Review of literature

A. Anogeissus latifolia (DC.)

1. Botany

*Anogeissus latifolia* (DC.) is medium sized deciduous tree belonging to the family combretaceae and it is commonly known as gahtti. It attains height of about 30-40 feet (Orwa *et al*., 2006). Leaves are opposite or sub-opposite. Bark is smooth with grey-white colour and exfoliating in irregular thin scales (Warrier, 1994). Flowers sessile, in dense heads. Fruit small, compressed, winged with beak, seed ovoid. Tree flowers and fruits in the month of Sept-March (Yadav and Sardesai, 2002).

2. Distribution

It is distributed throughout India (Warrier, 1994) and Ceylon (Kirtikar and Basu, 1975). The plant is common in dry deciduous forest, except E. Bengal and Assam. It is found in Sub-Himalayan tract, from the Ravi to Nepal, Bhihar, Chota Nagpur and ascends to south India (Chopra *et al*., 1956).

3. Ethnobotany

It is important timber and the leave and bark are used for tanning. The bark is effective in anaemic conditions and urinary
discharges, piles (Kirtikar and Basu, 1975). Stem bark is astringent, haemostatic, constipating, depurative and useful in vitiated conditions of *kapha* and *vata* (Warrier, 1994). According to Jain (1991) stem bark is useful in diarrhea, dysuria, cough, colic, liver complaints, snakebite and skin diseases. Tribals in Udaipur district of Rajasthan, use the bark of this tree in the treatment of fever (Nag, *et al.*, 2007). Bark is remedy for chronic cough called ‘Dangya Khokala’ (Patil, 2006). Tribal people residing in the forest of Gundlabranhmeswaram wild life sanctuary apply paste of stem bark on scorpion sting (Ratan and Raju, 2008). Decoction of bark, two spoons daily is useful as remedy against cough and leaf decoction is effective in epileptic fits (Pawar and Patil, 2008). Gum is used as tonic and generally consumed after delivery (Pawar and Patil, 2008). According to Jagtap *et al.* (2009) pawra tribes of Satpura hills, use the gum with a cup of water or milk during early morning for lactation. Leaf juice is given in purulent discharges from the ear while, fruit is astringent to bowels and cures kapha and biliousness (Kirtikar and Basu, 1975).

4. Phytochemistry

Reddy *et al* (1965) reported tannin, (+) leucocyanidin and ellagic acid from the bark, sapwood and heart wood, whereas, Deshapande *et al.* (1976) isolated 3,3′-di-O-methyle ellagic acid-4′-β-D-Xyloside and
3,4,3′-tri-O-methylflavellagic acid-4′-β-D-glucoside from stem bark. Steroid, β-sistosterol and a triterpenoid, 3-β-hydroxy-28-acetytaraxaren were isolated from the ethyl acetate fractions of stem bark of *A. latifolia* (Rahman *et al.*, 2007).

5. Pharmacology

Govindrajan *et al.* (2004) evaluated the wound healing potential of ethanolic extract of *Anogeisus latifolia* bark for treatment of dermal wounds in rats. They administrated ethanolic extract orally and found decreased epithelization period along with decrease in scar area. They noticed that tensile strength and hydroxyproline contents were significantly increased than nitrofurazone receiving rat, indicating its wound contraction ability. The ethanolic extract was also found inhibitory against human pathogenic bacteria *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonae* and *Escherisia coli* due to the presence of ellagic acid (Govindrajan *et al.*, 2004). Govindrajan *et al.* (2006) studied the antiulcer potential of *Anogeissus latifolia* against aspirin induced, cold resistance stress induced, pylorus ligated and ethanol induced ulcers. They observed that 50% ethanolic extract (200mg/Kg body weight) inhibited the ulcer formation induced by cold resistant stress and also reduced the lipid peroxidation and activity of superoxide dismutase along with increase in catalase activity.
in cold resistant stress induced ulcers. Parvathi et al. (2009) studied the hypolipidemic potential of *Anogeissus latifolia* in albino rats with respect to serum lipid levels. They noticed that treatment with gum ghaati significantly reduced the total cholesterol and triglyceride level at 500 mg and 750mg/kg of body weight in hyperlipidemic induced rats and dose of 750mg/kg of body weight also increased the high density lipoprotein cholesterol. Hepatoprotective activity of *Anogeissus latifolia* against carbon tetrachloride induced hepatotoxicity in albino rats of wistar strain was evaluated by Hulikere et al. (2009). They reported that CCl$_4$ (0.1mol/L) treatment depleted the viability of hepatocytes by 65%. *Anogeissus latifolia* treated hepatocytes showed concentration dependent protective effect and restored the viability of the hepatocytes. They further noticed that the effect was maximum at 1000µg/ml of *Anogeissus latifolia* extract while, *in vivo* hepatoprotective studies, CCl$_4$ treatment showed marked increase in the activities of the three enzymes, alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase. They also reported that oral administration of *Anogeissus latifolia* at 300mg/kg reduced the induced activities of the all the three enzymes as compared to CCl$_4$ treated rats. They observed that *A. latifolia* at a dose 300mg/Kg of body weight, showed significant decrease in lipid peroxidation while, extract of *Anogeissus latifolia* doses at 100 and 300mg/Kg of body weight minimised
degeneration of hepatocytes affected by CCl₄ treatment. Antioxidant potential of 50% ethanolic extract of A. latifolia was evaluated by Govindrajan et al. (2004) and reported dose dependent inhibition of nitric oxide, DPPH radical, hydrogen peroxide, superoxide radicals.

6. Biotechnology

Shekhawat et al. (2000) studied the micropropagation of Anogeissus latifolia using cotyledonary node and epicotyl explants from one month old seedlings germinated on half strength MS medium supplemented with 2% sucrose. They have inoculated cotyledonary segments on MS medium containing additives, 25mg/L each of adenine sulphate, arginine, ascorbic acid, citric acid and 1mM aspargine and 0.5mg/L BAP and noticed to produce 4-5 shoots than epicotyl explants producing 2 shoots only. With increasing concentration, number of shoots per plant also increased and at 1mg/L BAP, seven shoots were emerged while epicotyl produced only 3. They observed that MS medium containing additives (adenine sulphate, arginine, ascorbic acid, citric acid and 1mM aspargine) and 1.5mg/LBAP + 0.1mg/L IAA produced 9-10 shoots from cotyledonary node. Addition of 86mg/L (200µM) Fe-EDTA salt to this medium produced the maximum number of shoots per explant and higher concentration 300µM was found inhibitory to shoot induction (Shekhawat et al., 2000).
Bhatt (1987) improved the gum tapping method by ethephon treatment in trunk by injecting a syringe into holes made by increment borer. Gummosis is enhanced by ethephon application and 466 fold increases in gum yield was recorded in plants treated with 1600mg of active ethephon substance during April- May when plants becomes leafless. The ethephon application leads to ‘schizo-lysigenous’ formation of gum cavities in the axial parenchyma of sapwood and these results in the clogging of vessels of secondary xylem with gummy material.

B. *Crataeva religiosa* (Hook and Forst)

1. Botany

The name *Crataeva* is given in the honor of Crataevus, a Greek botanist, who was living in the time of Hippocrates and the name *religiosa* indicates its growth near the places of worship (Parkar, 1986). *Crataeva religiosa* is much branched deciduous tree belonging to the family capparidaceae, commonly called as Varuna (Williamson, 2002). The trade name given for this tree is three leaved capper (Pullaiah, 2006). The leaves are trifoliate, glabrous, and ovate. Flowers are whitish to milky white in colour in terminal dense corymbs (Williamson, 2002). Fruit is berry, globose or some times oblong with woody rind, embedding seeds in the yellow pulp (Parkar, 1986). The outer surface
of bark is wrinkled and grey-white in colour, covered with large number of lenticells. Tree flowers and fruits in the month of Dec-May (Yadav and Sardesai, 2002).

2. Distribution

_Ceataeva religiosa_ is globally distributed in India, Myanmar, Sri Lanka, Malaysia, Indonesia and China. In India, it is found in Peninsular India, Western India, Gangetic Plains, and Eastern India, up to Tripura and Manipur (Williamson, 2002). It is also found in Sikkim and Andman and Nicobar Island (Pullaiah, 2006). It is found mostly along the bank of the river and streams and near to temple side (Panda, 2004 and Warrier, 1995).

