Ficus benghalensis

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National Science Foundation
Colombo

January 2002

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I. Introduction: The Banyan Tree

The imposing banyan tree of poetry and legend is a store-house of invaluable remedies for some of the deadliest diseases. To scientists it is known as *Ficus benghalensis* Linn. Sp. Pl 1059.1753, the synonyms being *Ficus indica* Linn. Sp Pl. 1060. 1753[1] and *Ficus cotonaeifolia* Vahl., Enum. Pl. 2: 189.1806.[1]

It belongs to the family Moraceae. In Sinhala it is *Mahanuga* or *Nuga*, in Tamil *Al, Arla, Alam* [1,2] and in Hindi *Bar, Bor, Ber.*

Distribution: It is commọn in the low country dry regions of Sri Lanka up to an altitude of about 2000 feet. Also occurs in the sub-Himalayan forests and South India and naturalized elsewhere.

Morphology: A very large evergreen tree, 23-34 m tall, with huge spreading limbs supported by aerial roots which later form accessory trunks extending to a large area and stout, softly pubescent branchlets.[2]

Leaves:[2] Simple, alternate, 10-20 cm long, 5-12.5 cm broad, oval, ovate or orbicular-ovate to oblong, coriaceous, obtusely cuspidate, quite entire, glabrous or pubescent beneath, base rounded, subcordate or acute, basal veins strong, lateral veins 7-8 pairs, finely reticulate beneath, petioles 1.2-5 cm long, stipules 1.8-2.5 cm long, coriaceous.

Flowers:[2] Minute, unisexual, of 3 kinds, males, females and imperfect females (gall flowers) crowded along with bracteoles in the inner walls of fleshy receptacles which are sessile, globose, about 1.8 cm diam., puberulous, arising in axilliary pairs, basal bracts 3, orbicular, spreading. Male flowers: near the mouth of the receptacle, perianth 4, stamen 1, filament erect. Female flowers perianth as in the male but shorter, ovary superior, unilocular with a single pendulous ovule, straight or oblique, style excentric, stigma simple.

Fruit:[2] Fleshy pericarp and with achenes embedded in them. dark red in colour.

Use in Traditional Medicine. The medicinal properties of various parts of the tree have been well known to indigenous physicians. The milky juice is applied externally on pains, bruises, rheumatism, lumbago and on cracked and inflamed soles of feet. In India, the root is used to treat gonorrhoea,

biliousness, dysentery and inflammation of the liver. The tips of the aerial roots are also used to relieve persisting vomiting and dysentery. Infusion of the small branches are used for heamoptysis. The infusion of the bark is supposed to be a powerful tonic and is considered to have specific properties in the treatment of diabetes.[2.3a]

II. Phytochemistry

1. Compounds isolated from the milky juice

Early work on *Ficus benghalensis* reports the isolation of ficosterol from the **milky juice**[3a].

Although, the authors[3a] refer to the isolation of ficosterol from the milky juice, the original literature[3b] concerning this does not assign a structure to ficosterol. It reports the isolation of a compound (melting point 135^0C) from the unsaponifiable matter of the milky juice of *F. benghalensis* which has shown characteristic colour reactions of sterols in Liebermann Burchard and Salkowski's reaction. According to the iodine value and the formation of a dibromo compound, ficosterol is said to have one double bond. The acetyl, benzoyl and the 3,5-dinitro benzoyl derivatives have been prepared. Cupric oxide oxidation of ficosterol has yielded a ketone (ficostenone, m.p. 105^0C); thus showing the presence of a secondary OH group in ficosterol. The article also mentions that molecular weight determination by cryoscopic methods has given a molecular weight of 410 indicative of a molecular formula $C_{29}H_{50}$. This seems to be erroneous since ficosterol has been demonstrated as having a hydroxyl group.

2. Compounds isolated from the leaves

2.1 Hydrocarbons [4]

Surface lipids play a vital role in affecting transpiration and leaf surface properties. The composition of lipids and in particular hydrocarbon distribution may be a useful parameter in chemotaxonomy. The predominant occurrence of lower even numbered alkanes, viz. C_{14}, C_{16}, C_{18} and C_{20} from the same plant of *F. benghalensis* collected during two different months, is very striking. The occurrence of such lower hydrocarbons in greater proportions, from a dicotyledonous plant is unusual. (The preponderance of odd-numbered alkanes is what is normally seen in higher plants). The presence of branched chain alkanes in relatively higher proportion is also noteworthy.

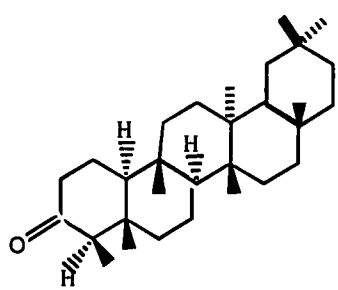
The above analysis has been carried out by GLC (10% SE-30 column with flame ionization detector) of the cold n-hexane extract of the leaves after initial purification by preparative TLC with carbon tetrachloride.

2.2 Triterpenoids and Sterols

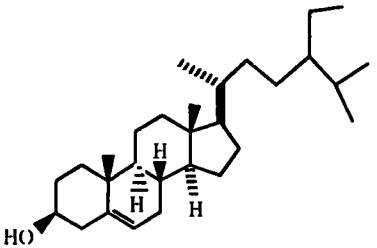
The triterpene, friedelin **(I)** has been isolated[5] from the neutral fraction of the petroleum ether extract of the leaves of the plant

following chromatography over alumina with petroleum ether and benzene as the eluant. The structure is said to have been proven by elemental analysis, mass spectral data and by mixed melting point and co-tlc with that of an authentic sample. β-sitosterol[5] (**II**) has been isolated from the column on elution with benzene. The compound has been characterized by elemental analysis, mass spectral data and by mixed melting point with a pure specimen.

