Research Article……!!!

INTERNATIONAL JOURNAL OF INSTITUTIONAL
PHARMACY AND LIFE SCIENCES

Received: 13-01-2013; Revised; Accepted: 25-10-2013

PHYSICOCHEMICAL AND PHYTOCHEMICAL SCREENING OF ETHANOLIC
EXTRACT OF LEAVES OF CLITORIA TERNATEA LINN. (FABACEAE)

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Keywords:
Clitoria ternatea, ash value, phytochemical screening, ethanol extract

ABSTRACT

Plants have been known to relieve various diseases in Ayurveda. A large number of plants are claimed to possess the anti-cancer, antimicrobials, anti-diabetic and antibiotic properties in the traditional therapeutic systems and also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Clitoria ternatea a valuable medicinal plant possess many bioactive principles which includes diabetes mellitus, chronic bronchitis, dropsy, goitre, leprosy, mucous disorders etc., The leaf of C. ternatea was investigated for its physicochemical and phytochemical properties and screened for its active chemical ingredients. Ash values - total ash (4.18 % w/w), water soluble ash (98.69 % w/w) and acid insoluble ash (1.01 % w/w) was studied from dry weight of crude drug. For qualitative and quantitative phytochemical screening ethanol extract of C. ternatea was prepared and by using conventional identification tests different classes of secondary metabolites were identified. The presence of these secondary metabolites signifies C. ternatea as a source of therapeutic agent.
INTRODUCTION
Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These are non-nutritive chemicals which possess protective or disease preventive properties. Some phytochemical studies have been shown to possess antioxidant activities, improving the effects of oxidative stress. They also have complementary and overlapping mechanisms of action in the body, including modulation of detoxifying enzymes, stimulation of the immune system, modulation of hormone mechanism and antibacterial and antiviral effect. Some of the most important phytochemicals includes alkaloids, flavonoids, tannins and phenolic compounds.

Phytochemicals with biological activity have great utility as pharmaceuticals and pharmacological actions. Many people are aware that eating plant based foods add much needed fiber, vitamins and minerals to the diet but what is less well known is the many benefits of the phytochemicals.

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society either directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. Since herbal medicines are prepared from materials of plant origin they are prone to contamination, deterioration and variation in composition. A lot of analytical techniques have been developed for quality control of drugs from plant origin. Therefore it is very important to undertake phytochemical investigations along with biological screening to understand therapeutic dynamics of medicinal plants and also to develop quality parameters.

Shankpushpi (Clitoria ternatea Linn) is a perennial twining herbaceous plant, belonging to the Fabaceae family. It is distributed throughout tropical equatorial Asia and latter was distributed widely in South and Central America, East and West Indies, Bangladesh, China and India, where it has become naturalized. It is now widely distributed throughout the humid, low land tropics, occurring both naturally and in cultivations. In traditional medicine, C.ternatea is used in treatment of various ailments like jaundice, migraine, sore throat, tumors, eye infections, skin diseases, asthma, fever, urinary tract infections, constipation and indigestion and for central nervous system disorders. Its root extracts are capable of curing whooping cough.
plant was used widely to cure sexual ailments, like infertility and gonorrhoea and to control menstrual discharge. It also acts as an aphrodisiac. Recent study showed that it has antihelmintic, antistress, anxiolytic, antidepressant, anticonvulsant, antipyretic, anti-inflammatory and antistress activity.

**MATERIALS AND METHODS**

**Collection and authentication of plant material**
Fresh leaf of *Clitoria ternatea* was collected from SKM Herbal Research Centre, Erode, Tamil Nadu, India. The plant was identified and authenticated by the taxonomic expert from the department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.

**Experimental Procedure**

**Physico-chemical analysis**
Shade dried coarse powder of *C.ternatea* was subjected to various physicochemical and phytochemical studies using method described by Ayurvedic Pharmacopeia of India.

**Ash values**
Ash values are helpful in determining quality & purity of crude drug in powered form.

**Determination of total ash**
Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators. About 1.0 g of powered sample was weighed accurately and evenly distributed in the crucible. Dried at 100 - 105°C for 1 hour and ignited to constant weight in a muffle furnace at 600 ± 25°C. The crucible was allowed to cool in desiccators. The percentage of ash with reference to the air dried substance was then calculated.

**Determination of water-soluble ash**
The ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water soluble ash and then the percentage of water soluble ash with reference to the air dried substance was calculated.

**Determination of acid-insoluble ash**
15 ml of water and 10 ml of hydrochloric acid were taken in the crucible along with the ash and it was covered with a watch glass. It was boiled for 10 minutes, filtered on an
ash less filter paper, washed with hot water until the filtrate was neutral, ignited to dull redness, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried substance.

Preparation of ethanol extract
Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites in to the extracting solvent. The leaves of \( C. ternatea \) was washed with distilled water and separately dried under shadow for several days. The shade dried leaves were coarsely powdered by mechanical grinder. The dried powdered samples were extracted with 70% ethanol in a soxhlet extractor. Extraction process was continued until the colour of the final drop of the extracts became colourless. The extracts were concentrated in vacuum at 60°C using a rotary evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50°C for 8 hours. The extracts so obtained, stored in air tight container for further studies.

Phytochemical analysis
Qualitative screening of ethanol extract of \( C. ternatea \) was performed for the identification of various classes of active chemical constituents like alkaloids, reducing sugars, flavonoids, glycosides, proteins, steroids etc., using different methods\(^{12,13,14}\). Total phenols, tannins and flavonoids were quantitatively measured according to the method\(^{15,16}\). Vitamin C was estimated by the method\(^{17}\). Total carbohydrate and total protein were determined by the method\(^{18,19}\) respectively.

RESULTS
Physico-chemical analysis
Dried coarsely powdered crude drug was used for the study of physico-chemical analysis. Results were shown in Table - 1.

