Chapter 05

Discussion and Conclusions
Many scientific investigations pertaining the screening of plant for bioactive compounds have been reported till date. Most of the research findings centre on the usage of stem, leaf, roots, seeds and fruits. The hypothesis put forth in the following study was to screen the barks for bioactive components, as bark is first defense to the outside environment, is more vulnerable to the insults (mechanical, chemical and biological attacks) of the surroundings. These insults might elicit the barks to produce diverse phytochemicals (for protecting itself viz. proanthocynidins to protect against UV light). No organic chemist can compete with nature as far as synthesis of organic compounds is concerned. Traditional approach to the discovery of new naturally active bioactive component is to screen. Screen is an assay procedure which includes isolation, characterization of bioactive component.

Ten medicinal plants and a formulation made of the plants were evaluated for their nutritional and invitro assay.

5.1 a) Proximate Analysis

i) Moisture content

The proximate analysis of these plants in form of moisture content was in the range of 3.2% to 8.6% on dry bases. The results are in agreement with analysis as done by Ahmed et al (2010) using the bark of Ficus racemosa 7.6%. Bakare et al, (2010) have reported a comparative evaluation of leaf, fruit and seeds for proximate analysis of M.charantia. The moisture content reported is 17.97, 10.74 and 20.69% on wet weight basis. Our reports are in proximity.

The results obtained in the proximate analysis of the plant showed that the dry matter of the plant is as high as 3.66%. This has always been the findings of most researchers and it is complemented by the reports of Harbinger (1994) and Pamplona-Roger (2000).
ii) Ash content

The ash value reported by Ahmed et al, 2010 for Ficus is 0.95 and 14.5 % wet and dry basis and values reported by Bakare et al, (2010) for M. charentia are 15.42, 7.36 and 9.73% for leaf stem and seed respectively our values were less than those reported for seeds. But the values of Ash were higher than reported in barks by Ajibade VA and Fagbohun ED, (2010). To validate the findings the tests were redone by the protocol as mentioned by TAPPI Test Method T211, "Ash in Wood and Pulp."

iii) Dietary Fiber content

The dietary fiber was in proximity of the reports of Ahmed et al, (2010) but was comparably lower than that reported by Enzmann et al, (1969). Dietary fiber gets its importance for its known use as therapeutic agent against cancer and also used as cholesterol lowering ability.

iv) Protein content

All the plant exhibited protein content on dry bases with Ficus benghalensis (1.46%) lowest and Syzygium cumini (2.43%) as highest. The protein content was in agreement to the value as reported by Enzmann et al, (1969). Seeds evaluated have given the values in proximity to those reported by Bakare et al, (2010) and Ajibade VA et al, (2010). However the protein content is seen to increase in dry samples.

v) Fat content

The lipid content was in agreement to Ahmed et al, 2010 as detected in F racemosa belonging to the same family (2.5%). The values are also in agreement to that of Bakare et al, (2010) and Ajibade VA et al, (2010). The fat content is much higher in leaf, fruit and seeds as compared to barks.
vi) Total sugars and starch

The total sugar and starch content are lower than those reported by Ajibade VA (2010) in the bark (the value reported by them is 45 but include both reducing and nonreducing sugars). For leaf, fruits and seeds these values reported are 32, 34 and 9 respectively. The values are in agreement to Bakare et al, (2010) and Ahmed et al, (2010).

The values reported for starch are also on the lower side. There may two reasons for this one barks are not good source, seeds tend to store lipids as reserve food and root of Withania contains a lot of minerals as for the leaves the lower values may be attribute to the method.

In starch determination many researchers use enzyme mixture of 1000 IU amylase and 5 IU amyloglucosidase to digest starch in plant tissue samples and estimate the total sugars (Paks Chow et al, 2004). Others make use of dilute sulfuric acid (0.005 N) for starch digestion than enzymatic hydrolysis. The acid breaks down structural carbohydrates, resulting in overestimates of starch content (Nicky Seager et al, 1993).

vii) Mineral content

The mineral content was in proximity to those reported by Ahmed et al, (2010), Ajibade VA (2010) and Bakare et al, (2010).

