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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF NIRGUNDI AND SACRED FIG FOR ANTIDIABETIC ACTIVITY

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ABSTRACT
For thousands of years the Indian traditional medicine, Ayurveda has been practiced in preventing and treating diseases and to maintain health. Some of these compounds may have therapeutic potential, while others may produce hypoglycemia as a side effect of their toxicity, especially hepatotoxicity. Several studies reported Nirgundi and Sacred fig used for treating various diseases and disorders. It is essential to investigate the properties of active phytocompounds. The solubility of the extract was higher in polar solvents like ethanol and water. It can be concluded from the above studies that nirgundi leaves possess potential bioactive compounds that have anti-inflammatory activity. Phytocompounds from the leaf of nirgundi plant can be used to discover bioactive phytodrugs that may serve as lead for development of a new antidiabetic drug. The significant antidiabetic and antioxidant activity indicates potential of this plant as a promising agent in the development of newer antidiabetic agent. It is also essential to identify and understand the molecular mechanism of its antidiabetogenic effect and to identify the bioactive compounds responsible for this effect. Our investigations confirmed the traditional use of this plant in the field of diabetes have clearly supported its use. These flavonoids are reported to possess good antidiabetic activity.

KEYWORD: Antidiabetic activity, Nirgundi, Sacred fig, Phytodrugs, Ayurveda.

INTRODUCTION
Nature is an inexhaustible source of natural compounds with interesting biological activities. The interesting chemicals identified as natural products are derived from the phenomenon of biodiversity in which the interactions among organisms and their environment formulate the diverse complex chemical entities within the organisms that enhance their survival and competitiveness. Natural products have been the major sources of chemical diversity for starting materials while driving pharmaceutical discovery over the past century. Today natural products are finding increasing use as probes to interrogate biological systems as part of chemical genomics and related researches.[1]

Human beings have been dependent on nature for their simple requirements as being the sources of clothing, flavours, fertilizers, medicines, shelters, food stuffs, fragrances and means of transportation throughout the ages.[2,3] For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use. The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing nations.[4,5]

Medicinal plants are rich in many organic compounds of great medicinal importance. The flavonoids are one of them and every group of flavonoids hascapacity to act as antioxidant, antitumor, anticancer agents and antimicrobial agents. Among them, flavones and catechin components act as the most powerful flavonoids.[6] The other flavonoid components such as quercetin, kaempferol, myricetin and rutin have antioxidant, anti-inflammatory, antibacterial, antiviral and antiallergic, as well as anticancer activities. Among Indian herbal plants, at least thirty have shown antitumor activities. Epidemiological studies have shown that many of the phytochemicals possess anti-inflammatory, anti-atherosclerosis, antitumor, anticancer, antimutagenic, anticarcinogenic, antibacterial and antiviral activities. Their use is also associated with reduced risk of cancer,

cardiovascular diseases, diabetes, rheumatoid arthritis and lower mortality rates of several human diseases.[7,8]

Diabetes mellitus

Diabetes mellitus is a metabolic disorder characterised by hyperglycemia arising as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both. This is a major and growing public health problem throughout the world, with an estimated worldwide prevalence of 171 million in 2000, expected to increase to 380 million people by 2035. In particular, the number of people with diabetes in India, currently around 40.9 million, is expected to rise to 69.9 million by 2030. India leads the world with largest number of diabetic subjects thus earning the dubious distinction of being termed as "Diabetes capital of the world". Although the prevalence of diabetes is consistently increasing, an effective treatment is still acking. Current pharmacotherapeutics insufficiently reverse hyperglycemia, having limited tolerability and induce side effects. Hence the identification of new pharmacological approaches to effectively prevent, treat and cure this metabolic disorder is of crucial importance. In recent times, many traditionally used medicinally important plants have been tested for their antidiabetic potential by various investigations in experimental animals.[9,10]