Physical data: Compound **I**: m.p. 235-238^{0}C, $[\alpha]_D = -22^0$ ($CHCl_3$). Pink colour with Liebermann-Burchardt reagent. Compound **II**: m.p. 135-137^{0}C, $[\alpha]_D = -35^0$ ($CHCl_3$). Positive test for sterols with Liebermann-Burchardt reagent



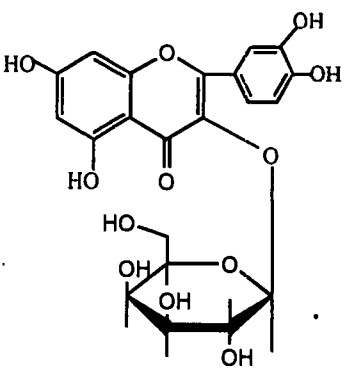
Friedelin (**I**)



β-sitosterol (**II**)

2.3 Flavonols[6]

The 70% ethanol extract of the finely powdered fresh tender leaves has been sequentially extracted into benzene, diethyl ether and ethyl acetate. The ethyl acetate extract yielded quercetin-3-galactoside (major compound, configuration of the sugar ring has not been specified) (**III**) and rutin (minor).

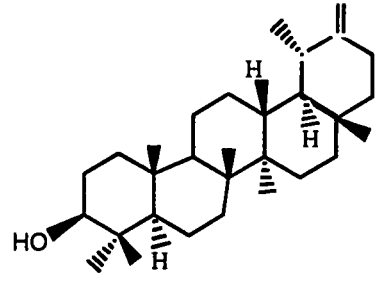


Quercetin-3-galactoside (**III**)

3. Compounds isolated from the heart wood

3.1 Tiglic acid ester of ψ-taraxasterol (**IV**) has been isolated[6] from hot benzene extract of the heartwood of the plant. The compound has been characterized by its infrared (IR) spectra and by mixed melting point and co-thin layer chromatography (tlc) of the alkaline hydrolysis products with authentic ψ-taraxasterol and tiglic acid.

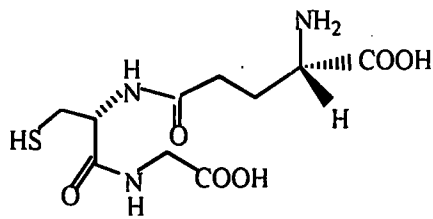
Physical data: Compound **IV**: m.p. 236-238^{0}C, $[\alpha]_D^{28} = +88.5^0$ ($CHCl_3$). IR (cm^{-1}): 2940, 1730, 1455, 1375, 1245, 1025, 980, 873 and 655. Alkaline hydrolysis (C_6H_6 medium) gave ψ-taraxasterol (m.p. 215-216^{0}C, $[\alpha]_D^{28} = +48.6^0$, acetyl, m.p. 240-242^{0}C. $[\alpha]_D^{28} = +53.2^0$, benzoate 270-274^{0}C) and tiglic acid (m.p. and mixed m.p. 62-64^0)



Taraxasterol (**IV**)

4. Compounds isolated from fruits and seeds

Early work reports the isolation of glutathione (V) from fruits.[3a]



Glutathione (V)

4.1 Amino acids[7]

The free and bound amino acid composition of fruits and seeds of some medicinal plants are reported. *F. benghalensis* is reported as one of the species having the highest level of amino acids in the fruit proteins. Of the medicinal plants that have been investigated (among these being *Ficus racemosa*, *Ficus religiosa* and *Ficus lacor*), cysteine was only found in the proteins of *F. benghalensis* and *F. religiosa*. Amino acids such as glutamine, methionine and tryptophan have not been detected in the protein hydrolysates of any of the other plant species. The essential amino acids arginine and methionine as well as citrulline and hydroxyproline were not present in the free state in the fruits of *Ficus benghalensis*

Amino acid analysis has been carried out by means of paper chromatography of the concentrated water extract of the fresh fruits. Proteins have been precipitated by addition of ethanol to the fruit extract. The protein mixture has been subjected to both acid and alkaline hydrolysis.

4.2 Polysaccharides[8]

The hot ethanol extract of the finely powdered plant material, when dissolved in water and analysed for low molecular weight carbohydrates by paper chromatography has shown the presence of **D**-glucose, **D**-galactose and **D**-fructose.

The water insoluble residue after extraction with chloroform has been dried and re-extracted into hot water. Concentrating the water extract and addition of ethanol has resulted in the precipitation of a xylan, which on hydrolysis with acid and analysis by paper chromatography revealed the presence of mainly **D**-xylose and **D**-arabinose together with traces of glucose and galactose.

The xylan on methylation followed by methanolysis and hydrolysis has afforded 2,3-di-*O*-methylxylose and 2,4-di-*O*-methylxylose together with 2,3,4-tri-*O*-methylxylose and 2-*O*-methylxylose The presence of the first two products indicated

that the xylose units in the xylan are linked by 1,4 and 1,3 bonds. The 2,3,4-tri-*O*-methylxylose has arisen from the terminal end groups. The presence of 2-*O*-methylxylose indicated branching.

5. Compounds isolated from the stem bark

5.1 Anthocyanidin derivatives

3',5,7-trimethylether of leucocyanidin **(VI)**, 3',5,7-trimethyl ether of delphinidin-3-*O*-α-**L**-rhamnoside(**VII**) and 3',5-dimethyl ether of leucocyanidin-3-O-β-**D**-galactosylcellobioside (**VIII**) have been isolated from the water insoluble fraction of the ethanolic extract of the defatted stem bark[9]. These compounds have been isolated from a silica gel column on elution with mixtures of chloroform/methanol followed by methanol itself.

Characteristics. Compound **VI**: m.p. 165^{0}C (crystallized from ethyl acetate-petroleum ether), $[\alpha]_D^{29} = +195^0$(MeOH).

Compound **VII**: m.p. 170-171^{0}C (Crystallized form ethyl acetate-petroleum ether), $[\alpha]^{29}_D = +232^0$(MeOH).

