are reputed to be effective in the treatment of lecoderma . Other coumarins include β-amyrin, lupeol, xanthotoxin, umbelliferone , isopimpinellin, imperatorin (El-Fishawy, 1994; El-Khrisy et al., 1994).

Stem bark: Flavonoids are chemical phenylbenzopyrones, which, usually conjugated with sugars, are present in all vascular plants (Zanoli et al., 2000). They are reported to be the major phytoconstituents of *Feronia limonia* heartwood which majorly contains flavanone glycosides like urosolic acid & aglucopyranoside (7-methyl-poriol-B-D-xylopyranosyl-D-glucopyranoside). Along with this a pyranoflavanone

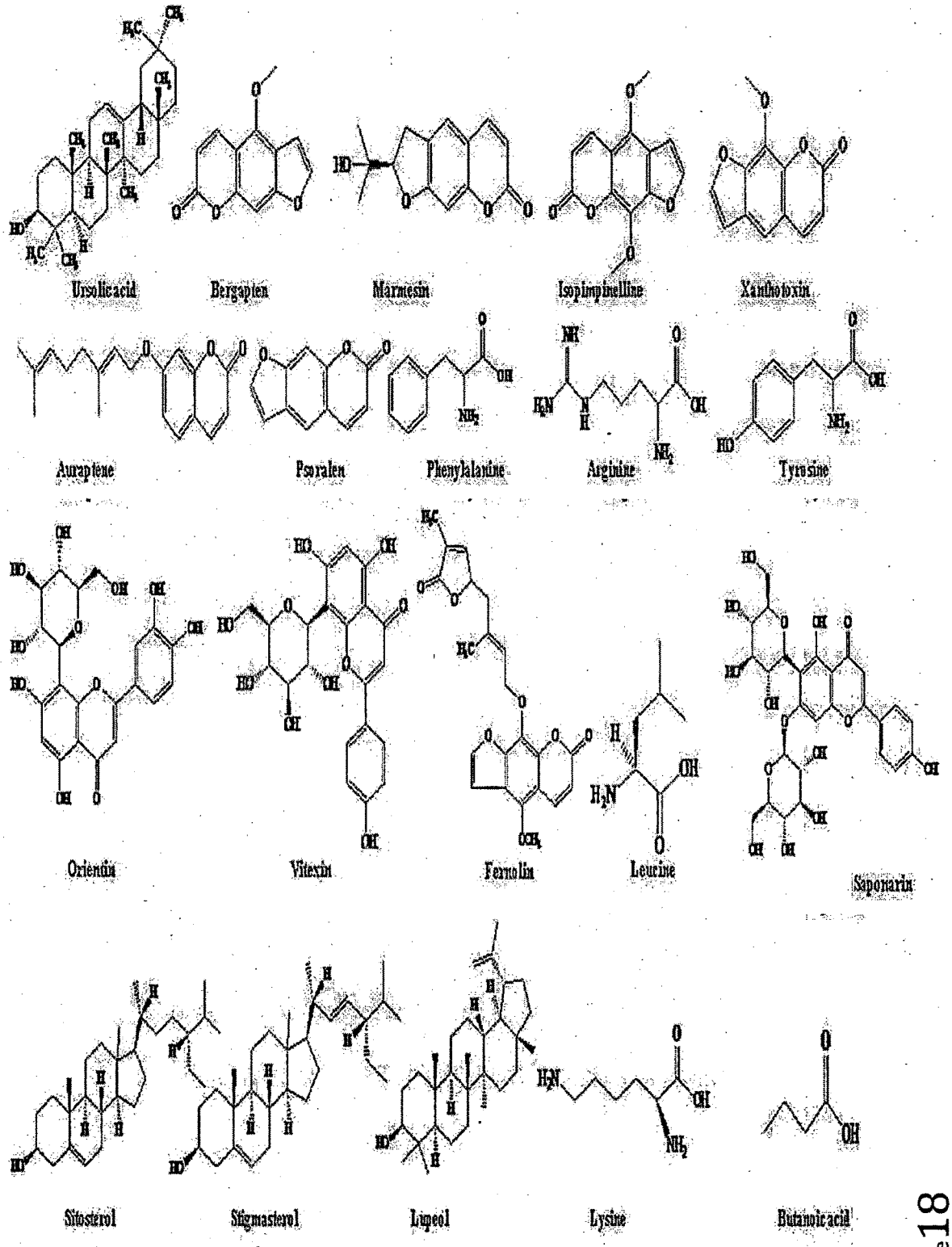
named (-)-(2S)- 5,3'- dihydroxy-4'-methoxy-6", 6"-dimethylchromeno-(7,8,2",3")-flavanone & a diprenylated flavanone 5,7,4;-trihydroxy-6,8-di-(3-methylbut-2-enyl)-flavanone had also been isolated (Rahman and Gray, 2002). Prenylated flavanones similar to the compound isolated by Rahman have been known (Miyamoto et al., 1998) to have oestrogenic effects and may be a useful in the treatment for oesteoporosis. It has been suggested (Ingram et al, 1997) that these types of phytoestrogens have anti-oestrogenic effects which may reduce the risks of hormone-related cancers when conventional steroid treatments for osteoporosis are used.
Apart from them stem bark also contains a feronolide, a ketolactone (Tiwari and Tiwari, 1964), tetratriterpinoid named acidissimin (limonoid) (Macleod and Moeller, 1989) and Steroids including stigmasterol & lupeol. Various coumarins, including bergapten, marmesin, psoralen (Talapatra et al., 1973; Wijeratne et al., 1992; Jain et al., 2010) and 7-hydroxy coumarin (dimethyl suberosin) were also isolated from the stem bark (Mukhlesur and Gray, 2002).

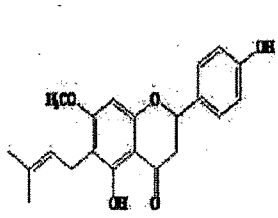
Root Bark: Root bark contains large percentage of coumarins; some of them are explored by researchers like xanthotoxin, osthenol , osthol , auraptan, isopimpinellin [95,96], & 6-methoxy7-geranyloxy (Talapatra et al., 1973). In addition, integriquinolone, a alkaloid is also isolated from the root bark of *Feronia limonia* (Wijeratne et al., 1992). Obacunone & acidissimin (Macleod and Moeller, 1989) belonging to the category of tetranortriterpenes along with a steroid, stigmasterol is also been explored out.

Root: A glycoside has been isolated from the roots of *Feronia limonia* namely 5,4'-dihydroxy-7-methoxyflavone-8-O-β-D-glucopyranoside (Intekhab et al., 2008) along with a wide variety of coumarins which includes marmesin, fernolin, aurapten, bergapten, xanthotoxin (Agarwal et al., 1989) and marmin (Wijeratne et al., 1992).

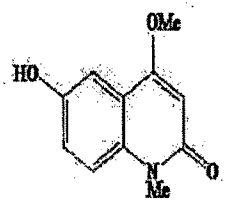
Structures of some important chemical compounds previously isolated from *Feronia limonia* are given below in Figure no 2.4

Figure 2.4 Chemical structures of isolated constituents from *Feronia limonia*

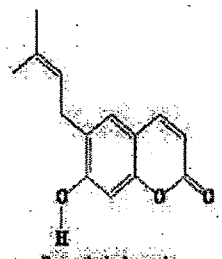




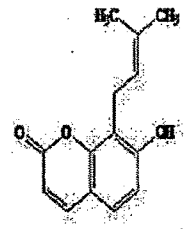
5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one



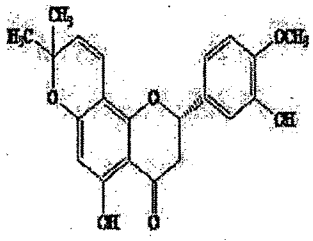
Integriquinolone



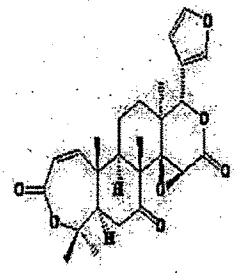
Demethylsuberosin



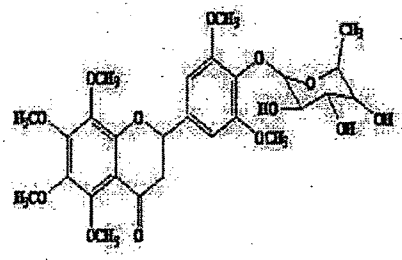
Osthenol



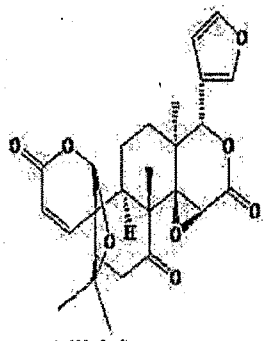
(-)(2S)-5,3'-dihydroxy-4'-methoxy-6",6"-dimethylchromeno(7,8,2",3")-flavanone



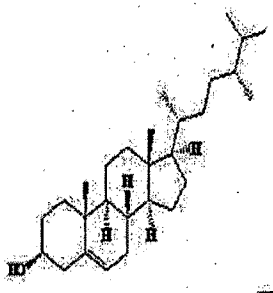
Obacunon



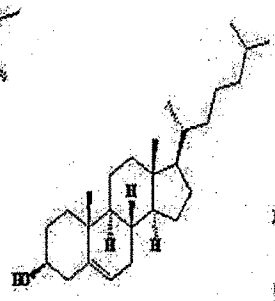
5,6,7,8,3',5'-hexamethoxy flavanone-4'-α-L-rhamnopyranoside



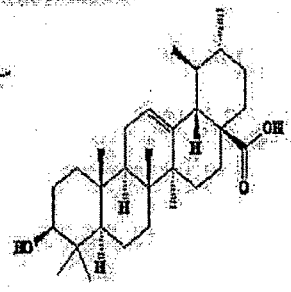
Acilissinin



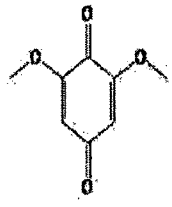
Campesterol



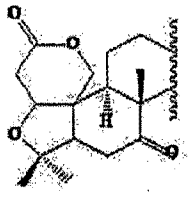
Cholesterol



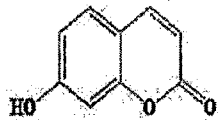
Beta-Amyrin



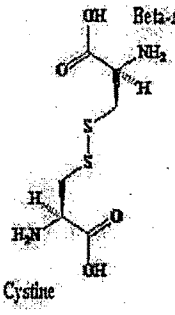
2,6-Dimethoxybenzoquinone



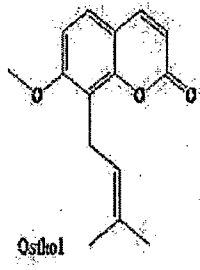
Limonin



Umbelliferone



Cystine



Osthol

2.1.4 Pharmacological aspects

The extracts and pure compounds derived from *Feronia limonia* show a wide spectrum of pharmacological activities, including hepatoprotective, snake bite, anti-tumour, antimicrobial, antidiabetic, anti-inflammatory, analgesic, antioxidant, anti mutagenic, anti malarial, and other activities. A summary of the findings of these studies performed is presented below:

Anti- tumour activity

An antitumor acidic heteropolysaccharide was isolated from the tropical angiosperm (ripe fruits) of *Feronia limonia* and was found to be active *in vivo* against Ehrlich ascites carcinoma (EAC) cell growth inhibition and in increasing macrophage number. For estimating the activity it was given in dose of 100mg/ kg body weight intraperitoneally (i.p.) to the adult female Balb/C mice (20-24gm) for 4 days. On day 4 animals were sacrificed & a significant *in vivo* EAC cell growth inhibition (75%) along with an increase in macrophage count (0.29+0.029) was observed, which is found to be responsible for strong anti-tumor activity of the pectic polysaccharide (Saima et al., 2000).