<table>
<thead>
<tr>
<th>Ash values</th>
<th>Values obtained percentage (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>4.18</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>98.69</td>
</tr>
<tr>
<td>Water insoluble ash</td>
<td>1.31</td>
</tr>
<tr>
<td>Acid soluble ash</td>
<td>98.99</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Qualitative phytochemical screening Phytochemical parameters are mainly used in judging the purity and quality of the powder drug. Analysis of various phytochemical constituents of ethanolic extract of \( C. ternatea \) was tabulated in Table - 2.
TABLE – 2 QUALITATIVE PHYTOCHEMICAL SCREENING IN ETHANOLIC EXTRACT OF LEAVES OF *C. TERNATEA*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Free amino acids</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Oils</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>Terpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: (+) Present; (-) Absent

Quantitative estimation of phytochemicals and nutrients

The quantitative analysis of different phytochemicals and nutrient in ethanolic extract of *C. ternatea* was depicted in Table - 3.

TABLE – 3 QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS AND NUTRIENTS IN ETHANOLIC EXTRACT OF LEAVES OF *C. TERNATEA*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Quantity present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flavonoids (mg RE/g extract)</td>
<td>20.48 ± 0.96</td>
</tr>
<tr>
<td>2.</td>
<td>Tannins (mg TAE/g extract)</td>
<td>78.75 ± 2.09</td>
</tr>
<tr>
<td>3.</td>
<td>Total Phenols (mg TAE/g extract)</td>
<td>245.14 ± 6.97</td>
</tr>
<tr>
<td>4.</td>
<td>Total carbohydrate (mg glucose/g extract)</td>
<td>176.03 ± 1.19</td>
</tr>
<tr>
<td>5.</td>
<td>Total protein (mg/g extract)</td>
<td>3110 ± 18.02</td>
</tr>
<tr>
<td>6.</td>
<td>Vitamin C (mg AAE/g extract)</td>
<td>118.83 ± 0.47</td>
</tr>
</tbody>
</table>

Values are means of three independent analysis of the extract ± standard deviation (n = 3). RE–Rutin Equivalents; TAE–Tannic Acid Equivalents, AAE–Ascorbic Acid Equivalents
DISCUSSION

Medicinal plants are the richest bio-resource for drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Ash value of a drug gives an idea of the earthy matter or inorganic composition and other impurities present along with the drug. The ash values obtained from the plant tissue (physiological) as well as from extraneous matter (non-physiological). The determination of the physiological ash and non-physiological ash together is called the total ash determination. Total ash may vary within wide limits for specimen of genuine drugs due to the variable natural ash, in such cases the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid – insoluble ash which represents most of the ash from the contaminating soil. Any significant deviation in the percentage of ash reported in this work may indicate adulteration or substitution of the drug.

Phytochemical study of the leaf extract of *C. ternatea* showed that leaf comprised a wide range of active chemical constituents such as alkaloids, flavonoids, free amino acids, glycosides, phenols, proteins, reducing sugars, steroids and tannins while saponins and oils were absent. These tests are helpful in finding chemical constituents in the plant materials that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound.

The quantitative estimation of ethanolic extract of *C. ternatea* found to contain major phytoconstituent total phenols (245.14 ± 6.97 mg TAE/g) relatively high compared to tannins (78.75 ± 2.09 mg TAE/g) and flavonoids (20.48 ± 0.96 mg RE/g). Plant-derived substances have recently become a source of great interest owing to their versatile applications. Recent researches has shown that phenols contribute to the prevention of cardiovascular diseases, cancers, osteoporosis and antioxidant character with potential health and benefits. They are also known to have a role in the prevention of neurodegenerative diseases and diabetes mellitus. In plants, flavonoids serve as protectors against a wide variety of environmental stress while, in humans flavonoids appear to function as “biological response modifiers”. It has been demonstrated to have anti-inflammatory, anti-allergenic, anti-viral, anti-aging and anti-carcinogenic activity. Phenols, flavonoids and tannins which may act as antioxidant, antimicrobial, antithelmintic and antidiarrhoeal activity.
*C. ternatea* also contains rich amounts of nutrients such as total proteins (3110 ± 18.02 mg/g), total carbohydrate (118.83 ± 0.47) and vitamin C (176.03 ± 1.19 mg AAE/g). Phytochemicals, working together with nutrients, may help to slow the aging process and reduce the risk of many diseases, including cancer, heart disease, stroke, diabetes mellitus, high blood pressure, cataracts, osteoporosis, and urinary tract infection. On the basis of the above results *C. ternatea* could serve as therapeutic agent for various ailments.

REFERENCES


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Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)
SCImago Journal Rank 0.129
Impact factor 0.47*

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ABSTRACT

Medicinal plants are of great importance to the health of individuals and communities. A large number of plants are claimed to possess the anti-diabetic, anti-fertility, anti-hyperlipidaemic, anti-inflammatory, anti-cancer, hepatoprotective and immunomodulatory activities in the traditional therapeutic systems. It is now believed that nature has given the cure of every disease in one way or another. *Clitoria ternatea* is a valuable medicinal plant possess many bioactive principles which includes diabetes mellitus, chronic bronchitis, goitre, mucous disorders and leprosy. The ethanolic extract of leaves of *C. ternatea* was investigated for its phytochemical properties and analysis for its active chemical ingredients. For qualitative and quantitative phytochemical analysis the ethanol extract of *C. ternatea* acts as a source of therapeutic agent.

