CHAPTER 2

ISOLATION AND CHARACTERIZATION OF ANTIDIABETIC ACTIVE PRINCIPLE FROM LIPPIA NODIFLORA L.

2.1. INTRODUCTION

Since ancient times, plants have formed the basis of traditional medical systems, such as Indian, Chinese and African ones. In recent years, the interest in folk medicine from different cultures, also known as traditional medicine, has increased significantly in industrialized countries, due to the fact that many prescription drugs worldwide have originated from the tropical flora (Nelson-Harrison et al., 2002).

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Traditional knowledge and historic literature on medicine play an important role in the discovery of novel leads from medicinal plants. Recently, the search for novel antidiabetic medicines has focused on medicinal plants because of their efficacy in human clinical trials and the minimal side effects of drugs derived from medicinal plants. In view of the increasing prevalence, there is a growing need to develop integrated approaches towards the management and prevention of diabetes mellitus by exploring the potential of traditional phytotherapy (Tag et al., 2012).

The plant kingdom is a potential source of new drugs. An alternate strategy for the fractionation of plant extracts rather than on a particular class of compound is necessary since not all the chemical compounds elaborated by plants are of equal interest to the pharmacognosis. The active principles are frequently alkaloids, steroids, flavonoids or glycosides and therefore, deserve special attention.
2.2. Phytoconstituents with hypoglycaemic potentials

Herbal products or plant products rich in phenolic compounds, flavonoids, terpenoids, steroids, coumarins and other constituents showed reduction in blood glucose levels (He et al., 2005; Jung et al., 2006; Ji et al., 2009). The introduction of these indigenous herbal compounds in the management of diabetes mellitus will greatly decrease the secondary complications and make it less expensive.

2.2.1. Flavonoids

Many kinds of flavonoids have been isolated from traditional plants for antidiabetic effects. Flavonoids are naturally occurring phenolic compounds that are widely distributed in plants. Over 5000 different flavonoids have been described to date and they are classified into at least 10 chemical groups (Harbone, 1993).

Due to the presence of phenolic hydroxyl groups, flavanoids have strong antioxidant properties. They are scavengers of reactive oxygen and nitrogen species and therefore, inhibit peroxidation reactions. They also protect macrophages from oxidative stress by keeping glutathione in its reduced form (du Thie and Crozier, 2000; Fuhrman and Aviram, 2001). Flavonoids have the capacity to inhibit enzymes such as cyclooxygenases and protein kinases involved in cell proliferation and apoptosis (Formica and Regelson, 1995).
Classification of flavonoids

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Flavonoids</th>
<th>Food source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavones</td>
<td>Apigenin, Luteolin, Isoorieentin</td>
<td>Apple skin, celery</td>
</tr>
<tr>
<td>Flavonols</td>
<td>Quercetin, Kaempferol, Myricetin</td>
<td>Onions, apples, tea</td>
</tr>
<tr>
<td>Flavanols</td>
<td>Catechin, Epicatechin, Epigallocatechingalate</td>
<td>Tea</td>
</tr>
<tr>
<td>Flavanones</td>
<td>Hesperitins, Naringenin</td>
<td>Citrus fruit, grapefruits</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>Cyanidin</td>
<td>Berries</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Genestin, Daidzein</td>
<td>Soy</td>
</tr>
</tbody>
</table>

2.2.2. Alkaloids

The alkaloids represent a group of natural products that have had a major impact throughout history on the economic, medical, political and social affairs of humans. They are a diverse group of low molecular weight nitrogen-containing compounds derived mostly from amino acids (Ziegler and Facchini, 2008). These secondary metabolites are found in about 20% of plant species and they are classified as true alkaloids, which have nitrogen atoms in heterocyclic rings, protoalkaloids, which do not have the nitrogen atom(s) in heterocyclic rings and pseudoalkaloids, which don’t derive from amino acids but may have nitrogen atoms in heterocyclic rings (Henriques et al., 2004). Several alkaloids are being used in therapeutics and as pharmacological tools. Alkaloids include hypoglycemic (Falcao et al., 2008), antimicrobial, anti-inflammatory, antitumor, diuretic, sympathomimetic, antiviral, antihypertensive, hypnoanalgesic, antidepressant, miorelaxant, antitussig properties and biological activities (Henriques et al., 2004).
A few compounds were isolated for diabetes from numerous Indian medicinal plants and investigated for their possible hypoglycemic activity in different animal models. They are; catharanthine, trigonelline, leurosine, lochnerine, tetrahydroalstonine, vindoline and vindolinine (Perez et al., 1998).