Compound **VIII**: Crystallized from MeOH-ether, m.p. 340^{0}C, $[\alpha]^{29}_D = +298^0$ (MeOH)

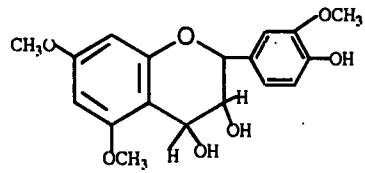
5,7-dimethylether of leucopelargonidin-3-*O*-α-L-rhamnoside (**IX**) has been isolated from the water soluble fraction of the ethanolic extract of the defatted stem bark after extraction into ethyl acetate.

Characteristics: Compound **IX**: m.p.190^{0}C (Crystallized from ethanol–ether), $[\alpha]^{29}_D = +260^0$(MeOH).

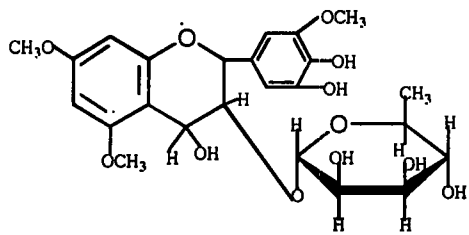
Starting with 2 kg of stem bark, 350 mg of compound **VI**, 500 mg of compound **VII**, 300 mg of compound **VIII** and 200 mg of compound **IX** have been isolated.

These compounds have been characterized by chemical tests which include demethylation, methylation, acetylation, degradation methods, use of colour reagents and elemental analysis and by ultraviolet spectroscopy.

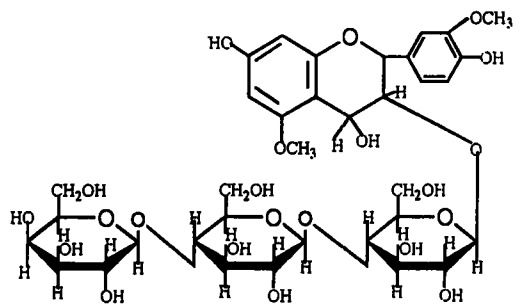
Although, melting points have been recorded no cross reference has been made[9] to that reported previously in literature for these compounds. No nmr data is reported. This same article is used as cross reference by other authors as well, who claim to have isolated these compounds from the stem bark of *F. henghalensis* with the aim of studying its pharmacological activity . However, they do not give any further data in support of the structures of the isolated compounds.



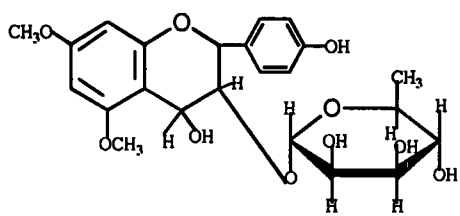
3',5,7-trimethyl ether of leucocyanidin **(VI)**



3',5,7-trimethyl ether of delphinidin-3-*O*-α-**L** rhamnoside**(VII)**



3',5-dimethyl ether of leucocyanidin-3-O-β-**D**-galactosylcellobioside **(VIII)**



5,7-dimethyl ether of leucopelargonidin-3-*O*-α-**L**-rhamnoside

(IX)

5.2 Other Compounds

β-sitosterol-α-**D**-glucose (**X**), 20-tetratriacontene-2-one (**XI**), 6-heptatriacontene-10-one (**XII**) and pentatriacontan-5-one(**XIII**) have been isolated[3a] from the hot petroleum ether extract of the **stem bark** and meso inositol (**XIV**) from the hot ethanol extract of the defatted stem bark.

Compound **X** was isolated by column chromatography on silica gel (eluant 1:1 v/v petroleum ether-benzene) of the crystalline compound that precipitated on standing of unconcentrated petroleum ether extract. Compounds **XI – XIII** were isolated by column chromatography on silica gel of the hot petroleum ether extract with petroleum ether : benzene 3:1 v/v – 1:1 v/v as eluant and purified by repeated chromatography with mixtures of CCl_4 and $CHCl_3$. Compound **XIV** was obtained by chromatography of the defatted ethanol extract of the stem bark on silica gel with methanol as eluant.

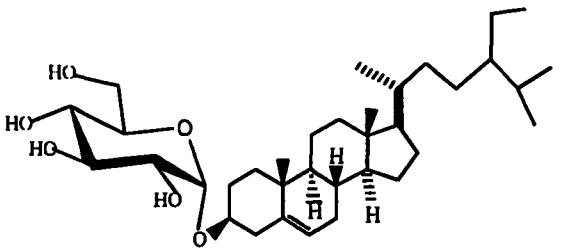
Physical data: Compound **X**: m.p. 283-284^{0}C, the compound on hydrolysis gave β-sitosterol and α-**D**-glucose which are said to have been identified by mixed melting point and co-chromatography and superimposable IR spectra.

Compound **XI**: m.p. 82-83^{0}C. IR(KBR)$_{max}$(cm^{-1}): 2920, 2850, 1720, 1450, 1375, 1160, 725, 705. NMR (δ in ppm): 0.9 (6H), 1.3 and 1.6 (CH_2 protons), 2.3 (2H, CH_2 next to CO), 4.65 (olefinic protons). Mass spectrum: *m/z* 490, 475, 447, 58, 43.

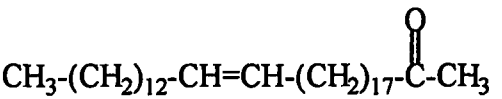
Compound **XII**: m.p. 93^{0}C. IR(KBR) ν_{max}(cm^{-1}):2817, 1709, 1407, 733, 722. NMR (δ in ppm) 0.4 (CH_3 protons), 1.2 (CH_2 protons), 2.6 (CH_2 protons next to CO function) and 4.58 (olefinic protons). Mass spectrum: *m/z* 532, 422, 407, 379, 168,153.