In another antitumor activity study, ethanolic extracts of 12 medicinal plants of Bangladesh, including the vincristine, vinblastine producing *Catharanthus roseus* was studied using the potato disk bioassay technique. Among these, 10 plants extracts at 25.0-mgrdisc exhibited significant inhibition of crown gall tumors caused by *Agrobacterium tumefaciens*. In this *Feronia limonia* shows 16.1% inhibition of crown gall tumors but found to be inactive because of the insignificant $\leq$ 20.0% inhibition of tumors (Haque et al., 2005).

Antimicrobial activity

Screening for antimicrobial activity was performed on a newly isolated (−)-(2*S*)-5,3′-dihydroxy-4′-methoxy-6″,6″-dimethylchromeno-(7,8,2″,3″)-flavanone moiety from stem bark, along with several known compounds including an alkaloid, five coumarins, a flavanone, a lignan, three sterols and a triterpene. Microdilution titre technique (Drummond et al., 2000) was used to determine the antimicrobial activities as well as minimum inhibitory concentrations (MICs) of the metabolites against gram positive and gram negative bacterias like *Staphylococcus aureus, Escherichia coli , Enterobacter eloacae , Klebsiella aerogenes* and the funguses *Candida albicans* and *Aspergillus niger* . The antimicrobial screening of compounds by a microdilution technique resulted in MICs in the range 25–100 mg/ml. In terms of molar concentration, the lignan was found to be the most potent against all test organisms with the diprenylated flavanone showing slightly less potency. The new flavanone was active against both gram positive and gram negative bacteria, but did not show

any antifungal activity. The remaining compounds revealed moderate activities. However, in comparison to standard antibiotics the activities of the compounds were not so promising, but the presence of this mixture may support the traditional use of the plant to treat minor bowel infections (Rahman and Gray, 2002).

Recently, a comparative evaluation of antimicrobial activity of crude methanolic extract of leaves of 5 medicinal plants viz., *Aegle marmelos, Chloris virgata, Collinsonia anisata, Feronia limonia* and *Cassia auriculata* were performed for their antibacterial activity against four bacterial pathogens. The tested bacterial strains were *Escherichia coli, Salmonella typhi, Proteus mirabilis* and *Klebsiella pneumoniae.* Among the five plants tested, the *Feronia limonia* showed more or less equal zone of inhibition or slightly greater against that pathogens when compared to each other. The antibacterial activities of the leaves were found to be due to the presence of various secondary metabolites.

Antidiabetic activity

Gupta et al evaluated the anti-diabetic activity the effect of ethanolic extract *Feronia elephantum correa* fruits on blood glucose level in normal & streptozotocoin- induced diabetic rats. For the purpose, extracts were orally fed to the experimental groups by metal canula at a single dose of 250 mg/kg body weight as prepared in 2% gum acacia suspension and control was fed only 2% gum acacia. Blood samples were then collected from tail vein at 0, 1, 3 and 4 hr interval for blood glucose level estimation. Results shows that the blood glucose levels was significantly lowered in fasted, fed & streptozotocin induced diabetic male albino rats. It also improved the oral glucose tolerance. Marked degranulation in B-cells of extract treated rats, associated with the blood glucose lowering was observed. It was assumed that, extract probably lowered the blood glucose concentrations by stimulating insulin secretogogue activity (Gupta et al., 2009).

Gastric ulcer

Ethanolic extract of *F. elephantum* fruit pulp was investigated against indomethacin-induced gastric ulcer in rats. The study was performed on Swiss albino rats & the parameters assessed were pH and acid concentration of gastric contents, and gastric ulcer index. Ranitidine was used as the reference anti-ulcer drug. Acute toxicity studies were also carried out. Results shows that the extract (500 mg/kg, p.o.)

inhibited indomethacin-induced gastric ulceration by decreasing acid concentration of gastric fluid while elevating its pH ($p < 0.01$), and compared well with the standard drug, ranitidine ($p < 0.001$). However, its anti-ulcer activity was not as potent as that of ranitidine. Acute toxicity studies showed that there was no mortality following the administration of the extract in a dose range of 250 - 5000 mg/kg, p.o. So it was concluded that *Feronia limonia* fruit pulp extract has potent antiulcer activity with low toxicity. Its anti-ulcer property probably acts via a reduction in gastric acid secretion. The results obtained support the use of this herbal material in folk medicine (Mishra et al., 2009).

A U.S. patent on novel synergistic herbal composition, obtained from the aqueos extract of root of Aegle marmelos, Withania somnifra and Blechnum orintale; the fruit of *Vitis vinifera, Feronia elephantum, Piper nigrum* and *Piper longum*; the fruit rind of *Punica grantum*; the rhizome of *Ziniber officnale*; and the bark of *Azadirachta indica* along with one or more pharmaceutically acceptable additives/carriers, is used for the treatment of gastric ulcer. Said composition can be formulated into tablets, capsules, syrup or any other form known in the art, to be administered orally, intra-muscularly, and by any other conventional methods, to prevent and treat gastric ulcer, such as gastric ulcer induced by cold restraint, aspirin, histamine, alcohol, or pyloric ligation (Rao et al., 2008).

Antioxidant activity and antimutagenic effect

Antioxidant activity and antimutagenic effect of phenolic compounds in *Feronia limonia* ripe fruit pulp was studied by analysing the total phenolic content by Folin–Ciocalteu method; antioxidant activity by the DPPH assay and antimutagenic potential by the Ames test. In the study, free and bound phenolic compound extracts were obtained by successive extractions of *Feronia limonia* ripe fruit pulp. The phenolic glycoside extract presented higher (229.0 mg/g, GAE) total phenolic contents followed by phenolic ester (37.5 mg/g) and free phenolics (11.0mg/g), whereas the antioxidant activity was found to be 88.7%, 11.8% and 3.8% respectively. Phenolic glycoside extract showed antioxidant activity higher than that of commercial antioxidant trolox (64.6%) and butylated hydroxytoluene (83.2%). At 2500 µl/plate a significant antimutagenic effect for digonistic mutagen sodium azide

was shown in the tester strains of Salmonella typhimurium by phenolic glycoside extract and the order of antimutagenic activity was found phenolic glycosides > phenolic esters > free phenolics (Phapale and Thakur, 2010).

Hepatoprotective activity

The hepatoprotective effect of a methanolic leaf extract of *Feronia limonia* was studied on albino rats & mice. Hepatic damage was induced by carbon tetrachloride significantly enhanced the biochemical markers like SGPT, SGOT, ALP, cholesterol, and bilirubin and reduced the levels of HDL in the rats & mice. Treatment with methanolic extract at the dose of 200, 400 and 600 mg/kg reduced the elevated levels of all the above mentioned biochemical indicators (except HDL level which increases with increased dose) and reduced the liver tissue GSH levels and increased the tissue peroxidation. Methanolic extract demonstrated dose dependant reduction in the *in vitro* and *in vivo* peroxidation induced by CCl4. Histopathological observations also revealed that pretreatment with methanolic extract protected the animals from CCl4 induced liver damage. Thus this study indicated the ability of ketha to reduce several parameters associated with liver injury (Kamat et al., 2003; Mansoor et al., 2008).

Antimalarial/ larvicidal activity

Larvicidal activity was performed & evaluated on *n*-hexadecanoic acid, a newly isolated compound from acetone fraction of dried leaves of *F. limonia*. Larvicidal activity on mosquito larvae was evaluated according to WHO method on forth instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* . For the purpose, fourth instar larvae were taken in five batches of 20, in 100 ml of water. Fractions were dissolved in acetone and made up to different concentrations. Tween 80 was used as an emulsifier & control was set up with acetone and Tween 80. After 24 h, the number of dead larvae was counted and the data analysed with to determine the LC50 and LC90 at 95% confidence limits. It was found that *n*-hexadecanoic acid is a potent mosquito larvicide & is effective against fourth instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*, with LC_{50} of 129.24, 79.58 and 57.23 ppm, respectively (Rahuman et al., 2000).

Antifungal activity

A comparative antifungal activity on the oils obtained from the leaves of 3 different

plants, namely *Feronia limonia, Ageratum conyzoides,* and *Blumea membranaceae* was evaluated, by performing a filter paper disk diffusion plate method. Spores of fungus *Fusarium moniliforme, Pyricularia setariae,* and *Helminthosporium oryzae* were grown on Sabouraud's agar & used for the purpose. Results of test showed that *Feronia limonia*possess antifungal activity but less as compared to the other two plants namely *Ageratum conyzoides* and *Blumea membranaceae* (Sharma et al., 1978).

Antibacterial activity

Similarly, the antibacterial activity of essential oil obtained from *Feronia limonia, Ageratum conyzoides*, and *Blumea membranaceae* had been evaluated by some researchers. Test was performed by using disk diffusion tests on different microorganisms by using benzylpenicillin & streptomycin sulphate as standards. Results show that *Feronia limonia* possess widest range of antibacterial activity as compared to other two plants (Sharma et al., 1979).