**KEYWORDS:** *Clitoria ternatea*, phytochemical screening, ethanol extract, anti-diabetic.

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INTRODUCTION

Plant medicines were regarded as highly important in the lives of our ancestors since they did not have any alternative therapy. Their dependence on the plants in their surroundings made them to acquire the knowledge about the medicinal properties of many plants by trial and error. They were also aware of the commercial value of these plants. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These are non-nutritive chemicals which possess protective or disease preventive properties. Some phytochemical studies have been shown to possess antioxidant activities, improving the effects of oxidative stress. They also have complementary and overlapping mechanisms of action in the body, including modulation of detoxifying enzymes, stimulation of the immune system, modulation of hormone mechanism and antibacterial and antiviral effect. Some of the most important phytochemicals includes alkaloids, flavonoids, tannins and phenolic compounds\textsuperscript{1,2,3}. Phytochemicals with biological activity have great utility as pharmaceuticals and pharmacological actions. Many people are aware that eating plant based foods add much needed fiber, vitamins and minerals to the diet but what is less well known is the many benefits of the phytochemicals.

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society either directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. Since herbal medicines are prepared from materials of plant origin they are prone to contamination, deterioration and variation in composition. A lot of analytical techniques have been developed for quality control of drugs from plant origin. Therefore it is very important to undertake phytochemical investigations along with biological screening to understand therapeutic dynamics of medicinal plants and also to develop quality parameters. \textit{Clitoria ternatea} Linn (family: Fabaceae) is a perennial twining herb found in India, China, Philippines and Madagascar but has been introduced to Africa, Australia and America It is now widely distributed throughout the humid, low land tropics, occurring both naturally and in cultivations\textsuperscript{4}. It is commonly called “Shankpushpi”. In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent\textsuperscript{5}. The root extracts are capable of curing whooping cough. It was used traditionally to cure sexual ailments, like infertility and gonorrhea, to control menstrual discharge, and also as an aphrodisiac\textsuperscript{6}. In traditional medicine, \textit{C.ternatea} is used in treatment of various ailments like jaundice, migraine, sore throat, tumors, skin diseases, asthma, fever, urinary tract infections, constipation and indigestion and for central nervous system disorders. Its root extracts are capable of curing whooping cough. This plant was used widely to cure sexual ailments, like infertility and gonorrhea and to control menstrual discharge. It also acts as an antioxidants\textsuperscript{7}. Recent study showed that it has anti-helmintic\textsuperscript{8}, anxiolytic, anti-depressant, anti-convulsant\textsuperscript{9}, anti-pyretic, anti-inflammatory and anti-stress activity\textsuperscript{10}.

MATERIALS AND METHODS

(i) Collection and authentication of plant material

Fresh leaf of \textit{Clitoria ternatea} was collected in the month of February from SKM Herbal Research Centre, Erode, Tamil Nadu. The plant was identified and authenticated by the taxonomic expert from the department of Botany, V.A.Chidamparamanadar College, Tuticorin. A voucher specimen of the herbarium has been deposited at the same department.

(ii) Ash values and extractive values

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs\textsuperscript{11}. Equally important in the evaluation of crude drug, is the determination of ash value and acid insoluble ash value. The total ash is
particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matters such as metallic salts and/or silica\textsuperscript{12}.

**Determination of total ash**
Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators. About 1.0 g of powered sample was weighed accurately and evenly distributed in the crucible. Dried at 100 - 105ºC for 1 hour and ignited to constant weight in a muffle furnace at 600 ± 25ºC. The crucible was allowed to cool in desiccators. The percentage of ash with reference to the air dried substance was then calculated.

**Determination of water-soluble ash**
The ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450ºC. The weight of the insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water soluble ash and then the percentage of water soluble ash with reference to the air dried substance was calculated.

**Determination of acid-insoluble ash**
15 ml of water and 10 ml of hydrochloric acid were taken in the crucible along with the ash and it was covered with a watch glass. It was boiled for 10 minutes, filtered on an ash less filter paper, washed with hot water until the filtrate was neutral, ignited to dull redness, cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried substance.

**Determination of sulphated ash**
1.0 gm of substance was ignited gently at first in a crucible, until the substance was thoroughly charred. Then the residue was cooled, moistened with 1.0 ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800 ± 25ºC until all the black particles were disappeared. The crucible was allowed to cool, a few drops of sulphuric acid was added and heated. Then it was ignited as before, cooled and weighed. The percentage of sulphated ash with reference to the air-dried substance was then calculated.

**Determination of water soluble extractive**
To 50 ml of water, 5.0 gm of the substance was added to a stoppered flask and allowed to stand for 10 minutes. 2.0 gm of Kieselghur was added, filtered and 5.0 ml of the filtrate was transferred to a tarred evaporating dish. Solvent was evaporated, dried for 2 hrs and the residue was weighed. Then the percentage of water – soluble extractive was calculated with reference to that of the air-dried substance.

**Determination of ethanol soluble extractive**
5.0 gm of the substance was macerated with 100 ml of ethanol in a closed flask for 24 hrs and agitated frequently for the first 6 hrs and allowed to stand for 18 hrs. It was filtered and 25 ml of the alcoholic extract was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ºC and the percentage of ethanol – soluble extractive with reference to that of the air-dried substance was calculated.

**(iii) Preparation of ethanol extract**
Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites in to the extracting solvent. The leaves of *C. ternatea* was washed with distilled water and separately dried under shadow for several days. The shade dried leaves were coarsely powdered by mechanical grinder. The dried powdered samples were extracted with 70% ethanol in a soxhlet extractor. Extraction process was continued until the colour of the final drop of the extracts became colourless. The extracts were concentrated in vacuum at 60ºC using a rotary evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50ºC for 8 hours. The extracts so obtained, stored in airtight container for further studies.

**(iv) Phytochemical analysis**
Qualitative screening of ethanol extract of leaf of *C. ternatea* was performed for the identification of various classes of active chemical constituents like alkaloids, reducing sugars, flavonoids, steroids, glycosides,
proteins etc., using different methods\textsuperscript{13,14,15}. Total phenols, tannins and flavonoids were quantitatively measured according to the method\textsuperscript{16,17}. Vitamin C was estimated by the method\textsuperscript{18}. Total carbohydrate and total protein were determined by the method\textsuperscript{19,20} respectively.