Compound **XIII:** m.p. 80^{0}C, IR(KBR) ν_{max}(cm^{-1}): 2900, 2830, 1750, 1725, 1700,1490,1480,1450,1275, 1175, 810, 725. NMR (δ in ppm): 0.8 (six CH_3 protons), 1.28 (CH_2 protons), 2.40(CH_2 protons next to CO). Mass spectrum: *m/z* 506, 464, 449, 421, 100, 85.

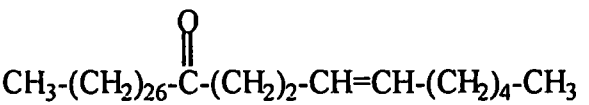
Compound **XIV**: m.p. 225-226^{0}C. It was identified by comparing the IR spectrum of an authentic sample.



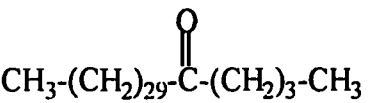
β-Sitosterol-α-**D**-glucose (**X**)



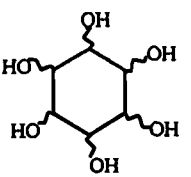
20-Tetratriacontene-2-one (**XI**)



6-Heptatriacontene-10-one (**XII**)



Pentatriacontan-5-one(**XIII**)



meso-inositol (**XIV**)

III. Pharmacology

1. Anti-tumor activity[10]

The chloroform extract of the fruit of *F. benghalensis* has shown toxicity in the brine shrimp (*Artemia salina*) bioassay (LC_{50} < 1000 μg/ml). It also possessed anti-tumor activity in the potato disc bioassay (% tumor inhibition >20%).

2. Anti-microbial activity[10]

The chloroform extract of the fruit of *F. benghalensis* has also shown inhibitory activity (0.5 mg/disc) against the bacterium *Micrococcus luteus* (18-26 mm diameter inhibition zone), which was not inhibited by kanamycin (100 μg/disc), streptomycin (100 μg /disc) or penicillin (5 μg/disc). *Streptococcus faecalis* and *Strep. faecium* were also inhibited by the fruit extract (17–20 mm inhibition zone). Other bacteria such as *Bacillus cereus*, *B. megaterium*, *Staphylococcus aureus*, *Staph. epidermis*, *Strep. lactis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were inhibited to a lesser extent (16-19 mm inhibition zone). The extract did not show any anti-fungal activity.

3. Anti-diarrhoeal activity[11]

The ethanol extract of the hanging roots of *Ficus benghalensis* has been evaluated for anti-diarrhoeal activity against different experimental models of diarrhoea in rats. The extract (400 mg/kg, orally) has shown significant inhibitory activity against castor oil induced diarrhoea (extract fed rats had 2.21±0.27 defecations per animal in 4 hr; control 4.00±0.33, $P < 0.001$) and PGE_2 induced enteropooling (for extract fed rats the value reported is 1.25±0.15 in terms of intestinal fluid; control 0.78±0.11, $P < 0.02$) in rats. The extract has also been significantly effective in reducing gastrointestinal mobility (extract fed rats: 50.2±2.7%; control 79.4±2.76% ,$P < 0.001$) in charcoal meal test in rats.

Summary of methodology: The coarse powder of the plant material was Soxhlet extracted into ethanol. The extract suspension to be administered to rats was prepared by using weighed amounts of the ethanol extract in 2%(w/v) aqueous tragacanth suspension.

Castor oil induced diarrhoea in rats: Albino rats (weighing between 180- 200 g) were fasted for 18 h. One group was given the 400 mg/kg dose. Another group received diphenoxylate (5mg/kg) orally in the form of a suspension as the standard drug for comparison. The control group received 2% w/v aqueous tragacanth suspension only.

After 1 h treatment each animal received 1 ml castor oil orally and was observed for defecation. Up to 4 h after the castor oil treatment the presence of diarrhoeal droplets were noted in the transparent plastic dishes placed in the individual rat cages.

Gastrointestinal mobility tests: Rats fasted for 18 h were administered orally with charcoal meal (3% deactivated charcoal in 10% aqueous tragacanth). Immediately afterwards one group was orally administered with 400 mg/kg of extract suspension. Another group received atropine (0.1 mg/kg i.p. (intra peritoneal)), the standard drug for comparison. A third group was treated with aqueous tragacanth suspension as the control. Thirty minutes later each animal was killed and the intestinal distance moved by the charcoal meal from the pylorus was cut, measured and expressed as a percentage of the distance from the pylorus to the caecum for each animal

PGE_2-induced enteropooling: Rats were deprived of food and water for 18 hours. One group of rats was treated with the extract at a dose of 400 mg/kg (orally). Another group was treated with 1 ml of 5% v/v ethanolic normal saline (i.p.) and aqueous tragacanth solution, which served as a vehicle control. Immediately after extract administration, PGE_2 was administered orally to each rat (100 μg/kg) in the first four

groups in 5% v/v ethanol in normal saline. A third group, treated with PGE_2 as well as tragacanth suspension served as PGE_2 control group. After 30 mins following administration each rat was killed and the whole length of the intestine from the pylorus to caecum dissected out and its contents were collected in a test tube and the volume was measured.

Each group of rats consisted of ten rats. Statistical significant tests have been performed by Student's t-test.

4. Anti-diabetic action and insulin raising effect

4.1. Compound VIII (3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside)[12]

Compound **VIII** isolated according to Subramanium and Misra[9] is reported as demonstrating antidiabetic activity. On oral administration of compound **VIII** (250 mg/kg body weight), the blood sugar decreased very significantly ($P < 0.001$) of both normal (26% fall) and moderately diabetic (19% fall) rats and serum insulin (39% rise) increased significantly in the latter at a dosage of 250 mg/kg for a 2 h period. During a month- treatment of the diabetic rats orally with compound **VIII**, at a dosage of 100 mg/kg, there was a significant ($P < 0.001$) decrease in the blood sugar (58% fall) and the urine sugar.