Wound Healing activity

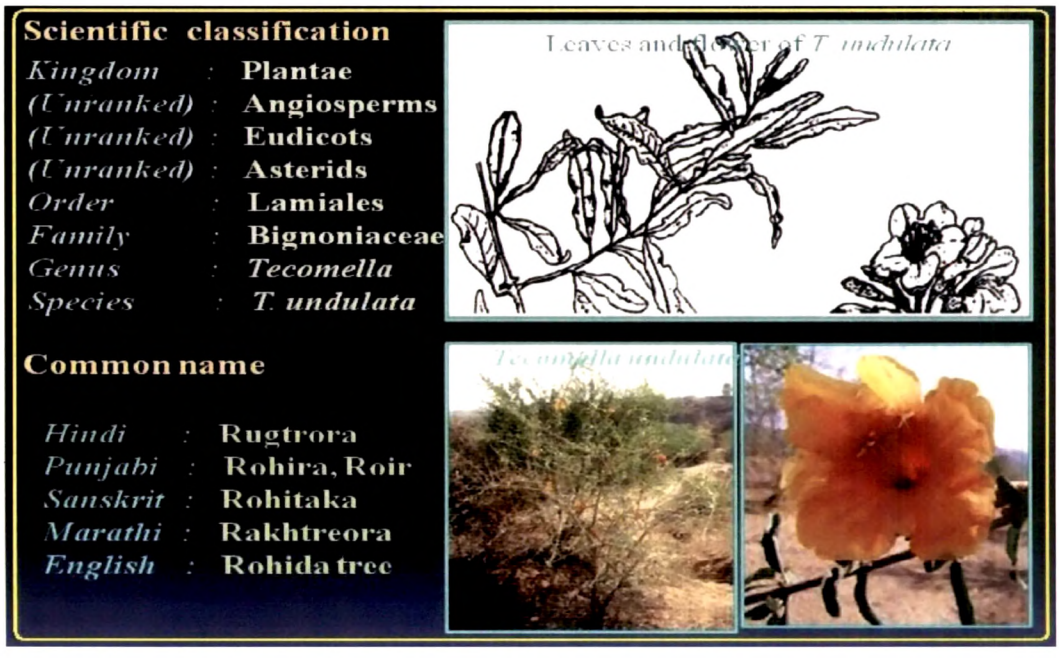
The wound healing activity of the methanolic extract of *Limonia acidissima* (MELA) fruit pulp *in incision, excision and dead-space wound models* were evaluated by Ilango et al. For the study, rats were divided into four groups, viz, wounded control, wounded rats administered standard drug, nitrofurazone (2 %), and wounded rats administered MELA 200 and 400 mg/kg, respectively. *In incision wound model, wound breaking strength and epithelization period* were evaluated. Wounds were created by making two longitudinal paravertibral incisions of 6 cm length through the skin and cutaneous muscle at a distance of about 1.5 cm from the midline on each side of the depilated back of rats. The parted skin was sutured 1 cm apart using surgical thread and curved needle. The wounds were left undressed. The extract was topically applied to the wound once a day as 5% w/w ointment in simple ointment Base I.P. until complete healing occurred. *Increased wound breaking strength, decreased epithelization period,* were observed in the various groups, and compared with the control group (Ilango K. and Chitra, 2010). The methanol extract of *L. acidissima* possesses significant dose-dependent wound healing and anti-oxidant activities; this supports traditional claims for the plant as a wound healer.

2.2 *TECOMELLA UNDULATA* SEEM.

Tecomella undulata seem. (Bignoniaceae) is a deciduous medium sized tree, which is commonly known as Rohitaka, Rohira and Rakta-Rohida in India. Its family comprises of 120 genera and nearly 800 species. These are mostly trees and shrubs, often climbers and rarely herbs found in tropical and subtropical areas (Oluwatoyin et al., 2000). In India, it occurs naturally in Rajasthan, Punjab, Haryana, Gujarat and Maharashtra. It is also distributed in sub-Himalyan tract from Gonda (Uttar Pradesh), eastward to Bengal, Sikkim and Assam west, in western ghat and Andmans. The species is mainly found to occur in western parts of Rajasthan such as Barmer, Jaisalmer, Jodhpur, Pali, Ajmer, Nagaur, Bikaner, Churu and Sikar districts. (http://en.wikipedia.org).

Its classical and common names in India includes *Rohitaka*, Shalmalikaa, Plihaari, Raktghna, Sadaapushpa (CCRAS has equated *Rohitaka* with *Tecomella undulata* seem, while the scientist of INSA, P. Ray et al, and the wealth of India, CSIR, with amoora rohituka . It is also known by the name Roheda, Tecoma undulata, *Bignonia undulata* (Khare, 2004).Its taxonomical classification and common names are given in Figure 2.6

Figure 2.5 Taxonomical classification, common names and photographs of leaves, Flower and plant of *Tecomella undulata*

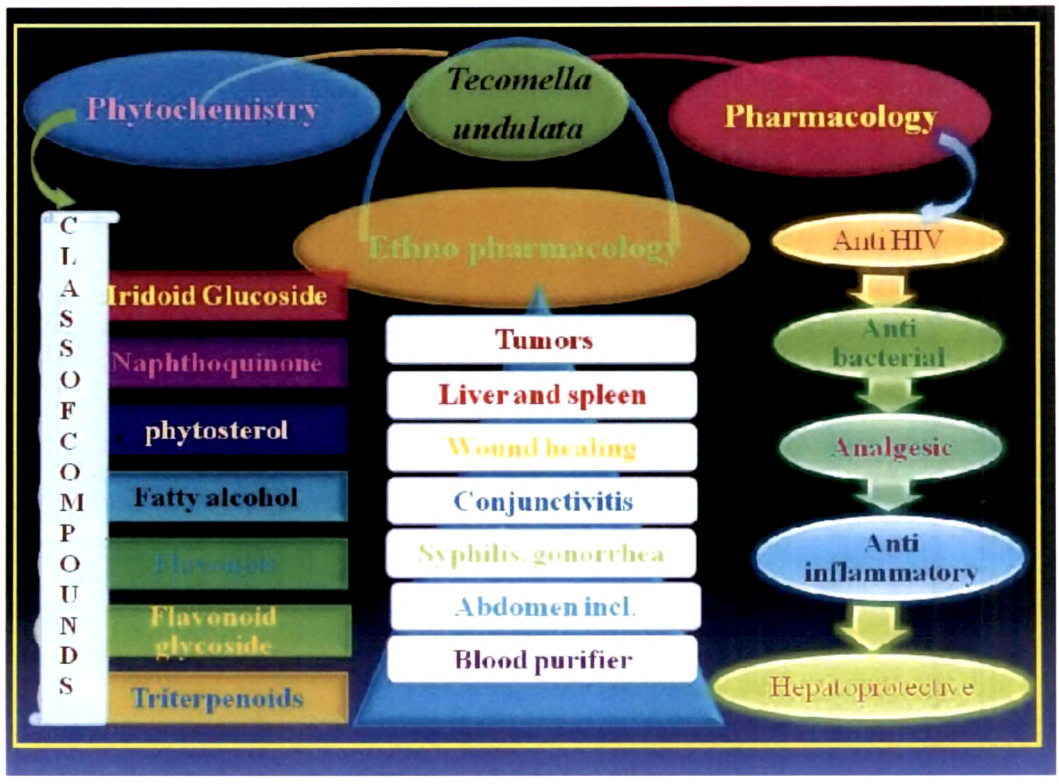


2.2.1 Description of Tecomella undulata

Tecomella undulata is a deciduous or nearly evergreen tree of arid and semi arid regions. It occurs on flat and undulating areas including gentle hill slopes and sometimes also in ravines. It is well adapted to drained loamy to sandy loam soil having pH 6.5-8.0. The species thrives very well on stabilized sand dunes, which experience extreme low and high temperatures. It grows in areas of scanty rainfall (annual 150-500mm) and high temperature (35 °C to 48 °C). It can withstand extreme low temperature (0 °C to −2 °C) during winter and high temperature (48 °C to 50 °C) in summers. The tree is a strong light demander. It is drought, frost, fire and wind hardy. At the time of flowering (December-February) it produces beautiful showy flowers in yellow, orange and red colours. Three types of flower bearing trees can be observed near to each other in the same vicinity (Jain et al., 1999; Indian Council of Forestry, 1996). It is rarely hardy and resistant to drought and used for forestation and landscaping of dry tracts. The tree is propagated from seeds and cuttings. Propagation is highly successful in well drained fibrous loam and it requires plenty of water in summer (The wealth of india, 2003). The leaves are elongated, alternate, rounded at the tips with opposite, entire; lamina elliptic-oblong to elliptic-lanceolate or linear-oblong, 35-95 x (8-) 10-20 mm, margin undulate, petiole 6-18 mm long (http://www.efloras.org). The wood is grayish or yellowish brown, close grained and mould with light streaks and is tough, strong and durable (The wealth of India, 2003). Bark of young plant is soft and greenish brown and it is hard and dark brown in tree. Its bark is up to 8 mm. thick in fully matured tree. Flowers are pale yellow or deep orange-red, showy, large, 6.5 - 7.5 cm. long in corymbose racemes, arrange in few flowered from short lateral branches. Fruit is a capsule, slightly curved, 15-20 cm long pods, 8 mm broad, thin, flattened and slightly crooked and seeds are winged, 2cm long and 8 mm broad. *Rohitaka* blooms in the month of April of May and bears fruits thereafter. Seeds of *Tecomella undulata* are winged (Pullaiah, 1917; Randhawa and Mukhopadhyay, 2004)

Graphical outline containing traditional uses, phytochemistry and pharmacology of *Tecomella undulata* is given in Figure 2.7

Figure 2.6 Graphical outline containing traditional uses, phytochemistry and pharmacology of *Tecomella undulata*



2.2.2. Ethnomeditional uses of Tecomella undulata

Tecomella undulata occupied a reputed position of having valuable medicinal properties in both folk and classical streams of indigenous medicinal systems. Medicinal usage on various plant parts of *Tecomella undulata* in indigenous system of medicines in India and other countries is outlined below:

Rohitaka is pungent, astringent and bitter in taste; it has post digestive effect and has cold potency. It alleviates *kapha* and *pitta* doshas. It has a special potency (*prabhava*) as *bhedana* – accumulation breaking herb, *plihasankocaka* – contracts the spleen and as a *bhutapidanasaka* – averts the evil powers. It possesses light and dry attributes. It is used in the diseases like ascites, liver and spleen disorders, obesity, tumors, blood disorders, flatulence, abdominal pain and cough (http://www.herbalcureindia.com).

