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Evaluation of Phytochemical and in *Vitro* Studies on Antioxidant, Anti-Diabetic Activities of *Gnetum ula*

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ABSTRACT
\Diabetes mellitus is the most common non-infective disease characterized by hyperglycemia. Formation of advanced glycation end products(AGEs) in long termed-hyperglycemia and oxidative stress are the key factors to accelerate complications. To *screen the potential candidates for treating diabetes, total phenolic content, total flavonoid content, antioxidant activity from crude extracts of Gnetum ula. The* plants were *primarily assessed, and the inhibiting potential of diabetes and its complications provided from Gnetum ula. This present study provided potential applications towards the prevention of diabetes. This fundamental information would be important for alternative drug discovery and nutritional recommendations for diabetic patients.*
Keywords*: Anti-diabetes. Antioxidant, Phytochemicals, Plant extract*

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INTRODUCTION

Medicinal plants include a various type of plants used in herbalism and some of these plants have a medicinal activity. These medicinal plants consider as a rich resource of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development of human cultures around the whole world [1].These include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin. Thousands of plants are used by rural and tribal communities to make crude drugs to cure various ailments. India is a highly populated country and it is difficult to provide medicine for all the people. However, the majority of the rural people use the plants as it is or their parts which are found in and around their locality as primary health care [2]. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Moreover, the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respectively.India has about 4.5 million plant species and among them estimated only 250,000-500,000 plant species, have been investigated phyto-chemically for biological or pharmacological activity [3].The interest in nature as a source of potential chemotherapeutic agents continues. Natural Products and their derivatives represent more than 50% of all the drugs in clinical use in the world today. Higher plants contribute no less than 25% of the total [4].The use of plants for treating diseases is as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. All over the globe, especially in South American countries, the use of medicinal plants has significantly supported primary health care [5].Diabetes mellitus (DM) is a metabolic disease of carbohydrates and fat due to deficiency of insulin secretion or varying degree of insulin resistance.It is a major public health problem and has become a menace globally.Presently, nearly half a billion people live with diabetes. Low- and middle-income countries carry almost 80% of thediabetes burden because they do not have adequate resources to provide preventive or medical care for their populations therefore, rely on traditional medicines. [6].In diabetes, herbal alternatives have proven to provide symptomatic relief and assist in the prevention of the secondary complications of the

disease.Some herbs have also been proven to help in the regeneration of b-cells and overcoming insulin resistance, while some herbs are also reported to possess antioxidant activity and cholesterol-lowering action in addition to their anti-hyperglycemic effect. Diabetes is a chronic systemic disease which is frequently associated with hyperglycemia, hyperinsulinemia and hypertriglyceridemia **[**7]Many studies have reported that phytochemicals such as phenolics offer potential therapeutic benefits in alleviating diabetes and obesity complications and inhibitory effects against α-amylase and α-glucosidase [8, 9, 10, 11] The present study to assess the phytochemical screening and *invitro*antioxidant, anti-diabetic activity of *Gnetum ula.*

MATERIAL AND METHODS

Plant collection

*Gnetum ula*plant was collected in and around Kolli Hills in Namakkal district, Tamil Nadu. Fresh leaves were collected from the plants, washed and used for the study. The leaves were dried in shade-dried, coarsely powdered and was extracted with ethanol.

Preparation of plant extracts

The plant material was dried in shade, coarsely powdered and passed through sieve and was used for the extraction. The grounded dried leaf powder of 30g was taken and extracted using the organic solvent (Ethanol) in a Soxhlet apparatus. The boiling point was set up at 61.15°C - 61.70°C. The solvent was recycled thereby extracting the compounds present in the sample. They were continuously extracted until the solvent losses its color. The extracts obtained was transferred to a Petri dish and concentrated to dry residue under reduced at room temperature. Concentrated residue was stored at 4°C and used for this study.