2.2.3. Terpenoids and steroids

Terpenoids and steroids glycosides, referred to collectively as saponins, are bioactive compounds present naturally in many plants and known to possess potent hypoglycemic activity (Rao and Gurfinkel, 2000). Terpenoids are many compounds differentiated by, monotriterpenoids, sesquiterpenoids, diterpenoids and triperpenoids. They are commonly found in essential oils. The pharmaceutical applications of triterpenes and steroids are considerable. Saponins from ginseng and liquorice exhibit many therapeutic effects. Triterpenoids and saponins are the promising compounds with potential to develop new drug for diabetes (Li et al., 2004; Mukherjee et al., 2006).

2.2.4. Polysaccharides

Many kinds of polysaccharides have been isolated from medicinal plants against diabetes. Most of these increased the level of serum insulin, reduced the blood glucose levels and improved tolerance of glucose. Examples are panaxan, laminaran, coixan, pachymaran, anemarn, moran, lithosperman, trichosan, saciharan, ephedran, abelmosan, atractin (Li et al., 2004).
2.2.5. Insulin like compounds, polypeptides and aminoacids

These substances are excellent for the treatment of diabetes. Examples include p-insulin (*Momordica charantia*), ginseng glycopeptides, α-ethylcyclopropylglycin and S-allylcysteinsulfoxide (Li *et al.*, 2004).

2.2.6. Sterols

The hypoglycemic effects of sterols are similar to sulfonylurea - like medicine. An example is charantin (*Momordica charantia*) (Li *et al.*, 2004). Charantin increases glucose uptake and glycogen synthesis in the liver, muscle and adipose tissue and improves glucose tolerance (www.tropilab.com).

2.2.7. Unsaturated fatty acids

The efficiency of antihyperglycemia is strong but the effectiveness is shown slowly. Examples are linoleic acid and trihydroxyljecoric (Li *et al.*, 2004).

2.2.8. Miscellaneous

Compounds with a sulphur, such as allicin and allylpropyl disulfide, 3-hydroxy-3-methylglutaric acid, sodium oxaloacetate, edyson are active compounds with varied structures (Li *et al.*, 2004).

2.2.9. Antidiabetic compounds isolated from medicinal plants

Some of the important anti-diabetic herbal plants and their active principles are given in table 2.1.
Table 2.1: Potential anti-diabetic herbal plants and their active principles