Bark: Bark of *Tecomella undulata* has great medicinal value and is used for medicinal purpose, externally as well as internally. Externally, the paste of its bark

skin is applied on traumatic wounds, associated with haematoma. It also promotes wound healing. In conjunctivitis, the juice is instilled into eyes, with great benefit. Internally, the powder of bark skin is given along with ghee in gaematoma. *Rohitaka* is specially recommended in ascites with hepatosplenomegaly. It is an excellent blood purifier and cholegogue, hence, rewarding in hepatitis. It is also used in curing urinary disorder, enlargement of spleen, gonorrhoea, leucoderma, liver diseases and remedy for syphilis (http://www.herbalcureindia.com).

According to ayurvedic classical texts *Rohitaka* is specially used for treating various abdominal ailments including Ascitis. *Charaka* prescribed powder bark, its decoction and extract in clarified butter in treating jaundice, enlarge spleen, anemia, intestinal worms, urinary disorders (Khare, 2004). Ladies of tribal communities of *Samahni valley* (Pakistan) take bark powder with hot milk for abortion (Muhammad and Khan, 2008). Also, in some parts of India, bark and wood of *Rohitaka* is soaked in water for two days, distillate then obtained is used for treating eczema (Herbal selection, 2) It is reported to be a potent blood purifier and is extremely useful in treating syphilis, gonorrhoea and gout. As a keen stimulant for digestive system, it is rewarding in the treatment for piles, anorexia, flatulence, tumors and worm infestations (http://www.herbalcureindia.com).

It is used for liver ailments and possesses pain relieving properties. Bark is used to treat skin disorder, jaundice, liver disorders, diabetes, cancer and obesity.

It is also used as tonic for animals for recumbent animal. The bark of the tree is ground to a powder and 100g of the same is administered daily till the animal recovers (Herbal selection, 2).

Seed: seeds crushed with pinus leaf extract are taken to cure haemorrhoids. It is also used against abscess (Muhammad and Khan, 2008).

Root: The paste of root was given internally in leucorrhoea some time its pulp is given along with rice water (Khare, 2004).

Flower: Traditionally in Musakhel, Pakistan its flowers are used for treating hepatitis (http://en.wikipedia.org).

Ayurvedic formulations

Various formulation of *Tecomella undulata* is available in market some of them are listed here: *Rohitakaarishta* (based on *Bhaishajya Ratnaavali*) is the only classical compound available over the counter and is being prescribed in liver and spleen diseases, oedema and anaemia. Other classical, compounds Rohtakaadya Churna, *Rohitaka* Ghrita, *Rohitaka*-lauha, are no more available. Ayurvedic brightening and fair complexion mask and lower back massage oil is prepared from this plant in combination with other plants. One patent was found on its medicinal application for immuno-compromised conditions (Muhammad and Khan, 2008).

2.2.3. Phytochemical aspects

Tecomella undulata has received particular attention by the researchers and, as a result, a significant number of articles have been published. Starting from the second half of the 20th century, several phytochemical studies were performed to investigate the composition of different plant extracts, leading to the isolation and identification of pharmacologically relevant compounds such as iridoid glucoside, naphthoquinone (Singh et al., 2008; Verma et al., 1986; Joshi et al., 1977), phytosterols, fatty alcohol, flavonoid glycoside, flavonol (Taneja et al., 1975), fatty acid (Khare, 2004) and triterpenoids (Mukerji, 1977). Some previously isolated chemical constituents and structures are given in Table 2.2 and Figure 2.7.

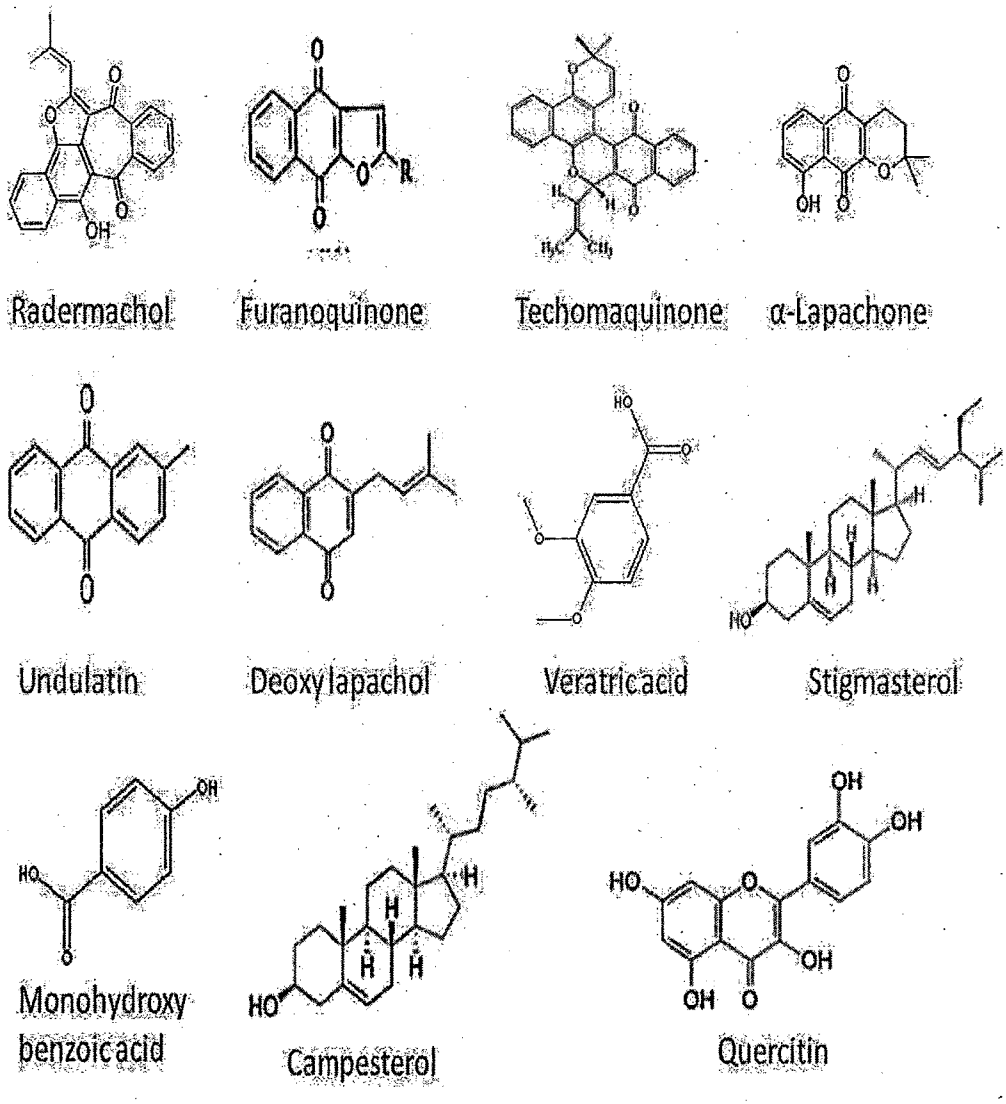
Table 2.2 Some important chemical constituents isolated from various parts of *T. Undulata*

Plant Part	Constituents isolated	Chemical formula	Reference
Heart wood	Radermachol	$C_{24}H_{16}O_4$/ 368.38	(Singh et at., 2008)
	2-Isopropenylnaphtho [2,3-*b*]furan-4,9-quinone	$C_{15}H_{10}O_3$	(Singh et at., 2008)
	Tecomaquinone-I	$C_{30}H_{24}O_4$/ 448.13	(Singh et at., 2008)
	Alpha-Lapachone	$C_{15}H_{14}O_3$/ 242.3	(Singh et at., 2008)
	Dehydro-alpha--Lapachone	$C_{15}H_{12}O_3$/ 240.254	(Singh et at., 2008)
	Cluytyl ferulate	$C_{38}H_{66}O_4$/ 586.92	(Singh et at., 2008)
	Undulatin	$C_{15}H_{10}O_2$/ 222.24	(Verma et al., 1986)
	Tectoquinone	$C_{15}H_{10}O_2$/ 222.23	(Verma et al., 1986)
	Deoxylapachol	C15H14O2/ 56-57c	(Verma et al., 1986)
	Lapachole	$C_{15}H_{14}O_3$/ 242.27	(Jabeen et al., 2009)
Heart wood and	Tectol	$C_{30}H_{26}O_4$/ 450.52	(Joshi et al., 1977)
	Dehydro-α- lapachone	C15H12O3/ 240.25	(Jabeen et al., 2009)