3. Ethnobotany

The plant part used for the medicinal purpose includes Leaves, stem bark and Root bark (Bhatachargee, 2001; Nadkarni, 1979 and Anonymous, 1987). These parts of _C. nurvala_ are commonly applied to regulate equilibrium among Vata, Pitta and Kapha in Ayurvedic system while the stem bark is used to promote the appetite and to decrease the secretion of the bile in unani medicines (Mhaskar _et al._, 2000). Recently Bopana and Saxena (2008) critically reviewed _C. nurvala_ for its ethnobotanical and pharmacological properties. Plant is used ethnopharmacologically as diuretic, laxative, lithonotriptic,
antirehumatic, antiperiodic, bitter tonic, rubifacient and counterirritant (Bhatachargee 2001 and Nadkarni, 1979). The bark is used in the urinary disorders including kidney and bladder stones, antiemetic, and calculous affections and as an antidote in snakebite (Bhatachargee, 2001). *C. religiosa* is valuable in treating vata (blood flow, waste elimination and breathing), Pitta- (fever and metabolic disorder) and Kapha (joint lubrication, skin moisture, wound healing, strength and vigour, memory loss, heart and lung weakness and weak immune system (Anonymous, 1987). A preparation called ‘Varunal’ contains *Crataeva* in combination with *Eclipts, Picrorrhiza, Achillea, Cichorium, Solanum, Arjuna, and Cassia* seeds is used against hepatitis, edema, ascites, urinary stones and arthritis (Warrier, 1997). The bark is contraceptive and cytotoxic and useful in kidney bladder stones, fever vomiting and gastric irritation (Gagandeep and Khadilkar, 2006). Roots and bark are laxative and lithontipic and increase appetite and biliary secretion (Malini *et al.*, 1995). Leaves are used as externally rubifacient and used in rheumatism. Leaves are given internally febrifuge and tonic (Walia *et al.*, 2007 and Sanayaima *et al.*, 2006).

According to Gurrero (http/www.bpi.da.gov.ph., 2009), In Philippines, leaves are useful in irregular menstruation and also in stomachic, whereas the bark is used to cure convulsions and tympanites. Sanyal and Ghose speculated that the crushed leaves are
applied in the form of paste for swelling of feet and also for a burning sensation in the soles of feet. The bark and the leaves are pounded and applied in the form of a poultice in rheumatism. The fresh leaves bruised with little vinegar, applied to skin. Bark and roots are rubifacient and vesicant. Decoction of bark is used in the disorders of urinary organs and urinary calculi. Roots and bark in the form of decoction are used as calculus affections (http/www.bpi.da.gov.ph., 2009). Traditionally, the plant is used as oxtocic, in rheumatic fever in kidney stones, bladder stone and as tonic (Ghani, 1998). It is useful as antipyretic, antilithitic, antihelminthic, demulcent, in blood and chest diseases (Dury, 1978). NR-AG-I is a polyherbal formulation containing \textit{Crataeva religiosa, Dolichos biflorus, Tribulus terrestris and Shilajit}. NR-AG-II is another herbal formulation containing \textit{Crataeva religiosa, Boerrhavia diffusa, Saccharum officinarum} and \textit{Butea frondosa}. Between these two, NR-AG-II is having good diuretic potential than NR-AG-I (Samiolla and Harish, 2000). A mixture containing-\textit{Tribulus terrestris} fruits (25%); \textit{Zinziber officinalis} roots(10%); \textit{Solanum xanthocarpum} whole plant (10%); \textit{Asparagus racemosus} roots (10%); \textit{Tephrosia purpurea} leaves (10%) and \textit{Crataeva religiosa} bark (25%) was prepared and 4gm of mixture given to patient twice daily with water in urinary disorder (Samy et al., 2008).
Berry like globose fruits of *Crataeva* are edible and used as astringent (Parker, 1999) and rind of the fruit is used as mordant in dying (Dury, 1978). In People living in Kango and Yurubas leaf paste in water is used for counter irritant purposes (Irvine, 1961). Quissumbing (1951) reported that fresh leaves of this plant have rubifacient and vasicant properties. According to Corner (1952), young shoots and fruits of *Crataeva religiosa* are eaten and used in curries. Fruits of this tree are used as spice because of its garlic taste (Seidemann, 2005). In Pallaypatty village of Tamil Nadu, people use the leaves and bark of this tree to cure jaundice, eczema, rabies (Ganesan et al., 2009). The bark of this tree is useful in family planning (http://www.milliontreedream.org/, 30/12/09). The bark is also diuretic (Krishnaraju, et al., 2005). The juice of fruit, leaves and bark is applied to cure snakebite, infected wounds and cuts. It increases appetite and controls other skin diseases (Sapkota, 2008). The decoction of the bark is useful in the treatment of urinary organs (Sreedharan, 2004) and leaves are used as vegetable and the dried leaves are smoked in caries of nasal bones, the smoke being exhaled through the nose in neurologic pains (Dastur, 1962).
4. Phytochemistry

Lakshmi and Chuhan (1997) isolated lupeol from the root bark of *C. religiosa*. It also contains lupeole acetate, varunaol, spinasterol acetate, taraxasterol and 3-epilupeol (Daniel, 2006).

A triterpene, diosgenin and two alkaloids cadabicine and cadabcine diacetate have been isolated the bark of *Crataeva religiosa* (Ahmed et al., 1987). By repeated chromatography technique, Enamual huque et al. (2009) isolated two triterpenoids phragmalin triacetate and lupeol from the ethyl acetate fractions of stem bark of *C. religiosa*. The stem bark also found to contain Epiafzelechin 5-glucoside (Sethi et al., 1984).

Chemical investigation of leaves by Gagandeep et al. (2006), showed presence of four compounds- Dodecanoic anhydride, methyl pentacosanoate, Kaemferol-3-O-α-D glucoside and quercitin-3-O-α-D-glucoside. Leaves contain isovitexin, proanthocyanidins, myricetin and phenolic acids, p-hydroxyl benzoic acid vanillic acid, ferulic acid and sinapic acid (Daniel, 2006).

Sethi et al. (1978) reported glucocaparin from the fruits of *Crataeva religiosa*. Gagandeep et al. (2009), first time reported four chemical compounds- pentadecane, octanamide, 12-tricosanonoe and friedelin from the fruits of *C. religiosa*. 
5. Pharmacology

Sahoo et al. (2008) studied the antifungal activity against the Candida albicans, Candida tropicalis, Candida krusei, Cryptococcus marinus, and Aspergillus niger. They noticed that aqueous extract of bark inhibited Cryptococcus marinus and Aspergillus niger whereas aqueous and ethanolic extract did not showed any effect against Candida albicans, Candida tropicalis, Candida krusei and Cryptococcus marinus. While ethanolic extract, the chloroform extract showed better results against the test fungal pathogens and indicates the presence of higher contents of phytochemicals having antifungal activity.

The pharmacology division of Central Drug Research Institute Lucknow has carried out detailed pharmacological and chemical studies on this plant. According to Varalakshmi et al. (1991), decoction of bark also lowers the intestinal Na+ and K+ATPase levels. Reports of Singh et al. (1999) showed the effectiveness of Crataeva bark in prophylaxis of oxalate urolithiasis induced by simultaneous administration of sodium oxalate and methionine in guinea pig. Alan et al. (2006) studied the antinociceptive effects of crude ethanolic extract of C. religiosa on mice by acetic analgesic test method. Crude ethanolic extract of Crataeva religiosa bark produced dose dependent significant antinociceptive effects against chemically induced
nonciceptive pain stimuli in mice. Further results of Alam et al. (2006), suggested that the effects are peripherally and centrally mediated.

A pentacyclic triterpane, lupeol isolated from the stem bark and its ester derivative lupeol linolate were tested for their anti-inflammatory activity in Freund’s adjuvant induced arthritis in rats (Geetha et al., 1998). The effect of lupeol linolate was found to be better in this respect when compared with lupeol (Geeta et al., 1998). According to Geeta and Varalakshmi (1999), lupeol linolate reduces the foot-pad thickness and complement activity in arthritis in rats, was greater than unestrified lupeol and indomethacin. Shirwaikar et al. (2004) investigated the role of lupeol in reducing the blood urea nitrogen, creatinine and lipid peroxidation with corresponding increase in glutathione and catalase activities in cisplatin induced nephrotoxicity indicating its antioxidant nature. Hippocratic screen carried out by Molone and Robichaud (1962) revealed the ability of crude bark extract to minimize acetic acid induced writhing at higher doses. According to Vidya and Varalakshmi (2000), the total administration of lupeol and its analogue betulin to hyperoxaluric rats minimized tubular damage and reduced markers of crystal deposition in kidney. In this connection the lupeol was found to be effective than betulin. Anti-inflammatory, antinociceptive anti-pyretic and ulcerogenic properties of lupeol and lupeol linolate were studied by Geetha and Varalakshmi (2001) in
comparison with commonly used nonsteroidal anti-inflammatory drug indomethacin. Lupeol and lupeol linolate caused reduction in paw-swelling in adjuvant arthritis. Singh and Kapoor (1991), reported antiurolithiatic activity of ethanolic extract of bark extract in albino rats by foreign body insertion method using glass bead. A triterpenoid compound, Lupeol isolated from bark of this plant showed prophylactic and curative activities in albino rats when studied by foreign body insertion method (Anand et al., 1989). Lupeol also showed significant anitoxaluric (Bhaskar et al., 1996) and anticalciuric (Anand et al., 1994) effects in rats against hydroxyproline induced hyperoxaluria. Vidya et al. (2000) studied the antioxaluric effects of number of lupeol derivatives against hyoeroxaluria in rats. The effect of bark decoction on calcium oxalate urolithiasis induced by 3% glycolic acid has been studied in rats (Varalkashmi et al., 1990). Bark decoction significantly prevented the deposition of calcium and oxalate in the kidney by inhibiting the glycolic acid oxidase activity in liver. Sunita et al. (2001) investigated the hepatoprotective properties of Lupeol and lupeol linolate against cadmium induced toxicity in rats. The cadmium toxicity caused elevated level of malondialdehyde and decreased level of enzymic and non-enzymic antioxidants in rat liver. Oral administration of lupeol and lupeol linolate significantly reverted the tissue redox system to normal level by scavenging free radicals and improved the antioxidant status.
of the liver. Lupeol linolate had better effect than lupeol on antioxidant status of the liver. Preetha et al., (2006) studied the effect of lupeol on aflatoxin (AFB-1) induced peroxidative hepatic damage in rats. They compared the hepatoprotection of lupeol with silymarin, a standard hepatoprotectant drug. AFB-1 treatment caused hepatic damage by increasing the level of lactate dehydrogenase, alkaline phosphatase, alanine and aspartate aminotransferase along with increase in the lipid peroxide level while enzymic and non-enzymic antioxidant levels were found to be decreased in hepatic tissue. Oral treatment of lupeol normalized the altered mechanism of the hepatic tissue system and the effect was comparable to silymarin, indicating potent hepatoprotectant (Preetha et al., 2006). In experimental model of urolithiasis, Deshpande et al. (1982) noticed decrease in weight of kidney stone when treated with bark of the plant. He also observed that, the bladder mucosa had less edema and ulceration, and also the cellular infiltration was intense than control group not treated with C. religiosa bark. According to Malini et al. (2000), lupeol administration to stone forming rats reduced the renal excretion of the oxalate, a causative agent of stone formation and reduced the stone formation in animals preventing crystal induced tissue damage to kidney eluting stone forming constituents from kidney and restricted oxalate and crystal induced peroxidative alterations to renal tissue (Malini et al., 1998). According
to Deshpande et al., (1982) stem bark decoction in water is useful in the treatment of prostatic hypertrophy and hypotonic bladder and positively affects the patients from incontinence, urinary frequency, pain and urine retention.