Diabetes mellitus is a disorder of metabolism of carbohydrate, protein and fat associated with a relative or absolute insulin secretion and with various degrees of insulin resistance. It is recognized as one of the leading causes of morbidity and mortality in the world. Seghrouchni reported that about 0.5-3 % of the world's population is suffering from this disease, a portion which in some countries reaches to 7% or more. In all its form, at least 200 million people in the world are affected and this number is expected to double by the year.[11] Fully developed clinical expression is characterized by fasting hyperglycemia. Mehring and Minkowski in the late 19* century demonstrated that pancreatectomy resulted in diabetes in experimental animals. Earlier Langerhans (1869) recognized the structural differences of islets from remainder of the pancreas in rabbit and these islets were subsequently known as islets "of Langerhans". Recognized the islets of Langerhans as endocrine tissue. Prepared an alcohol soluble extract from dog pancreas whose duct had been ligated several weeks earlier. They injected the alcohol soluble extract to pancreatectomised dogs, which alleviated diabetes. This anti-diabetic hormone was named insulin. Published literature on the comparative physiology of the pancreatic islets. Although major phenotypic differences in types of clinical diabetes have been appreciated for centuries, it is only in the last 30 years that newer knowledge of the cause and pathogenesis of diabetes are understood. Though the knowledge is incomplete it is imperative that diabetes is a heterogeneous group of disorders. Thus diabetes is not a single disease but a syndrome.[12,13]

Diabetes mellitus sub types[14,15,16]

Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced.There are three main types of diabetes mellitus:

- Type 1 diabetes results from the pancreas's failure to produce enough insulin due to loss of beta cells. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The loss of beta cells is caused by an autoimmune response. The cause of this autoimmune response is unknown.
- Type 2 diabetes begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses, a lack of insulin may also develop. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The most common cause is a combination of excessive body weight and insufficient exercise.
- Gestational diabetes is the third main form, and occurs when pregnant women without a previous history of diabetes develop high blood sugar levels.

Collection of Plant Material

Plants namely Nirgundi and Sacred fig were used for this study. The different parts of the medicinal plant were dried leaves and bark of Sacred fig and Nirgundi were procured in the month of June. The leaves were dried under shade and after that, powdered to fine texture and 100g each of the dried leaves were extracted with water and alcohol. The extracts were concentrated under vaccum and the residue were used in the experiments.

Solvent Extraction (Soxhlet method)

The powdered plant material (100 g) was treated successively with different solvents in the increasing order of polarity- Hexane, chloroform, methanol for 48 hours in the soxhlet apparatus and finally it was boiled with distilled water for 6 hours. Soxhlet extraction is required where the desired compound has a limited solubility in a solvent and the impurity is insoluble in that solvent. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.[17,18]

Preparation of the extracts

Prior to any isolation and purification work, natural products have to be extracted (or released) from the biomass. This could be with a view to isolate a known metabolite or to isolate and characterize as many compounds as possible (some of unknown structure) in the context of a systematic phytochemical investigation.[19] Several approaches can be employed to extract the plant material. Although water is used as an extractant in many traditional protocols, organic solvents of varying polarities are generally selected in modern methods of extraction to exploit the various solubility of plant constituents. In the present study ethanolic extracts of Sacred Fig and Nirgundi of were prepared by soaking

them in ethanol, overnight and then refluxing for three hours, thrice with ethanol. The colour, consistency and percentage yield of each extract was presented in table 1.1.

Table 1.1: Physicochemical Evaluation.

S. No	Physicochemical Evaluation	Nirgundi	Sacred fig
1.	Colour	Dark greenish	Dark brown
2.	Consistency	Semi solid	Semi solid
3.	Yield	8.5 g /100g of dried powder	6.2 g /100g of dried powder

Loss on drying

The loss on drying was 7.5% and 8.5% for nirgundi and for sacred fig respectively. The average values are expressed as percentage of air-dried material. The percentage of active chemical constituents in crude drug is mentioned on air-dried basis. Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. The moisture content of dry powder of nirgundi was 7.5% which is not very high, hence, Bhattacharya and Zaman reported that if moisture content is very low, it will not allow the growth of microbes. The moisture content of dry powder of sacred fig was 8.5% which is not very high, hence, Reported that if moisture content is very low, it will not allow the growth of microbes.[20]