Root			(Verma et al., 1986)
Heart wood and Bark	Lapachol	$C_{15}H_{14}O_3$/ 242.27	(Joshi et al., 1976)
	β-Sitosterol	$C_{29}H_{50}O$/ 414.72	(Joshi and Singh, 1977)
	Veratric acid	$C_9H_{10}O_4$/ 182.173	(Khare, 2008)
	n-Triacontanol	C30H62O/ 438.8	(Gujral et al., 1979)
	Tecomelloside	C24H30O13/	(Gujral et al., 1979)
Heart wood, bark and leaf	Stigmasterol	$C_{29}H_{48}O$/ 412.69	(Singh et at., 2008)
Bark	Tecomin	C15H14O3/ 242.27	(Pandey et al., 1971; Birkhauser, 1970)
	Undulatoside A		(Gujral et al., 1979)
	Undulatoside B		(Verma et al., 1986)
	Tecoside		(Verma et al., 1979)
	β -Sitosteryl acetate	C31H52O2/ 456.74	(Ahmed and ahsana)
	p-Hydroxybenzoic acid	$C_7H_6O_3$/ 138.12	(Ahmed and ahsana)
	β -Amyrin	$C_{30}H_{50}O$/ 426.73	(Ahmed and ahsana)
	Campesterol	C28H48O/ 400.68	(Ahmed and ahsana)
	Alkanols		(Khare, 2008)
	Alphanamixinin		(Khare, 2004)
	β -Sitosterol	$C_{29}H_{50}O$/ 414.72	(Taneja et al., 1975; Singh et at., 2008)
Leaves	Deterpene		(Khare, 2004)
	Aphanamixol		(Khare, 2004)
	Triacontanol	$C_{30}H_{62}O$/ 438.81	(Mukerji, 1977)
	Betulinic acid	$C_{30}H_{48}O_3$/ 456.7	(Mukerji, 1977)
	Oleanolic acid	$C_{30}H_{48}O_3$/ 456.7	(Mukerji, 1977)
	Ursolic acid	$C_{30}H_{48}O_3$/ 456.70	(Mukerji, 1977)
	n-Octacosanol	$C_{28}H_{58}O$/ 410.76	(Gujral et al., 1979)
	Campesterol	C28H48O/ 400.68	(Gujral et al., 1979)
	α-Amyrin	$C_{30}H_{50}O$/ 426.72	(Gujral et al., 1979)
	Oleanolic acid	$C_{30}H_{48}O_3$/ 456.7	(Gujral et al., 1979)
	Triacontanol	$C_{30}H_{62}O$/ 438.81	(Bhaul et al., 2007)
	Cirsimaritin	$C_{17}H_{14}O_6$/ 314.28	(Bhaul et al., 2007)
	Cirilineol		(Bhaul et al., 2007)
	Pentatriacontanol	$C_{35}H_{72}O$/ 508.94	(Bhaul et al., 2007)
Root	6-O-veratrylcatalposide		(Joshi et al., 1977; Joshi et al., 1975)
Flowers	Rutin	$C_{27}H_{30}O_{16}$/ 610.51	(Taneja et al., 1975)
	Quercetin	$C_{15}H_{10}O_7$/ 302.23	(Taneja et al., 1975)

	Luteolin-7-glucoside	$C_{21}H_{20}O_{11}$/ 448.37	(Taneja et al., 1975)
Seed	Alimonoid		(Khare, 2004)
	Rohitukin		(Khare, 2004)
	Linoleic acid	$C_{18}H_{32}O_2$/ 280.45	(Khare, 2004)
	Oleic acid	$C_{18}H_{34}O_2$/ 282.46	(Khare, 2004)
	Stearic acid	$C_{18}H_{36}O_2$/ 284.48	(Khare, 2004)
	Palmitic acid	$C_{16}H_{32}O_2$/ 256.42	(Khare, 2004)
Fruit shell	Aphanamixin lactone		(Khare, 2004)
	Aphanamixolide		(Khare, 2004)

Figure 2.7 Chemical structures of isolated constituents from *Tecomella undulata*



2.2.4. Pharmacological aspects

In recent years many researchers have examined the effect of *Tecomella undulata* used traditionally by indigenous healers and herbalists to support function of various body parts and treat diseases in human and animals. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action as well as reaffirming the therapeutic effectiveness of plant or plant extracts in clinical studies.

Anti microbial activity

Biochemical analysis indicated that *T. undulata* leaves have oleanolic acid, ursolic acid and betulinic acid, compounds that are strong HIV prohibitors. Octadimethyl succinate derivatives of oleanolic acid and betulinic acid have been reported to be 24 times more active than AZT, a drug that is currently used for checking the spread of AIDS (Azam, 1999). Other compounds isolated from the leaves of *T. undulata* are sitosterol, triacontanol, cirsimaritin, cirilineol, pentatriacontanol and 4,5-dihydroxy-3,6,8-trimethoxy flavone. Both aqueous and alcoholic leaf and stem extracts of *T. undulata* showed growth inhibition of *Salmonella typhi*, a causal organism of typhoid fever.

Sumitra Chandra et al. works on antibacterial activity with methanolic and aqueous extracts of *Tecomella undulata*. They found that plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria. The most susceptible bacteria were *B. subtilis*, followed by *S. epidermidis*, while the most resistant bacteria were *P. vulgaris*, followed by *S. typhimurium*. The antibacterial activity of aqueous and methanol extracts was determined by agar disk diffusion and agar well diffusion method. The methanol extracts were more active than the aqueous Extract (Giridhar et al., 1980; Rao et al., 1989).

Central analgesic activity

Tecomella undulata has significant analgesic activity. Whole plant of *Tecomella undulata* was extracted with absolute methanol by Ahmad F. et al. using the hot water tail immersion test in mice and carrageenan induced pedal edema in rats, both extracts were tested for oral analgesic potential. Result showed that *T. Undulata* had analgesic potential when compared with aspirin (Ahamad et al., 1994). This extract probably act

on opoidergic receptors and appear to be promising analgesic agent. However, further experiments will possibly define this pharmacological effect. If confirmed it, may become of importance for human clinical treatments (Almeida et al., 2001).

Hepatoprotective activity

Stem bark of *Tecomella undulata* have strong activity against thioacetamide induced hepatotoxicity. Oral administration of *Tecomella undulata* at 1000 mg/kg resulted in a significant reduction in serum aspartate aminotransaminase (35% and 31%, respectively), alanine aminotransaminase (50% and 42%, respectively), gamma glutamyl transpeptidase (56% and 49%, respectively), alkaline phosphatase (46% and 37%, respectively), total bilirubin (61% and 48%, respectively) and liver MDA levels (65% and 50%, respectively), and significant improvement in liver glutathione (73% and 68%, respectively) when compared with thioacetamide damaged rats. Histology of the liver sections of the animals treated with the extracts also showed dose-dependent reduction of necrosis (Khatri et al., 2009).

Immune modulator activity

Tecomella undulata possesses diverse biological activities and have bio- modulatory and immunomodulatory functions. Its alcoholic extract influence immune system viz. increase phagocytic activity of macrophages, stimulating the production of antibodies and cytokines, increase accumulation of NK cells into tissue and activation and mobilization of T and B cells (Rajkapoor et al., 2006).

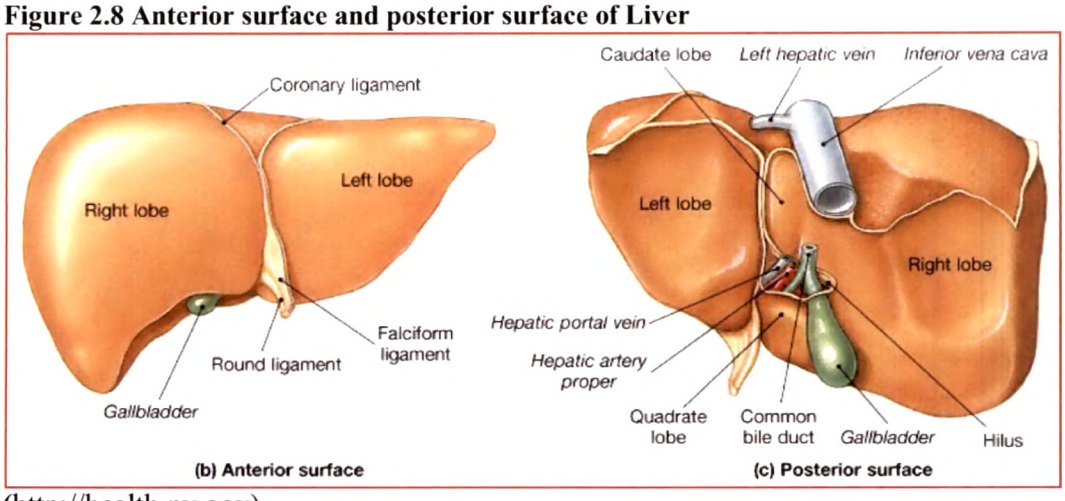
Tecomella undulata with herbal combination of *Moringa oleifera, Boerhavia diffusa, Onosma bracteatum, Bauhinia variegata, Spheranthus indicus, Chlorophytum borivilianum, Ficus racemosa,* and *Cyperus rotundus* is effective for the treatment a wide range of physiological and pathological conditions in the human body resulting from a weakened or deteriorating immune system. This combination herbal preparation has been found to be particularly useful in maintaining the normal physiological functions of the immune system, in regulating the immunological functions and all the aberrations that occur due to the subtle immunological imbalances and reduced immunity, and to restore and improve the immune function in individuals exhibiting a weakened or deteriorating immune response. It have beneficial effects and to improve the quality of life in individuals experiencing all

types of cancer, especially those that directly weaken the immune system, in individuals affected with HIV and AIDS, in individuals exhibiting failing immunity due to old age, and all other conditions of the human body that negatively affect the immune system through the following mechanisms: (1) by stimulating the production of growth factors responsible for production of the cells of the immune apparatus, like lymphocytes, macrophages, Langerhans cells, histiocytes, etc.; (2) by enhancing the immune response due to the production of new cells and replacing the aging and functionally incompetent cells of the immune system; (3) by mopping up the free radicals generated by the metabolism of cancer cells, the anti-retroviral metabolism in cells of individuals affected with HIV or AIDS, and during the aging process (i.e. antioxidant effect); and (4) by stimulating the immune apparatus to produce antibodies and to form immune complexes (i.e. immunostimulatory effect). It can also be used as a chemo protective or radio protective agent in individuals affected with cancer, wherein it can be used as an adjuvant to conventional treatments, such as chemotherapy and radiotherapy, to reduce the adverse side effects of these therapies. The combination herbal preparation also exhibits radio sensitizing and chemo sensitizing effects in cancer patients by enabling the tumor to become more sensitive to the effects of these two standard modalities of conventional anticancer therapy. Improved sensitivity of the tumor to radiotherapy and chemotherapy also helps in effectively reducing the required dosage of these therapies in order to achieve the prescribed therapeutic effects, thereby reducing and alleviating the powerful and devastating adverse toxic effects exerted by radiotherapy and chemotherapy in cancer treatment (Managoli et al., 2008).

2.3 Liver

It is an organ in the upper abdomen that aids in digestion and removes waste products and worn-out cells from the blood. The liver is the largest solid organ in the body. The liver weighs about three and a half pounds (1.6 kilograms). It measures about 8 inches (20 cm) horizontally (across) and 6.5 inches (17 cm) vertically (down) and is 4.5 inches (12 cm) thick (http://www.medterms.com). Anatomy of liver is shown in Figure 2.8.