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Class of compound</th>
<th>Antidiabetic compound</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abelmoschus moschatus</em></td>
<td>Flavanoids</td>
<td>Myricetin (3,5,7,3’,4’,5’,-hexahydroxyflavone)</td>
<td>Antidiabetic effect (Liu et al., 2007)</td>
</tr>
<tr>
<td><em>Agarista Mexicana</em></td>
<td>Triterpenoids</td>
<td>12-ursene and 23,24-dimethyl-24-ethyl-stigmast-25-ene</td>
<td>Antihyperglycemic effect (Perez and Vargas, 2002)</td>
</tr>
<tr>
<td><em>Alpinia galanga</em></td>
<td>Polysaccharides</td>
<td>Protein-bound polysaccharide</td>
<td>Increases serum insulin levels, reduces blood glucose levels and improves glucose tolerance (Quanhong et al., 2005)</td>
</tr>
<tr>
<td><em>Andrographis paniculata</em></td>
<td>Diterpenoids</td>
<td>Andrographolide</td>
<td>Hypoglycemic activity (Yu et al., 2003)</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>Steroid</td>
<td>β-sitosterol</td>
<td>Hypoglycemic activity (Chattopadhyay et al., 1987)</td>
</tr>
<tr>
<td><em>Catharanthus roseus</em></td>
<td>Alkaloid</td>
<td>Catharanthine and Vindoline</td>
<td>Hypoglycemic activity (Chattopadhyay et al., 1999).</td>
</tr>
<tr>
<td>Plant/Component</td>
<td>Constituent</td>
<td>Activity</td>
<td>Source</td>
</tr>
<tr>
<td>-----------------------</td>
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<td>----------------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>Dietary fibers</td>
<td>Fiber</td>
<td>Inhibits the activity of alpha-amylase and decreases the rate of glucose absorption (Chau et al., 2003).</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>Ferulic acid</td>
<td>4-hydroxy-3-methoxycinnamic acid</td>
<td>Hypoglycemic effect on stereoptozotocin induced diabetic mice and Kk-Ay mice (Ohnishi et al., 2004). Stimulates insulin secretion in pancreatic RIN-5F cell (Nomura et al., 2003).</td>
</tr>
<tr>
<td>Gymnema sylvestre</td>
<td>Triterpenoid</td>
<td>Gymnemic acid IV</td>
<td>Hypoglycemic activity (Sugihara et al., 2000).</td>
</tr>
<tr>
<td>Momordica charantia</td>
<td>Sterol</td>
<td>Charantin</td>
<td>Insulin like activity and</td>
</tr>
<tr>
<td><strong>Plant</strong></td>
<td><strong>Compounds</strong></td>
<td><strong>Actions</strong></td>
<td><strong>Effects</strong></td>
</tr>
<tr>
<td>-----------</td>
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<tr>
<td><em>Punica granatum</em></td>
<td>Tanins</td>
<td>Gallic acid</td>
<td>Enhanced cardiac PPAR-γ mRNA and cardiac glucose transporter (GLU)-4 (the insulin – dependent isoform of GLUTs mRNA (Huanga <em>et al.</em>, 2005).</td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em></td>
<td>Alkaloid</td>
<td>Berberine</td>
<td>Hypoglycemic</td>
</tr>
</tbody>
</table>
Several species of herbal drugs have been described in the scientific and popular literature as having antidiabetic activity (Valiathan, 1998). Due to their perceived effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are prescribed (Verspohl, 2002). Many traditional plant treatments for diabetes mellitus are used throughout the world. Only few of the traditional plant treatments for diabetes have received scientific scrutiny, and the World Health Organization has recommended that this area warrants attention (WHO, 1980). This chapter reports the isolation and identification of the active principle responsible for hypoglycemic potential of *L. nodiflora*.

<table>
<thead>
<tr>
<th>Herbal Drug</th>
<th>Active Principle</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tribulus terrestris</em></td>
<td>Imidazoline compounds, Harmane, norharmane and pinoline</td>
<td>Increases insulin secretion (Nadkarni, 1976; Cooper <em>et al.</em>, 2003).</td>
</tr>
</tbody>
</table>
2.3. Materials and methods

1. Plant material

As given in 1.3.1

2.3.2. Plant extraction

As given in 1.3.2

2.3.3. Liquid-liquid partition of active methanol extract

Ninety-seven grams of crude methanol extract of L. nodiflora were dissolved in 200 ml of methanol and diluted with 300 ml of water. This solution was partitioned with diethyl ether (DEE) in a 1:1 ratio using a separating funnel. Both diethyl ether soluble (54 gm) and insoluble (43 gm) parts were concentrated in a rotor evaporator to obtain the extracts and they were screened for their antidiabetic property.

2.3.4. Column chromatography and compound isolation

Fifty four grams of DEE soluble part was chromatographed on a silica gel column (Merck 100–200 mesh, 750 g 3.5 i.d.x60 cm) and successively eluted with stepwise gradient of hexane: ethyl acetate solvent system (5 %, 25 %, 50 %, 75 % and 100 %). 184 fractions were collected and finally five major fractions were obtained based on similar Thin Layer Chromatography (TLC) profiles. A light green colour precipitate was obtained in fraction 2, eluted with 75 % ethyl acetate and 25 % hexane solvent system. This was finally washed with ethyl acetate and crystallized from hexane; it was tested by over TLC (pre-coated silica gel (60
F254) s 0.25 mm thick TLC plate, Merck) using toluene: ethyl acetate (9:1) solvent system. Fraction 2 was subjected to Liberman’s-Burchard test for sterol.

2.3.6. Source of fine chemicals

Streptozotocin was procured from Sigma chemicals Co., St. Louis, MO, USA. Glucose-oxidase peroxidase Kit was purchased from Dialab, Austria.

2.3.7. Experimental animals

As given in 1.3.5.

2.3.8. Induction of diabetes mellitus

As given in 1.3.6.

