Nutritional Properties and Chemical Composition of *Corallocarpus epigaeus* (Arn.) Cl : As Remedy to Control Diabetes mellitus

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ABSTRACT

Medicinal climber *Corallocarpus epigaeus* (Arn.) Cl, family Cucurbitaceae was evaluated for its antidiabetic activity and nutritional properties from tubers. The tuber of plants was sequentially extracted using organic solvent. Out of these, methanolic extract showed maximum extractive yield is 3.28 %. For determination of nutritive value, nutritive elements per 100 grams of methanolic extracts of tuber contain 46.43 mg of carbohydrates, 21.17 mg of protein, 5 mg crude fibres, 0.225 mg calcium, 0.124 mg magnesium, 0.049 mg phosphorus, 0.051 mg vitamin C and 288.4 calori. Alpha Amylase inhibition assay by DNS method revealed that the methanolic extract inhibited $\alpha$-amylase enzyme activity 75.76, 81.52 and 98.55% at 0.1, 0.5 and 1 mg/ml concentration respectively and is comparable to standard reference of acarbose. Alpha amylase inhibitory effect of methanolic extract was found to be competitive. Phytochemical constituents present in the methanolic extract are tannins, alkaloids, saponins, steroids and phenolic compound. GC-MS and LC-MS analysis of methanolic extracts for characterization of bioactive compound. The bioactive compound(s) revealed that the amylase inhibition in the methanol extract of tuber could possibly contribute to the antidiabetic activity.

Keywords: *Corallocarpus epigaeus*, Cucurbitaceae, tuber, nutritive value, antidiabetic activity

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia (fasting blood glucose level > 126 mg/100 ml (6.1mmol/l) with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1, 2]. More than 300 million people have diabetes; representing 6% of the world's adult population. Currently, the overall global prevalence of diabetes is estimated to be between 3.0% and 3.6% of the population, of which 90% is type 2 diabetes [3]. The prevalence of diabetes is reported to be higher in men than in women; however,
there are more women than men with diabetes. Population growth, urbanization, increasing prevalence of obesity and physical inactivity are thought to be the main factors responsible for the increasing prevalence of type 2 diabetes mellitus [3]. It is urgent need to control this disease other wise create the problem in the human population and day by day death ratio are increases in the world population. If these trends continue, by 2030, some 552 million people, or one adult in 10, will have diabetes. Medicinal plants have long back been used as nutritional additional parts and in the treatment of many diseases without proper knowledge of their function [4]. In recent year, consumers have begun to look at food not only for basic nutrition, but also for health benefits. The functional food and nutraceutical industry is responding to a continuing increase in consumer understanding of a link between diet and disease. Currently there is an increased global demand due to the recognition that “nutraceuticals” play a major role in health related problem [5]. Dietary therapy is the key role for the control of gestational diabetes mellitus. Although there is world wide use of herbal dietary supplements that are believed to control the diabetes mellitus [6].

Corallocarpus epigaeus (Arn.) Cl. (Cucurbitaceae) known as Jungali suran in Gujarat. It is distributed in tropical Africa, Persian Gulf region and India (Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal). Monoecious, up to 4m long with tuberous root climber. Stem angular-sulcate and tendrils simple, elongated and glabrous. Leaves 3 lobed, 20-60mm long, cordate, finely hairy on both surfaces and lobes obovate to oblanceolate. Petiole 10-30mm long, glabrous to shortly hairy. Male peduncle 4-6cm long, 5-15-flowered, pedicels 1-5mm long, filiform. Calyx lobes lanceolate, 1mm long. Corolla greenish yellow, obtuse and 1mm long. Female flowers often solitary, on contracted axillary branch, pedicel 1-5mm long, thickened in fruit; calyx-tube campanulate, 2 mm long; petals 1.5-2.5mm long, 1-1.5mm broad, reflexed. Fruit ovoid or ellipsoid, beaked and glabrous. Seeds asymmetrically pyriform, smooth, yellowish and turgid. Flowers and fruiting on June to October [7]. The root contains a bitter principle allied to bryonin. A p-hydroxybenzoyl ester, named epigaeusyl ester, a sesterpene lactone, viz. corallocarpascalaride, a pyridine carboxylic ester, designated as corallocarpeonyl ester [8]. It has a bitter and sub-acid taste and is credited with alterative and laxative properties, and is used in syphilitic rheumatism, later stages of dysentery. Deccan and Mysore the root has repute as a remedy for snakebite administered internally and applied to bitten part [9]. The present study was undertaken to investigate the C. epigaeus plant for its nutritional value and antidiabetic activity.