Urinary electrolytes Calcium, Sodium and magnesium play very important role in the pathology of urolithiasis. Deshpande et al., (1982) estimated the urinary electrolytes after treatment of bark decoction of Crataeva religiosa and noticed reduced excretion of urinary calcium while that of sodium and magnesium increased significantly, altered the relative proportion of urinary electrolyte responsible for kidney stone formation (Deshpande et al., 1982).

According to Deshpande et al. (1982), decoction of Crataeva religiosa bark helps in passing out the kidney stones in crystalluria patients. ‘Uriflow’, a commercial herbal formulation containing C. religiosa bark has been found to be useful in initiating the passage of renal and bladder stones (http://www.uriflow.com, 2005).

Urinary tract infection includes urethritis, cystitis, nephritis or pyelonephritis. Four day treatment with C. religiosa bark decoction minimized these symptoms of urinary tract (Deshpande et al., 1982). A formulation patented by Oneal and White (2004) contain cratavin (extract of root bark and stem bark), is used to treat the E. coli infection

Chakraborty *et al.* (2004) studied the potential of a polyherbal formulation Himplasia (Himalaya herbal healthcare). Daily use of himaplasia reduced the prostate size and increased the flow rate. It also reduced the prostrate volume, postovid residual urine volume, urinary flow time, latent period and increased the peak flow rate significantly (Modi and Kolhapure, 2004). Clinical study with 98-BDH patients ‘Himaplasia’ treatment relived the symptoms of BDH by increasing the urinary flow rate and reducing the postovid residual volume through reduction in prostrate size (Arrora *et al.*, 2003; Shhu *et al.*, 2003). Mehar and *et al.* (2001), studied the effect of *Tribulis terrestris* and *Crataeva religiosa* against gentamycin induced nephrotoxicity in albino rats. Aqueous extract of these drugs protected
the rats from the nephrotoxicity and showed the maximum level of nephroprotective action. Lupeol and its derivative known as Lupeol-EPA is a fatty acid, lowered the increased activities of the lysosomal and glycoproteins in the rats to normal level when compared to control edomethacin treatment (Lata et al., 2001). Possible cardioprotective effects of lupeole isolated from the *Crataeva religiosa* bark against cyclophosphamide (CP) induced oxidative stress have been studied by Sudarshan et al. (2004). CP used in cancer treatment causes fatal cardiotoxicity, and increases the activities of enzymes lactate dehydrogenase, creatine phosphokinase in serum and decreases in cardiac tissue. Cyclophosphamide treatment in albino rats caused intermuscular hemorrhage in histology, increased the lipid peroxide level and decreased the enzymic and non enzymic oxidant level in hearts. Oral administration of lupeol and lupeol linolate reversed the alterations caused by cyclophosphamide induced oxidative stress (Sudarshan et al., 2005).

High cholesterol diet caused increase in total cholesterol and triglyceride levels in plasma along with elevation in the low density protein (LDL), very low density protein (VLDL) and cholesterol content and decreased level of high density lipoprotein. The lupeol and its derivative normalized the impaired lipid profile by decreasing the lipid peroxidation levels and stabilizing the antioxidant defense system by
increasing the enzymic and non enzymic antioxidant status (Sudhahar et al., 2005). Lupeol also posses anti-malerial potential against chloroquine resistant *Plasmodium falciparum*. Among 96 lupeol derivatives synthesized, 15 posses higher activity than lupeol, and other have potential equivalent to lupeol or nil effect against the pathogen (Shrinivasan et al., 2005). An herbal formulation (Herbmed-constitutes Bark of *Crataeva religiosa* and stem of *Mussa paradisiaca*) prepared by Patankar et al. (2008) helps to dissolve the renal calculi and facilitate their passage out, thereby minimizing the pain due to renal and ureteric calculus disease. Saleem et al. (2009) evaluated the anti-inflammatory and anticancer activities of lupeol in Benzoyl peroxide induced tumor formation in murine skin. Benzoyl peroxide administration increased the microsomal peroxidation and hydrogen peroxide generation. Activities of the antioxidant enzymes catalase, peroxidase glutathione reductase, glutathione peroxidase, and glutathione S-transferase were found to be decreased along with decrease in coetaneous glutathione level and increase in ornithin decarboxylase activity and thymidin uptake in the DNA synthesis. Administration of lupeol 1 hr. before the Benzoyl peroxide treatment prevented the oxidative damage due to Benzoyl peroxide (Saleem et al., 2009).
6. Biotechnology

Walia et al. (2003), regenerated *C. religiosa* by using seedling derived explants- cotyledonary nodes, epicotyl nodes, hypocotyl segments, first pair of leaves, cotyledons and root segments. MS medium supplemented with 0.5mg/L BAP and 0.02mg/L or 0.1mg/L was found suitable for production of multiple shoots and good rooting. Walia et al. (2007) successfully regenerated the *C. religiosa* by using the nodal explants from the 30 year old *Crataeva religiosa*. MS medium containing 2.22 µM of BAP successfully produced multiple shoots which elongated satisfactorily on the same medium. Sanayaima et al. (2006) investigated the cryopreservation of *in vitro* grown axillary shoot tips. Axillary buds from 4 weak old *in vitro* cultures produced shoots on MS medium supplied with 0.1mg/L BAP. The shoots were rooted on MS medium supplied with 0.02mg/L NAA. Shirin and Maravi (2006) studied the clonal propagation of *Crataeva religiosa* by using the stem node segments from axillary branches. MS medium containing 10micro M BA and 15 micro M IAA was effectively producing the shoots and rooting the plants.
C. *Pterocarpus marsupium* (Roxb.)

1. Botany

*Pterocarpus marsupium* (Roxb.) is a deciduous tree, commonly called as Indian Kino tree or Malabar Kino, belonging to the family fabaceae. It is a medium to large sized tree reaching height up to 15-20 meter with dark brown to grey bark having swallow cracks. The bark exudes a red gummy substance called ‘Gum Kino’ when injured. Leaves are compound and imparipinate. Flowers are yellow in terminal panicles. Fruit is circular, flat, winged pod. Seed is convex and bony (Warrier, 1995). Tree flowers and fruits in the month of March to June (Yadav and Sardesai, 2002).

2. Distribution

*Pterocarpus marsupium* is distributed in deciduous forest throughout the India (Varghese, 1996). It is found to grow in parts of states such as Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamilnadu, Uttar Pradesh, West Bengal (Caius, 1989) and Goa (Sanjappa, 2000).

3. Ethnobotany

Leaves are used for food and manure. The flowers are used in the treatment of fever. *Pterocarpus marsupium* is a multipurpose
leguminous tree. Heart wood is astringent, bitter, acrid, cooling, anti-inflammatory, depurative, haemostatic, revulsive, anthelmintic, constipating and rejuvenating (Warrier, 1997). The wood is useful in chest pain, body pain, and indigestion (Bressers, 1951). The paste of seed and wood is useful in diabetic anaemia (Trivedi, 2006). The paste of heart wood is useful in body pain and diabetes (Yesodharan and Sujana, 2007). Wood of the tree is useful in making the water glasses of the diabetic patients (Reddy et al., 2008).