Phyto and Biochemical Analysis

This chemical process was used to determine the phytochemicals present in the extracts. Test such as alkaloids, flavonoids, saponins, glycosides, sterioids, terpenoids, phenol, tannins, thoils, carbohyrates, amino acids and protein are determined from previously reported methods [12,13, 14]

Antioxidant assay

The antioxidant activity of the extract was determined with DPPH radicals using the method of Bhandari [20]. To 1 ml of 100 µM DPPH solution in ethanol, equal volume of the test sample in ethanol of different concentration was added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance of using a spectrophotometer at 514 nm. 1 ml of ethanol instead of test sample was added to the control tube. Different concentration of ascorbic acid was used as reference compound. Percentage of incubation was calculated from the equation [(Absorbance of control – Absorbance of test)/Absorbance of control)] X 100. IC_{50} value was calculated using Graph pad prism 5.0.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical scavenging activity of the test sample was estimated by the method of Ruch et al., [15]. A solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). 200 µl of sample containing different concentration was mixed with 0.6 ml of H_2 O_2 solution. Absorbance of H_2O_2 was determined 10 minutes later against a blank solution containing phosphate buffer without H_2O_2. A test tube containing 200 µl of phosphate buffer and processed as described above served as the control tube. Different concentration of ascorbic acid was used as reference compound [14, 16].

In vitro Inhibition of α-amylase enzyme

A starch solution (0.1% w/v) was obtained by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartrate solution and 3, 5 di nitro salicylic acid solution 96 mM. Both control and plant extracts were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25◦C. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5-nitro salicylic acid. This reaction is detectable at 540 nm.

Calculation of 50% Inhibitory Concentration (IC50)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

$$I\ \% = (Ac\text{-}As)/\ Ac\ X\ 100$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The result of preliminary qualitative phytochemical analysis on leaves of ethanol solvent extract of *Gnetum ula* were showed in (Table 1). The result showed the presence of Alkaloids, Carbohydrates,

Flavonoids, Proteins, Amino acids, Phenols, Saponins, Tannins, Glycosides, Thiols in ethanol extract *Gnetum ula,* the result of qualitative phytochemical analysis of leaves of ethanol extract. The phytochemical screening of the ethanol of *Gnetum ula* showed the presence of carbohydrates, flavonoids, alkaloids, phenols and tannins. Total phenolic and flavonoid assay reveals about the plant therapeutic property which must be explored [17].Free radicals occur in living system and it causes extensive damages to the cells, tissues and organs which may lead to various disease statuses, especially degenerative disorders and inflammatory diseases. Antioxidant offer resistance to cells and prevent the diseases through scavenging the free radical molecules, by inhibiting lipid per-oxidations and many other mechanisms [18].Many researchers continuously exploit the medicinal plants and their extracts in order to discover the potential drugs with reduced toxicity. In this work we have reported the significant *in vitro* antioxidant and antidiabetic activity of ethanol solvent extracts of *Gnetum ula* leaves.

In anti-oxidant activity, different concentrations ranging from 20-320 µg/ml of the ethanol extract of leaves of *Gnetum ula* were tested for their antioxidant activity in different *in vitro* models. The percentage of inhibition was observed and found that the free radicals were scavenged by the test compounds in a concentration dependent up to the given concentration in all the models. DPPH radical scavenging activity of the leaves extract was presented in Table2 and Figure 1. The percentage of inhibition in DPPH in different concentration like 20, 40, 80, 160, 320 µg/ml were observed in 24, 37, 55, 73, 86 respectively whereas the percentage inhibition of ascorbic acid in concentration like 20, 40, 80, 160, 320 µg/ml were found to be 32, 45, 64, 79, 96 respectively. The IC 50 values for DPPH scavenging activity for ethanol extract of leaves of *Gnetumula* and ascorbic acid were 20 µg/ml and 320µg/ml respectively. The DPPH scavenging activity of the extracts evaluated against the positive control ascorbic acid and the DPPH reduction is directly proportional to the antioxidant content in the extract the leaf extracts showed the potential scavenging of DPPH radicals similar to that of ascorbic acid, its likely due to the proton donating ability of *Gnetum ula* leaf and stabilizes the free radicals in association with a number of hydroxyl groups. The result of this study suggests that this crude extract of *Gnetum ula* leaves contain phytochemicals that are capable of donating hydrogen to free radical [19].