2.3.9. Experimental design and treatment schedule to screen the DEE

Rats were divided into 7 groups (n=6)
Group 1 Normal control rats + Vehicle alone (2% tween 80)
Group 2 Normal control rats + DEE soluble extract (200 mg/ kg /b.wt)
Group 3 Normal control rats + DEE insoluble extract (200 mg/ kg /b.wt)
Group 4 Diabetic control
Group 5 Diabetic rats + DEE soluble extract (200 mg/ kg /b.wt)
Group 6 Diabetic rats + DEE insoluble extract (200 mg/ kg /b.wt)
Group 7 Diabetic rats + Glibenclamide (600µg/ kg /b.wt)
At the end of the 15th day, the rats were sacrificed and fasting blood glucose level was estimated.

2.3.10. Screening of fractions isolated from diethyl ether active part

2.3.10.1 Assessment of hypoglycemic activity by OGTT in mild diabetic rats

Forty eight overnight fasted mild diabetic model rats were divided into seven groups of six rats each. Group I served as normal control and received vehicle (distilled water only); Group II served as diabetic control, whereas the groups III, IV, V, VI and VII received fractions 1, 2, 3, 4 and 5 at 20 mg/kg/b.wt respectively. Group VII served as a positive control and received a dose of 600µg/kg/b.wt of a known antidiabetic drug glibenclamide, as reference drug (El-Hilaly et al., 2006). A dose of 2 gkg−1 of glucose was then given orally to all the groups. Blood Glucose Level (BGL) was further checked at 0, 60, 120 and 180 min.

2.3.11. Biochemical estimation

2.3.11.1 Separation of Plasma

As given in 1.4.1.

2.3.11.2 Estimation of Fasting Plasma Glucose levels (Glucose Oxidase peroxidase method – Trinder, 1969)

As given in 1.4.3.

2.3.12. Identification of active compound by spectroscopic methods

Melting point was found out by open capillary method on a Gallenkamp heating block instrument. The IR spectra were taken on Perkin-Elmer FT-IR grating spectrophotometer in KBr disc. $^1$H-NMR and $^{13}$C-NMR were taken on a Bruker instrument in CDCl$_3$ at 400 MHz and 100
MHz. The chemical shift values are given in δ scale with TMS as the internal standard. GC-MS was taken on a Shimadzu instrument equipped with a CPB-capillary column (0.5 mm i.d.×50 m length) mass spectrometer (ion source 200 °C, RI 70 eV) programmed at 40–280 °C with a rate of 4 °C/min. Injector temperature was 280 °C; carrier gas was He (20 psi). Flow rate was 5 ml/min.
Fig. 2.1: Schematic representations for Extraction, isolation and identification of active principle from Lippia nodiflora (whole plant)

Lippia nodiflora (Whole plant)

Hexane extract, Ethyl acetate extract, Methanol extract (Active)

Methanol extract partitioned with diethyl ether in a 1:1 ratio using a separating funnel

Diethyl ether insoluble part

Diethyl ether soluble part (Active)

Column chromatography

184 fractions were collected

Fr 1 Fr 2, (Green Powder) Fr 3 Fr 4 Fr 5

All the fractions were screened for their hypoglycemic property

Fraction 2 showed significant glycemic control and showed single spot in TLC

Fraction 2 was subjected to GC-MS, NMR, IR, MASS spectroscopy

COMPOUND WAS IDENTIFIED
Statistical analysis:

All the data were statistically evaluated using SPSS program; version 11.5 software package. The values were analyzed by one way analysis of variance (ANNOVA) followed by Student’s t-test. All the results were expressed in mean ± S.E.M for six rats in each group. \( p \leq 0.05 \) was considered significant.

2.4. RESULTS

2.4.1. Antihyperglycemic effect of Diethyl ether soluble and insoluble parts of methanol extract of *L. nodiflora*.

Both diethyl ether soluble and insoluble parts were screened for their glucose lowering effect in diabetic rats. Significant increase in the blood glucose level was observed in diabetic control group when compared with normal control. Oral administration of 200 mg/kg/b.wt diethyl ether soluble and insoluble parts for 15 days separately was done. Diethyl ether soluble part significantly decreased (-53%) the fasting blood glucose level, whereas only low (-14 %) reduction was observed in diethyl ether insoluble part when compared with diabetic control (Table1). Diethyl ether soluble part was selected for further studies.