Bark is useful in vitiated condition of *kapha* and *pitta*, elephantiasis, erysipelas, urethrorrhea, rectalgia, ophthalmopathy, hemorrhages, dysentry, cough and grayness of hair. Aqueous infusions of the bark possess antidiabetic potential (Anonymous, 1969). The powdered bark is mixed with *Schleichera oleosa* and taken with cold water to treat dysentery (Mohanta et al., 2006). The juice of the bark is applied in the mouth (Prusti and Behara, 2007). Tribal people residing in the Jodhalal forest of Karnataka use stem bark to treat the wounds, fever, stomachache, diabetes and elephantiasis (Mankani et al., 2005). Bark is useful in urinary discharge and piles. The gum Kino is externally applied to leucorrhoea (Pullaih, 1999). Gum Kino is used in the treatment of polyurea and inordinate night sweat and phthisis plumonalis. The Kino powder may be dusted on ulcers and bleeding surfaces (http://www.henriettesherbal.com 16/12/09). The gum is used
in the toothache (Chopra, et al. 1956) and leaves paste is applied in wounds (http://www.milliontreedream.org. 30/12/09). Bruised leaves are useful in boils sores, skin diseases, stomachic, cholera (Jain, 1991). Leaf juice is given in purulent discharges from ear, plant is useful in snakebite and scorpion sting. Fruit cures biliousness and kapha (Kirtikar and Basu, 1975). Flowers are bitter, sweet, cooling, appetizing and febrifuge (Warrier, 1995) and used in fever (Pullaih, 1999).

4. Phytochemistry

Mitra and Joshi (1982) isolated an isoflavon glycoside from the heart wood of the Pterocaepus marsupium and identified it as 5, 4’-dimethoxy-8-methylisoflavone. Three isoflavon glycosides namely retusin 7-glucoside, irisolidone 7-rhamnoside and 5, 7-dihydroxy-6-methoxyisoflavone-7-rhamnoside were isolated by Mitra and Joshi (1983). A eudesmane type sesquiterpene alcohol, selin-4(15)-en-1β, 11-diol was reported from the heart wood of the P. marsupium (Adinarayana and Syamsundar, 1982). Suba Rao and Mathew (1982), characterized a naturally occurring hydrobenzoin, marsupol, 4, 4’-dihydroxy-a-methylhydrobenzoin and a novel 2-hydroxy-2-benzylcoumaranone, carpucin, characterized as 2-benzyl-2,4’,6-trihydroxy-4-methoxybenzo(b) furan-3(2H)one from the P. marsupium
heart wood. From the heart wood, propterol-B-1-(2, 4-dihydroxyphenyl)-3-(4-hydroxyphenyl) propan-2-ol identified by Mathew and Suba Rao (1883). Suba Rao et al. (1984), isolated propterol: A 1, 3-bis (4-hydroxyphenyl) propan-2-ol as one of the extractive of heart wood. Bezuidenhoudt et al. (1987) reported two flavonoid analogue, 8-C-β-D-glucopyranosyl-3, 7, 4-trihydroxyflavone and 3, 7, 4′-tetrahydroxyflavone from the heart wood which are representatives of the first 5-deoxy- C-C-coupled flavonol glucosides, and rare 3′-C-β-D-glucopyranosyl-α-hydroxydihydrochalcone. A novel 6,7,3′,4′-tetraoxygennated homoisoflavonoid, which has been characterized as 6-hydroxy-7-O-methyl-3-(3-hydroxy-4-O-methylbenzyl) chronan-4-one from ether soluble fractions of *Pterocarpus marsupium* heart wood (Jain et al., 1997). 6-hydroxy-3, 5, 7, 4′-tetramethoxyflavone 6-O-rhamnopyranoside, a flavonol glycoside was characterised from the root (Yadav and Singh, 1998). An aqueous extract of heart wood yielded a isoaurone C- glycoside (Handa et al., 2000).

Grover et al. (2004) reported two interconvertible disteriomeric epimers 2α / 2β-hydroxy-2-P-hydroxybenzyl-3(2H)-benzofuranone-7-C-β-D-glucopyranoside from the heart wood. Five new glycosides reported from the aqueous extract of *P. marsupium* by Maurya et al. (2003) are -
1) 6-hydroxy-2-(4-hydroxybenzyl(1)-benzofuran-7-C-β-D-glucopyranoside.

2) 3-(α-methoxy-4-hydroxybenzylidene)-6-hydroxy-benzo-2(3H)-furanone-7-C-β-D-glucopyranoside.

3) 2-hydroxy-2-P-hydroxybenzyl-3(2H)-6-hydroxybenzofuranone-7-C-β-D-glucopyranoside.

4) 8-(C-β-D-glucopyranosyl)-7,3’,4’-trihydroxy flavone

5) 1,2-bis-(2,4-dihydroxy-3-C-glucopyranosyl)-ethane dione.

Eight compounds, pterostilben, isoliquiritigenin, liquiritigenin, carpucin, propterol, propterol-B, oleanolic acid and marsupol were isolated from the heart wood of *Pterocarpus marsupium* (http://www.silbinol.com. 23-7-2009). Mohan and Joshi (1989) analyzed flower of *P. marsupium* and reported two aurone glycosides, 4, 6, 4’-trihydroxyaurone 6-O-rhamnopyranoside and 4, 6, 4’-trihydroxy-7-methylaurone 4-O-rhamnopyranoside. They also reported another two aurone glycoside from the heart wood and characterised as 6,4’-dihydroxy-7-methylaurone 6-O-rhamnopyranoside and 4,6,3’,4’-tetrahydroxyaurone 6-O-rhamnopyranoside. From the roots of this plant, Tripathi and Joshi (1988) isolated two flavone glycosides, 7-hydroxy-6, 8-dimethyl flavanon-7-O-α-L-arbinopyranoside and 7,8,4’-trihydroxy-3’,5’-dimethoxyflavanone-4’-O-beta-D-glucopyranoside.
Shrikrishna and Mathew (2009), synthesized a dimethyl ether of marsupin

5. Pharmacology

In the Indian system of medicine many plants have been used to treat diabetes; of which *Pterocarpus marsupium* popularly known as Bijasar is one of the most potent species (Rastogi and Mehrotra 1989; Varier 1995; Sivarajan and Balchandran 1994 and Grover et al., 2002). Its heart wood is official part used as antidiabetic drug (Shah, 1967). Many workers have studied the antidiabetic potential of *Pterocarpus marsupium*. According to Joshi et al. (2004), *P. marsupium* decreased the blood glucose levels both in normal and non-insulin dependent diabetic (NIDDM) rats. In NIDDM rats the propensity was increased to gastric ulcer which was induced by cold resistant stress, aspirin, and ethanol and pylorus ligation. They observed that the *Pterocarpus marsupium* did not show significant protection from the gastric ulcer in case of normal rats due to above inducers, but it protected the mucosa in NIDDM rats by affecting the mucosal offensive and defensive factors. Ahamad et al. (1991) studied the hypoglycemic activity of the wood. Vats et al. (2002) found that absolute ethanol extract fraction dissolved in ethyl acetate was protective in lowering the blood sugar level and increased the insulin
level in the blood sugar in alloxan diabetic rats while, aqueous extract of *P. marsupium* lowered blood sugar level from 72.32±5.62 to 61.35±1.2mg in alloxan diabetic rats. The drug also lowered the blood glucose level from 202±5.44 to 85.11±11.28mg when administrated daily (Vats et al., 2002). Kar et al. (2003), also evaluated the hypoglycemic activity of vacuum dried 95% ethanolic extract, when administrated at a dose 250mg/ounce, twice or thrice daily found effective in lowering the glucose level in the blood to normal in alloxan diabetic rats. Vats et al. (2002) reported the anticataract activity of the *P. marsupium* and *Trigonella foenum* seed extract. They noticed that administration of aqueous extract of *P. marsupium* decreased the opacity index, indicating anticataract potential of the plant. Further, they also noticed that in cataract examined rats, it showed significant effect on body weight and blood glucose values.