According to Pravin K Bhoyar, (2011), mineral remedies consist of Bangabhasma (calcinated tin), Jistbhasma (calcinated zinc), Abrhab-hasma (mica ash), and Lohabhasma (calcinated iron).

The commercial preparations (e.g., Nowojar) containing above bhasmas are very effective in NIDDM; in case of IDDM, they help in reducing dose of insulin. Some complexes with metformin and tolbuta-mide with zinc, cadmium, cobalt and copper have some complexes with the exception of complexes have shown good
hypoglycemic effect. Zinc complexes have shown to have good blood-sugar-lowering activity.

Shilajit, an organo-mineral preparation found in nature, has been used as a tonic in diabetes mellitus. Rasayana therapy is widely used in diabetes mellitus. Rasayana is an important branch of Ayurveda. Many of the drugs used in Rasayana therapy in diabetes mellitus have excellent antioxidant properties, like *Phyllanthus emblica*, *Azadirachta indica*, *Ocimum sanctum* and *Tinospora cordifolia*. The Rasayana approach to treat diabetes consists of -

- Aeara Rasayana (antistress)
- Ajasrika Rasayana (dietary control)
- Osad Rasayana (antistress)
- Kamya Rasayana (preventive)
- Naimittika Rasayana (hypoglycemic)

It is observed that potassium was the most abundant mineral present followed by calcium and phosphorous. The bark was a good source of iron, zinc and copper.

The calcium, iron and phosphorus contents are lower than the values reported by Hussain, (1985).

viii) Vitamin A and vitamin C

The purpose of determine vitamin A in the plants evaluated was for the role it plays in vision. Recently it has also shown to have antioxidant property and its consumption along with vitamin E and C reduces risk of Diabetes (The Hindu, 26th October, 2012). However in most of the plants it was not detected. The reason could be plant part used. The barks is not a good source, it has other functions to perform. Mostly orange yellow color parts are rich in carotenes.

In plants Vitamin A exists as the precursor carotenoid family. β carotene is cleaved to retinyl esters and retinoic acid in the enteroctye of the small intestine,
packaged into chylomicrons for transport to liver for storage as vitamin A (retinol form) in hepatic stellate cells. When needed, retinol is supplied to the tissues bound to retinol binding protein, a zinc dependent protein. Zinc deficiency disturbs the normal metabolism of retinol and supplementation of zinc treats retinol resistant night blindness (Neeld JB Jr, 1963).

Natural ascorbic acid is vital for body performance. It is an antioxidant and acts as electron donor to 8 human enzymes; 3 of which participate in hydroxylation of collagen, one is necessary for biosynthesis of catecholamine, noradrenalin, one for amidation of peptide hormone, one is involved in tyrosine metabolism and two in carnitine biosynthesis. It also promotes absorption of soluble heme iron by chelation or maintaining the iron in reduced form (Hussain T, 1985).

Lack of ascorbic acid impairs normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine. In retinopathy the above pathological changes weaken the endothelial walls of the capillaries due to reduction of the amount of intercellular substances. Exposure to high levels of copper and iron destroys vitamin C (Reza M et al, 2010).

**5.1 b) Secondary metabolites**

i) **Polyphenols**

These are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. These are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. Fruits like grapes, apple, pear, cherries and berries contains up to 200–300 mg Polyphenols per 100 grams fresh weight (Kanti Bhooshan Pandey and Syed Ibrahim Rizvi, 2009).

Epidemiological studies have repeatedly shown an inverse association between the risk of chronic human diseases and the consumption of polyphenolic rich diet.
The phenolic groups in Polyphenols can accept an electron to form relatively stable phenoxy radicals, thereby disrupting chain oxidation reactions in cellular components. It is well established that Polyphenol-rich foods and beverages may increase plasma antioxidant capacity (Joshua DL et al, 2010).

Polyphenols are generally regarded safe as these have been consumed since long, however increased consumption has been correlated to have adverse reactions (Chow HH et al, 2003). Relatively high doses (600 -1800mg/day) have been reported high incidents of hepatotoxicity in humans (Mazzanti G et al, 2009). Liver biopsies have indicated inflammation and necrosis (Stevens T et al, 2005), (antinutrient) but neuraceutical.