2.4.2. Isolation and purification of active compound

The crystalline solid obtained from fraction 2 showed melting point at 145 °C (lit m.p.147-148°C) (Jain *et al.*, 2009). \([\alpha]_D\) = -42.0 °C (CHCl₃) (lit value -41.13). It showed single spot on TLC (\(R_f = 0.45\)) with toluene: ethyl acetate 9:1 as the developing system. The spot turned blue with vanillin – sulphuric acid agent on heating the plate at 110 °C for 5 minute. The
yield of the compound was 800 mg. It answered Liberman’s-Burchard test for sterol giving purple colour on treatment with acetic anhydride and concentrated sulphuric acid in chloroform.

2.4.2. Assessment of hypoglycemic activity of isolated fractions and compound from Diethyl ether soluble part of *L. nodiflora* methanol extract by OGTT in mild diabetic rats.

Hypoglycemic activity of different fractions and compound (Fraction 2) of Diethyl ether soluble part of *L. nodiflora* methanol extract was evaluated. Single dose of 20 mg/kg/b.wt was administered orally to the mild diabetic rats. At the end of 180 min the diabetic control group showed 76.78 % increase in the blood glucose level when compared with normal control. At the same time fraction 2 significantly decreased the blood glucose level by -44.58 % when compared with diabetic control. Values of -9.55 %, -12.44 %, -7.66 % and -1.8 % decreases were observed in fractions 1, 3, 4 and 5 respectively at the end of the 180 min. Significant decrease (-30.29 %) was recorded in the glibenclamide treated group when compared with diabetic control. This study showed that fraction 2 had more potent action than the positive control glibenclamide (Table 2).

2.4.3. Identification of active compound (fraction 2) by spectroscopic methods

Gas chromatography–mass spectrometry (GC–MS)

GC-MS of the active compound showed single peak (100 %) (Fig: 2.2). The compound was identified as γ-sitosterol (Fig: 2.7) by comparison of the mass spectrum with that of the reference compound in the GC-MS library (SI=91 %).
**IR**

IR ν KBr (max): 3418 [hydroxyl], 2932, 2854, 1637 (trisub. double bond), 1462, 1379, 1254, 1164, 1024, 839, 801 (trisub. double bond) (Fig: 2.3).

**¹H NMR**

¹H NMR (δ, CDCl₃, 400 MHz): 0.68 (3H, S, H-18), 1.02 (3H, S, H-19), 0.82 (3H, d, J=6.5 Hz, H-26) 0.84 (3H, d, J=6., H-27), 0.85 (3H, t, J=7.0Hz, H-29), 0.92 (3H, d, J=6. 5Hz, H-21), 3.50 (1H, m, H-3), 5.35 (1H, m, H-6) (Fig: 2.4).

**¹³C NMR**

¹³C NMR(δ, CDCl₃, 100 MHz): 37.25 (C-1), 31.899 (C-2), 71.79 (C-3), 42.29 (C-4), 140.74 (C-5), 121.70 (C-6), 31.65 (C-7), 31.65 (C-8), 50.12 (C-9), 36.13 (C-10), 21.07 (C-11), 39.77 (C-2), 42.29 (C-13), 56.759 (C-14), 26.07 (C-15), 28.23 (C-16), 56.05 (C-17), 11.84 (C-18), 19.38 (C-19), 36.49 (C-20), 19.02 (C-21), 33.93 (C-22), 26.07 (C-23), 45.82 (C-24), 29.14 (C-25), 18.77 (C-26), 19.81 (C-27), 23.05 (C-26), 12.13 (C-29) (Fig:2.5, 2.6).

**EI-MS**

EI-MS m/z (rel.int %): 414 (M⁺, C₂₉H₅₀O, 48.9), 396 (34.0), 381(26.3), 329 (41.7), 303(32.4), 275 (8.8), 255 (25.6), 213 (39.2), 199 (17.0), 163 (31.1), 161 (32.8),159(34.2), 147 (30.9), 145 (53.6),135 (31.5),131 (23.3), 121 (31.0),119 (36.9),109 (30.8), 107 (57.8),105 (52.5), 95 (56.0), 93 (42.3), 91 (39.9), 81 (54.0),79 (34.4),71 (29.9), 69 (39.7), 67 (33.4), 57 (56.4), 55 (60.8), 43 (100). (Fig: 2.2). Molecular formula is C₂₉H₅₀O.
The above physical and spectroscopic data are comparable with those reported in the literature (Jain et al., 2009; Manoharan et al., 2005) it was identified as \(\gamma\)-sitosterol Figure 2.1 shows the overall outline of isolated active compound from \textit{L. nodiflora}.