Administration of three Phenolic compounds in hyperglycemic rats significantly minimized the blood sugar level. Marsupin and pterostilben are more effective than Pterospin and when compared with metoformin (Manikam et al., 1997). ICMR study group (1998) also studied the antidiabetic potential of *P. marsupium* at multi-center level and hypothesized that, the plant significantly reduced blood glucose level without any side effects in non-insulin dependent diabetes mellitus or newly diagnosed mellitus. Indian Council of Medical
Research group (2005), have evaluated the efficacy of Vijaysar (*P. marsupium*) in newly non-insulin dependent diabetes mellitus. They reported that blood glucose level and mean HbA1c levels were decreased significantly from 151-216mg/dl to 32-45mg/dl and 9.8 to 9.4 % respectively indicating the utility of Vijaysar in NIDDM patients. Rizvi et al. (1995), reported the insulin like activity of (-) epicatechin by studying the effect on erythrocyte osmotic fragility. Though the mechanism of action of both (-) epicatechin and insulin are different, they illict their protective role on red cell osmotic fragility (Rizvi et al., 1995). Ahamad et al. (1989) also described the insulin like effects of (-)epicatechin. According to Ahamad et al. (1991) (-) epicatechin increases the c-AMP content of the islets and insulin release. They observed that the conversion of proinsulin to insulin have been due to (-) epicatechin and the effect of (-) epicatechin was more in one month old rats than mature (12 month old rats). Sheeshan et al. (1983) studied antidiabetic potential of epicatechin in alloxan diabetic rats. In this rats no measurable effects have been noticed in control and (-) epicatechin treated rats and in already attained diabetic condition, the effect of (-) epicatechin was found to be nil. Gayathri and Kannabiran (2008), evaluated the ameliorative potential of aqueous extract of *P. marsupium* bark in streptozotocin (STZ) induced diabetic rats. Oral administration of aqueous extract normalized the glycosylated
hemoglobin, total cholesterol, triglycerides and LDL-cholesterol. Increased levels of various enzymes such as aspartate transaminase, alanine transaminase, alkaline phosphatase, glutamyle transferase and ceratine kinase were brought to normal level. They also indicated that the prominent effect of metabolic alterations in experimentally induced diabetes mellitus was due to restoration of the plasma insulin and liver glycogen levels. Rizvi and Zaid (2001) studied the effect of insulin and (\(-\) epicatechin on glutathione content in normal and Type-2 diabetic erythrocytes. They found that the glutathione content was lower in Type-2 diabetic erythrocytes than normal while, the insulin treatment both at 1mm and 10mm increased the glutathione level in normal and diabetic Type-2 patient. They also noticed that the (\(-\) epicatechin treatment also increased the glutathione content at 1mm but did showed dose dependant effect like insulin and was ineffective below 1mm concentration. The effect of (\(-\) epicatechin was remarkable at 1mm and 10mm when compared to insulin (Rizvi and Zaid, 2001). The effect of aqueous extract of P. marsupium on glycogen content of tissue was studied by Grover et al. (2002). According to Grover et al. (2002), increase in glycogen content in renal and decrease in glycogen content in hepatic and skeletal muscle was partly prevented by aqueous extract of Pterocarpus treatment. Alterations in the activities of hexokinase, glucokinase and
phosphofructokinase in diabetic and control were corrected by *Pterocarpus marsupium* extract (Grover *et al.*, 2002). Zaid *et al.* (2002) reported that lowered activities of erythrocytic membrane Ca**++**-ATPase leads to cardiomyopathy indicated by reduction in contractibility, relaxation, cardiac work and diastolic complications in Type-2 diabetes mellitus. When the normal and diabetic type -2 patients treated with 1mm (-) epicatechin, the Ca**++**-ATpase activity increased both in normal and diabetic type-2 patients (Zaid *et al.*, 2002). Apart from this many other researchers proved the antidiabetic nature of the *Pterocarpus marsupium* (Sharma, 2007).

Anti-hyperlipidemic effect of ethanolic extract of heartwood of *Pterocarpus marsupium* and its flavonoid constituents marsupin, pterosupin, and liquiritigenin are studied by Jahromi and Ray (1993). They observed that ethanol extract decreased the serum triglyceride, total cholesterol and LDL and VLDL cholesterol levels without affecting the HDL cholesterol level. They found significant effect of liquiritigenin and pterosupin in lowering the serum cholesterol, LDL cholesterol and antherogenic index while, pterosupin was satisfactory in reducing the triglyceride level. Cardiotonic activities of aqueous extract of heart wood have been evaluated by Mohire *et al.* (2007). The extract *Pterocarpus marsupium* protectes cardiac muscle at 4mg/ml, as compared to standard drug Digoxin (0.5mg/ml). 5, 7, 2-4
terahydroxy isoflavone 6-6-glucosides which is protective in cardiovascular diseases (Mohire et al., 2007).

Anti-cancerous potential of pterostilben has been studied by (Pan et al., 2007). Further, stilbens isolated from berries and grapes posses anticancer properties and used to cure colon cancer in men and women (Rimando and Suh, 2008). Mankani et al. (2005) studied the hepatoprotective activity of aqueous and methanolic extract of marsupium wood against carbon tetrachloride induced hepatotoxicity. They found marked increase in total bilirubin, serum transaminase and serum alkaline phosphatase activity caused due to carbon tetrachloride toxicity were restored by aqueous and methanolic extract and later it was more effective in restoring the altered levels of these parameters (Manakani et al., 2005). Rajlakshmi et al. (2008) studied the antioxidant activity of P. marsupium on isolated frog heart and found that the plant extract protected the cardiac muscles from oxidative stress induced by H₂O₂. While, the cardiac arrest time was prolonged by 14 minutes in the presence of plant extract than control, indicating the antioxidant activity of the methanolic extract of marsupium bark.
6. Biotechnology and Physiology

The natural regeneration of *Pterocarpus marsupium* takes place by means of the seed but the germination percentage is only 30% and very low (Kalimuthu and Lakshaman, 1995). The native natural stands of tree are fast disappearing (Wilkins, 1991). The conventional seed and vegetative propagation of the tree has not been very successful due to hard fruit coat, less germinability together with poor viability (Venkataramaiah *et al.*, 1980). Bharmukh and Nikam (2008) studied the seed germination of *Pterocarpus marsupium* and reported that 30 min. scarification treatment with concentrated H$_2$SO$_4$ is found fruitful to induce seed germination up to 85% with highest vigour index of 3.3. Due overexploitation of the tree for its various useful applications coupled with low germinability, *Pterocarpus marsupium* has been included in the list of depleted plant species (Choudhuri and Sarkar 2002). Inevitably, therefore, its propagation and multiplication through tissue culture technique is urgently needed (Anis *et al.*, 2005). There are some earlier reports on the regeneration of *Pterocarpus marsupium* employing cotyledonary node explants, but reports lacked in the data for number of roots, and their length (Chand and Singh 2004 and Anis *et al.* 2005). Chand and Singh (2004) raised this plant *in vitro* by using cotyledonary nodal explants from 20 day old axenic seedling. They obtained the regeneration frequency 85% and 9.5 shoots per explant
Husain et al. (2007) developed a protocol to regenerate the *Pterocarpus marsupium* using ‘Thidiazuron’. MS medium supplemented with 0.1-10μM thidiazuron successfully produced the multiple shoots from cotyledonary nodes and obtained highest regeneration frequency 90% and much maximum number of shoots 15.2±0.20 per explant on MS medium supplied with 0.4μM thidiazuron. Distabanjong and Geneve (1997) inoculated the nodal explant on the MS medium showing differential response to all the three cytokinins (BA, Kin and 2iP) and noticed that nodal explant taken from the 18 day old axenic seedling showed better response for shoot induction as compared to 6, 12 and 24 days old seedlings. They also noticed that the age of the seedlings plays an important role in morphogenesis. Husain et al. (2008) propagated this plant through tissue culture technique using nodal explants from 18 day old axenic seedling. They obtained highest regeneration frequency (85%) and maximum number of multiple shoots (8.6). The length of the shoot was increased on MS medium supplied with 4.0μM 6-benzyl-adenine (BA), 0.5μM indol three acetic acid (IAA), and 200μM adenine sulphate (ADS).

Raju and Raju (1999) studied the distribution patterns of rare earth metals, thorium and uranium in three species of *Pterocarpus-* *P. santalinus, P. marsupium* and *P. dalbergioides*. They reported that,
concentration of these metals was highest in the heart wood than the leaves. The concentration of uranium and thorium was quite high in the heart wood of *P. santalinus* than other two species.

**D. Terminalia arjuna** (Wight & Am.)

1. **Botany**

   The name *Terminalia* is derived from latin ‘teminalis’ due to terminal crowding of the leaves in many species of the genus *Terminalia* (Parekar, 1999). It belongs to the family Combretaceae. It is a large deciduous tree with buttressed roots, and reaches up to 60-70 feet. Stem is with white-grey bark which changes colour to pink according to season and age of the bark, and flakes off in large flat species from the trunk (Warrier, *et al.*, 1995). Leaves are opposite with glands on the stalk. Flowers are pale-yellow to milky-white, crowded at the tip of the branches in short panicles or axillary spikes (Parekar, 1999). Fruits are winged with 5 wings, 1-2 inch long woody hard (Kirtikar and Basu 1935). It flowers between March to June and fruits between September to November (Yadav *et al.*, 2002).

2. **Distribution**

   *T. arjuna* is distributed throughout India, Burma and Sri Lanka. It is common throughout India especially in the Sub Himalayan tract and
eastern India, Uttar Pradesh, Madhya Pradesh, Maharashtra, Bihar and Deccan regions (Jain et al., 2009). It mainly grows along the banks of the river and streams (Kirtikar and Basu, 1975).

3. Ethnobotany

Paste of root in water is applied on headache whereas leaf paste with sugar and milk is given in the treatment of spermatorrhoea (Shikarwar et al., 2007). *Arjuna* is potent due to its cardiotonic properties since ancient times (Yeshodharan and Sujana, 2006). Leaves soaked in water over night used in burning sensation and dyspepsia (Anisuzzaman et al., 2007). Leaves of *Terminalia* pounded together with the leaves of *Syzygium cuminii* and *Acacia catechu* given to cattle for the treatment of diarrhoea.