Total Ash Content

The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts, silica etc. Determination of ash residues plays a significant role for standardization of the indigenous crude drugs. Total ash value of the leaf crude powder of nirgundi and sacred fig was 8.5%and 8%. Low amount of total ash showed that the metal salts and silica were absent or very low, which was in accordance to. In our study the total ash value was 8.5% and 8%. In this test we conclude that total ash was very less percentage which shows that absence of foreign inorganic matter.[21,22]

Solubility Test

The Solubility test is useful to find the bioactive substances present in the plant material. Sharma reported that high alcohol soluble and water soluble extractive values reveal the presence of polar substance like phenols, tannins and glycosides. In solubility test, various solvent such as petroleum ether, chloroform, ethyl acetate, ethanol, and water were used. The extractive yield percentage was calculated by using various solvents. Ethanol showed higher amount of extraction 22.8% and 35.5% than the other solvent. Water yield was 24.1%. The petroleum ether had comparatively showed low level of 14.8%. In this study, we examined that the crude powder was soluble in various solvents but the yield percentage will vary from solvent to solvent. In high polar solvents, the percentage of yield was higher than other solvents.[23]

Extract recovery percentage

The most commonly and widely used extraction technique for the extraction of natural product is Soxhlet extraction using Soxhlet apparatus. The results of the extraction recovery percentage were given. The maximum yield was obtained in ethanol which was 8.5 g /100g of dried powder respectively. The minimum yield was obtained in ethyl acetate solvent yield 6.2 g/100g. Chloroform solvent yield 7.2 g /100g. The yield percentage was in lower polar solvents like Petroleum ether and chloroform comparatively very low than high polar solvents like ethyl acetate and ethanol. The result conclude that the leaf may contain more polar compounds than non-polar compounds.[24]

Table 1.4: Parameters of Crude Powder of Nirgundi.

S.No	Parameters	Value (W/W)
1.	Loss On Drying	7.5%
2.	Total Ash	8.5%
3.	Petroleum Ether Soluble Extraction	15.7%
4.	Ethanol Soluble Extraction	21.5%
5.	. Water Soluble Extractive	25.5%

Table 1.3 Parameters of Crude Powder of Sacred Fig.

S.No	Parameters	Value (w/w)
1.	Loss on drying	8.5%
2.	Total ash	6.5%
3.	Petroleum ether soluble extraction	12.7%
4.	Ethanol soluble extraction	18.5%
5.	Water soluble extractive	23.5%

The combined administration of Allaxon leads to the development of moderate and stable hyperglycemia with reduced pancreatic insulin stores. It leads to a partial loss of the cell mass which occurs by necrosis and/or apoptosis, induced by the relatively specific β cytotoxic effect of allaxon, which is partially counteracted by allaxon. This model appears close to human NIDDM with regard to insulin responsiveness to glucose. Animals were injected with allaxon at a dose to induce stable hyperglycemia which mimics diabetic syndrome.[26]

Table 1.6: Effect of extracts on body weight.

Treatment	Dose/day (p.o)	Body weight (g)			
		On day 1	On day 10	On day 20	On day 30
Normal control	5 mL/kg	182.67±5.87	200.50±4.50	217.17±3.78	236.00±4.74
diabetic rats treated with standard drug	2 mL/kg	176.50±6.20	158.50±9.51	161.67±14.79	150.67±10.91
diabetic rats induced with alloxan (100mg/kg).	100 mg/kg	176.17±8.99	182.00±11.58	194.83±12.18	207.67±9.05
alcoholic extract of nirgundi treated with 100mg/kg.	100 mg/kg	239.17±5.79	237.50±6.51	226.00±5.21	219.33±3.95
alcoholic extract of nirgundi treated with 200mg/kg.	200 mg/kg	211.50±12.13	179.17±11.29	161.50±14.15	* 183.17±10.66
alcoholic extract of nirgundi treated with 300mg/kg.	300 mg/kg	215.50±6.83	189.17±13.89	191.50±9.32	177.17±17.17
alcoholic extract of sacred fig treated with 100mg/kg.	100 mg/kg	186.17±7.78	177.50±6.65	179.67±18.86	188.00±13.12
alcoholic extract of sacred fig treated with 200mg/kg.	200 mg/kg	240.16±9.88	182.00±8.11	187.00±6.48	157.50±9.88
alcoholic extract of sacred fig treated with 300mg/kg.	300 mg/kg	194.83±4.81	187.00±6.48	156.16±5.98	229.16±15.94