2.5. DISCUSSION

Streptozotocin is well known for its selective pancreatic islet \(\beta\)-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio \textit{et al.}, 2000). In all diabetic patients, treatment should aim to lower blood glucose to near normal level (ADA, 1997). The present investigation fulfills this by producing a significant fall in blood glucose levels in the diabetic rats treated with diethyl soluble part and its fraction 2. The standard drug, glibenclamide, has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic \(\beta\)-cells (Tian \textit{et al.}, 1998). It may be suggested that the mechanism of action of diethyl ether soluble part and fraction 2 is similar to glibenclamide.

\(\gamma\)-sitosterol is a sterol; the hypoglycemic effect is similar to sulphonylureas. Sterols are present in large number of clinically important medicinal plants including \textit{Momordica charantia}, \textit{Azadirachta indica}, \textit{Phyllanthus emblica} and \textit{Trigonella foenum-graecum}. Extraction and decoction of these medicinal plants have been traditionally used to treat various disorders including cardiovascular disease, liver disease, cancer and diabetes. This is the first report of the isolated compound \(\gamma\)-sitosterol from \textit{L. nodiflora}.

**Antidiabetic properties of sterol**

Charantin, a sterol obtained from \textit{Momordica charantia} is known to have insulin like activity, responsible for its hypoglycemic effect (Ng \textit{et al.}, 1986). \(\beta\)-sitosterol, a steroid, obtained
from *Azadirachta indica*, was responsible for its hypoglycemic property (Chattopadhyay *et al.*, 1987). Phytosterol present in *Aloe ferox* leaf gel showed promise in alleviating symptoms associated with/or prevention of cardiovascular diseases, cancer, neurodegeneration and diabetes (Loots *et al.*, 2007).

Tanaka *et al.*, (2006) isolated and identified five antidiabetic sterols from *Aloe vera* gel; they are lophenol, 24-methyl- lophenol, 24-ethyl lophenol, cycloartanol and 24-methylene-cycloartanol. These showed significant antihyperglycemic effects in type 2 diabetic BKS.Cg (+/+ Lepr (db/db) mice. Antihyperglycemic steroidal sapogenins were isolated from Jamaican bitter yam, *Dioscorea polygonoides*. They are Delta 3 diosgenin, diosgenin, pennogeninphytosterol and β-sitosterol. They decreased fasting blood glucose and intestinal amylase and ATPase in streptozotocin- induced diabetic rats (McAnuff *et al.*, 2005).

Lee *et al.*, (2004) have reported that fucosterol is a main antidiabetic principle from the marine alga *P. siliquosa*. Bark of the cashew plant, *A. occidentale*, exhibited a hypoglycemic effect probably due to the presence of the sterol compound stig mast-4-en-3-one (Alexander-Lindo *et al.*, 2004).

**Other medicinal properties**

Plant sterol esters are used as food supplements to reduce vascular diseases like, endothelial function and stroke or the artherogenesis (Weingartner *et al.*, 2008). Takaku *et al.*, (2001) have reported the anticancer activity of both β-siosterol and ergosterol, aphytosterol, due to direct inhibition of angiogenesis induced by solid tumors.

Withaferin A, a major chemical constituent of *Withania somnifera*, reportedly showed cytotoxicity in a variety of tumor cell lines (Malik *et al.*, 2007). *Borago officinalis* L.
Chapte...2

(Boraginaceae) contained the highest number of sterols and they possessed in vivo antiinflammatory and in vitro antioxidant activities (Conforti et al., 2008).

Kim et al., (2008) have reported that phytosterols decreased the cholesterol accumulation in hypertensive patients. Lactose-beta-sitosterol and β-sitosterol have been reported for their cure of asthma and lung inflammation (Yuk et al., 2007). Ezetimibe, simvastatin, atorvastatin, and ezetimibe-statin therapies on non-cholesterol sterols decreased the cholesterol absorbtion in patients with primary hypercholesterolemia (Assmann et al, 2008).