The presences of polyphenolics are less than that reported by (Chunhou Zhou et al, 2011).

For at the higher concentration these compounds act as antinutrients and in lower concentration is health benefiting Neutraceutical. As for example A.catechu in small dose has proven anticancer property but at elevated levels it acts as carcinogen. Determination of threshold concentration between health promoting and cancer can be an active research work.

ii) Proanthocynidins

Proanthocyanidins represent a group of polyphenolic bioflavonoids diverse in chemical structure, pharmacology and other characteristics. These have been reported to exhibit a wide range of biological effects including anti-inflammatory, antiarthritic, antiallergic, prevent heart attack and skin aging.

Proanthocyanidins have been shown to serve as free radical scavengers and antioxidants both in vitro and in vivo models. (Bagchi D,1998).

Sabry M. Attia, Saleh A. Bakheet and Nouf M. Al-Rasheed have shown that proanthocyanidins produce significant attenuation of doxorubicin-induced
mutagenicity via suppression of oxidative stress. Pretreatment of mice with proanthocynidins (100 mg/kg/day, orally) for 7 days and simultaneously with doxorubicin (12 mg/kg, i.p.) for another day, significantly reduced the frequency of bone marrow DNA strand breaks and micronucleated polychromatic erythrocytes compared to doxorubicin-treated mice alone (Sabry M. Attia, 2010).

They are pioneers to report that proanthocynidins have a protective role in the abatement of doxorubicin-induced mutagenesis and cell proliferation changes in germinal cells of mice that reside, at least in part, in their radical scavenger activity. To improve treatment efficacy, health beneficial potential of proanthocynidins can be harvested (Sabry M. Attia, 2010).

iii) Flavonoids

Flavonoids belong to a broad family of polyphenolic compounds having antioxidant property. Over 4,000 flavonoids have been identified from various plants (E Middleton Jr et al, 2000). The radical scavenging ability of Flavonoids resides in its molecular structure, the presence of hydroxyl groups, can stabilize the phenoxy radicals with hydrogen bonding, electron delocalization and their metal chelating capacity.

According to Xiaoyun Meng, (2010) flavonoids have been known as plant pigments for over a century and belong to a broad class of polyphenolic compounds (Xiaoyun Meng, et al, 2010).

Flavonoids are among the most secure and reliable substances available for preventing various diseases including maladies related to the oxidative-stress and neurodegeneration (Xiaoyun Meng, et al, 2010).
5.2 HPTLC

The availability of state-of-the-art instrumentation for all steps of the TLC process, the use of HPTLC plates, and the availability of the powerful winCATS-Planar Chromatography Manager software enable modern planar chromatography to stand strong among today's high-performance separation techniques.

Due to its off-line principle, TLC can offer enormous flexibility and an almost unlimited number of choices in the selection of individual parameters. Unfortunately, there is up to now no agreement about what analysts consider as "standard parameters" or "standard procedure".

This fact often limits the repeatability of published TLC methods and has caused some concerns about the reliability of qualitative and quantitative TLC data in general. On the other hand, if methods are properly documented, validated and executed, precise and accurate results can be obtained (Peter Jänchen, 2004 Camag flash US).

The method used was standardized for separation and quantitation of flavonoids. Anchrom laboratory and Camag Biliography service have reported the separation techniques for flavonoids from Withania, [F-08 - HPTLC Fingerprint of Ashwagandha (Withania somnifera)]. The results obtained are very similar to ours.

5.3 FRAP assay

The antioxidant potentials of the ten medicinal plant extracts were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). Antioxidant activity increased proportionally to the polyphenol content. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (Oktay M et al, 2003). Our reports are similar to those reported by Bushra Sultana et al (2009).
5.4 DPPH and ABTS

There has been a notable increase in the research involving screening and identifying plant based antioxidants. Assays based on the use of \( \text{O}_2^{\cdot\cdot} \) and \( \cdot\text{OH} \), DPPH, ABTS\(^{+}\) are among the most popular spectrophotometric methods for determination of the antioxidant capacity of foods, beverages and plant extracts. DPPH and ABTS\(^{+}\) scavenging methods have been the most commonly used to evaluate the antioxidant potential of compounds due to their simple, rapid, sensitive, and reproducible procedures (Matthew S, Abrahim TE, 2006).