Figure 2.8 Anterior surface and posterior surface of Liver



(http://health.nv.gov)

The liver has a multitude of important and complex functions. Some of these functions are to:

- ✓ Manufacture (synthesize) proteins, including albumin (to help maintain the volume of blood) and blood clotting factors
- ✓ Synthesize, store, and process (metabolize) fats, including fatty acids (used for energy) and cholesterol
- ✓ Metabolize and store carbohydrates, which are used as the source for the sugar (glucose) in blood that red blood cells and the brain use
- ✓ Form and secrete bile that contains bile acids to aid in the intestinal absorption (taking in) of fats and the fat-soluble vitamins A, D, E, and K.
- ✓ Eliminate, by metabolizing and/or secreting, the potentially harmful biochemical products produced by the body, such as bilirubin from the breakdown of old red blood cells and ammonia from the breakdown of proteins
- ✓ Detoxify, by metabolizing and/or secreting, drugs, alcohol, and environmental toxins

2.3.1 Liver disorder/Hepatic damage

It plays a key role not in metabolism and disposition of exogenous toxins or therapeutic agent, but also in the biochemical reaction. It is continually exposed to a

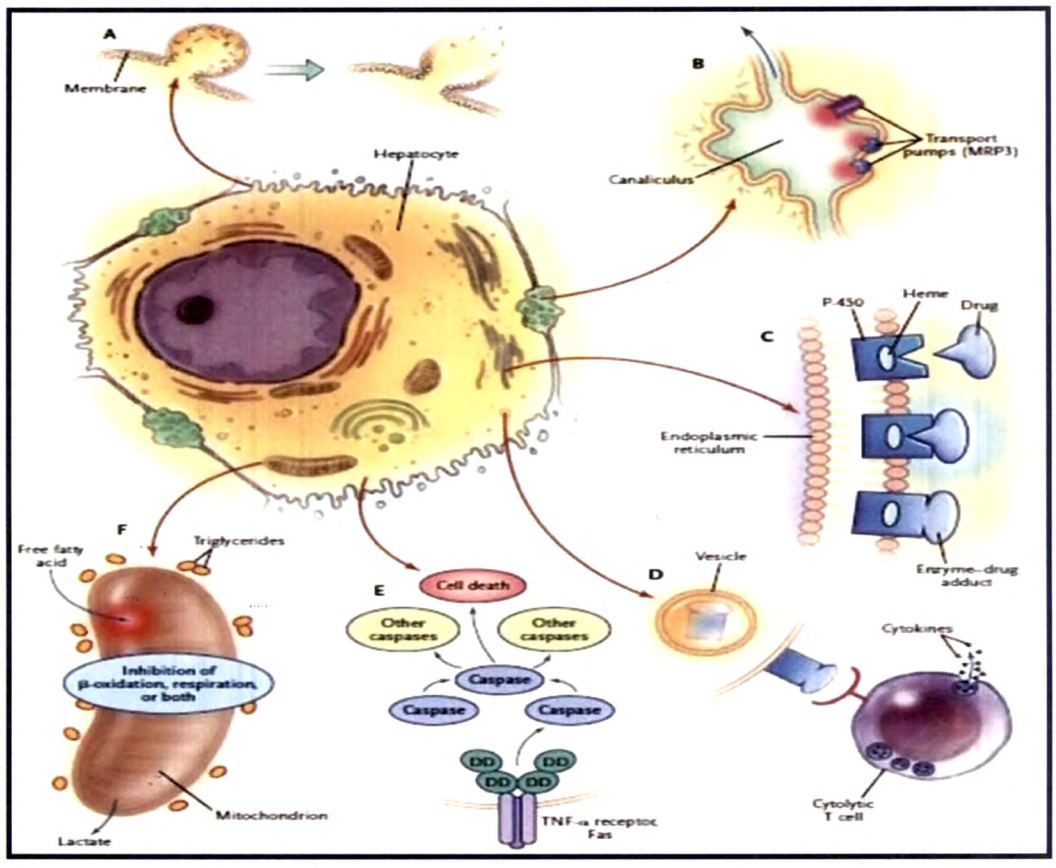
wide variety of xenobiotics and therapeutic agents due to inadequately controlled environmental pollution and to drugs which results in liver damage. Liver diseases are categorized both by the cause and the effect they have on the liver. Causes may include infection, injury, exposure to drugs or toxic compounds, an autoimmune process, or a genetic defect that leads to the deposition and build-up of damaging substances such as iron or copper. Effects may include inflammation, scarring, obstructions, clotting abnormalities, and liver failure (http://www.liver-damage).

Mechanisms of injury to hepatocytes at cellular level

Various mechanisms of injury to hepatocytes are given in figure 2.9. Injury to liver cells occurs in patterns specific to the intracellular organelles affected. The normal hepatocytes shown in the center of the Figure may be affected in at least six ways, labeled A through F. (A) Disruption of intracellular calcium homeostasis leads to the disassembly of actin fibrils at the surface of the hepatocyte, resulting in blebbing of the cell membrane, rupture, and cell lysis. In cholestatic diseases, disruption of actin filaments (B) may occur next to the canaliculus, the specialized portion of the cell responsible for bile excretion. 11 Loss of villous processes and the interruption of transport pumps such as multidrug-resistance–associated protein 3 (MRP3) prevent the excretion of bilirubin and other organic compounds. Many hepatocellular reactions involve the heme-containing cytochrome P-450 system (C), generating high-energy reactions that can lead to the covalent binding of drug to enzyme, thus creating new, nonfunctioning adducts. These enzyme–drug adducts migrate to the cell surface (D) in vesicles to serve as target immunogens for cytolytic attack by T cells, stimulating a multifaceted immune response involving both cytolytic T cells and cytokines. 12 Activation of apoptotic pathways by tumor necrosis factor *a* (TNF*a*) receptor or Fas may trigger the cascade of intercellular caspases (E), which results in programmed cell death with loss of nuclear chromatin. 13 Certain drugs inhibit mitochondrial function by a dual effect on both *b* -oxidation (affecting energy production by inhibition of the synthesis of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, resulting in decreased ATP production) and the respiratory-chain enzymes (F). Free fatty acids cannot be metabolized, and the lack of aerobic respiration results in the accumulation of lactate and reactive oxygen species. The presence of reactive oxygen species may further disrupt mitochondrial DNA.

This pattern of injury is characteristic of a variety of agents, including nucleoside reverse-transcriptase inhibitors, which bind directly to mitochondrial DNA, as well as valproic acid, tetracycline, and aspirin. 14 Toxic metabolites excreted in bile may damage bile-duct epithelium (not shown). DD denotes death domain (William and Lee, 2003).

Figure 2.9 Figure depiciting various mechanisms of Injury to hepatocytes



Some of the commonly observed liver diseases are as follows (http://en.wikipedia.org):

- **Hepatitis,** inflammation of the liver, caused mainly by various viruses, but it may also be caused by poisons, alcohol, autoimmunity (autoimmune hepatitis) or hereditary conditions.

- **Non-alcoholic fatty liver disease,** a spectrum in disease, associated with obesity and characterized as an abundance of fat in the liver; may lead to a hepatitis, i.e. steatohepatitis and/or cirrhosis.
- **Cirrhosis** is the formation of fibrous tissue in the liver from replacing dead liver cells. The death of the liver cells can be caused by viral hepatitis, alcoholism or contact with other liver-toxic chemicals.
- **Haemochromatosis,** a hereditary disease causing the accumulation of iron in the body, eventually leading to liver damage.
- **Cancer** of the liver (primary hepatocellular carcinoma or cholangiocarcinoma and metastatic cancers, usually from other parts of the gastrointestinal tract).
- **Wilson's disease,** a hereditary disease which causes the body to retain copper.
- **Primary sclerosing cholangitis,** an inflammatory disease of the bile duct, likely autoimmune in nature.
- **Primary biliary cirrhosis,** autoimmune disease of small bile ducts.
- **Budd-Chiari syndrome,** obstruction of the hepatic vein.
- **Gilbert's syndrome,** a genetic disorder of bilirubin metabolism, found in about 5% of the population.
- **Glycogen storage disease type II,** the build-up of glycogen causes progressive muscle weakness (myopathy) throughout the body and affects various body tissues, particularly in the heart, skeletal muscles, liver and nervous system.

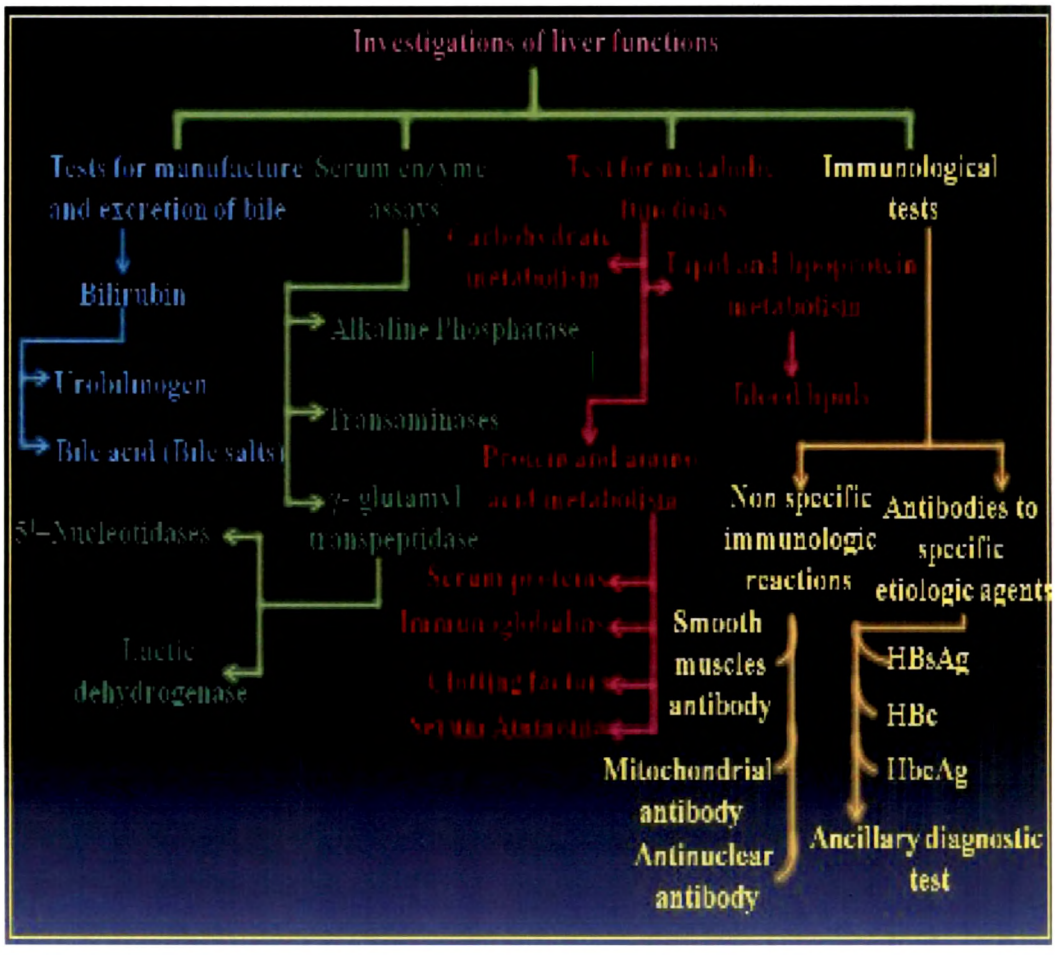
2.3.2. Investigations of liver functions

When the liver is diseased, one or more but not necessarily all of its functions are impared. There can be no test for liver functions as a whole. The various "liver functions test" (LFTs) are test of derangements of individual functions of the liver. Since many tests give similar abnormal results in a particular liver disease, it may be possible to extend a conclusion drawn from a single test. Liver biopsy results may not be comparable with the LFTs since many functional changes are not mirrored by obvious structural changes in the liver cells (Praful, 1996). Flow diagram of various liver function tests are depiceted in Figure 2.10.