The mechanism of action has been suggested as the stimulatory action of compound **VIII** on the sources of endogenous insulin. Compound **VIII** is structurally related to tannin. There are reports[13] that insulin can complex to tannins and remain biologically active.

Summary of methodology: Short term effect of compound **VIII** on normal and on diabetic rats have been determined as follows. Rats weighing 115-155 g were made diabetic by subcutaneous injection of an aqueous solution of alloxan (250 mg/kg body weight). After a week rats having blood sugar in the range 200-250 mg/100ml were taken for the study.

Two groups of normal and two groups of diabetic rats (each group with six rats each) were fed on rat feed. Their fasting blood sugar was determined according to the method of Asatoor and King[14] from a sample obtained from their eyes (venous pool). The leucocyanidin derivative (compound **VIII**) suspended in saline (10 ml/kg wt of rats) was administered to the rats as follows: 1..normal control rats fed saline (10 ml/kg wt of rats) 2. normal rats treated with compound **IV** (dose 250 mg/kg), 3. diabetic control rats fed saline 4. diabetic rats treated with the same doses of compound **VIII**. Blood samples were collected at 2 h intervals for blood sugar analysis. All groups

were sacrificed and their whole blood was collected for serum insulin assay.[15]

The long term effect of the same was determined as follows. Three groups were used. A normal group of rats was fed saline (10 ml /kg/day), a diabetic group fed on saline and diabetic group fed on leucocyanidin derivative (compound **VIII**) (100 mg/kg/day). Fasting blood sugar[14], urine sugar[16] and body weight of the diabetic rats at weekly intervals and for the normal rats only their initial and final fasting blood sugar and body weights were determined.

4.2. Compound IX (5,7-dimethylether of leucopelargonidin-3-O-α-L-rhamnoside)

a) Compound **IX** has been isolated from the water soluble portion of the ethanol extract of the defatted stem bark of *F. henghalensis* according to the procedure of Subramanium and Misra[9]. The anti-diabetic effect[17] of compound **IX** (250 mg/kg single dose and 100mg/kg/day long term study) has been compared with that of glibenclamide (2 mg/kg and 0.5 mg/kg/day respectively) in moderately diabetic **rats**. The single dose glycoside treatment decreased ($P < 0.001$) fasting blood glucose by 19% and improved glucose tolerance by 29%. The corresponding effects of glibenclamide were 25% and 66% respectively over the control values. On one month treatment,

the fasting blood glucose levels had dropped almost to half the pretreatment levels in both the groups and their glucose tolerance improved by 41% ($P < 0.001$) in the glibenclamide treated group and by 15% ($P < 0.02$) in the glycoside treated group. Urine sugar decreased to traces in both groups.

In vitro studies have shown that insulin secretion by beta cells was more in the presence of compound **IX** than with compound **VIII** (leucocyanadin derivative).

Summary of methodology: Similar methodology to that for compound **VIII** in section 4.1

Serum insulin (as determined by radio immunoassay) had increased[18] by 41% ($P < 0.01$) in the group treated with compound **IX** as compared to 51% ($P < 0.001$) in the glibenclamide treated group in moderately diabetic rats.

In one month period, while the blood glucose of the diabetic control group had increased ($P < 0.01$) by 34%, that of the glycoside (compound **IX**) and glibenclamide treated groups decreased significantly ($P < 0.01$) by 39% and 53% respectively. The corresponding serum insulin variations in these groups were also significant. In the control group it decreased by 27% and in the glycoside and glibenclamide treated groups it increased by 43% and 56% respectively ($P < 0.01$).

The body weight of the treated groups has remained steady while that of the (diabetic) control decreased very significantly by 27%.

Summary of Methodology: Methodology is given in section 6.1

b) The serum insulin raising and hypoglycemic effects of the leucopelargonidin derivative (compound **IX**) has also been demonstrated[19] in normal and alloxan diabetic **dogs**. A blood glucose decrease of 15% in normal dogs in the first hour and a 19.5% decrease ($P < 0.01$) in the second has been reported[19]. Corresponding to this there has been a significant increase in serum insulin by 62% in the first hour and by 76% ($P < 0.001$) in the second hour. In the control group there has been no significant variation in the blood glucose or in the serum insulin level.

The leucopelargonidin derivative significantly decreased the blood glucose by 18% in the first hour and by 29% in the second hour. There was correspondingly an increase of serum insulin by 60% in the first hour and 100% in the second hour ($P < 0.001$).

Acute and chronic administration in single doses of 0.2-1.8 g/kg to different groups of **mice** and daily administration of 100, 250 and 500 mg/kg to rats for a period of one month

respectively had no toxic effect and the compound was not lethal even at the high dose of 1.8 g/kg in experimental animals.

In the chronic toxicity study, the only significant effect observed was a 12% blood glucose fall in the $2.5ED_{50}$ treated group and a 14% blood glucose fall in $5ED_{50}$ treated group after one month treatment. There has been no notable change in the food intake, body weight gain or in the behaviour as compared to the control group. Urine analysis has not shown even traces of sugar, albumin or any abnormality on microscopic analysis. Haematological studies including Hb content, total WBC count and differential WBC count, have also not shown any significant changes in them. Histopathological study of the intestine, liver and kidney of the $5ED_{50}$ group has shown that the chronic administration of the drug did not damage the organs. There has been no significant changes in the levels of blood urea and enzymes studied in the chronic test group.

Summary of methodology: *Studies on the antidiabetic effect* : Six normal dogs were kept in an animal house and were maintained on a uniform diet. A pilot study with 100 mg/kg ED_{50} dose of the compound on dogs had shown that the hypoglycemic action attains its peak at 2 h. The same group of dogs served as the control (placebo fed) and later, after a week,

as the test group (fed with leucopelargonidin (compound **IX**)). Placebo feed was empty gelatin capsules whereas in the second week the capsules were filled with compound **IX** (500-600 mg/capsule). In both the control and the test group the blood of each group of dogs fasted for 18 h was collected from the veins of legs and the samples were used to determine the blood glucose by glucose oxidase[20] method and serum insulin by immunoassay[15].