According to Adeolu A Adedapo et al, (2008) Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals.

The 2,2'-azinobis- 3- ethylbenzothiazoline-6-sulfonic acid (ABTS) activities of the 2 plant extracts were similar and comparable to that of BHT. Higher concentrations of the extracts were more effective in quenching free radicals in the system. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann J et al, 1979).

Although the DPPH radical scavenging abilities of the extracts were significantly lower than those of ascorbic acid and BHT, it was evident that the extracts did show the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants (Yu. L et al, 2002).

The scavenging of the ABTS\(^{+}\) radical by the extracts was found to be higher than that of DPPH radical. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals Wang et al, (2002)
found that some compounds which have ABTS+ scavenging activity did not show DPPH scavenging activity. In this study, this was not the case. The results indicated that a higher percent of DPPH scavenging is correlated to a higher antioxidant activity as reported by Sultana, B et al, (2007).

5.5 TBARS assay

Assays based on the use of O2•− and ·OH, DPPH, ABTS+, are among the most popular spectrophotometric methods. ·OH scavenging activity of antioxidants can be accomplished through direct scavenging or preventing of ·OH formation through the chelation of free metal ions (TBARS).

The scavenging ability of antioxidants can be determined by Gutteridge, 1987; method, which is monitored in the Fe3+-EDTA–H2O2–deoxyribose system. The extent of deoxyribose degradation by the ·OH formed can be measured directly in the aqueous phase by thiobarbituric acid reactive species (TBARS) assay at 532 nm. This method is based on the fact that the degradation of deoxyribose by ·OH forms a reactive species malondialdehyde (MAD), which forms an adduct with thiobarbituric acid.

The adduct, MDA-TBA, has an absorption at 532 nm that can be assayed spectrophotometrically. By this assay, the ability of several antioxidants to scavenge ·OH has been studied and compared with that of DMTU, uric acid, trolox and mannitol (Floriano-Sanchez et al, 2006 and Haces et al, 2008).

The modification done in our investigation was to use erythrocyte ghosts. The fatty acids in the cell membrane get converted to MAD which was determined by TBA.
5.6 Cytotoxicity assay

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines or primary patient cells. In this chapter the protocol of the assay is described including important considerations relevant for each step of the assay as well as its limitations and possible applications.

Plant based pharmaceuticals exhibit excellent efficacy against certain ailments but at the same time have shown Cytotoxicity, therefore evaluation of Cytotoxicity or cell viability becomes a must. These values are in agreement to previously reported values Sharma et al, (2010).

5.7 Intestinal absorption assay

The evereted intestinal absorption method has been describe in David Plummer, 1990 a practical manual for Biochemistry. The method makes use of mice intestine and has been utilized by many researchers to investigate the amino acid absorption.

After comparing the goat and mice intestinal absorption of amino acids (Not reported in thesis) the method has been standardized for the evaluation of absorption of Polyphenolics, flavonoids and proanthocynidins.

The Flavonoid absorptions are in agreement to the reported data by Sharma et al, (2010).
5.8 Comet assay

We are the pioneer to use Methylene blue for staining of Comets in Comet assay. Methylene blue being a basic dye and carrying positive charge binds the negatively charged DNA. The use of Methylene blue instead of Ethidium bromide ameliorates the use of costly fluorescent microscopes.

Pasquini R et al, (2002) has reported the antimutagenic potential of chloroform, acetone, methanol, methanol + HCl, diethyl ether, and ethyl acetate extracts of *Terminalia arjuna bark* against the model mutagen 4-nitroquinoline-N-oxide (4-NQO) using the Salmonella/ microsome, comet, and micronucleus (MN) tests. The results of the current are in agreement.