Advantages of Liver function tests: (http://elearning.medicine.swu.ac.th)

- ✓ sensitive, noninvasive method of screening liver dysfunction
- ✓ pattern of laboratory test abnormalities to recognize type of liver disorder
- ✓ assess severity of liver dysfunction
- ✓ follow cause of liver disease

Figure2. 10 Flow diagram of various liver function tests



2.3.3 Common liver function test

Liver function tests (LFTs or LFs), are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver. The parameters measured include PT/INR, albumin, bilirubin (direct and indirect) and others. Liver transaminases (AST/ALT (SGOT/SGPT)) are not liver function tests but

are biomarkers of liver injury in a patient with some degree of intact liver function. Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. Commonly used liver function test are given below:

- ALT: alanine aminotransferase (SGPT)
- AST: aspartate aminotransferase (SGOT)
- Alkaline Phosphatase
- Bilirubin
- Total Protein and serum albumin

a) ALT: Alanine aminotransferase (SGPT)

ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the two parts of the alanine cycle.It is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. When used in diagnostics, it is almost always measured in international units/liter (U/L). While sources vary on specific normal range values, most show between 5-60 U/L as being normal.

- ✓ Found primarily in hepatocytes
- ✓ Released when cells are hurt or destroyed
- ✓ Normal levels depend on the reference range which actually differs lab to lab
- ✓ Considered normal between 5-40 U/L
- ✓ Probably should be half of this (5-20)

b) AST: Aspartate aminotransferase (SGOT)

AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health.

AST (SGOT) is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health. It has also been shown to be a marker for chronic alcoholism. When used in diagnostics, it is almost always measured in international units/liter (U/L). While sources vary on specific normal range values, most show between 6-40 U/L as being normal.

- ✓ Found in many sources, including liver, heart, muscle, intestine, pancreas
- ✓ Not very specific for liver disease
- ✓ Often follows ALT to a degree
- ✓ Elevated 2 or 3:1 (vs. ALT) in alcoholics
- ✓ Normal range: 8-20 U/L

c) Alkaline Phosphatase

An alkaline phosphatase (ALP) test measures the amount of the enzyme ALP in the blood. ALP is made mostly in the liver and in bone with some made in the intestines and kidneys. It also is made by the placenta of a pregnant woman.

The liver makes more ALP than the other organs or the bones. Some conditions cause large amounts of ALP in the blood. These conditions include rapid bone growth (during puberty), bone disease (osteomalacia or Paget's disease), or a disease that affects how much calcium is in the blood (hyperparathyroidism), vitamin D deficiency, or damaged liver cells.

- ✓ Found in liver (especially biliary tract), bones, intestines, & placenta
- ✓ "Fractionated" or "isoenzymes" to source
- ✓ Liver AP rises with obstruction or infiltrative diseases (i.e., stones or tumors)
- ✓ Normal range: 20-70 U/L

d) Bilirubin

Bilirubin is a yellowish pigment found in bile, a fluid made by the liver. A small amount of older red blood cells are replaced by new blood cells every day. Bilirubin is left after these older blood cells are removed. The liver helps break down bilirubin so that it can be removed by the body in the stool. Large amounts of bilirubin in the blood can lead to jaundice. Jaundice is a yellow color in the skin, mucus membranes, or eyes.

Bilirubin metabolism begins with the breakdown of red blood cells in many parts of the body. Red blood cells contain hemoglobin, which is broken down to heme and globin. Heme is converted to bilirubin, which is then carried by albumin in the blood to the liver.

In the liver, most of the bilirubin is chemically attached to another molecule before it is released in the bile. This "conjugated" (attached) bilirubin is called direct bilirubin; unconjugated bilirubin is called indirect bilirubin. Total serum bilirubin equals direct bilirubin plus indirect bilirubin.

It is normal to have some bilirubin in your blood. Normal levels are:

- ✓ Direct (also called conjugated) bilirubin: 0 to 0.3 mg/dL
- ✓ Total bilirubin: 0.3 to 1.9 mg/dL

e) Albumin (Alb)

Albumin is a protein made specifically by the liver, and can be measured cheaply and easily. It is the main constituent of total protein; the remaining fraction is called globulin (including the immunoglobulins). Albumin levels are decreased in chronic liver disease, such as cirrhosis. It is also decreased in nephrotic syndrome, where it is lost through the urine. Poor nutrition or states of impaired protein catabolism, such as in Ménétrier's disease, may also lead to hypoalbuminaemia. The half-life of albumin is approximately 20 days. Albumin is not considered to be an especially useful marker of liver synthetic function; coagulation factors (see below) are much more sensitive.

While sources vary on specific normal range values, most show between 3.5 to 5.3 g/dLas being normal.

2.3.4 Evaluation of hepatoprotective activity

Until recently it had been accepted almost as dogma that there was not and could not be any screening method for standardization and evaluation of hepatoprotective drugs since most of the available methods do not simulate the clinical hepatic diseased conditions. Therefore evaluation of any compound with hepatoprotective claims in a single model does not suffice the purpose and needs to be based on multi models, which are in grea demand today. A review of literature reveals that several chemical substances and drugs having specific actions on liver are used as hepatotoxins in experimental animals to simulate ideal disease conditions.

Hepatotoxins may be grouped into direct and indirect types depending upon their intrinsic capability, host susceptibility and circumstances of exposure. Generally direct toxins injure many tissues including liver (eg. CCl_4), and indirectly affects

particular metabolic pathways (eg. Galactosamine). Thus the hepatotoxins affect the liver in a number of ways as:

1. Interference with hepatic bilirubin uptake, conjugation and excretion eg: Rifampcin.
2. Dose and time dependent reactions.
   a. Acute toxic hepatitis eg. Paracetamol
   b. Fatty liver eg. Tetracycline
3. Dose independent reactions.
   a. Diffuse hepatocellular damage eg. Isoniazid
   b. Cholestatic hepatitis eg. Chlorpromazine
   c. Granulomatous infilteration eg. Phenytoin, chlorpropamide

Thus hepatoprotective activity can be most easily evaluated / screened with the aid of several model systems of liver damage in experimental animals.

In all test model systems, conditions for liver damage are implemented and an attempt is made to counteract this toxicosis with substance/ preparation under test. The magnitude of the protective effect can be measured by estimating the enzyme activity and the rate of survival and can be verified histologically. The available methods are in *in vivo, ex vivo* and *in vitro* methods (Visen, 1993). All these methods are used to study the protective or curative effects of any compound under test. In order to test for hepatoprotective activity the test substance and the hepatotoxins are administered simultaneously whereas in case of antihepatotoxic or curative activity, the test substances are generally administered after induction of hepatotoxicity.

A) *In vitro methods:*

In these methods hepatocytes are generally isolatd by using in-situ, two step recirculating collagenase perfusion technique. These are then seeded in small containers and exposed to test samples and toxins. After a specified time period the degree of toxicity or protection is assessed by viability tests and enzyme levels such as SGOT and SGPT.

In vitro methods employing primary culture hepatocytes using carbontetrachloride, galactosamine, thioacetamide, ethanol and paracetamol etc. as hepatotoxins have been deviced. These have anumber of advantages over in vivo methods such as their ability

to dispose numerous samples at a time, low cost with a small size, little variation and reproducibility of results.

The major disadvantage is that some time it may not reflect the events which occur in animals.

B) *Ex vivo models:*

In this after completion of pre selected in vivo test protocol hepatocytes are isolated and percentage of viable cells and biochemical parameters are determined as liver function test. These methods are some better correlated to clinical models than in vitro or in vivo method.

C) *In vivo methods:* These are of two types:

i) Based on serum parameters:

In vivo methods are used not only to study the nature of the given compound but also to study the mechanism of the toxicants. Hepatotoxicity is produced in experimental animals by the administration of known dose of hepatotoxins like carbontetrachloride, paracetamol etc. which produce marked measurable effects, the magnitude of which can be measured by carrying out various liver function tests viz. morphological, metabolic or functional, biochemical and histopathological determinations. Although it is a very convenient laboratory method, reproducibility of results is rather poor.

ii) Based on bile parameters: The compounds having hepatoprotective claims are also evaluated in general for their choleretic or anticholestatic activity in order to known whether the liver disorder is due to an abnormality of bilirubin metabolism or not. Choleretics are those agents which increase the outputs of bile by stimulating the liver where as anticholestatics are those which correct the retention and accumulation of bile due to intrinsic and extrinscic factors in the liver. These activities are evaluated by studying bile flow content in conscious and anaesthetized animals for % hours.

2.3.5 In vivo experimental model for hepatoprotective screening

A toxic or repeated dose of a known hepatotoxin is administered to induced liver damage in experimental animals. Hepatoprotective agents are those compounds, which mitigate the liver injury caused by hepatotoxic agents. Hepatoprotective effects

of plant drugs and herbal formulations are studied against chemicals (alcohol, CCl_4, alcohol-CCl_4, beta galactosamine, thioacetamide) and drugs (paracetamol, nimusalide, antitubercular drugs like isoniazid, rifampicin etc.) induced hepatotoxicity in rats and mice as they virtually mimic any form of naturally-occurring liver disease.