Traditional healers of the Kancheepuram district of Tamil Nadu apply the paste of fruit topically on wounds while, Bark powder boiled with water and inhaled to cure headache and to kill worms in the teeth (Muthu et al. 2006). The Malamalasar tribe of Parambikulam use the fresh leaf juice to treat earache and powdered bark is used to treat heart troubles (Yeshodharan and Sujana, 2007). According to Kumar and Jain (1998) people living in the South Surguja district of Madhya Pradesh use the bark of *T. arjuna* in the treatment of fever and high blood pressure. Peoples living in the Malkangiri district of Orissa,
chew the fresh bark of *Terminalia arjuna* and the juice is used as antacid (Prusti and Behera, 2007). In Rajasthan, people use the bark powder with ghee or milk to treat the heart diseases (Sharma and Kumar, 2007). According to Sikarwar *et al.*, (2008) tribal communities residing in the Chitrakoot district of Madhya Pradesh use the leaf of *Terminalia arjuna* along with the leaves of *Syzygium cuminiin* and *Acacia catechu* pounded together and give to cattle to check diarrhoea. The tribals apply root paste on headache and tender leaves paste with sugar and milk is given once a day for 20 days for the treatment of spermatorrhoea. Fruits are found useful as tonic (Kirtikar and Basu, 1975) whereas leaves are used to treat earache (Anisuzzaman *et al.*, 2007). The bark of the arjuna can be used in the treatment of the chest pain (Nishikanta, 2006). Bark of arjuna boiled in water and inhaled to cure headache and as germicide in teeth problems (Muthu *et al.* 2006). Decoction of the bark also used in the treatment of the fever and high blood pressure (Kumar and Jain, 1998). Decoction of the bark is used as ulcer wash while bark ash is used in the treatment of the snakebite and scorpion sting (Yeshodharan and Sujana, 2007). Paste of bark in water applied externally on the site of fractures and helps in the early healing. It is also useful in traumatic injuries associated with odema-swelling. Paste is applied to check the bleeding from the wounds effectively (http//www.herbalcureindia.com 16/12/09). Decoction of the
bark is also useful in the asthma, cleaning sores and dysentery. An ointment prepared by mixing powdered bark and honey is applied to treat the acne (http://www.ayurvediccure.com 16/12/09). The bark is also used in anaemic condition, leucoderma and excessive perspiration. The bark is externally and internally used in gleets and urinary discharges (http://www.eco-planet.com 16/12/09). Bark is also useful as expectorant, aphrodisiac, tonic and diuretic (http://www.hort.purdue.edu 16/12/09).

4. Phytochemistry

Many useful phytoconstituents have been isolated from *T. arjuna*. Tannins and flavonoids are responsible for its anticancer properties and triterpenoids are mainly responsible for cardiovascular properties (Jain *et al.*, 2009)

Upadhaya *et al.* (2001) identified a triterpene glycoside, arjunetoside (3-O-beta-D-glucopyranosyl-2alpha, 3beta, 19alpha-trihydroxyolean-12-en-28-oic acid, 28-O-beta-D-glucopyranoside) from the root bark of *Terminalia arjuna*. Oleanolic acid and arjunic acid have also been isolated from the root bark of arjuna (Upadhyay *et al.*, 2001). Yadav and Rathore (2001) isolated a cardenolide from the roots of *Terminalia arjuna* and identified as 16,17-dihydrorneridienone 3-O-

There are some chemical substances present in plants that may act as anticarcinogens or antimutagens by blocking or trapping ultimate carcinogen electrophile in a nucleophilic chemical reaction to form innocuous products. A study done by Kaur et al. (2002) found that the acetone and methanol fractions of T. arjuna also possesses antimutagenic effects. The bark of T. arjuna is rich in polyphenols (60–70%) including flavones, flavanols and tannins. The high content of tannins and polyphenols are mainly responsible for anticancer activity. The antimutagenic effect of benzene, chloroform, acetone and methanol fractions from T. arjuna was determined against Acid Black Dye, 2 aminofluorene (2AF) and 4-nitro-O-phenylenediamine (NPD) in TA98 frame shift mutagen tester strain of Salmonella typhimurium. Tannins arjunin, ellagic acid, and gallic acid were isolated from the bark (Kandil and Narsar 1998; Tripathi and Singh, 1996). Flavonoid leucocyanidin from bark was reported by Tripathi and Singh (1996);
and Nagar et al. (1997) and Row and Rao (1996). Flavonoid, luteolin have been reported from stem bark (Kandil and Narsar, 1998). A compound arjunetin has been isolated from the bark of stem by Tripathi and Singh (1996). Alam et al. (2008) identified two oleanane type triterpene glycoside named as Termiarjunoside I and Termiarjunoside II from the stem bark of *Terminalia arjuna*. King et al. (1954) and Row et al. (1970) isolated arjunolic acid from the stem bark and heartwood of arjuna. From the stem bark, Tripathi and Singh (1996) isolated arjunic acid. Pawar and Bhutani (2005) and Honda et al. (2001) isolated arjungenin from the stem bark. Sharma et al. (1982) identified arjunglycoside-1 from stem bark. An arjunoglucoside-3 has been isolated by Sharma et al. (1982) from stem. Arjaneyulu and Prasad (1982) identified arjunoside-1 and Arjunoside-2 from stem bark. Tripathi and Singh (1996) identified arjunolitin from stem bark. Pawar and Bhutani (2003) separated arjunetoside from stem bark of arjuna. Lupeol from stem bark by Tripathi and Singh (1996), King et al (1954) and Honda et al. (2001). Triterpenoids arjunoglucoside-2 and β-sitosterol-D-glucoside have been isolated from stem bark (Tripathi and Singh, 1996). Asif et al. (2003) isolated Olean 3β,6β,22,triol,12ene,28oic acid-3-O-βD-glucopyranosyl (1→4)-β-D-glucopyranoside from stem bark. Terminoside A, an oleanane triterpene isolated from the acetone fraction of the ethanolic extract of

Tannin arjunin, ellagic acid, and gallic were isolated by, Kandil and Narsar (1998) and panicalin by (Pettit *et al.*, 1996). Many workers identified tannin, casuarinin from leaves (Cheng *et al.*, 2002; Chen *et al.* 2004 and Link and Chang, 2005).

Agrawall and Dutt (1936) isolated arjunic acid from the fruit of arjuna. Terminolitin (23-deoxyarjunolitin), a triterpene diglycoside has been isolated from the fruits of *Terminalia arjuna* (Singh *et al.*, 1997). Triterpenoids arjunoglucoside-2 and β-sitosterol-D-glucoside have been isolated from fruit (Tripathi and Singh, 1996). Tannin arjunin, ellagic acid and gallic were isolated by Kandil and Narsar (1998). Tripathi and Singh (1996) located gallic acid and ellagic acid from fruit. Tripathi and Singh (1996) and Nagar *et al.* (1979) reported occurrence of two flavonoids cerasidin and quercetin-7-O-Rhamnoside in fruit tissue.

5. Pharmacology

Two herbal formulations- Himax ointment and lotion, containing bark extract of *arjuna* tree are reported to possess wound healing potential accompanied by its wound contracting ability, epithelization
period, tensile strength and regeneration of the tissue at the wound area (Mukherjee et al., 2003). 50% ethanolic extract and tannin isolated from the *Terminalia arjuna* bark have been shown to possess wound healing potential in rats (Rane and Mengi, 2003). Tannin treatment effectively increased the tensile strength and reduced the wound size as compared to control and ethanol extract (Rane and Mengi, 2003).