5.9 Angiogenesis Assay

There is no published data of angiogenic activity reported for the plants evaluated in the current studies. Shanshan Wang et al, (2004), reported angiogenic activity in 24 herbs traditionally used in china. The herbs we selected have a strong angiogenic activity and hold a promise for further investigation as the potential candidates for not only diabetic retinopathy but also anticancer drugs.

Procedure wise a lot of development in angiogenic protocols is taking place and the most latest is software. This software requires the photographs of the results of angiogenic assay and computationally calculates not only the number of blood vessels but also their internal, external diameter, thickness of the endothelial walls of blood vessels and leakage. The software is going to be available in 3-5 years down the line. Such softwares are going to be boon for retinopathy research for year to come.
Individual plant extracts have not shown any significant angiogenesis but formulation has exhibited a cumulative action. This may be because of synergism.

5.10 Pericyte culture

Several factors are thought to be involved in pericyte recruitment during vascular development and maintenance, including angiopoietin-1 and its receptor tyrosine kinase Tie-2, vascular endothelial growth factor (VEGF)-A and its receptor flk-1, tissue factor, and the platelet-derived growth factor PDGF-B/PDGFR-receptor β system. (Benjamin et al, 1999; Carmeliet et al, 1996; Leeven et al, 1994; Hirschi et al, 1998; Betsholtz et al, 1995).

PDGF-B is critically involved in the recruitment of pericytes to a variety of vascular beds such as brain, kidney, heart, lung, and adipose tissue (Hellstrom et al, 1999). Studies using PDGF-B- and PDGF-receptor β-deficient mice lead to the concept that mesenchymal progenitor cells are initially recruited around vessels through PDGF-independent mechanisms, while subsequent sprouting involves PDGF-B-dependent co migration/proliferation events (Lindahl et al, 1997).

This proliferation is critically hampered in excess blood glucose [25mM]. Streptozotocin induced diabetic mice lack microvascular pericytes and form capillary microaneurysms. (Hellstrom et al, 2001).

Withania somnifera and the formulation did allow the proliferation of pericytes in high concentration of glucose. The morphology of pericytes as seen microscopically also gave significant results indicating that this can be a promising area of research to be explored as far as signal transduction is concerned.
5.11 GCOP [Goat corneal opacity permeability assay]

In all the publications pertaining to the corneal opacity and permeability assays, investigations are done on Bovine corneas. We are reporting for the first time the use of goat corneas. The availability of goat corneas: as the byproduct of the local slaughter house, all through the year and low cost make it a suitable substitute for bovine source (as bovine source is also considered sacred in India) and the material of choice for experimentation.

However the results that were obtained for few chemicals investigated (not reported in the thesis i.e. Amway fabric softener, Cyclohexanone, Johnsons baby shampoo) are in affirmation to that of results reported in bovine corneas (as confirmed by Balls et al (1999).

Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals. In the Draize rabbit eye test, 100 μl of the test substance is introduced into the conjunctival sac of each animal’s eye. Alternatives to the Draize test have been explored to reduce the possibility of pain and distress during the test procedure. No test was found capable of replacing the Draize rabbit eye test, but GCOP assays showed considerable promise as screens for ocular irritancy.

The onset of retinopathy is because of four reasons- Oxidative stress, hyperglycemia, improper angiogenesis and weakening of pericytes the smooth muscle cells lining the vascular endothelium in retina. So the aim of the current research was to formulate an herbal formulation that will act as preventive remedy against Diabetic retinopathy.

The very purpose of the research was to develop and test the innovative formulation for its ability to overcome the four problems associated with diabetic retinopathy.
i. Oxidative stress.

ii. To control and or regulate the blood glucose.

iii. To promote angiogenesis and

iv. To impart strength to pericytes.

i. Oxidative stress

The formulation contains, secondary metabolites (Polyphenolics, Proanthocynidins and Flavonoids) derived from five plants, A. catechu, E. officinalis, F. benghalensis, F. glomerata and Terminalia arjuna to overcome oxidative stress.