**RESULTS**

**Ash values and extractive values**
Dried coarsely powdered crude drug was used for the study of Ash values and extractive values. Results were shown in Table - 1.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Physical evaluation</th>
<th>Values obtained (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Loss on drying</td>
<td>23.5</td>
</tr>
<tr>
<td>2.</td>
<td>Moisture content</td>
<td>11.52</td>
</tr>
<tr>
<td>3.</td>
<td>Ash values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total ash</td>
<td>4.18</td>
</tr>
<tr>
<td></td>
<td>Water soluble ash</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>Acid insoluble ash</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>Sulphated ash</td>
<td>6.97</td>
</tr>
<tr>
<td>4.</td>
<td>Extractive values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water soluble extractive</td>
<td>20.41</td>
</tr>
<tr>
<td></td>
<td>Alcohol soluble extractive</td>
<td>8.57</td>
</tr>
</tbody>
</table>

**Preliminary phytochemical screening**
Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary prerequisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Analysis of various phytochemical constituents of ethanolic extract of leaf of \textit{C. ternatea} was tabulated in Table - 2.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Free amino acids</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Oils</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>Terpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

*Note: (+) Present; (-) Absent*

**Quantitative estimation of phytochemicals and nutrients**
The quantitative analysis of different phytochemicals and nutrient in ethanolic extract of \textit{C. ternatea} was depicted in Table - 3.
DISCUSSION

Medicinal plants are the richest bio-resource for drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs\textsuperscript{21}. The total ash value of leaf of \textit{C. ternatea} is 4.18% respectively. The ash value is indicative of the impurities present in the drug. Since the ash value is constant for a given drug, this value is also one of the diagnostic parameters of the drug. The sample has more water soluble ash than acid insoluble ash. These ash values are generally considered as the index of the purity as well as identity of the drug. Extractive values are useful for the evaluation of phytoconstituents especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the active constituents present in a crude drug. Phytochemical study of the leaf extract of \textit{C. ternatea} showed that leaf comprised a wide range of active chemical constituents such as alkaloids, flavonoids, free amino acids, glycosides, phenols, proteins, reducing sugars, steroids and tannins while saponins and oils were absent. HPTLC analysis also confirmed the presence of alkaloids, flavonoids, steroids, glycosides and saponins in the studied plant. These tests are helpful in finding chemical constituents in the plant materials that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound. The quantitative estimation of ethanolic extract of \textit{C. ternatea} found to contain major phytoconstituent total phenols (245.14 ± 6.97 mg TAE/g) relatively high compared to tannins (78.75 ± 2.09 mg TAE/g) and flavonoids (20.48 ± 0.96 mg RE/g). Plant-derived substances have recently become a source of great interest owing to their versatile applications. Recent researches has shown that phenols contribute to the prevention of cardiovascular diseases, cancers, osteoporosis and antioxidant character with potential health and benefits\textsuperscript{22,23,24}. They are also known to have a role in the prevention of neurodegenerative diseases and diabetes mellitus\textsuperscript{25}. In plants, flavonoids serve as protectors against a wide variety of environmental stress while, in humans flavonoids appear to function as “biological response modifiers”. It has been demonstrated to have anti-inflammatory, anti-allergenic, anti-viral, anti-aging and anti-carcinogenic activity\textsuperscript{26,27,28}. Phenols, tannins and flavonoids which may act as antioxidant, anti-microbial, anti-diarrhoeal and anti-helmintic activity\textsuperscript{29}. \textit{C. ternatea} also contains rich amounts of nutrients such as vitamin C (176.03 ± 1.19 mg AAE/g), total proteins (3110 ± 18.02 mg/g) and total carbohydrate (118.83 ±

\begin{table}[h]
\centering
\caption{Quantitative estimation of phytochemicals and nutrients in ethanolic extract of leaves of \textit{C. ternatea}}
\begin{tabular}{|l|c|}
\hline
Parameters & Quantity present \\
\hline
Flavonoids (mg RE/g extract) & 20.48 ± 0.96 \\
Tannins (mg TAE/g extract) & 78.75 ± 2.09 \\
Total Phenols (mg TAE/g extract) & 245.14 ± 6.97 \\
Vitamin C (mg AAE/g extract) & 118.83 ± 0.47 \\
Total carbohydrate (mg glucose/g extract) & 176.03 ± 1.19 \\
Total protein (mg/g extract) & 3110 ± 18.02 \\
\hline
\end{tabular}
\end{table}

Values are means of three independent analysis of the extract ± standard deviation (n = 3).

RE - Rutin Equivalents; TAE - Tannic Acid Equivalents; AAE – Ascorbic Acid Equivalents
0.47). Phytochemicals, working together with nutrients, may help to slow the aging process and reduce the risk of many diseases, including cancer, heart disease, stroke, diabetes mellitus, high blood pressure, cataracts, osteoporosis and urinary tract infection\textsuperscript{10}. On the basis of the above results, 	extit{C. ternatea} could serve as therapeutic agent for various ailments.

**REFERENCES**


Studies on the Synergetic Effect of *Trichosanthes dioica* and *Clitoria ternatea* Leaf Extract on the Streptozotocin-Induced Diabetic Rats

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ABSTRACT

The study evaluated the effect of combined leaf extracts of *Trichosanthes dioica* (TDL) and *Clitoria ternatea* (CTL) on the streptozotocin (STZ) induced diabetic Wistar rats. Forty eight (48) albino rats were divided equally into 8 groups. Group I and II which served as normal (NC) and diabetic (DC) controls respectively, received placebo treatment. The diabetic test groups III to VII were treated with either alone and combined leaf extracts of TDL and CTL (200 and 400 mg/kg b. w., p.o.) and group VIII was treated with glibenclamide, (600 µg/kg, s.c.) for 28 days. Thereafter, the animals were sacrificed and blood was collected for the estimation of blood glucose, serum insulin, glycosylated hemoglobin, urea, creatinine, total protein, albumin, globulin, SGOT and SGPT to demonstrate its therapeutic effects on the STZ-induced diabetic albino rats. Changes in animal body weight were also measured within the period. From the results it was revealed that both the combined extracts and glibenclamide significantly increased the animals’ body weight. In the same order, serum glucose significantly decreased (p<0.05) after the 28-day treatment compared to DC. The extent of reversal of hyperglycaemia in the combined extract treated animals compared well with the glibenclamide treated group.