The body needs constant nourishment to ensure that the cardiovascular system operates efficiently. Many herbal drugs are used to cure cardiovascular disorders the most common one of which is *T. arjuna*. *Terminalia arjuna* has been used in the treatment of heart disease since ancient times by Ayurvedic physician (Kumar and Prabhapkar, 1987). Singh et al. (1982) studied the effect of aqueous extract of *Terminalia arjuna* to understand the mechanism of cardiovascular disease. The oleanane triterpenoids (*arjunic acid, arjunoglycosides, arjunone, arjunolic acid* etc.) present in this plant are mainly responsible for the cardio protective effect. This plant is effective in many cardiac disorders like angina, myocardial infraction, hypertension, hypercholesteremia, cardiac arrest etc. (Rose and Treadway 2000 and Khan and Balick, 2001). Intravenous administration of the extract reduced blood pressure and heart rate. The extract also inhibited carotid occlusion response induced due to I. V. administration
of norepinephrine and electrical stimulation of preganglionic fibers of
the abdominal splanchnic nerve (Kumar and Prabhapkar, 1987).
According to Dewivedi and Agrawal (1994) *Terminalia arjuna* lowered
systolic blood pressure and body mass index significantly in stable
angina cases without any impairment in the normal function of the
kidney and liver, and reported its effectiveness in stable angina
pectoris. Many other workers also reported the antianginal and
 cardioprotective properties of *Terminalia arjuna* (Chopra, 1994 and
Anand, 1994). Bharani *et al.* (1995) noticed that *Terminalia arjuna* at a
dose 500mg/kg of body weight when given to refractory chronic
congestive heart failure (class IV NYHA), extract improved the signs
and symptoms of the NYHA class while It decreases the echo-left
ventricular enddiastolic and endsystolic volume indices with increase in
the left ventricular ejection fractions. Its long term evaluation in patients
showed continuous improvement in the symptoms, signs, efforts
tolerance of NYHA class patients (Bharani *et al.*, 1995). Ram *et al.*
(1997) evaluated the hypocholesterolaemic effects in diet induced
hyperlipidemic rabbits. They observed that ethanolic extract of bark
reduces the total cholesterol LDL cholesterol, HDL cholesterol,
triglyceride level and cholesterol LDL and LDD/HDL without adverse
effects on the renal and kidney functions. Dewivedi and Jauhari (1997)
studied the efficacy of bark powder in coronary artery diseases. They
showed that reduction in angina frequency in both postmyocardial infarction angina and ischemic cardiomyopathy is dose dependent. They noticed that this treatment improved the left ventricular injection fraction and reduced the left ventricular mass giving relief from the coronary heart failure whereas long term administration of drug did not alter the normal renal and kidney physiology (Dewivedi and Jauhari, 1997). ‘Lipistat’, an herbal formulation with arjuna bark reduces the loss of myocardial HEP stores and lactate accumulation in myocardial tissues in isoproterenol induced myocardial necrosis and also protects the ischemic heart disease (Seth et al., 1998).

Shaila et al. (1998) evaluated the hypolipidemic activities of the three Terminalia species T. arjuna, T. belarica and T. chebula in experimentally induced atherosclerosis in rabbits by feeding cholesterol rich diet. Among the three botanicals studied, arjuna was found effective hypolipidemic agent and inhibited rabbit atheroma partially indicating its antiantherogenic role. Kumar et al. (1999) studied the efficacy of ‘Harton’ an herbal formulation against stable angina pectoris. 80% patient with Harton treatment showed relief from the stable angina pectoris as compared to those with isosorbide mononitrate (70%). Rate of heart attack reduction was quite higher than ISMN. The formulation improved the blood pressure response to the stress without disturbing the renal and hepatic functions. Oral
administration of *T. arjuna* for 12 weeks in rabbits was shown to cause augmentation of myocardial antioxidants, superoxide dismutases, catalyze and glutathione along with induction of heat shock protein 72. *In vivo* ischemic reperfusion injury induced oxidative stress, tissue injury of heart and haemodynamic effects were prevented in *T. arjuna* treated rabbit hearts. In *T. arjuna* treated rabbits the normalization of left ventricular end diastolic pressure occurred in 10 min of reperfusion and the extent of myocardial lipid peroxidation was also found to be less in *T. arjuna* treated rabbits (Bharani *et al.*, 2002). Gupta *et al.* (2001) studied the antioxidant and hypocholesterolaemic effect of arjuna in coronary heart disease. They noticed that Arjuna treatment reduces the total cholesterol and LDL cholesterol levels significantly with decrease in lipid peroxide level. Ischemic mitral regurgitation (IMR) is a frequent complication of acute myocardial infraction and a progress factor for morbidity and mortality. The powder of *T. arjuna* the bark given to healthy (volunteers less than 70 years old) have shown to reduce IMR and there was improvement in E/A ratio (E: Early Echocardiographic phases and A: Late Atrial Phase of ventricular filling) and reduction in anginal frequency (Dwivedi *et al.*, 2005). Dried and pulverized bark powder increased the thiobarbeturic reactive species with corresponding increase in superoxide dismutase, reduced glutathione content catalase levels which are endogenous antioxidants
and prevents the heart from oxidative stress induced by ischemic-reperfusion injury (Gauthaman et al., 2001). Antioxidant studies among Vitex nigunda, Cassia fistula and Terminalia arjuna, T. arjuna shows the highest antioxidant activity accounting its use in cardiovascular disease (Munasinghe et al., 2001). According to Sumitra et al. (2001), arjunolic acid, a triterpenoid, arrests decrease in antioxidant system, ceruloplasmin, alpha tocopherol, reduced glutathione, lipid peroxide and ascorbic acid levels and protect the heart from damage caused due to myocardial necrosis induced by isoproterenol. Terminalia arjuna decreased the ischemic mitral regurgitation in acute myocardial infraction at 500mg doses administrated orally for three months and reduced the anginal frequency considerably (Dewivedi et al., 2005). Mary et al. (2003) reported that alcoholic extract at a dose 0.75mg/Kg of body weight normalized the in vivo myocardial injury induced due to isoproterenol in rats. In double blind, placebo-controlled study carried out with chronic stable angina cases, Terminalia improved clinical and treadmill exercise parameters when compared to placebo therapy are similar with the isosorbide mononitrate therapy (Bahraini et al., 2002). 6mg/Kg dose of 70% alcoholic extract of bark produced the hypotension effect in anaesthetized dogs which was blocked by propranolol, indicating the active constituents present in ten extract might be possesing the adrenergic-betaz2-receptor potential acting
directly on the heart muscle showing its cardiovascular use in Ayurveda (Nammi et al., 2003).

Tannins and flavones in the bark, stem and leaves of the Mauritius medicinal plant *T. arjuna* were reported to be mainly responsible for anticancer activity (Saxena et al., 2007). Pettit et al. (1996) studied the effects of gallic acid, ethyl gallate and luteolin and reported their efficacy in inhibiting the cancer cell line. Ellagic acid isolated from the bark has been found to be effective against S9-dependent 2-aminofluorene. Kaur et al. (1997) evaluated the antimutagenic effects of ellagic acid in TA98 and TA100 strains of *Salmonella typhimurium* against direct and indirect acting mutagens. A fraction called tannin; from *T. arjuna* inhibited the mutagenicity in *Salmonella typhimurium* (Kaur et al., 2000). In *invitro* studies, methanolic and acetone extract of bark inhibited the growth of transformed cells of human normal fibroblast, osteosarcoma and glioblastoma (Nagpal et al., 2000). Kaur et al. (2001), reported antimutagenic property of triterpenoid glycoside against sodium azide in TA98 and TA100 *Salmonella typhimurium* indicating anticarcinogenic activity. Pasquini et al., (2002), reported highest antimutagenic activity by ethyl acetate extract against the mutagen 4-nitroquinolin-N-oxide (4NQO) than chloroform, acetone, methanol, diethyl ether and methanol-HCl extract where as acetone extract was found effective
against the damage by 4NQO suggesting that the drug contains some polar and non-polar compounds reflecting its antimutagenic property. Chloroform insoluble fractions mainly of polyphenols, showed maximum inhibition of 98% and 101.55% against 2-aminofluorene (2AF) than direct acting mutagen 4-nitro-o-phenylenediamine (NPD) and sodium azide, are more effective than soluble fractions (Kaur et al., 2002). Acetone and ethanol extract of arjuna bark reported to have antimutagenic properties against acid black dye, 2-aminofluorene (2AF) and 4-nitro-o-phenylenediamine (NPD) (Kaur et al., 2002). Mary et al. (2003) studied the antimutagenic effect of Terminalia arjuna herbal preparation containing Terminalia arjuna bark and reported that the formulation has a potential to act as antioxidant and it showed platelet antiaggregatory property and hypolipidemic activities in rats. Antimutagenic assay of acetone, methanol, methanol-HCl, chloroform, ethyl acetate and ethyl ether extracts of arjuna bark carried out by using the ‘comet’ assay and micronuclei test revealed that, acetone and methanol extracts are effective than other extract in reducing the DNA damage induced by 4NQO (Scassellati et al., 1999). Kuo et al. (2005) reported the anticancer effect of casuarinin in the human breast adenocarcinomas MCF-7 cells and human non small cells (Kuo et al., 2005) and lung cancer AS49 cells by blocking the progress of the cell cycle. Increased levels of plasma and the liver glycolytic enzymes and
decreased level of glucose-6-phosphatase was reverted to normal level by depleting the energy metabolism and inhibiting the cancer growth accounting its anticancer potential (Shivalokanathan et al., 2005). Arjunin an ellagitannin isolated from the ethanolic extract of the leaves of *T. arjuna* extract showed moderate cytotoxic activity against BT-20 human breast cancer cells with IC50 value 6.5 µg/ml (Kandil and Narsar, 1998). Arjunic acid was significantly active against the human oral (KB), ovarian (PA1) and liver (HepG-2 and WRL-68) cancer cell lines. Arjunic acid was converted into seven semi-synthetic ester derivatives. 2-O-Palmitoyl arjunic acid showed two times more activity, while 2, 3-di-O-acetyl-, 2-O-p-anisoyl-, 2, 3-di-O-benzoyl and 2, 3-di-O-p-nitrobenzoyl arjunic acid showed 1.7–2.3 times less activity than the cytotoxic drug vinblastine against the liver cancer cell lines HepG-2 and WRL-68, respectively (Saxena et al., 2007). Luteolin, a flavone isolated from the butanol fraction of *T. arjuna* was found to be effective in inhibiting a series of solid tumors (Renal A-549, ovary SK-OV-3, Brain SF-295, Leukemia P 388, SKMEL-2, XF-498, HCT 15, Gastric HGC-27). It also acted as an antitumour promoter and had antimutagenic properties (Pettit et al., 1996).