In living organisms the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damage to lipids, proteins, enzymes, and nucleic acids leading to cell or tissue injury implicated in the processes of aging as well as in wide range of degenerative diseases including inflammation, cancer, atherosclerosis, Diabetes, liver injury, Alzheimer, Parkinson, and coronary heart pathologies, among others.

The ROS and the RNS include diverse reactive entities namely superoxide ($O_2^-$), hydroxyl ($OH^-$), peroxyl ($ROO^-$), peroxinitrite ($ONOO^-$), and nitric oxide ($NO^+$) radicals, as well as non free radicals species as hydrogen peroxide ($H_2O_2$), nitrous acid ($HNO_2$), and hypochlorous acid ($HOCl$).

On the other hand, the aerobic organisms developed antioxidant defense mechanisms that arrest the damage caused by ROS and RNS entities. The defense mechanisms can be enzymatic and nonenzymatic.
In the enzymatic mechanisms are included, for instance, superoxide dismutase, catalase, and glutathione reductase and peroxidase, and nitric oxide synthase enzymes, among others.

On the contrary, in the non-enzymatic mechanisms are comprised antioxidants and trapping agents such as ascorbic acid, α-tocopherol, β-carotene, glutathione, flavonoids, uric acid, cysteine, vitamin K, serum albumin, bilirubin, and trace elements as zinc and selenium, among others. Both processes can contribute to prevent the damage caused by oxidative reactions.

Since the natural antioxidant mechanism in mammals under some circumstances can be inefficient, a dietary intake of antioxidant compounds becomes an alternative, once it has been suggested that there is an inverse relationship between dietary intake of antioxidants and the incidence of diseases caused by the deficiency on these substances.

In recent years, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are added to food preparations because they are good free radical scavengers, even though there are some experimental evidences that they induce DNA damage.

Therefore, there is an increasing interest in searching antioxidants from natural origin to scavenge free radicals to prevent human body from oxidative stress produced by ROS and RNS species.
From the results (Fig 5.1) it can be said as there is increase in the concentration of polyphenolics, proanthocynidins and flavonoids, there is increase in antioxidant power and radical scavenging ability.

ii. To control and/or regulate the blood glucose.

The insulin like polypeptides of *Momordica charantia* [Karela], Bitter compounds of *Syzgium cumini* [Jambool beez], *Tinospora cordifolia* [Gulvel], and Saponins of *Trigonella foenum* [Methi], help in maintaining blood glucose [For their hypoglycemic properties].

A study carried out by E N Sundaram et al (2009) reports that Diabetes was induced in rats by single dose of streptozotocin (30 mg/kg i. p.) oral doses of 250 mg and 500 mg/kg/d for 30 d. Glibenclamide (300 μg/kg/d) was used as a reference drug. They have further shown that Streptozotocin diabetic rats showed a significant increase in serum glutamic oxaloacetate transminase and serum glutamic pyruvate transminase activities and serum urea concentration but
a significant decrease in serum total protein and albumin concentrations and albumin/globulin ratio.

Oral administration of alcoholic extract of *Momordica charantia*, *Aegle marmelos* and *Eugenia jambolana* in daily doses of 250 mg and 500 mg/kg for a period of 1 mo produced dose- and duration-dependent decrease in serum glutamic oxaloacetate transminase and serum glutamic pyruvate transminase activities as well as decrease in serum urea concentration and restored the serum total protein and albumin concentration and albumin/globulin ratio to a great extent in streptozotocin diabetic rats.

The beneficial effects of these plants in 500 mg/kg dose in streptozotocin diabetic rats were comparable to that of glibenclamide (300 µg/kg), a standard oral hypoglycaemic drug used in clinical practice (E N Sundaram et al, 2009).

Akanksha et al, (2009), have reported a hyperglycemic compound FB isolated from aerial roots of *F benghalensis* lowered blood glucose by 19.4% at a dose of 100mg/Kg in normoglycemic model.

