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In Vitro Anti-Diabetic Activity of Ethanolic Extract of *Artabotrys hexapetalus* (L. F.) Bhandari. Leaves

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ABSTRACT

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. Plants are indispensable to man, the knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that today we possess many effective means of ensuring health care. As per the World Health Organisation (WHO), diabetes mellitus (DM) is defined as a heterogeneous metabolic disorder characterised by common feature of chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism. *Artabotrys hexapetalus* known as Manoranjitham (Vernacular name) is a medium size climbing shrub producing extremely fragrant flowers. Due to its great therapeutic value, *Artabotrys hexapetalus* (AH) has been used for various ailments. The present study was conducted to evaluate the antidiabetic activity of ethanolic extract of *Artabotrys hexapetalus* leaves by *in vitro* methods such as alpha- amylase inhibition assay and beta-galactosidase inhibition assay. The present study suggested that the ethanolic extract of *Artabotrys hexapetalus* leaves possesses significant antidiabetic activity.

INTRODUCTION

As per the WHO, Diabetes Mellitus (DM) is defined as a heterogeneous metabolic disorder characterised by common feature of chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism. The number of individuals with diabetes is rising rapidly throughout the world. Both genetic and environmental factors contribute to its pathogenesis, which involves insufficient insulin secretion, reduced responsiveness to endogenous or exogenous insulin, increased glucose production, and/or abnormalities in fat and protein metabolism. The resulting hyperglycaemia may lead to both acute symptoms and metabolic abnormalities. DM is a leading cause of morbidity and mortality worldwide. It is estimated that approximately 11.8 % of population suffers from DM in India. The incidence is rising in the developed countries of the world at the rate of about 10% per year, especially of type 2 DM, due to rising incidence of obesity and reduced activity levels. DM is expected to continue as a major health problem owing to its serious complications, especially end-stage renal disease, Ischemic Heart Disease (IHD), gangrene of the lower extremities, and blindness in adults. It is anticipated that the number of diabetics will exceed 250 million by the year 2010. [1-2]

The plant *Artabotrys hexapetalus* (L. f.) Bhandari (Annonaceae) is a medium size climbing shrub 8-10 ft long, producing flowers that are greenish in colour and fade to yellow with age, and are extremely fragrant. In different parts of the world, it is largely known as ornamental plant. This species is native to South India and largely cultivated in India, Sri Lanka, Burma, Southern China, and Taiwan. It is also known by its common name in India as “Manoranjini”. It has absolutely intoxicating fragrance, once picked they are very long lasting and hold their scent for days, if kept in water, permeating an entire room. Flowers have three outer and three inner greenish yellow petals – hence the name *hexapetalus*.

VERNACULAR NAMES [3-4]

Tamil : Manoranjitham

Malayalam : Madanakameswari

Hindi : Hari champa

English : Tail grape

Telugu : Aku sampenga

Kannada : Apurva champaka

Manipuri : Chini Champra

It has fruity sweet smell- the Manipuri name *Chini Champra*, meaning sugar lemon, is indicative of that. Bark dark wide, acute or almost so at base, short -acuminate at the tip, not glossy. Lateral veins are 8-16 pairs. Flowers are bisexual, fragrant, about 2.5-3 cm across, on a hooked peduncle, pedicles glabrous, about 10-15 mm long. Fruits are 3-4 cm long when ripe, ovoid and smooth. Seeds 1-2, oval-oblong brown, thin, branchlets puberulous, glabrescent or glabrous when mature. The leaves are simple, alternate, lanceolate-elliptic to oblong, 6-15 cm long, 2-4.5 cm wide, are usually 3-4 times as long as, brown, deeply grooved on one side. [5-6]

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *Artabotrys hexapetalus* was collected from Tiruchirappalli District. The Morphological study identification was carried out and further processed for authentication. The authentication was done by Dr. V. Nandagopalan, Controller of Examination, Associate Professor in Botany, National College, Tiruchirappalli.

Preparation of extract

The leaves of *Artabotrys hexapetalus* (L. f.) Bhandari were collected, Shade dried and the dried material was ground into the coarse powder. Dried powdered leaves were defatted with petroleum ether (60-80°C) and extracted with ethanol using Soxhlet apparatus. A brown coloured residue was obtained. Then it was stored in desiccator. The crude extract was suspended in ethanol before use.

Preliminary Phytochemical screening of AH Leaves

Various qualitative tests were performed for the detection of phytochemical constituents present in the extract for the presence of carbohydrates, tannins, phenols, flavonoids, steroids, glycosides, alkaloids, and saponins etc.

Phytoconstituents [5]

Alkaloids, terpenoids, anthraquinones, butyrolactones, flavonoids, neoligans, phenolic compounds, isoamericanin A, isoamericanol, artabotricinol, americanin, artabotriol, β-unsaturated-β-butyrolactones, artabotrine, apigenin-7-o-apiosylglucoside, glucoluteolin, arapetaloside taxifolin.