2. MATERIAL AND METHODS

2.1 Plant Material

Corallocarpus epigaeus (Arn.) Cl. plant species tuber were selected and collected between June to July, 2011 form Viramgam village of Gujarat and surroundings of the region. The tubers of healthy and disease free plants were used to test the medicinal properties and nutritive value. The plant specimens were identified by Dr. Kalpesh Ishnava (Plant Taxonomist) at Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India.
2.2 Drying and Grinding the Plant Material

The plant material is collected and kept to dry on trays at ambient temperature and in a room with adequate ventilation. Plant materials were sliced into small pieces and distributed evenly to facilitate homogeneous drying. Protection from direct sunlight is advised to minimize chemical reactions (and formation of artifacts) induced by ultraviolet rays [10].

2.3 Preparation of Plant Tubers Extracts

Preparation of plant tubers extraction according to the Satyajit et al. (2009) described followed with slightly modification [11].

2.4 Analysis of Nutritional Value


2.4.1 Determination of mineral and heavy metals content by inductively coupled plasma - optical emission spectrophotometer (ICP-OES)

The method described by Indrayan et al., (2005) was employed to determine the mineral and heavy metal contents in the samples [12]. Minerals and heavy metals analysis was performed using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICPOES, OPTIMA 2100DV, Perkin Elmer, USA).

2.4.2 Determination of vitamin C

Vitamin C was quantified in the methanol extract of C. epigaeus tuber using high performance liquid chromatography. 100 mg/ml of sample was prepared in methanol.

The peak of plant extract obtained on carrying out the analysis by HPLC (with RT 2.81 min.) matched with the peak of standard vitamin C obtained using the same analysis (with RT 2.7 min.). Vitamin C present in the plant extract was quantified using the following equation:

\[
\% \text{ Concentration} = \frac{\text{Area of peak of plant extract}}{\text{Area of peak of sample}} \times \frac{0.002}{\text{Mass of plant extract loaded}} \times 92.09
\]

2.5 Antidiabetic Activity

The inhibition assay was performed using the chromogenic DNSA method. The IC_{50} values were defined as the concentration of the extract, containing the α-amylase inhibitor that inhibited 50% of the PPA activity. The other quantifiers were calculated as follows:

Percentage inhibition = 100 - % reaction, whereby the % reaction = (mean maltose in sample/mean maltose in control) × 100

2.5.1 Enzyme inhibitor effectiveness

The effectiveness of extracts showing strong enzyme inhibitory activities was evaluated in terms of their IC_{50} values and compared with those known synthetic enzyme inhibitors. The IC_{50} value is half maximal inhibitory concentration of the inhibitor and it is commonly used as a measure of inhibitor effectiveness [13].

2.5.2 α-amylase inhibitor effectiveness

Alpha amylase was pre-mixed with methanol extracts (which demonstrated the strongest alpha amylase inhibitory activities during screening) at various concentrations (62.5 - 200 ml/ml) and starch as a substrate was added as a 0.5% starch solution in phosphate buffer to start the reaction. The reaction was carried out for 20 minutes.
and terminated by adding 2 ml of DNS reagent (1% 3,5-dinitrosalysalic acid and 12% sodium potassium tartarate in 0.4 M NaOH). The reaction mixture was heated for 15 minutes at 100°C and alpha amylase activity was determined by measuring absorbance at 540 nm.

IC50 values were determined from dose-response curve of percentage inhibition versus methanol and acetone extracts concentration and compared with the IC50 of the synthetic inhibitor of alpha amylase (acarbose) determined under similar conditions.

2.5.3 Mode of inhibition on α-amylase activity
Mode of inhibition of methanol extract of the C. epigaeus towards alpha amylase activity was determined according to the method described by Kim et al., (2005) [14].

2.6 Phytochemical Analysis
2.6.1 Qualitative phytochemical analysis
Qualitative phytochemical analysis of tannins, alkaloid, saponin, cardiac glycosides, terpenoids, steroids and phenolic compounds according to the method described by Parekh and Chanda (2007) [15].