Hydrogen peroxideradical scavenging activity

The activity of Hydrogen Peroxide radical scavenging of the leaves extract was presented in Table 3. The percentage of inhibition in Hydrogen peroxide in different concentration like 20, 40, 80, 160 and 320 µg/ml were observed in 24, 42, 65,83 and 98 respectively whereas the percentage inhibition of ascorbic acid in concentration like 20, 40, 80, 160, 320 µg/ml were found to be 32, 49, 72, 86 and 107 respectively. The IC_{50} values for Hydrogen peroxide scavenging activity for ethanol extract of leaves of *Gnetum ula*and ascorbic acid were 20 µg/ml and 320 µg/ml respectively.

Antidiabetic Activity

There was a dose- dependent increase in percentage inhibitory activity against alpha- amylase enzyme at a concentration of 0.2 ml of plant extract showed a percentage inhibition 35.12% and for 1.0 ml plant extract showed inhibition of 85.13% (Table 4)

Nonenzymatic glycation of proteins or Maillard reaction is increased in diabetes mellitus due to hyperglycemia. Numerous synthetic and natural compounds with different glycation inhibition mechanisms are reviewed in literature. However, owing to the side effects of synthetic molecules, all future expectations rely on natural antiglycation compounds. The potential effects of *Gnetum ula* in terms of protein glycation and glucose diffusion inhibition were evaluated in the present study [20].

Table 1: Preliminary phytochemical analysis of hexane extract of *Gnetum ula* (+ Present; - Absent)

S. No	Phytoconstituents	Ethanol extract of *Gnetum Ula*
1	Alkaloid	+
2	Flavonoids	+
3	Glycosides	-
4	Terpenoids	-
5	Saponins	-
6	Phenol	+
7	Phytosterol	+
8	Tannins	+
9	Carbohydrate	+
10	Protein	+
11	Thiols	+
12	Amino acid	-

Table 2: DPPH Radical Scavenging Activity of Ethanol extract of leaves of *Gnetum ula*

Group	Concentration (µg/ml)	% of Inhibition
Ethanol extract of leaves of *Gnetum ula*	20	24
	40	37
	80	55
	160	73
	320	86
Ascorbic acid	20	32
	40	45
	80	64
	160	79
	320	96

Table 3: Effect of *Gnetum ula* ethanol plant extract on Hydrogen Peroxide Radical Scavenging Activity

GROUP	CONCENTRATION (µg/ml)	% of Inhibition	IC50
Ethanol extract	20	25	
	40	42	
	80	65	
	160	83	
	320	98	
Ascorbic acid	20	32	
	40	49	
	80	72	
	160	86	
	320	107	

Table 4: *IN VITRO* ANTIDIABETIC ACTIVITY OF ALPHA- AMYLASE METHOD

S.No	Concentration Of Sample (Ml)	% OF INHIBITION
1	0.2	35.12
2	0.4	45.62
3	0.6	55.65
4	0.8	78.9
5	1.0	85.13

CONCLUSION

The phytochemical analysis revealed the presence of alkaloids, flavonoids, phytosterol, tannins, carbohydrates and thiols respectively. It could be suggested that herbal product is not only interesting source of medicinal activities but also potential source of phytochemicals. Evaluated in vitro alpha amylase and alpha glucosidase activity of crude ethanol extract of *Gnetum ula* leaves. the ethanol extract of*Gnetum ula* exhibits a very potential antioxidant and anti-diabetic effect. These results can be the strong scientific evidence for the use of this plant as a useful source of antioxidant, and anti-diabetic agents.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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