When Compound FB was administered in sucrose at a dose of 50mg/kg STZ induced rats blood glucose lowered by 35.6%, P<0.01 comparable to metformin (37.8%, P <0.01) In db/db mice compound FB exhibited the results (shown in table 5.1). Different parts of the plant *Ficus glomerata* Roxob extracted with water and organic solvents. The effects of these extracts on blood sugar level of streptozotocin induced diabetic rats have been studied. The pet ether extract of the stem bark of the plant have shown to reduced the blood sugar level significantly.

<table>
<thead>
<tr>
<th>Dose 50mg/Kg</th>
<th>Compound FB</th>
<th>Metformin</th>
</tr>
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<tbody>
<tr>
<td>3rd Day</td>
<td>18.7%(P &lt; 0.05)</td>
<td>17.8%(P &lt; 0.05)</td>
</tr>
<tr>
<td>5th Day</td>
<td>27.1%(P &lt; 0.05)</td>
<td>30.5%(P &lt; 0.05)</td>
</tr>
<tr>
<td>7th Day</td>
<td>40.0%(P &lt; 0.01)</td>
<td>39.4% (P &lt; 0.01)</td>
</tr>
<tr>
<td>10th Day</td>
<td>51.6%(P &lt; 0.01)</td>
<td>52.5% (P &lt; 0.01)</td>
</tr>
</tbody>
</table>

Table 5.1 Antidiabetic action of FB compound (Akanksha et al, 2009)
Extracts from fruit and latex of the plant have not shown any significant effect on blood sugar level of these diabetic rats as reported. The pet ether extract of the stem bark have been reported to completely inhibit the enzymes glucose-6-phosphatase and arginase and activated the enzyme glucose-6-phosphate dehydrogenase from rat liver. Extracts from fruit and latex inhibited only glucose-6-phosphatase but not arginase from rat liver (N. N. Rahman et al, 1994).

**GII at a** dose of 50 mg/kg bw per day brought down the elevated FBG levels in the untreated subdiabetic (FBG 96.6 ± 7 mg/dl), moderately diabetic (150.1 ± 14 mg/dl) and severely diabetic rabbits (427 ± 46 mg/dl) to normal in 12, 15 and 28 days of treatment has been reported. It has been further demonstrated to improve serum HbA1C and insulin levels also in these rabbits. D Puri et al, (2000) have further demonstrated that intermittent therapy once a week for 6 weeks with GII at the same dose brought down the FBG values to normal in the subdiabetic (FBG 96.0 ± 2 mg/dl) and in the moderately diabetic rabbits to 133.0 ± 12 mg/dl.

After stopping therapy of the sub diabetic and moderately diabetic rabbits whose FBG values came to normal after treatment with GII 50 mg/kg bw, the values remained normal for 1 week and showed a tendency to increase only after 15 days. If these animal studies are applicable to humans these results indicate that a diabetic person need not take GII daily when once the FBG value comes to normal or near to normal. Patients might be able to take GII only when the FBG value shows tendency to increase. So, intermittent therapy is possible with the potent product GII of the fenugreek seeds which is of a great advantage. (D Puri et al, 2012).

Fenugreek seed extract IND01 (100 mg/kg, oral) and glyburide (10 mg/kg, oral) treatment have shown significant reversal of n-STZ-induced changes (rise in SG, decline in body weight and rise in HBA1c) by Chetan PK et al, (2012). They have further reported histology sections of pancreas from the rats treated with IND01 (but not glyburide) showed increase in number and size of pancreatic islet β-
cells. IND01 showed a potential to ameliorate symptoms of DM during progressive deterioration and improved glycemic functions in n-STZ induced diabetic rats (Chetan PK et al, 2012).

Oral administration of MCSEt1 and MCSEt2 *M. charantia* extracts resulted in a significant reduction in the levels of cholesterol, phospholipids, triglycerides and free fatty acids in plasma and tissues of STZ-induced diabetic rats as reported by D Sathish et al (2006). The study further demonstrated that the altered fatty acid composition in liver and kidney were restored by the treatment. In their study Glibenclamide was used as a reference drug to compare the efficacy of the seeds extract hypolipidemic effect of *Momordica charantia* (D. Sathish Sekar et al, 2006).