Reactive oxygen species play an important role in the pathophysiology of various cardiovascular disorders. Arjungenin and its glucoside isolated from the stem bark of *T. arjuna* exhibited moderate
free radical scavenging activity Arjungenin was tested for its free radical scavenging property in 1,1-diphenyl 2-picrylhydrazyl (DPPH) assay. Arjungenin was found to have moderate activity with an IC50 value of 290.6 µg/ml as compared to standard vitamin C. Arjungenin also exhibited greater inhibitory action on the hypochlorous acid production from human neutrophills (Pawar and Bhutani, 2005). A naphthanol glycoside from bark is antioxidant in nature and inhibits nitrous oxide production (Ali et al., 2003a). Terminoside A (1), an oleanane triterpenoid from bark inhibited the nitric oxide production and decreased the inducible nitric oxide synthase level in lipopolysaccharide stimulated murine macrophages (Ali et al., 2003b). Significant increases in lipid peroxidases were observed while the levels of enzymatic and non-enzymatic antioxidants were decreased when subjected to DEN induction. The enzyme levels were ameliorated significantly by administration of ethanolic extract of *T. arjuna* bark at a concentration of 400 mg/kg in drug treated animals by the *Terminalia arjuna* treatment indicating its protective role in N-nitrosodiethylamine (DEN) induced liver cancer (Shivlokanathan et al., 2006). They also reported efficacy of *Terminalia arjuna* in inhibiting the proliferation of the human hepatoma cell lines (HepG2) also lowered the GSH content in HepG2 cells. Arjuna bark treatment significantly decreased the thiobaraturic acid reactive substances and increased the reduced
glutathione content superoxide dismutase and catalase levels in vivo ischemic reperfusion injury (Karthikeyan et al., 2003). According to Manna et al. (2006), carbon tetrachloride induced oxidative stress causes liver injury by producing the free radicals, increasing glutamate pyruvate transaminase and alkaline phosphatase levels with decrease in reduced glutathione content, lowering superoxide dismutase, catalase and GST levels in kidney and liver. Oxidative stress induced by smoking causes endothelial disruption which triggers atherosclerosis. Arjuna therapy reversed the impaired endothelium in smokers when compared to standard placebo therapy (Bharani et al., 2004).

Rani et al., (2004) studied the anti-enteric properties of the Terminalia arjuna bark against a multidrug resistant Salmonella typhi bacterium. Casuarinin isolated from bark has been found to be effective in preventing H₂O₂ induced oxidative damage of DNA by depleting the intracellular GSH content in Casuarinin treated Madin-darby canine kidney (MDCK) cells (Chen et al., 2004).

T. arjuna bark extract at the dose of 500 mg, given 8 h to patients with stable angina with approvocable ischemia on tread mill exercise, led to improvement in clinical and treadmill exercise parameters as compared to placebo therapy. These benefits were
comparable to isosorbide mononitrate (40 mg/day) therapy and the extract was well tolerated (Bharani et al., 1995). High level of low density lipoproteins (LDL) and low levels of high density lipoproteins (HDL) cholesterol are important coronary risk factors. The ethanol extract of the bark of *T. arjuna* was found to be very effective in lowering LDL cholesterol levels at the dose of 100 mg/kg body weight. The total cholesterol level was also reduced at the dose of 500 mg/kg body weight (Alpana et al. 1997). Oral administration of *T. arjuna* in ischemic reperfusion injury of rabbits lead to augmentation of antioxidant enzyme system thereby induces heat shock protein 72 (HSP72) and prevents the oxidative damage induced by ischemic reperfusion injury (Gauthaman et al., 2005). Aqueous extract of arjuna positively prevented alterations due to carbon tetrachloride oxidative stress, acting as free radical scavenger. *Terminalia* bark extract at doses of 21.42mg and 42.84mg/ kg of body weight decreased the level of thyroid hormone and cardiac lipid peroxides protecting the heart from free radicals (Parmar et al., 2006). A dose of 500mg/kg body wt. increased, total sulphedryl group, protein sulphedryl group, the enzymic and non-enzymic antioxidant levels and decreased the lipid peroxidation in liver and kidneys of alloxan induced diabetic rats and showed its antioxidant nature (Raghavan and Kumari, 2006). Devi et al. (2007a) studied the gastroprotective properties of arjuna in Diclofenac
sodium induced gastric ulcer in rats due to its free radical scavenging and cytoprotective nature. Methanol extract showed dose dependent antiulcer and ulcer healing activity against 80% ethanol, diclofenac sodium and dexamethasone ulcer models. Gastroprotective effect might be due to its ability to maintain the membrane integrity by its antilipid peroxidative activity (Devi et al., 2007). *Terminalia arjuna* at 50mg/kg of body wt. dose, significantly decreased the increased levels of all the antioxidant due to carbon tetrachloride induced oxidative stress along with increase in reduced glutathione content, decreased the lipid peroxidation and its products (Manna et al., 2007a). A triterpenoid saponin, arjunin isolated from the arjuna plant ameliorates arsenic induced cyto-toxicity in isolated murine hepatocytes by normalizing the altered enzymic and non-enzymic levels (Manna et al., 2007b). Sun et al. (2008) evaluated free radical scavenging of activity arjunic acid isolated from the fruits of *Terminalia arjuna*. Another triterpenoid saponin, arjunolic acid, prevented the arsenic induced testicellular damage in mice. Treatment with Arjunolic acid at 20mg/kg of body wt. protected the oxidative stress injury to the testis, spermatocytes and seminiferous tubules (Manna et al., 2008). According to Singh et al. (2008), butanolic fractions of bark of arjuna tree plays important role in Doxorubicin (DOX) induced cardiotoxicity. Aqueous extract of bark balances the impaired activities of antioxidant
defense system inhibiting lactate dehydrogenase activity and thereby regulating anaerobic metabolism in lymphoma bearing mice indicating anticarcinogenic behavior (Verma and Vinayak, 2009). DNA damage caused by adriamycin treatment leads to formation of the micronuclei in human blood lymphocytes. Reddy et al., (2008) reported that *Terminalia arjuna* treatment arrested the micronuclei formation at 15µg/ml inducing inhibition of free radical formation. Sinha et al. (2008), investigated the antioxidative potential of ethanolic extract against the sodium fluoride (NaF) induced oxidative stress in murine hearts. Extract protected the murine hearts from NaF oxidative stress by enhancing cardiac intracellular antioxidant system. Malik et al. (2009) revealed the antithrombotic properties of the *Terminalia arjuna* which decreases the platelet activation and inhibits the platelet aggregation. According to Kumar et al. (2009), oral administration of aqueous extract at 63,125, and 250mg/kg of body wt. prevented the decline in the endogenous antioxidant level and increased oxidative stress and prevented the myocardial fibrosis induced by isoprenaline.

antifungal property against fungi *Aspergillus niger, Candida albicans* and *Bacillus oryzae* at 25 and 50 ppm concentrations by filter paper disk method which found to be was comparable with Griseofulvin. Studies of Aggarwal and Dutt (1936) shows that *T. arjuna* bark extract exhibited antibacterial activity against *Escherichia coli, Plasmodium vulgaris and Plasmodium aerogenes*. Their study indicated that the bark of *T. arjuna* in dichloromethane and aqueous fraction showed activity against the bacteria at 1,000–5,000ppm dosage. They observed that high activity was observed at 4,000 and 5,000ppm against Plasmodium aerogenes. Casuarinin, a hydrolysable tannin posseses antiviral property against Herpes simplex type-2 preventing the attachment of virus to cell. It inhibits the viral penetration and reduces the viral titers at a virucidal concentration 25µM (Cheng et al., 2002). Arjunic acid, arjungenin, arjunetin, and crude ethanolic extract evaluated for their antifeedant, growth inhibitory and oviposition-deterrent activities in lepidopterous insects, *Spilarctia obliqua*. Among these, arjunetin found potent growth inhibitor with feeding deterrent properties acting as antifeedant at 188.5 and 287.1 ml/ kk diet accounting its glycosidation linkage (Singh et al., 2004).
6. Biotechnology

Thomas et al. (2003) produced the whole plant directly by using the terminal bud and mature explants. They noticed that shoots produced from mature nodal explants were higher in number than the shoots produced from terminal bud while the medium supplemented with 2mg BA was effective in producing more shoots. IAA dose 1mg/L enhanced greater rooting in plantlet than IBA and NAA (Thomas et al., 2003). Isa et al. (1993) vegetatively generated the plants through stem cutting treated with auxin. Pandey and Jaiswal (2002) produced the multiple shoots using cotyledonary explants from 21 day old seedling. They found that MS medium containing 0.5 mg/L of BAP and 1mg/L IBA was effective in generating the more shoots (44-55) from single cotyledonary node.