Key Words: *Trichosanthes dioica*, *Clitoria ternatea*, diabetes mellitus, streptozotocin.

INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and postprandial blood sugar levels. An estimated 285 million people across the world are affected by diabetes in 2010; the number is expected to grow to 438 million by 2030. The total number of people in India with diabetes is currently around 50.8 million in 2010, raising to 87.0 million by 2030. India has been named the diabetes capital of the World, followed by China with 43.2 million. The largest age group currently affected by diabetes is between 40 – 59 years (IDF, 2009). The major concerns are that much of this increase in diabetes will occur in developing countries, due to population growth, aging, unhealthy diets, obesity and sedentary life style (Chan et al., 2009). Diabetes can be associated with serious complications including heart disease, kidney failure, blindness, nerve damage and amputation (Hungley, 1997). The lesions in the pathophysiology of diabetes are multiple and therefore it would require more than a single drug agent to reverse all or majority of the aspects of the disease. The effective therapeutic approach should be multimodal and in this light, several traditional medicinal herbs have been preferred given the plethora of active ingredients present in a single herb (Tiwari and Rao, 2002).

The use of plant derived natural compounds as part of herbal preparations for alternate source of medicament continues to play major roles in chemotherapy especially in third world countries (Joy et al., 1998). Several studies carried out have shown that traditional medicines could provide better glycaemic control than currently used conventional drugs (Rates, 2001). Plants by means of secondary metabolism contain a variety of herbal and non-herbal ingredients that can ameliorate a disease condition by acting on a variety of targets (various modes and mechanisms) in the host organism. On the basis of the above, polyherbal therapy is considered the preferred therapeutic approach to management of diabetes.
mellitus given its multi-factorial pathogenicity. Polyherbal therapy which is the use of a combination of various agents from different plant sources for therapeutic purposes is a current pharmacological principle and has the advantage of producing maximum therapeutic efficacy with minimum side effects (Ebong et al., 2008). This enhanced efficacy is thought to derive from phytosanthes dioica endowed traditional medicinal plants, since they present exciting opportunities for the development of new types of therapeutics for the management of diabetes mellitus. The leaf extract of Trichosanthes dioica (TDL) and Clitoria ternatea (CTL) were well studied for its antidiabetic activity (Sharmila et al., 2007; Daisy and Rajathi, 2009). Hence, in the present investigation, combination of TDL and CTL extracts were selected to evaluate the antihyperglycaemic effectiveness in STZ-induced diabetic rats.

In the present investigation, the first plant Trichosanthes dioica Roxb (family: Cucurbitaceae) is a dioecious perennial plant, grown throughout India and it is often called green potato and also known as the pointed gourd. It is mainly cultivated as a vegetable crop. It has been used for overcoming problems like constipation, fever, skin infection and wounds. The fruits are used in traditional system of medicine since ancient times (Singh, 1989). The fruits are used as a remedy for spermatorrhoea and also used for cooling and as a laxative (Kirtikar and Basu, 1975). The leaves and tender shoots are used for preparation of syrup for treating cough, cold and bronchitis. It is used as a vegetable crop. It has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent (Mukherjee et al., 2008). The root extracts are capable of curing whooping cough. It was used traditionally to cure sexual ailments, like infertility and gonorrhoea, to control menstrual discharge, and also as an aphrodisiac (Fantz Paul 1991).

Preparation of ethanol extracts
Leaves of Trichosanthes dioica and Clitoria ternatea were washed with distilled water and separately dried under shadow for several days. The shade dried leaves were coarsely powdered by mechanical grinder. The dried powdered samples were extracted with 70% ethanol in a soxhlet extractor. Extraction process was continued until the colour of the final drop of the extracts became colourless. The extracts were concentrated in vacuum at 60ºC using a rotary evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50ºC for 8 hours. The dried residues were administered intragastric catheter tube (IGC) and the dosages were selected based upon its earlier antidiabetic studies.

Experimental animals
Healthy male adult albino rats of Wistar strain approximately of same age, weighing around 150-200 g were procured from Nandha College of Pharmacy, Erode, Tamil Nadu. The entire experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) (Proposal No. NCP/IAEC/PHD/01/2007-2008). The animals were kept in clean and dry polycarbonate cages and maintained in a well ventilated animal house at 24 - 28ºC temperature with 12 hours light – 12 hour dark cycle (Nagappa et al., 2003). The animals were fed with standard pellet diet (purchased from Sai Durga Feeds, Bangalore.) and water was given ad libitum throughout the period of experiment. Prior to each study, the animals were made to fast for 12-14 hours but had free access to water. All the animal experiments were conducted at Dr.Samsung Immuno Clinical Research Laboratory, Tirupur, Tamil Nadu.

Chemicals
Streptozotocin (STZ) was purchased from Sigma Chemical Company (USA). Glibenclamide was obtained from Aventis Pharmaceuticals Limited (India). Insulin radioimmunoassay kit was obtained from Crystal Cheminc, USA. All the chemicals and reagents used in the experiments were of analytical grade obtained from BDH (England and India), E.Merck (Germany), Sigma Chemical Company (U.S.A), LOBA – Chemie Indo Austronel Co., (India) whenever necessary the solvents were redistilled before use.