2.6. CONCLUSION

Methanol extract of L. nodiflora (whole plant) suspended in water was partitioned using diethyl ether. The diethyl ether soluble part showed antidiabetic activity and it was subjected to coloumn chromatography. Five fractions were obtained. When all the five fractions were screened, fraction 2 showed significant hypoglycaemic property. Fraction 2 was obtained as green powder. Structural determination was done by using physical and spectroscopic datas (IR, $^1$H NMR, $^{13}$C NMR and GC-MS). The compound was identified as γ-sitosterol.
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www.tropilab.com/bittermelondatabase.html


Table 1: Effect of oral administration of Diethyl ether (DEE) soluble and insoluble methanol extract of *L. nodiflora* on plasma glucose levels in STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting Blood Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero day</td>
</tr>
<tr>
<td>Normal control</td>
<td>91.44 ± 0.57</td>
</tr>
<tr>
<td>Normal + DEE soluble (200 mg/kg/b.wt)</td>
<td>86.63 ± 1.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal + DEE insoluble (200 mg/kg/b.wt)</td>
<td>85.12 ± 1.39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>232.72 ± 3.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + DEE soluble (200 mg/kg/b.wt)</td>
<td>223.76 ± 2.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + DEE insoluble (200 mg/kg/b.wt)</td>
<td>225.94 ± 1.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic +glibenclamide (600µg/ kg/b.wt)</td>
<td>229.76 ± 2.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are (mg/dl) mean ± S.E.M for six animals;<sup>a</sup> values deviate significantly from normal control group by comparison with normal rats (P≤0.05);<sup>b</sup> values deviate very significantly from diabetic control groups.
Table 2: Effect of fractions of soluble Diethy ether extract of methanol extract of *L. nodiflora* on blood glucose level in mild diabetic rats using GTT.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma glucose level (mg/dl)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>30 (min)</td>
<td>60 (min)</td>
<td>120 (min)</td>
<td>180 (min)</td>
</tr>
<tr>
<td>Normal control</td>
<td>73.45 ± 2.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.82 ± 1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.95 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.64±0.96&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>182.21 ± 2.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>362.30 ± 5.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>344.37 ± 8.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>307.18±4.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Fraction 1 (20 mg/kg/b.wt)</td>
<td>183.56 ± 2.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>319.84 ± 2.43&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>311.45 ± 4.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>293.76±2.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Fraction 2 (20 mg/kg/b.wt)</td>
<td>183.56 ± 1.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>321.35 ± 2.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>190.82 ± 4.74&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>180.78±1.46&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>Diabetic + Fraction 3 (20 mg/kg/b.wt)</td>
<td>181.55 ± 2.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>316.72 ± 6.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>301.53 ± 7.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>274.05±9.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Fraction 4 (20 mg/kg/b.wt)</td>
<td>183.95 ± 3.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>333.18 ± 7.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>317.96 ± 7.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>306.54±6.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Fraction 5 (20 mg/kg/b.wt)</td>
<td>187.95 ± 1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>350.48 ± 14.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>338.04 ± 14.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>328.54±48.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + glibenclamide 600µg/kg/b.wt</td>
<td>179.68 ± 2.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>340.52 ± 4.55&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>240.03 ± 2.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>161.72±3.29&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are (mg/dl) mean ± S.E.M for six animals;
<sup>a</sup> values deviate significantly from diabetic control group by comparison with normal rats (P≤0.05); <sup>b</sup> values deviate very significantly from diabetic control groups.
Fig. 2.3. IR spectrum of γ-sitosterol.
Fig. 2.4. 1H NMR spectrum of γ-sitosterol
Fig. 2.5. $^{13}$C NMR spectrum of γ-sitosterol
Fig. 2.6. $^{13}\text{C}$ NMR of γ-sitosterol (A) DEPT, (B) proton decoupled
Fig. 2.2. GC-MS spectral analysis of γ- sitosterol isolated from *L. nodiflora*

A- GC-MS chromatograph of γ- sitosterol
B- Mass spectrum of γ- sitosterol
Fig 2.7. Structure of γ- sitosterol