2.6.2 Quantitative phytochemical analysis
2.6.2.1 Alkaloid determination using harborne method
Quantitative phytochemical analysis of alkaloid determination, estimation of total phenols and total flavonoid assay according to method described by Parekh and Chanda, 2007 [15].

2.6.3 HPLC analysis
HPLC analysis was performed on a Perkin Elmer (Series, 200), USA system consisting of quaternary gradient system pump, RI (Range 1.00 to 1.75 RIV) detector, auto-sampler along with DGU 20A3 degasser and with Class VP software. Separations were achieved using column C-18, RP-18 and PI-Gel, 5 μm particle size and mobile phase of 0.1% formic acid in water and 0.1% formic acid in acetonitrile water in the ratio of 60:40 at a flow rate of 1 ml/min. The sample was run for the analysis. Each analysis was repeated twice.

2.6.4 HPTLC analysis
The methanol extract was applied on HPTLC (Camag, Switzerland) aluminum sheet pre-coated with silica gel 60 (1.05547 E Merck) was used as the adsorbent. Methanol:Chloroform (3:17) were used as the mobile phase. The chromatographic development chamber was saturated with mobile phase for 10 min prior to placement of the plates. The plates were run up to 8 cm height and derivatized (10% H2SO4) in methanol. The derivatized plates were heated at 100°C for 4 min, bands were observed and scanned at 366 nm and photographs were taken for record.

2.6.5 Gas chromatography-mass spectroscopy (GC-MS) and LC-MS
The GC-MS analysis was done by electron impact ionization (EI) method on Auto system XL gas chromatography (Perkin Elmer Instrument, Germany) coupled to a Turbo mass spectrophotometer (Perkin Elmer Instrument, Germany). The column was fused silica capillary column, 30 × 0.25 mm ID; coated with D-I, 0.25 μm film thickness. The temperature of column was programmed at 70 to 250 °C at the rate of 10 °C /min increase, injection port temperature at 250 °C. Helium was used as carrier gas at constant pressure of 100 kpa and flow rate of 20 ml/min. Samples which dissolved in chloroform was run fully at
range of 60-550 amu and the results were compared by using NIST 107 Spectral library search programme.

2.6.6 NMR spectroscopy

$^1$H NMR spectra were recorded in CDCl$_3$, using a BRUKER and 400 MHz for proton NMR spectrometer at the Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Vallabhbhidyanagar, Gujarat.

3. RESULTS AND DISCUSSION

Plants continue to be important sources of new drugs. Important plant-derived drugs are still obtained commercially by extraction from their whole plant sources [16]. Plant based many of these metabolites have been showed to be active against different medicinal properties [17]. The tuber of *C. epigaeus* was sequentially extracted using hexane, chloroform, methanol and distilled water and then extracts were used to investigate their nutritional value and anti-diabetes activity.

3.1 Extractive Yield (%) of *C. epigaeus* Tuber

Before evaluating the nutritive value, knowledge regarding the yield of extract from each herb is important. Lower extract yielding plants are not commonly preferred by the pharmaceutical industry though they are rich in their potency [18]. So, the work was carried out with yield calculation. We have selected chloroform, hexane, methanol and distilled water solvent in the ratio of 1:5 to extract the plant constituents. The organic solvent (Methanol) eluted most of the phytoconstituents from the plant. By increasing the polarity of the solvent system with water, the elution of glycosides from plant can be improved. Hebber et al. (2004) [18] reported the same plant collected from Tamilnadu and used leaves and root for extraction using 70 % ethanol as solvent. The methanol extract (3.28 %) showed maximum extractive yield compare to other solvent extracts like chloroform, hexane and distilled water, which are 1.29 %, 0.75 % and 0.85% respectively.