Experimental induction of diabetes in rats
Streptozotocin (STZ) is well known for its -cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. Single intraperitoneal injection of STZ at a dose of 60 mg/kg body weight. The STZ was freshly dissolved in 0.1 M citrate buffer (pH 4.5).
The injection volume was prepared to contain 1.0 ml/kg (Murali et al., 2002). The control rats were injected with saline only. Prior to this the rats were fasted for 12 hrs. After one week, blood glucose content was measured by glucose oxidase method using a blood sample from the tail vein of rats. The animals with blood glucose levels above 250 mg/dl were considered to be diabetic and used for the experiment (Cetto et al., 2000).

**Experimental animal group**

The animals were randomly divided into eight groups of 48 rats each to determine the toxicity and antidiabetic effects of TDL and CTL extracts and to approve its traditional usage for controlling diabetes mellitus.

**Group I**
Normal control.

**Group II**
Diabetic control.

**Group III**
Diabetic rats treated with TDL extract (200 mg/kg b.w/day) orally for 28 days consequently by IGC.

**Group IV**
Diabetic rats treated with TDL extract (400 mg/kg b.w/day) orally for 28 days consequently by IGC.

**Group V**
Diabetic rats treated with CDL extract (200 mg/kg b.w/day) orally for 28 days consequently by IGC.

**Group VI**
Diabetic rats treated with CDL extract (400 mg/kg b.w/day) orally for 28 days consequently by IGC.

**Group VII**
Diabetic rats treated with TDL extract (200 mg/kg) and CDL extract (200 mg/kg b.w/day) orally for 28 days consequently by IGC.

**Group VIII**
Diabetic rats treated with TDL extract at b.w/day orally for 28 days consequently by IGC. After 28 days of treatment, the fasted animals from each group were sacrificed by cervical dislocation. Blood was collected from the heart by cardiac puncture and allowed to clot and the serum was separated by centrifugation at 3500 rpm for 10 min. Serum was assayed either immediately or stored at -20°C. Liver and kidney were dissected out and skimmed off the adherent tissues and kept at -20°C till the biochemical and hormonal estimations.

**Biochemical Estimation**

Blood glucose was estimated by glucose oxidase method (Hjelm and de Verdier, 1963). Insulin was estimated by the method of Anderson et al., (1993). Glycosylated hemoglobin was determined by the method of Karunanayake and Chandrasekharan (1985). Urea and creatinine were described by the method of Marsh et al., (1965) and Owen et al., (1954). Serum protein, albumin and globulin were estimated by the method of Lowry et al., (1951). SGOT and SGPT were assayed by the method of Reitman and Frankel (1957), respectively.

**Statistical analysis**

The data were analyzed using student’s t-test statistical methods. For the statistical tests a ‘p’ values of < 0.01 and 0.05 was taken as significant.

**RESULTS**

There was no mortality in any experimental group throughout the investigation period. Table - 1 (cited in pg no.17) shows the body weight of control and experimental groups of rats. Body weight of diabetic control animals was decreased significantly by -12.67% (p<0.05) when compared to the initial body weight, whereas the normal control and the treated groups showed a significant increases (p<0.05) in the final body weight. The extent of increase in body weight of the TDL and CTL extract treated animals (11.61 and 12.03%) was similar (p>0.05) in the high dose treated groups. On the other hand the low dose leaf extract (TDL and CTL) treated group (6.73% and 7.88%), normal control (7.51%) and standard drug (glibenclamide) treated group (8.62%) have shown similar trend in the elevation of the body weight after 28 days of the treatment. The combined leaf extracts (TDL and CTL) treated groups have shown a significant (P<0.01) elevation in their body weight and this elevation was achieved even in the low dose. Table - 2 (cited in pg no. 18) shows the levels of blood glucose, plasma insulin and glycosylated hemoglobin levels of normal and experimental rats. There was a significant elevation in blood glucose and glycosylated hemoglobin levels, while the plasma insulin level decreased significantly in STZ-induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of leaf extracts of TDL and CTL either single or combination of two extracts (Group III to Group VII) and glibenclamide (Group VIII) tends to bring the parameters significantly towards the normal. The effect of TDL and CTL extract at the dose of 200 mg/kg body weight was significantly in restoring to normalcy and therefore, the same dose was used for the combined studies also. The effect of combined low dose leaf extract treated groups indicated an excellent revival of both the parameter to normalcy indicating that the hypoglycaemic effect of both the leaf extracts was dose dependent.
The levels of blood urea and creatinine; total protein, albumin and globulin; and liver marker enzymes such as SGPT and SGOT in the serum of normal and experimental rats are summarized in Table – 3 (cited in pg no. 19), Table – 4 (cited in pg no. 20) and Table - 5 (cited in pg no. 21). The diabetic rats (Group II) had decreased levels of serum total protein, albumin, globulin and elevated levels of blood urea, creatinine and liver marker enzymes when compared with normal control rats (Group I). After treatment with leaf extracts of TDL and CTL either single or combination of two extracts (Group III to Group VII), after 28 days, blood urea, creatinine, total protein, albumin, globulin, and liver marker enzymes were brought back to near normal levels. The known drug treated group have shown the similar trend as in the case of leaf extract treatment.

DISCUSSION
The results from this study revealed a significant loss of body weight of untreated diabetic rats compared to non-diabetic animals. This may be due to the loss of muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids. Glycosuria is known to cause a significant loss of calories for every gram of glucose excreted and presumably this loss results in severe weight loss in spite of increased appetite, especially when it is coupled with loss of muscle and adipose tissue due to excessive breakdown of protein. Weight loss is one of the symptoms of diabetes mellitus occurring especially when glycaemic control is poor. Studies have equally reported significant weight reduction in untreated diabetic rats (Ahmed et al., 2005).

In the present investigation, the animals treated with plant extracts (TDL and CTL) tended to gain more weight than the standard drug treated group due to improved glycaemic control by the extracts promotes weight gain by decreasing both metabolic rate and glycosuria. Such severe weight loss was prevented in the extract treated group probably due to interaction of several bioactives. However the extracts treated animals showed appreciable increase in weight compared to the diabetic control group. This appreciation in weight indicates that the treatment allowed the tissues to access the glucose both to supply energy and spared some to build tissue materials required for growth.