Effects on alloxan diabetic dose. The dogs were rendered diabetic by intravenous injection of alloxan monohydrate (60 mg/kg). The dogs were maintained on the same diet as before. After a fortnight, when the dogs were found to be moderately diabetic (200-300 mg/100 ml glucose levels) and after fasting them for 18 hours, blood samples were collected. A placebo was administered to each of them and their blood was again withdrawn at 1 and 2 h and the blood glucose and serum insulin levels were determined in all the samples. A ED_{50} dose of compound **IX** (100 mg/kg) was administered to the same group after a period of one week and the effect of the compound on blood glucose and serum insulin level has been determined.

Acute Toxicity:. Albino mice weighing between 20-26 g were divided into 5 groups of 8 each. The drug (compound **IX**) was dissolved in normal saline and administered to groups of 18 h

fasting mice in doses of 200, 400, 800, 1200 and 1800 mg/kg body weight. Each group received the drug at these doses in a volume of 10 ml/kg body weight. The animals were closely observed for 3 h and the mortality if any was noted during and after 24 h.

Chronic Toxicity: This was studied on male albino rats weighing 125-150 g which were divided into four groups of 12 rats each. The control group was fed on normal saline daily (1ml/rat) for a period of one month. The drug (compound **IX**) was administered orally in 1 ml normal saline daily at a low dose of 100 mg/kg to the second group and at an intermediary dose of 250 mg/kg to the third group and at a high dose of 500 mg/kg to the fourth group of rats for a period of one month. These doses were at ED_{50}, $2.5ED_{50}$, and $5ED_{50}$ levels. The fasting blood glucose values and body weight were recorded initially and again on the 15^{th} and 30^{th} day. Their urine was collected for detection of albumin, sugar and microscopic examination on the 30^{th} day. All the rats were killed the next day after overnight fasting and blood samples were collected for estimation of glucose and urea and also haematological examination. Serum alkaline phosphotase[21]; glutamate pyruvate transaminase, glutamate oxaloacetate transaminase[22] and serum albumin: globulin ratio[23] were estimated. Gross pathological

study of the liver, spleen, heart, lungs and kidneys have also been done.

4.3 Compound VII (3',5,7-trimethylether of leucodelphinidin-3-O-α-L-rhamnoside)[24]

Compound **VII** has been isolated by a similar procedure to that of Subramanium and Misra[9].

Compound **VII** has demonstrated hypoglycemic action at a dosage of 250 mg/kg given both in normal and alloxan diabetic rats. Its action was closely similar to that of an effective dose of glibenclamide (2mg/kg) tested under the same conditions. Both compound **VII** and glibenclamide decreased significantly ($P < 0.01$-0.001) the fasting blood sugar of normal and diabetic rats by 20 to 24% at two hours. However, compound **VII** improves the glucose tolerance in diabetic condition only by 35% whereas glibenclamide does so by 70% .

Summary of methodology. Rats were made diabetic by a subcutaneous injection of alloxan monohydrate (160 mg/kg). After one month, rats with blood glucose range of 200-250 mg/100 ml were selected. Rats were fed on a laboratory diet. Blood was collected from the venous pool of the eyes of 18 hr fasting rats for glucose estimation by the glucose oxidase method[20].

Drugs were administered with a stomach tube after the determination of their fasting blood glucose (FBG).

1. Normal control rats were given saline (10 ml/kg). 2. Normal rats were administered compound **VII** (250 mg/kg) 3.Normal rats were administered glibenclamide (2 mg/kg). 4. Diabetic rats were given saline (10 ml/kg). 5. Diabetic rats were administered compound **VII** (250 mg/kg). 6. Diabetic rats were administered glibenclamide (2 mg/kg). After two hours of administration blood glucose was estimated as above .

Effects of Glucose tolerance: The three groups (groups 4,5 and 6) of rats were given a solution of glucose (100 mg/ml) at a dosage of 3 g/kg. After administration of the drug, blood glucose was determined every 30 mins for 2.5 hours. The mean percentage rise in each group was calculated and statistically analysed.

5. Insulin Sparing Action

5.1. Compound VIII (3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside)[25].

Compound **VIII** in median effective dose (ED_{50}) of 100mg/kg

has demonstrated 11% or more hypoglycemic action in normal rats. A low dose of insulin in combination with compound **VIII** at ED_{50} for a long term treatment in alloxan diabetes equaled in response to the effects brought about by a double dose of insulin in respect of body weight, urine and blood sugar. It has also excelled in its amelioration action of serum cholesterol and triglycerides as compared to that with a high dose of insulin.

Summary of methodology: 1). A control group of diabetic rats were subcutaneously injected daily with 0.1 ml normal saline. 2) A second group of diabetic rats were similarly injected with a high dose of plain insulin (10 units/kg). 3) A third group of diabetic rats were similarly injected with a low dose of plain insulin (5 units/kg). 4) A fourth group of diabetic rats were similarly injected with a low dose of plain insulin plus leucocyanidin (compound **VIII**) (100 mg/kg/day).
All groups were maintained on a rat feed. After 15 days treatment, body weight, urine sugar[26] and fasting blood glucose[20] for all groups were determined. The treatment on rats were continued for another two weeks , and the above parameters were determined again.

5.2. Compound IX (5,7-dimethylether of leucopelargonidin-3-*O*-α-L-rhamnoside)[27]

Compound **IX** has shown its median effective dose (ED_{50}) as 100 mg/kg, in exhibiting about 12% hypoglycemic activity in normal rats.