All values are expressed as mean. n=6
n=Number of animals per treatment
Significantly different from diabetic control *p<0.05

Body weights of allaxon injected rats were found to be statistically less (p<0.01) as compared to normal rats and associated with observations of diabetic symptoms like polyuria, polydipsia and polyphagia. Induction of diabetes by allaxon leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins. The failure of diabetes animals to gain weight during the course of time is due to continuous excretion of glucose because of the defect in peripheral uptake and impairment of liver's capacity to synthesize glycogen. Body weights of all the animals were measured on day 1st, 10th, 20th and 30th day and the results are presented in table 5.6. There was no lethality or toxic reaction in diabetic and treated animals during the course of study at the administered dose level. Normal rats gained body weight significantly throughout the experimental period, while both diabetic control and extract treated diabetic animals had significantly lower body weights when compared to normal animals. The loss in body weight in rats resulted from hyperglycemic state has not been improved by the treatment with the extracts. However, the body weight of the treated group showed a significant improvement after the completion of treatment. But the diabetic control group showed a significant weight loss at the end of the experiment.

Histopathological studies

After blood sampling for the biochemical analysis, the animals were sacrificed, pancreas, liver and kidney were quickly dissected, perfused with normal saline and fixed in 10% formalin. The samples were processed in tissue processor and embedded in paraffin. The paraffin blocks were cut using microtome and stained with Haematoxylin and Eosin and studied under microscopy at 100X magnification.

The photographs of the histopathological examination carried out on pancreas, liver and kidney of normal rats, diabetic rats and diabetic rats treated with various test preparations are shown in the following figures. Pancreas Histology of the pancreas of control rat showed normal pancreatic pattern. Islet cells were distributed evenly with their nucleus lightly stained than the acinar cells. Sections of the pancreas of extract treated diabetic rats showed normal pancreatic acini in lobules separated by thin bands of hyalinised fibro collagenous tissue. Islet cells could not be made out in case of diabetic control group. Smaller lymph nodes were seen adherent to the pancreas which showed nonspecific reactionary changes.

Liver

The liver of the control rats showed normal portal triads and hepatic veins. Hepatocytes showed normal morphology and they were arranged in cords. Hepatocytes of the diabetic rats appeared smaller in size, but they were arranged in cords and sinusoidal spaces appeared dilated. The diabetic rats treated with standard drug showed normal portal triads and hepatic veins. Hepatocytes showed normal morphology and they were arranged in cords. Kupffler cells and sinusoidal spaces appeared normal. We used the procedure from **Lucchesi et al., (2015)**.[27]

Kidney

Examination of the kidney of normal control rats showed normal glomeruli and bowman's capsule. Afferent and efferent arterioles were normal. Renal tubules showed normal morphology. Diabetic control rats showed hypo cellular glomeruli, some of the glomeruli appeared totally atrophied and no glomeruli tufts seen. Renal tubules were lined by normal cuboidal cells. Interstitial tissue showed hemorrhage and scattered inflammatory

cells. The standard treated rats showed normal glomeruli with normal glomerular tufts. Bowman's capsule also appeared normal. Interstitial tissue showed hemorrhage, fibrosis and inflammatory reaction. Renal tubules were lined by low cuboidal epithelial cells. Interstitial tissues showed hemorrhage, some area showed fibrosis and inflammation. A few glomeruli (about 5%) showed decreased cellularity with widened bowman's capsule. Renal tubules were lined by low cuboidal epithelial cells. Interstitial tissues showed hemorrhage. A few (about 2%) glomeruli showed decreased cellularity. Renal tubules were lined by cuboidal cells. Interstitial tissue appeared normal.[27]