The result from the present study shows the significant changes in biochemical parameter during the experimentally induced diabetes. The administration of ethanolic extracts of TDL and CTL decreases the blood glucose level whereas serum insulin level was increased in treated rats compared to control rats. The hypoglycaemic effect of TDL and CTL either alone or combined was found to be inducing insulin release from pancreatic β-cells of diabetic rats. It is evident from this study that, there was an increase in insulin levels in diabetic rats treated with plant extract and this study was augmented with many other studies on the antihyperglycaemic effect of medicinal plants and their insulin release stimulatory effects (Sharma and Garg, 2009; Ahmed et al., 1991; Ayoola et al., 2009; Awobajo and Olatunji-Bello, 2010).

A significant elevation in blood urea, creatinine and SGOT and SGPT were observed in STZ-induced diabetic rats (Group II), when compared to control rats. On the other hand, a significant reduction in serum protein, albumin and globulin were also observed in STZ-induced diabetic rats (Group II), when compared to control (Group I). The ethanol extract of TDL and CTL were administered orally (Group III-VII) to rats for twenty eight days, reversed the urea and creatinine and liver marker enzymes level to near normal. The administration of glibenclamide also decreased the levels of urea and creatinine to some extent. These results were in accordance with the effect of Wartakaka volubilis leaf in diabetic rats (Manthupandian et al., 2000). The increased level of serum protein, albumin and globulin in STZ-induced diabetic rats are presumed to be due to increased protein catabolism and gluconeogenesis during diabetes (Palanivel et al., 2001).

The animals treated with STZ developed hepatic damage which was evident from the increase in the enzyme activities. Pre-treatment with ethanol extracts of TDL and CTL and glibenclamide resulted in a decrease of transaminase activities in STZ-treated rats. The serum GOT and GPT levels increases as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes (Chalasani et al., 2004). Similarly in the present study, it was observed that the levels of SGPT and SGOT in STZ-induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of STZ (Stanely et al., 1999). The serum GOT and GPT were used as markers to assess the extent of liver damage in STZ-induced diabetic rats (Hwang et al., 2005). In this study, the ethanol extracts of TDL and CTL regulated the activity of SGPT and SGOT in liver of rats intoxicated with STZ. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study (Preethi and Kuttan, 2009).

Ethanol extracts from the leaves of Trichosanthes dioica and Clitoria ternatea when combined produced a very good reduction in glucose and glycosylated hemoglobin level when compared to the treatment with single leaf extract. Plant extracts are known to contain phytochemicals including tannins, saponins, polyphenols and alkaloids and dietary fibre which are said to contribute to their
blood sugar lowering effect. Micronutrients such as vanadium which have insulin-like activities are present in some plant extracts as well and this can equally account for their hypoglycaemic potency. Majority of plant extracts exert their blood sugar lowering effects via insulin-like micronutrients present in the extracts. Besides the presence of phytochemicals and micronutrients in plant extracts which exert direct effect on blood sugar, the proliferation/regeneration of the pancreatic islet cells which was evident in this study could also account for the reduction in blood glucose of treated animals. Treatment with glibenclamide significantly reduced the blood glucose and glycosylated hemoglobin level below to normal control. This is in accordance with reports by Rother, (2007) that treatment with insulin is usually a common cause of hypoglycaemic attacks in diabetic subjects. It is very clear from our study that ethanolic extracts of TDL and CTL is exhibiting higher degree of antihyperglycaemic activity. With regard to the mechanisms, it cannot be excluded that TDL extract and CTL extract may contain some biomolecules that may synthesize the insulin receptor to insulin or stimulate the beta cells of islets of Langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the establishment of normal glucose levels. Another important finding of this study was significant and higher degree of antihyperglycaemic efficacy was achieved with combination (200 mg/kg of TDL + 200 mg/kg of CTL) when compared to the extent of efficacy that was obtained with 400 mg/kg dose of individual plant extracts of TDL and CTL. This is going to be beneficial in the clinical point of view.

CONCLUSION

Many herbal medicines, as single agents or in different combined formulations have been recommended for diabetes mellitus, as they are less toxic than oral hypoglycaemic agents and insulin. Antidiabetic herbal therapy is less expensive and have oral route of administration, when compared to insulin preparations. In the present study, a combination of two different herbs have been used, each of which have been individually used as hypoglycaemic agents in traditional medicine, which are available abundantly. The added advantage is that the combination may act at multiple levels to bring about the therapeutic effects.

Table 1: Comparative effects of ethanol extract of leaves of *Trichosanthes dioica* and *Clitoria ternatea* on the body weight of normal control and experimental animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Normal control (Group I)</td>
<td>213±8.45</td>
<td>229±9.24</td>
</tr>
<tr>
<td>Diabetic control (Group II)</td>
<td>221±8.67</td>
<td>193±8.83**</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL (Group III)</td>
<td>208±7.42</td>
<td>222±9.15***</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg TDL (Group IV)</td>
<td>198±6.87</td>
<td>221±8.34**</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg CTL (Group V)</td>
<td>203±10.58</td>
<td>219±9.11</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg CTL (Group VI)</td>
<td>216±9.85</td>
<td>242±8.36**</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL+ CTL each (Group VII)</td>
<td>209±8.32</td>
<td>246±9.55</td>
</tr>
<tr>
<td></td>
<td>197±7.95</td>
<td>214±8.48</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for six rats in each group

Statistical comparison: *: Normal control to diabetic control and drug treated groups