3.2 Nutritive Value of Methanol Extract

Tuber were found to be of high nutritive value, on a dry matter (DM) basis (moisture 5.6%), which support their medicinal use. The results obtained from the nutritive value analysis of *C. epigaeus* tubers established the fact that it can be ranked as carbohydrate and protein rich tubers due to their relatively high content. These results can be favorably compared with that reported by [11], containing carbohydrates (48.48%) and crude protein (2.5 %) [11]. Carbohydrate and Glycosides play an important role in immunomodulatory reactions and their free radical scavenging activity has earlier been reported by [19]. The high ash content is a reflection of the mineral contents reserved in the tubers. The value of the crude fats (2%) can be compared with that reported by Morelli et al., (2003) [20]. A diet providing 1-2% of its caloric energy as fat is said to be appropriate for human beings, as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging.

Fibre content of *C. epigaeus* was found to be 5%. According to available data, when compared to all root and tuber reported by Onianwa et al., (1999), the fibre content of *C. epigaeus* is very high [21].

Calcium helps in bone formation and blood coagulation. Iron is important as a constituent of haem, an essential part of haemochromagens that are important in respiration reported by Gopalan et al., (2004) [22]. The presence of these minerals has contributed to its medicinal value as well as
physiological activity reported by Antia et al., (2006) [23].

The percentage of various mineral elements and nutritional parameters are analyzed in methanol extract of tuber. Nutritive value of methanol extract of tuber is 288.4 cal/100g. The carbohydrate content of tuber is very high (46.43 mg/100 gm) compare to other nutritive components. The analysis revealed that it contains 21.17 % Crude protein, 19.4 % Ash and 2.0 % fat. Other minerals were detected in reasonable amounts such as Calcium (0.225 mg/100g), Magnesium (0.124 mg/100mg), Iron (0.0061 mg/100mg) and Phosphorous (0.049 mg/100g).

3.3 Antidiabetic Activity of Methanol Extracts of *C. epigaeus* Tuber

3.3.1. α-amylase inhibitory effects of methanol extracts of *C. epigaeus* tuber

In the study, the alpha amylase inhibition by DNSA assay revealed that the methanolic extract of tuber showed significant inhibition of α-amylase enzyme activity (Figure 1). Methanolic extracts at 0.1, 0.5 and 1 mg/ml concentration showed 75.76, 81.52 and 98.55 percentage inhibition of α-amylase activity respectively and IC<sub>50</sub> value. However, acarbose was used as reference standard, and showed 18.75, 29.06 and 58.45 percentage inhibition of α-amylase activity at concentrations of 10, 40 and 100 μg/ml and the calculated IC<sub>50</sub> value was found to be 83.33 ± 0.34 at 0.312 μg/ml. The methanolic extract showed good inhibitory activity compare to the reference standard of acarbose. The sample of methanolic extract can be compared with the absorbance against maltose concentration for the estimation of residual maltose concentration. The K<sub>m</sub> value for both control and plant extract remained same while the V<sub>max</sub> value changed. Thus, it can be concluded that the mode of inhibition is competitive. The alpha amylase inhibitory effects of methanol extracts are relatively good compared to the control. WHO, (1999) [1] reported the review of the diabetic activity of medicinal plants including some plant of curbitaceae family. It can be depicted that methanolic extract of tuber showed comparatively better alpha amylase inhibitory activity. Thus, data presented here indicate that methanolic extract of tuber possesses significant anti-diabetic activity. The mechanism by which tuber exerted anti-diabetic action may be due to its action on carbohydrate binding regions of α-glucosidase enzyme, α-amylase, endoglucanases that catalyse hydrolysis of the internal α-1, 4 glucosidic linkages in starch and other related polysaccharides have also been targets for the suppression of postprandial hyperglycemia. Since α-amylases play an important role in starch break down in human beings and animals, the presence of such inhibitors in food stuffs may be responsible for impaired starch digestion [24, 25]. Thus, this α-amylase inhibitor may be of value as novel therapeutic diabetic agents.
3.4 Phytochemical Analysis of Methanol Extract

The presence of alkaloids in the investigated plants of the wild cucurbits indicates that they have medicinal values and some of the use as an anti-diabetic like e.g. *Momordica charantia* (Bitter gourd). Alkaloids have powerful effect on the physiology of animals. They play some metabolic role and control development in living system [26, 27, 28, 29]. Different antioxidant and radical scavenging activity may be partly due to wide variety of antioxidant constituents such as phenolics, ascorbate and carotenoids [30, 31, 32, 33]. The different spraying reagents were used for the conformation of the particular chemical constituents. The spraying of dragendroff reagent gave orange coloured reaction; vanillin sulphuric reagent spray gave blue colour and vanillin-phosphoric acid reagent spray gave green colour indicating the presence of particular bioactive compound like alkaloids, saponins and terpenoids, respectively (Table 1).