Hepatotoxicity from drugs and chemicals is the commonest form of iatrogenic disease. Some of the inorganic compounds producing hepatotoxicity are arsenic, phosphorus, copper and iron. The organic agents include certain naturally-occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins. The synthetic group of organic compounds is a large number of medicinal agents. In addition, exposure to hepatotoxic compounds may be occupational, environmental or domestic that could be accidental, homicidal or suicidal ingestion. The paper discusses various models used for screening hepatoprotective drugs.

1. **CCl_4 model**

   Chronic administration of carbon tetrachloride to rats induces severe disturbances of hepatic function together with histologically observable liver fibrosis. A number of CCl_4 model are devised depending upon its dosage through different routes of administration.

a) Acute hepatic damage: Acute liver damage, characterised by ischemia, hydropic degeneration and central necrosis is caused by oral or subcutaneous administration of CCl_4 (1.25 ml/kg). The maximum elevation of biochemical parameters are found to be 24 hours after the CCl_4 administration normally administered as 50% v/v solution in liquid paraffin or olive oil (Rage et al., 1989)

b) Chronic reversible hepatic damage: Adminstration of CCl_4 (1ml/kg s.c.) twise weekly for 8 weeks produce chronic, reversible liver damage (Saraf et al., 1991)

2. **Thioacetamide model**

   Thioacetamide (100 mg/kg s.c.) induces acute hepatic damage after 48 hrs and of administration by causing sinusoidal congestion and hydropic swelling with increased mitosis (saraf et al., 1992)

3. **D-Galactosamine model**

   D-Galactosamine 800 mg/kg i.p. induces acute hepatotoxicity after 48 hours of administration with diffused necrosis and steatosis (Kiso et al., 1983)

4. **Paracetamol model**

   Paracetamol induces acute hepatotoxicity depending upon its dosage through different routes of administration, such as

   a) Paracetamol 800 mg/kg i.p. induces centrilobulor necrosis without steatosis (Rachmilewtiz et al., 1950)

   b) Paracetamol at asingle dose of 3 g/kg p.o. stimulate acute hepatic damage. It takes 48 hours to induce the toxicity (Handa and Anupama, 1990).

5. **Chloroform model**

   It produces hepatotoxicity with extensive central necrosis, fatty metamorphosis, hepatic cell degeneration and necrosis either by inhalation (for 1 hours in atmosphere) or by subcutaneous administration (0.4- 1.5 ml/kg) (Goldschmidt et al., 1939).

6. **Ethanol model:**

   Ethanol induces liposis to a different degree depending upon its dose, route and period of administration as follows-

   a) A single dose of ethanol 1ml/kg induces fatty degeneration (Goodell et al., 1944).

   b) Administration of 40% (V/V) ethanol 2 ml/ 100 gm/day p.o. for 21 days produces fatty liver (Thripati et al., 1991).

   c) Administration of country made liquour 3ml/ 100gm/ day p.o. for 21 days produces liposis (Gulati et al., 1991)

2.3.6 Mechanisms of Chemical-induced Liver Injury

Carbon tetrachloride (CCl_4)

Liver injury due to carbontetrachloride in rats was first reported in1936 and has been widely and successfully used by many investigators. Carbontetrachloride is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with

the formation of $CCl_3O^{\cdot}$, a reactive oxidative free radical, which initiates lipid peroxidation.

$$CCl_4 \rightarrow CCl_3O^{\cdot} + O^{\cdot}$$

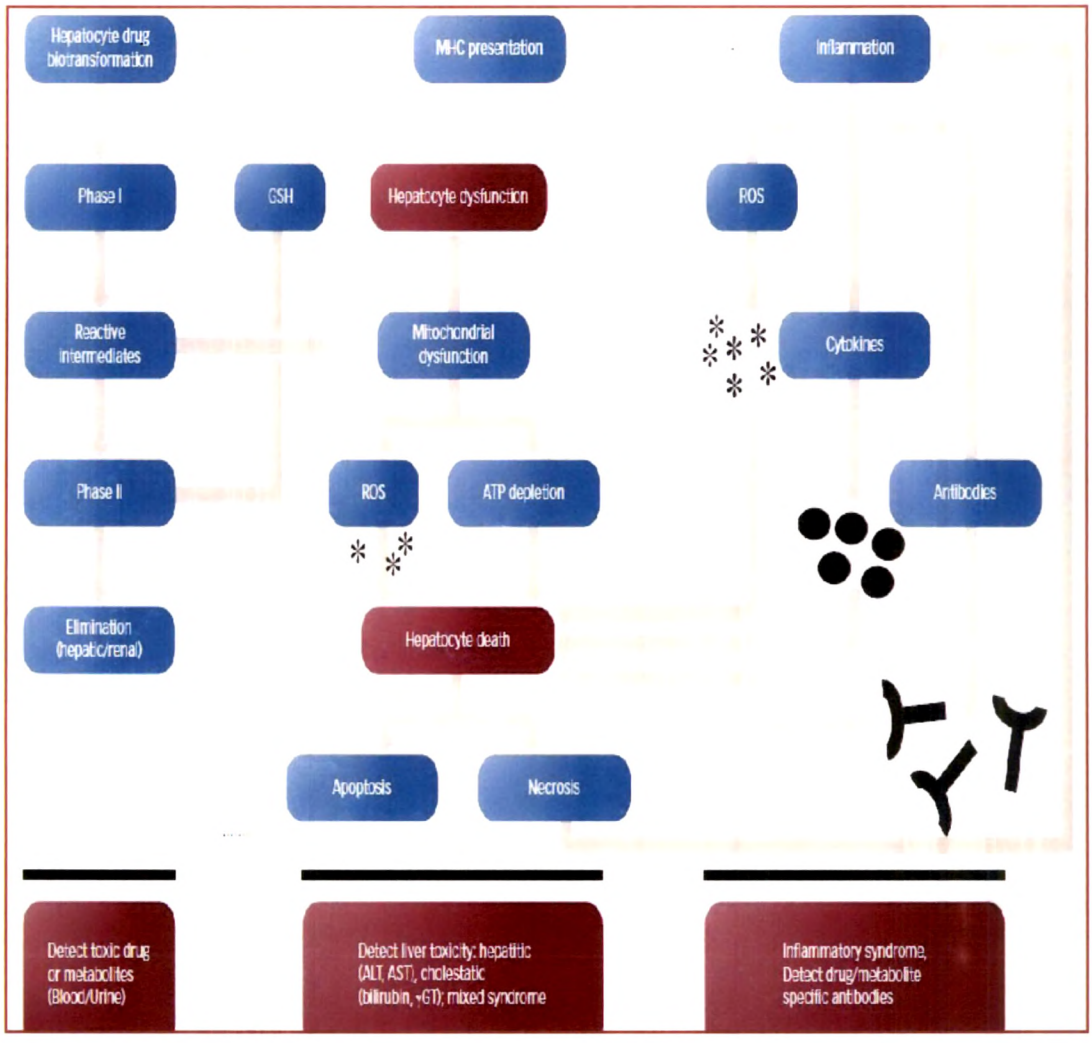
Administration of a single dose of CCl_4 to a rat produces, within 24 hrs, a centrilobular necrosis and fatty changes. The poison reaches its maximum concentration in the liver within 3 hrs of administration. Thereafter, the level falls and by 24 hrs there is no CCl_4 left in the liver. The development of necrosis is associated with leakage of hepatic enzymes into serum. Dose of CCl_4: 0.1 to 3 ml/kg I.P.

2.3.6 Mechanisms of drug-induced Liver Injury

Drug-induced liver injury (DILI) is common and nearly all classes of medications can cause liver disease. Most cases of DILI are benign, and improve after drug withdrawal. It is important to recognize and remove the offending agent as quickly as possible to prevent the progression to chronic liver disease and/or acute liver failure.Drug induced hepatotoxicity is a potentially serious adverse effect of the currently used antitubercular therapeutic regimens containing Isoniazid (INH), Rifampicin and Pyrazinamide.

Drugs are metabolized by the liver p450 system in a series of phase I and phase II reactions (left column). Toxic intermediates can illicit hepatocyte damage and death by inducing apoptosis ornecrosis (center column). Drugs that bind to cellular membranes can elicit an immunologic reaction upon presentation to major histocompatibility complex (MHC) particles, resulting in inflammation (right column).

Figure2.11 Mechanisms of Drug-induced Liver Injury



2.4 Research envisaged

The present studies were proposed with the purpose, first to develop the method for standardization of selected plant materials and thereby to provide a means for true identification and secondly to evaluate biological activity mentioned in traditional literature in order to justify their role in therapeutics. The detailed steps to undertake studies were planned as follows:

A. Pharmacognostic studies and proximate analysis
   1. Collection and identification of plant material
   2. Macroscopic evaluation
   3. Microscopic evaluation
   4. Determination of ash value
   5. Determination of extractive value
   6. Estimation of in-organic elements
   7. Fluorescence analysis
   8. Estimation of total phenolic content
   9. Estimation of total flavonoid content
   10. Determination of total Flavonols content

B. Phytochemical studies
   1. Successive solvent extraction of plant drugs
   2. Qualitative evaluation of successive extracts
   3. TLC studies of successive extracts
   4. HPLC studies on methanolic extract

C. Preparation of selective extracts , fractions and compound isolation
   1. TLC fingerprinting of extracts, fractions and isolated compounds
   2. TLC studies of bioactive extracts and fractions
   3. Development of HPLC and HPTLC methods for isolated compounds
   4. Characterization of isolated compounds

D. Biological studies
   1. *In vitro* screening of extracts, fractions and isolated compound for hepatoprotective activity
      a. *In vitro* Cytotoxicity assay (MTT assay)

b. *In vitro* CCL_4 induced hepatotoxicity in HepG2 cells
c. Morphological analysis of HepG2
d. Acridine orange/ Ethedium bromide staining in HepG2 cells

2. *In vivo* screening of selected extracts, Fractions or isolated compound for hepatoprotective activity
   a. *In vivo* Cytotoxicity studies
   b. *In vivo* CCL_4 induced hepatotoxicity in Albino rats
   c. Hepatic lipid peroxidation and antioxidant studies
   d. Histopathological studies