The degree of blood glucose control by the high dose of insulin and low dose of insulin plus compound **IX** combined therapy on alloxan treated diabetic rats was not significantly different from each other at both 15 days and 30 days treatment. However, such effects brought about by the combined therapy were better than those by a low dose of insulin ($P < 0.001$). Body weight and urine sugar have also behaved in the same way.

Treatment with insulin and insulin plus leucopelargonidin (compound **IX**) controlled very effectively the serum cholesterol and triglyceride ($P < 0.01$- 0.001) contents.

In both 15 days and 30 days treatment it has been found that insulin plus leucopelargonidin combination had a better control on serum cholesterol and triglycerides ($P < 0.01$- 0.001) than that with a high or low dose of insulin. There has been no significant difference between the effects of a high dose of insulin and low dose of insulin plus lecupelargonidin combination on the tissue levels of these parameters.

The mechanism of action of this compound is thought to be partly dependent on its insulin releasing action from beta cells and insulin complexes and partly on its hypolipidemic and other pharmacological actions.

Summary of methodology: Similar to that of compound **VIII** in section 5.1

Free hydroxyl groups on carbons 3,5,7,3' and 4' are necessary for the biological activity of flavonoids and omission of hydroxyl at C-5 or methylation at C-7 reduces the activity.[19] Flavonoids have been reported[28] to be potent therapeutic agents with a wide spectrum of pharmacological action, flavonoids with more OH groups are feebly toxic. The leuco compounds contain many OH groups, some of which are methylated. Further, the methylated leuco compounds isolated from *F. benghalensis* do not contain a taxophore group (CO-CH=CH-O-) characteristic of toxic flavonoids.[29] Flavonoids possessing these groups, such as quercetin (3,3',4',5,7-pentahydroxy flavone) kaempferol (3,4',5,7-tetrahydroxy flavone) are powerful mutagens.[30]

6. Hypolipidemic Action

6.1 Compound IX (5,7-dimethylether of leucopelargonidin-3-O-α-L-rhamnoside)[18]

Compound **IX** is reported to have produced the maximum cholesterol lowering effect on kidneys, triglyceride lowering effect on liver and phospholipid lowering effect on aorta, whereas in the glibenclamide treated group the maximum cholesterol lowering effect has been observed in serum and triglyceride and phospholipid lowering effects in the liver.

The serum fatty acids had also decreased[18] very significantly ($P < 0.001$) by 39% in the glycoside (compound **IX**) treated group and by 52% in the glibenclamide group. In the diabetic condition the fecal excretion of sterols and bile acids had decreased by 28% and 33% respectively as compared to reported normal values. The glycoside significantly enhanced the fecal excretion of sterols (12%) and bile acids (25%) while glibenclamide had no such effect even though both controlled hypercholesteremia.

Summary of methodology: Rats were made moderately diabetic in the usual way.

Blood glucose has been estimated by glucose oxidase method[20] and the urine has been tested qualitatively.[16] Serum insulin has been estimated by radio immunoassay.[15] Blood has been taken from the tip of the tails of fasting rats for various estimations before and after the drug administration. Experiment 1: Three groups of overnight fasting rats containing six in each were subjected to the study as follows: Group 1 (Control): Rats were fed normal saline (10 ml/kg) . Group 2: Rats were fed with glycoside in normal saline (250 mg/kg). Group3: Rats were fed glibenclamide in normal saline (2mg/kg). After 2 hr, their blood was collected for estimation of blood glucose and serum insulin. Experiment 2: After a period of 2 weeks the same groups were used for long term treatment. Their fasting blood glucose, urine sugar serum insulin and body weight were recorded before the treatment was carried out using minimal doses of the drug as follows: The control remained the same, but the rats in group 2 and 3 were fed with 100 mg/kg/day glycoside and 0.5 mg/kg/day glibenclamide respectively.

After 1 month treatment the body weights, urinary sugar, blood glucose and serum insulin levels were again recorded in fasting condition for each group. After the last day of treatment, their feces were collected for 24 hr. The rats were sacrificed and their serum, liver, heart, aorta, and kidney were collected for estimation of triglyceride glycerol, cholesterol and

phospholipids[31,32] and also for serum insulin[15] and fatty acids[33] using standard methods. Fecal sterols[34] and bile acids[35] have also been determined.

6.2. Compound VIII (3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside)[12]

During the one month treatment of the diabetic rats orally with compound **VIII**, cholesterol and triglyceride contents of both tissues and serum and phospholipids of tissues were found to increase significantly as compared to normal values[12]. On treatment with compound **VIII**, with the exception of phospholipid in serum and cholesterol in heart, the cholesterol, triglyceride and phospholipid contents of serum and tissues (liver, aorta, kidney and heart) decreased significantly as compared to the diabetic controls (Table 1).

After the expiry of one month all the rats were sacrificed and their blood, liver, heart, intestine and kidney were separated. Analysis of cholesterol[34], triglyceride glycerols[36] and phospholipid[37] in serum, liver, aorta, kidney and heart were carried out according to previously published procedures.

Table 1. Percentage decrease in cholesterol , triglyceride and phospholipid content in serum and tissues.

	Cholesterol	Tiglycerides glycerols	Phospholipids
Serum	17%	9%	-
Aorta	22%	29%	23%
Liver	20%	21%	14%
Kidney	24%	19%*	7.4%
Heart	-	25%**	9.7%

Values are significant at $P < 0.001$ except *$P < 0.02$ and ** ($P < 0.01$)

7. Effects on glucose-6-phosphate, hexose kinase and HMGCoA reductase enzyme activity[12]

7.1 Compound VIII (3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside)

Compound **VIII** decreased the glucose-6-phosphatase activity in the liver by 38%($P < 0.001$) as compared to the diabetic controls[12].

The activity of HMGCoA reductase in the liver (by 12.5%) and intestine (by 23%) and that of hexose kinase (by 29%) in the liver increased significantly ($P < 0.01$) in diabetic condition. A higher ratio of HMGCoA/mevalonate was indicative of the

lower activity of HMGCoA reductase. On administering compound **VIII** these values increased significantly and the intestinal HMGCoA reductase activity was within the normal range.