Table 1. Phytochemical analysis of qualitative analysis of methanolic extract of *C. epigaeus.*

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Spraying reagent</th>
<th>Colour observed for presence of compound</th>
<th>Colour expected for presence of compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>Blue-black precipitation</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendroff</td>
<td>Orange-brown</td>
<td>Orange</td>
</tr>
<tr>
<td>Saponins</td>
<td>Vanillin-Sulphuric acid</td>
<td>Blue/ blue-violet/ green</td>
<td>Blue</td>
</tr>
<tr>
<td>Cardiac</td>
<td>-</td>
<td>Green-blue</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>Blue - green ring</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>Vanillin-Phosphoric acid</td>
<td>Blue/Pink/Yellow/green</td>
<td>Green</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Vanillin-Phosphoric acid</td>
<td>Blue/Pink/Yellow/green</td>
<td>Green</td>
</tr>
</tbody>
</table>

Figure 1. Mode of inhibition of alpha amylase activity by methanolic extract
3.4.1 Qualitative analysis

The phytochemical constituents present in the methanolic extract are tannins, alkaloids, saponins, steroids and phenolic compound. The cardiac glycosides are absent in the methanolic extract (Table 1).

3.4.2 Quantitative analysis

Phytochemical constituents of the methanolic extract of *C. epigaeus* tuber were quantitatively analyzed for the presence of % chemical constituents in plant under study. The maximum phytochemical present is alkaloid (60 %) in the methanolic extracts. The phenol and flavanoid are in relatively less quantity in the methanolic extracts.

3.4.3 HPTLC analysis of methanol extract of *C. epigaeus* tuber

In our study, total 9 bands were obtained in the sample. The plant material is collected from semi deciduous forest of Gujarat. Environmental factors may have significant effect on the production of secondary metabolites. The methanolic extract showing two major peaks out of 9 bands. The peak no. 1 exhibited maximum % of area which was 98.88% and minimum % of area was present in the peak no. 2 which was 1.12%. The peak no.1 was collected from the HPLC for further characterization of this compound. The maximum % of height was obtained in the peak no 5 which was 2516.0 and Rf value was 28.21 in methanol extract. Parimala and Rama subramania (2009) [34] reported the same plant collected from Tamilnadu, India using the mobile phase (Toluene: Methanol (8:2)) separating 11 bands.

3.4.4 GC- MS and LC-MS analysis of methanolic extract of *C. epigaeus* tuber

The GC-MS data can be used to identify major bioactive, phytochemical constituent corresponding to major peak with an area of 23.20 %. The peak show maximum percentage area at RT 23.20 in GC-MS analysis and scan 4.7e6 through mass spectrophotometer. The LC-MS data identify major bioactive, phytochemical constituents. Total 20 peaks were obtained. The maximum peak obtained is 662.46 mass spectra on RT are 0.12 that were 100 relative. These data can be useful for the identification of bioactive chemical constitution. The identifications of phytochemical compound were based on the peak area, retention time and molecular formula. LC-MS analysis of methanolic extract of *C. epigaeus* tuber has been shown in Figure 2.
3.4.5 NMR analysis

The NMR data of methanol extracts of *C. epigaeus* tuber showed the major peak at 4.768. Some other minor peaks present in sample were 3.218, 3.214, 1.313, 1.297, 0.818 and 0.801. The major peak is of the pure compound which was eluted using HPLC.

4. CONCLUSION

*Corallocarpus epigaeus* (Cucurbitaceae) tubers of different extract have a great medicinal potential. Also, the methanol extracts have good nutritional potential. The plant material is rich source of carbohydrates, protein and different vitamins.

The alpha amylase inhibition assay by DNS method revealed that the methanol extract showed inhibition of α-amylase enzyme activity at low concentration. The presence bioactive compound(s) conferring the amylase inhibition in the methanol extract of tuber could possibly contribute to the anti-diabetic activity. The bioactive compound present in methanol extract of tuber can, thus, be identified and characterized to utilize its enormous medicinal potential.

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