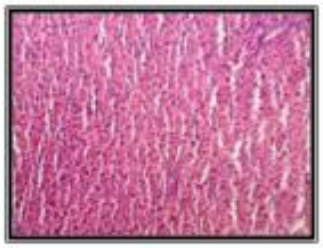

FIGURE 5.1 Group1

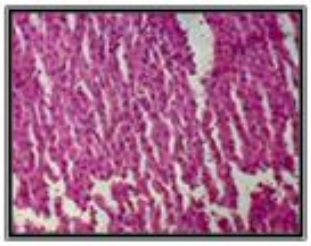

FIGURE 5.2 Group2

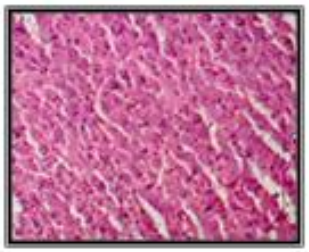

FIGURE 5.2 Group3

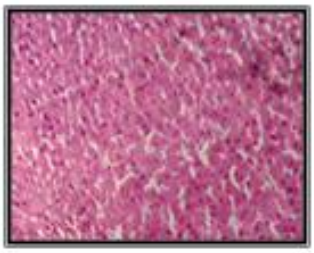

FIGURE 5.2 Group 4

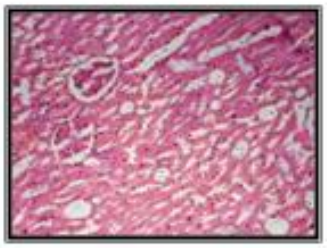

FIGURE 5.2 Group 5

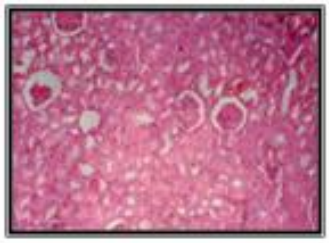

FIGURE 5.2 Group6

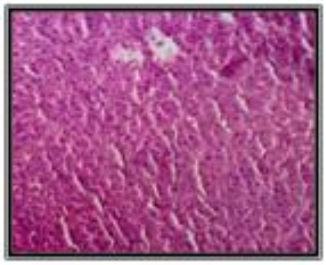

FIGURE 5.2 Group 7

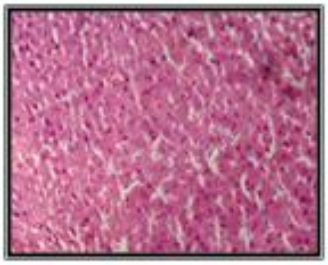

FIGURE 5.2 Group 8

CONCLUSION

Herbal drugs are the oldest known healthcares available to mankind, enlisted in naturopathic, ayurvedic, homeopathic and other medicine systems obtained from natural sources. Being obtained from natural sources the toxicity profile is low for herbal drugs and possesses characteristics like low/minimum cost, complete accessibility and enhanced tolerance. In physicochemical analysis, crude powder of nirgundi leaves was free from heavy metals. The highest extractive value was obtained

from ethanol. The solubility of the extract was higher in polar solvents like ethanol and water. It can be concluded from the above studies that nirgundi leaves possess potential bioactive compounds that have anti-inflammatory activity. Phytocompounds from the leaf of nirgundi plant can be used to discover bioactive phytodrugs that may serve as lead for development of a new antidiabetic drug. The significant antidiabetic and antioxidant activity indicates potential of this plant as a promising agent in the development of newer antidiabetic agent. It is also essential to identify and understand the molecular mechanism of its antidiabetogenic effect and to identify the bioactive compounds responsible for this effect. Our investigations confirmed the traditional use of this plant in the field of diabetes have clearly supported its use. These flavonoids are reported to possess good antidiabetic activity. The chronic study was evaluated by using the rats. in histopathological studies, abnormalities induced by diabetic agens including allaxon was caused at for intervals in heart pancreas and kidney. The aqueous extract of both the plant were found to be effective.

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Conflict of Interest

The authors declare no conflict of interest.

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