IN VITRO ANTI-DIABETIC ACTIVITY

Alpha-amylase inhibition assay [7]

Succinctly, 125,250, 375, 500 and 1000 µg of plant extract made up to 0.2 ml with distilled water, and 400 µl of starch solution were mixed. The reaction started by the addition of 200 µl of the enzyme solution (pancreatic extract) and the tubes were incubated at 25°C for 5 min at room temperature. 200 µl of DNS colour reagent (50.68 g sodium potassium tartrate dissolved in 70 ml of 2 M NaOH with 0.026 mM of 3,5-dinitrosalicylic acid) and placed in a water bath maintained at 85–90°C for 15 min. The mixture in each tube was diluted with 900 ml of distilled water and the absorbance was measured at 540 nm. For each concentration of the extract used, blank incubation was prepared by replacing the enzyme solution with distilled water (200 µl) at the start of the reaction, to correct for the absorbance generated by the plant extract. Control incubations, representing 100% enzyme activity,

Percentage inhibition (PI) = 100 − % reaction (at min),

Where % reaction = mean glucose in sample × 100/mean glucose in control.

Beta-Galactosidase Inhibitory Assay [8]

2-Nitrophenyl β-D-Galactopyranoside as substrate, which is hydrolysed by β-Galactosidase to release 2-nitrophenyl (a coloured agent; which can be monitored at 410 nm). Briefly, a mixture of 150 µl of the samples at different concentrations (0.5-5 mg/mL) and 100 µl of sodium phosphate buffer 0.1 M (pH=7.6) containing the enzyme β-Galactosidase solution (µl *E. coli* cell lysate) was incubated at 37°C for 10 min. After preincubation, 200 µl of gala solution 1 mM in sodium phosphate buffer 0.1 M (pH=7.6) was added. The reaction mixtures were incubated at 37°C for 30 min. After incubation, 1 ml of 0.1 M of Na_2CO_3 were added to stop the reaction and the absorbance was recorded at 405 nm using the spectrophotometer. The β-Galactosidase inhibitory activity was expressed as percentage inhibition and calculated using

$$\text{Percentage inhibition (PI)} = \frac{\text{Control-Test}}{\text{Control}} \text{X100}$$

RESULT AND DISCUSSION

Table No.1: Effect of EEAHL on α-amylase inhibition assay

Conc of Sample (µg/ml)	**Conc of Sugar released (mg/ml**	**Conc of Standard (µg/ml)**	**Conc of Sugar released (mg/ml)**
0.05	0.26	0.25	0.18
0.1	0.20	0.5	0.16
0.15	0.15	0.75	0.14
0.2	0.10	1.00	0.06
0.25	0.03	–	–
Mean	0.148	**Mean**	0.135

Untreated Sample Conc of Sugar Release (mg/ml) = 0.24

% of inhibition (Sample) = 61 %

% of inhibition (Standard) = 56.25%

IC_{50} Value of Sample = 0.427

IC_{50} Value of Standard = 1.38

The intestinal digestive enzyme alpha-amylase plays a vital role in the carbohydrate digestion. Alpha amylases are enzymes that catalyses the hydrolysis of the internal α-1, 4-glycosidic linkages in starch, converting starch into low-molecular weight products such as glucose, maltose, and maltotriose units.[9] The inhibition of this enzyme prevents the conversion of starch into glucose and leads to antidiabetic activity. The *in vitro* alpha-amylase inhibitory study demonstrated that *Artabotrys hexapetalus* leaves has significant antidiabetic activity. At 0.25, 0.2, 0.15. 0.1, 0.05 µg/ml concentration of crude plant extracts shown concentration dependent decrease in concentration of sugar release as shown in **Table No. 1.**

Table No. 2: Effect of EEAHL on β-galactosidase inhibition assay

Conc of Sample (µg/ml)	% of inhibition	Conc of Standard (µg/ml)	% of inhibition
0.125	10	0.25	20
0.250	15	0.50	20
0.375	30	0.75	40
0.500	40	1.00	40
1.00	50	_	_

IC_{50} Value of Sample = 0.61

IC_{50} Value of Standard = 0.90

β-galactosidase is an enzyme that breaks down the more complicated sugar lactose into two simple sugars glucose and galactose by breaking the glycosidic bond.[10]

It also has transgalactosylation activity to synthesize galacto-oligosaccharides. The inhibition of this enzyme prevents the conversion of lactose into glucose and possesses antidiabetic activity. The *in vitro* beta-galactosidase inhibitory study demonstrated that *Artabotrys hexapetalus* (L. f.) Bhandari leaves has well antidiabetic activity. The percentage inhibition at 0.125, 0.25, 0.375. 0.5, 1.0 µg/ml concentration of crude plant extracts shown concentration dependent increase as shown in **Table No. 2.**

CONCLUSION

Ethanolic fraction of *Artabotrys hexapetalus* (L. f.) Bhandari leaves showed anti-diabetic activity, which was comparable to a standard drug, i. e., Acarbose (50 mg/kg). As reported earlier, *Artabotrys hexapetalus* contains many bioactive compounds and majority of these compounds are alkaloids and flavonoids that are responsible for the health benefits. Therefore, this study explores the use of *Artabotrys hexapetalus* leaves in the treatment of diabetes. Further study can be explored to isolate the active constituents and evaluate the mechanism of antidiabetic activity of *Artabotrys hexapetalus* leaves.

CONFLICT OF INTEREST

No conflict of interest for the above work.

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