Rajlaxmi et al, 2009 have reported the oral administration of various extracts (hexane, ethyl acetate and methanol) of *Tinospora cordifolia* stem (TCS) were found to have potent antidiabetic activity that reduces blood sugar level in streptozotocin-(STZ) induced diabetic rats. They investigated the chronic (100 days) antihyperglycemic effect of the extracts at a dose of 250 mg/kg b.w.p.d of TCS. In their study, insulin was used as a reference drug at a dose of 3 I.U/kg.b.w.p.d. They evaluated fasting blood glucose, glycosylated hemoglobin (HBA1C), serum insulin, C-peptide and liver enzymes levels in normal, diabetic and treated rats. Supplementation of methanol extract significantly reduces the fasting blood glucose level as compared to other 2 extracts. In diabetic control (p < 0.001) the TCS treated groups, the insulin and C-peptide levels were improved which shows the regeneration of β-cell which secretes insulin, histopathological studies of pancreas of TCS methanol extract treated groups also exhibits the regenerating capacity of extract.

Oral administration of the hydro-methanolic (20:80) extract of leaves of *Emblica officinalis* Gaertn. (HMELEO) at a concentration of 100, 200, 300 and 400 mg/kg
b.w. daily for 45 days showed a significant (P<0.05) decrease in fasting blood glucose and increase insulin (Nain P et al, 2012).

iii. To promote angiogenesis

There is no published data of angiogenic activity reported for the plants evaluated in the current studies. Shanshan Wang et al, (2004), reported angiogenic activity in 24 herbs traditionally used in china. The herbs we selected have a strong angiogenic activity and hold a promise for further investigation as the potential candidates for not only diabetic retinopathy but also anticancer drugs.

Flavonoids apigenin, luteolin, orientin, quercetin, vitexin, and isovitexin; free amino acids, calcium and iron; saponins of Trigonella foenum helps in proper blood vessel formation.

iv. To impart strength to pericytes.

The alkaloids nicotine, somnine, somniferine, somniferinine, withanine, withaninine, pseudowithanine, tropane, pseudotropane, choline, anaferine of Withania somnifera, not only imparts strength to pericytes but also allows them to proliferate and divide at an alleviated blood glucose levels [25mM] which is the case in diabetes.

Since the study is recommending edible formulation, the cytotoxicity [MTT assay] was performed. The drug was evaluated for its absorption via intestine [using goat intestine model]. Safety of the drug can be guaranteed for it is reducing lipid peroxidation [TBARS assay], No DNA damage [Comet assay], and Imparts strength to vascular pericytes [Pericyte culture and CAM assay]. The results of the entire assays are promising and the drug can be safely consumed.
5.13 Conclusion

From the experimentations it can be concluded that the plant extract selected are rich in flavonoids, the formulation is safe for consumption as evaluated by cell viability assay. It has a good antioxidant potential as illustrated by DPPH assay, it also has a good intestinal absorption.

The formulation not only combats lipid peroxidation but also prevents DNA Damage as checked by Comet assay. Cam assay also exhibits promising results and Pericyte cell cultivation in high concentration of glucose [25 mM] were cells do under go division and proliferation are of immense significance if we look at Pathophysiology of diabetic retinopathy. Prevention is always better than cure so here we suggest the daily consumption of just 0.5 mg of the formulation will keep the retinopathy away.

However the clinical significance of the formulation is under way. A total of 1532 diabetic patients are given taking the formulation, the study is underway. The results will truly evaluate the potential of the formulation.

END NOTE: The benefits of medicinal plants are immense and need of the day is in situ and ex situ conservation of plants. Together lets promote the idea of cultivating, growing and preservation of the germplasm. Our responsibility is even more for we (India) are within the top ten biodiversity hotspots (2nd) of the world.

"If all of these natural remedies are so safe and their side effects so uncommon, why has western medicine not employed them in the battle against diabetes and other degenerative diseases?"

The fact is, medical science has become overwhelmingly dependent on the pharmacological approach to disease management. So enamored are western medical practitioners with the allure of high-tech medicine that they lack interest in the more down-to-earth cures offered by Mother Nature.