Glucose-6-phosphatase[38] and hexose kinase[39] in liver, HMG CoA reductase[40] in both liver and intestines have been carried out according to previously published procedures.

8. Antioxidant effect

8.1 Compound IX (5,7-dimethylether of leucopelargonidin-3-*O*-α-L-rhamnoside) and Compound VIII (3',5-dimethyl ether of leucocyanidin-3-*O*-β-D-galactosylcellobioside [41]

Lipid peroxidation has been implicated in the pathogenesis of a number of diseases such as diabetes mellitus and coronary heart diseases. Changes in the levels of free radicals have been suggested as a derogatory condition in atherogenic process, myocardial infarction, emotional stress, radiation exposure etc. High fat diet is a risk factor in atherosclerosis and during an atherogenic process, the production of free radicals increase. Compounds that can scavenge the activated oxygen species may therefore be useful as chemoprotective agents against arterial disease.

The antioxidant effects of compound **VIII** and **IX** in cholesterol fed rats have been compared with those of a structurally similar synthetic flavanoid quercetin with known antioxidant action.

Cholesterol feeding significantly decreased ($P < 0.05$) the normal activities of glutathione peroxidase and glutathione reductase in tissues. Treatment of these rats with compound **VIII, IX** and quercetin had significantly elevated these parameters only in the heart and liver. Other parameters studied were the malodialdehyde, hydroperoxides, conjugated dienes and glutathione contents as well as the activities of superoxide dismutase and catalase. In all these instances quercetin showed a slightly better effect than compound **VIII** or **IX**.

The decrease in the levels of lipid peroxides observed in flavonoid treatment of hypolipidemic rats suggests that these compounds can counteract the deleterious effects of a cholesterol diet.

The activities of superoxide dismutase and catalase have been significantly lowered in rats maintained on a cholesterol diet as compared to those fed on a normal control diet. This was in agreement with previous reports[42] which have suggested that the feeding of a hyperlipidemic diet to experimental animals depresses their antioxidant systems resulting in the production of toxic intermediates. Therefore, the increase in the levels of

glutathione on treatment of cholesterol fed rats with compounds **VIII** and **IX** and quercetin is noteworthy.
The slightly better action of quercetin observed on antioxidant enzymes was thought to be due to its structural advantages over compounds **VIII** and **IX** with relation to the number of hydroxyl groups. Methylation of the two hydroxyl groups in the *Ficus* flavonoids may have slowed down their antioxidant action but conferred on them a degree of non-toxicity. These compounds do not contain the toxic group CO-CH=CH-O- as is the case with quercetin. Quercetin is not advised for human consumption as it is a toxic flavonoid.

Summary of methodology: Male albino rats were divided into 5 groups of 6 rats each.
Group 1- Rats fed control diet (normal control)
Group 2- Rats fed 2% cholesterol diet (cholesterol control)
Group 3 - Rats fed 2% cholesterol diet + leucopelargonidin derivative (compound **IX**) (100 mg/kg body wt/day)
Group 4 -Rats fed 2% cholesterol diet + leucocyanidin derivative (compound **VIII**) (100 mg/kg/ body weight/day)
Group 5- Rats fed 2% cholesterol diet + quercetin (100 mg/kg body weight/day).
Composition of the control diet (g/100g diet) was

Corn starch-71; Casein –16; Groundnut oil-8; salt mixture 4, vitamin mixture –1

Composition of the cholesterol diet (g/100 g diet) was

Corn starch-62; Casein –16; Coconut oil -15; Cholesterol –2; salt mixture 4, vitamin mixture –1

All drugs were administered as suspensions in normal saline through a gastric tube. The rats were treated for a period of 90 days. At the end of the experimental period, the rats in each group were deprived of food overnight and sacrificed. Blood and tissues (liver, heart and kidney) were removed for estimation of serum lipids[33,36,37,43], malondialdehyde[44], hydroperoxides[45],and conjugated dienes[46] in tissues. The activities of superoxide dismutase[47], catalase[48], glutathione proxidase[49] and glutathione reductase[50] have been assayed according to previously published procedures. The concentration of reduced glutathione[51] in the tissues have also been determined.

IV. Summary

It is clear from the literature that the banyan tree contains compounds with a wide range of biological activities which can be utilized in the treatment of some common major diseases. In particular the following have been identified.

Activity	Part of the tree	Active Ingredient
Anti-tumour	fruits	Not isolated. (chloroform extract)
Anti-microbial	fruits	Not isolated. (chloroform extract)
Anti-diarrhoeal	hanging roots	Not isolated. (ethanol extract)
Anti-diabetic Action and Insulin raising effect	stem bark	3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside
---do-----	-do-	5,7-dimethylether of leucopelargonidin-3-O-α-L-rhamnoside
---do-----	-do-	3',5,7-trimethylether of leucodelphinidin-3-O-α-L-rhamnoside
Insulin sparing action	Stem bark	3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside
---do----	-do-	5,7-dimethylether of leucopelargonidin-3-O-α-L-rhamnoside

Hypolipidemic effect	Stem bark	5,7-dimethylether of leucopelargonidin-3-O-α-L-rhamnoside
--do--	-do-	3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside
Effects on glucose-6-phosphate, hexose kinase and HMGCoA reductase enzyme activity	Stem bark	3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside
Antioxidant effect	Stem bark	5,7-dimethylether of leucopelargonidin-3-O-α-L-rhamnoside
--do--	-do-	3',5-dimethylether of leucocyanidin-3-*O*-β-D-galactosylcellobioside

Further research and careful study of side effects will be necessary before these compounds are perfected for human use. It is, nevertherless, satisfying to note that the potential of a widely accessible tree in the tropics as a treasure-house of remedies is being explored extensively.

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