*P < 0.05  **P<0.01

Statistical comparison: a: Diabetic control to drug treated groups

a: P<0.05  aa: P<0.01

NS: Not significant
Table 2: Comparative effects of ethanol extract of leaves of *Trichosanthes dioica* and *Clitoria ternatea* on blood glucose, serum insulin and Glycosylated hemoglobin levels in normal control and experimental animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (IU/l)</th>
<th>Glycosylated hemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (Group I)</td>
<td>79.56±3.21</td>
<td>13.56±1.21</td>
<td>3.68±0.11</td>
</tr>
<tr>
<td>Diabetic control (Group II)</td>
<td>214.55±16.59***</td>
<td>4.31±0.93**</td>
<td>10.16±1.14**</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL (Group III)</td>
<td>131.56±3.93*</td>
<td>8.56±1.14**</td>
<td>5.31±0.34***</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg TDL (Group IV)</td>
<td>121.33±4.16a</td>
<td>11.21±1.39a</td>
<td>4.23±0.14a</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg CTL (Group V)</td>
<td>112.16±2.92a</td>
<td>10.11±1.08a</td>
<td>5.28±0.19a</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg CTL (Group VI)</td>
<td>81.53±2.17aa</td>
<td>13.46±1.19aa</td>
<td>3.96±0.06aa</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL+CTL each (Group VII)</td>
<td>92.11±1.94aa</td>
<td>12.86±1.56aa</td>
<td>4.14±0.12aa</td>
</tr>
<tr>
<td>(Group VIII)</td>
<td>79.16±1.93a</td>
<td>12.98±1.13a</td>
<td>3.93±0.07aa</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for six rats in each group
Statistical comparison: *: Normal control to diabetic control and drug treated groups
*P < 0.05  **P<0.01
Statistical comparison: a: Diabetic control to drug treated groups
a: P<0.05     aa: P<0.01
NS: Not significant

Table 3: Comparative effects of ethanol extract of leaves of *Trichosanthes dioica* and *Clitoria ternatea* on serum urea and creatinine levels in normal control and experimental animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (Group I)</td>
<td>16.31±0.83</td>
<td>0.58±0.03</td>
</tr>
<tr>
<td>Diabetic control (Group II)</td>
<td>38.56±1.21**</td>
<td>1.56±0.14*</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL (Group III)</td>
<td>21.16±0.93</td>
<td>1.21±0.11</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg TDL (Group IV)</td>
<td>23.69±1.19</td>
<td>1.53±0.15</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg CTL (Group V)</td>
<td>19.96±1.24NS</td>
<td>0.89±0.06*</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg CTL (Group VI)</td>
<td>17.91±1.03*</td>
<td>1.02±0.09NS</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL+CTL each (Group VII)</td>
<td>18.16±0.84a</td>
<td>0.94±0.05a</td>
</tr>
<tr>
<td>enclumide (Group VIII)</td>
<td>15.84±0.84**</td>
<td>0.77±0.03a</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for six rats in each group
Statistical comparison: *: Normal control to diabetic control and drug treated groups
*P < 0.05  **P<0.01
Statistical comparison: a: Diabetic control to drug treated groups
a: P<0.05     aa: P<0.01
NS: Not significant
**Table 4: Comparative effects of ethanol extract of leaves of *Trichosanthes dioica* and *Clitoria ternatea* on total protein, albumin and globulin levels in normal control and experimental animals**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (Group I)</td>
<td>8.11±0.13</td>
<td>4.61±0.11</td>
<td>3.50±0.31</td>
</tr>
<tr>
<td>Diabetic control (Group II)</td>
<td>5.36±0.14</td>
<td>3.20±0.63</td>
<td>2.16±0.15</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL (Group III)</td>
<td>7.12±0.11</td>
<td>4.02±0.51</td>
<td>3.10±0.71</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg TDL (Group IV)</td>
<td>7.84±0.20</td>
<td>4.26±0.34</td>
<td>3.58±0.22</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg CTL (Group V)</td>
<td>7.94±0.26</td>
<td>4.11±0.26</td>
<td>3.83±0.41</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg CTL (Group VI)</td>
<td>8.18±0.19</td>
<td>4.21±0.19</td>
<td>3.99±0.32</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL+ CTL each (Group VII)</td>
<td>8.06±0.21</td>
<td>4.12±0.13</td>
<td>3.94±0.23</td>
</tr>
<tr>
<td></td>
<td>7.89±0.21</td>
<td>4.31±0.11</td>
<td>3.58±0.33</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for six rats in each group
Statistical comparison: *: Normal control to diabetic control and drug treated groups
P < 0.05
**P<0.01
Statistical comparison: a: Diabetic control to drug treated groups
a: P<0.05
aa: P<0.01
NS: Not significant

**Table 5: Comparative effects of ethanol extract of leaves of *Trichosanthes dioica* and *Clitoria ternatea* on serum SGOT and SGPT levels in normal control and experimental animals**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (Group I)</td>
<td>21.51±0.36</td>
<td>13.61±0.14</td>
</tr>
<tr>
<td>Diabetic control (Group II)</td>
<td>49.31±1.31</td>
<td>41.31±0.74</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL (Group III)</td>
<td>31.56±1.08</td>
<td>23.14±0.54</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg TDL (Group IV)</td>
<td>26.11±1.26</td>
<td>28.11±0.39</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg CTL (Group V)</td>
<td>23.16±1.56</td>
<td>18.56±0.14</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg CTL (Group VI)</td>
<td>21.11±1.07</td>
<td>14.11±0.73</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg TDL+ CTL each (Group VII)</td>
<td>26.16±1.23</td>
<td>13.56±0.14</td>
</tr>
<tr>
<td></td>
<td>20.7±1.34</td>
<td>13.26±0.27</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for six rats in each group
Statistical comparison: *: Normal control to diabetic control and drug treated groups
* P < 0.05
**P<0.01
Statistical comparison: a: Diabetic control to drug treated groups
a: P<0.05
aa: P<0.01
NS: Not significant
REFERENCES
27. Ayyoola GA, Ngene IE, Awobajo FO, Olatunji- Bello II and Odugbemi TO. Hypoglycaemic effect of the aqueous


