

# Evaluation Of Antidiabetic And Anticancerous Activities Of *Hydnocarpus Macrocarpus* (Bedd.) Warb. (Achariaceae) - An Endangered Species


Mariyaraj J, Anand Gideon V, John Britto S and Steffy Francis

**ABSTRACT:** The present study evaluates the antidiabetic and anticancer activity of the *Hydnocarpus macrocarpus*. Antidiabetic activity was evaluated using  $\alpha$ -amylase Inhibitory Assay by DNSA Method and anticancer activity using in vitro ant proliferative effect determination By MTT Assay. The Antidiabetic  $IC_{50}$  for the leaf extracts acetone was found to be 57.82  $\mu$ g/ml and Antidiabetic standard acarbose  $IC_{50}$  value was found to be 92.87 $\mu$ g/ml respectively. The anti-cancerous studies of cervical cancer cell line of acetone leaf extract showed almost moderate level of inhibition activity. The result obtained in the in vitro methods suggests that *H. macrocarpus* may be administered for its antidiabetic and anticancerous activity.

**Keywords:** Anticancer, Antidiabetic activity,  $\alpha$ -amylase, *H. macrocarpus* and MTT assay.

## 1. INTRODUCTION

Plants are the basic primary producers in food webs and food chains. As a "waste" product of photosynthesis, plants have been releasing oxygen to the atmosphere for hundred millions of years creating a key life, supporting atmosphere for humans and other aerobic organisms. The family Achariaceae APG IV comprises of species of *hydnocarpus* some 32-33 genera and 155 species including herbs, shrubs and trees. Trees of Achariaceae are evergreen, deciduous growing up to 15 m height and bear fruits between the months of August and September. *Hydnocarpus macrocarpus* was collected from Idukki district in Kerala. It is a densely forested, considered Endemic to the Western Ghats.

Medicinal Rating	
Habit	Evergreen Tree
Height	15.00 m
Pollinators	Insects
Cultivation Status	Wild

## MATERIALS AND METHODS

**Collection and authentication** The plant material was collected from Idduki, Kerala and authenticated by Dr. S. John Britto S. J at The Rapinat Herbarium and Centre for Molecular Systematics (RHT), St. Joseph's College (Autonomous), Tiruchirappalli. The voucher Specimen (RHT68237) was deposited at RHT for future references. **Extraction** The leaf was shade dried and powdered using mechanical grinder.

The powder sample was stored in an air tight container and the portion of the powder was taken in test tubes and solvents (Acetone, Ethanol, Methanol and Aqueous) were added to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of muslin cloth and filtered extracts were taken and acetone extract used for the following test. **Anti-Diabetic assay ( $\alpha$ - Amylase Inhibitory Assay)** By DNAS Method Procedure  $\alpha$ -amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH6.8) at a concentration of 0.1 mg/ml. Various concentrations of sample solutions (0.25 ml) were mixed with  $\alpha$ -amylase solution (0.25 mL) and incubated at 37°C for 5 min. Then the reaction was initiated by adding 0.5 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37°C for 3 min, the reaction was stopped by adding 0.5 ml DNS reagent (1% Dinitrosalicylic acid, 0.05%  $Na_2SO_3$  and 1% NaOH solution) to the reaction mixture and boiling at 100°C for 5 min. After cooling to room temperature, the absorbance (Abs) at 540 nm was recorded by a spectrophotometer. The inhibition percentage was calculated by the following equation:

$$\text{Percentage Inhibition} = \frac{[Abs_1 - Abs_2]}{Abs_1} \times 100$$

Where,  $Abs_1$  = Sample and  $Abs_2$  = Control.

### Hela cell line

Hela cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Himedia). The cell line was cultured in 25 cm<sup>2</sup> tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100 $\mu$ g/ml), and Amphotericin B (2.5 $\mu$ g/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO<sub>2</sub> incubator (Galaxy<sup>®</sup> 170 Eppendorf, Germany). The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

### Preparation of plant extracts and compound stock:

1 mg of each plant extract or compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After

- Affiliated to Bharathidasan University
- Mariyaraj, Research Scholar, Department of Botany, Bishop Heber College (Autonomous), Tiruchirappalli – 17.
- Dr. V. Anand Gideon, Associate Professor & Head, Department of Botany, Bishop Heber College (Autonomous), Tiruchirappalli – 17.
- Dr. S John Britto, Director and Head of The Rapinat Herbarium and Centre for Molecular Systematics (RHT), St. Joseph's College (Autonomous), Tiruchirappalli. 2.
- Steffy Francis, Research Scholar, Department of Botany, Bishop Heber College (Autonomous), Tiruchirappalli – 17.

that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

#### Anti proliferative effect Evaluation:

After 24 hours the growth medium was removed, freshly prepared samples in 5% DMEM were five times serially diluted by two fold dilution (6.25µg, 12.5µg, 25µg, 50µg, 100µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Anti proliferative effect by Direct Microscopic observation: Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Labomed TCM-400 with MICAPS™ HD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

#### Anti proliferative effect by MTT Method:

Fifteen mg of MTT (Himedia, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilisation Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilise the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm (Laura B. Talarico et al., 2004).

**Table 1** Antidiabetic Activity of *Hydnocarpus macrocarpus*

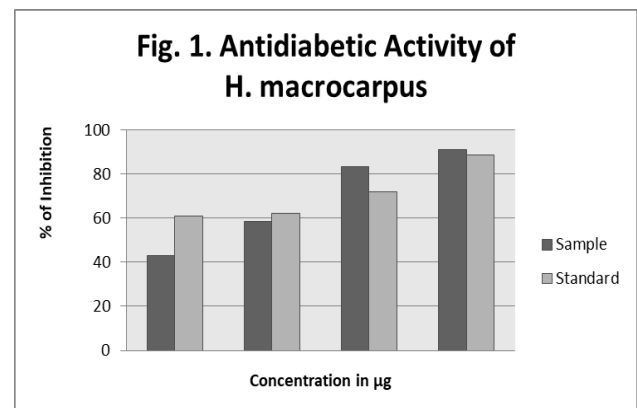
S. No	Percentage of Inhibition (%)		
	Concentration (µg/ml)	<i>H. macrocarpus</i> (Leaf)	Standard
1	25	42.79±1.04	60.7±0.01
2	50	58.42±0.79	62.3±0.03
3	75	66.18±0.35	71.9±0.02
4	100	77.83±0.95	88.4±0.21
IC <sub>50</sub> values (µg/ml)		57.82	92.87

Values were expressed as Mean ± SD (n = 3)  
Values are significantly different from control at (P<0.0001).  
IC<sub>50</sub> values (µg/ml) was calculated by IC<sub>50</sub>calculator

## Result and Discussion

### Antidiabetic activity

leaf extract of *H. macrocarpus* was investigated for in vitro antidiabetic activity with respect to inhibition of α-amylase. The concentration of samples used for testing the inhibitory activity is 25, 50, 75 and 100 (µg). The inhibition activity of test samples showed moderate activity when compared to commercial drug. The maximum inhibition shown at 100 µg concentrations was 77.83±0.95% with the IC<sub>50</sub> value 57.82µg and minimum inhibition shown at 25µg concentrations was 42.79±1.04%. (Table 1, Fig. 1).



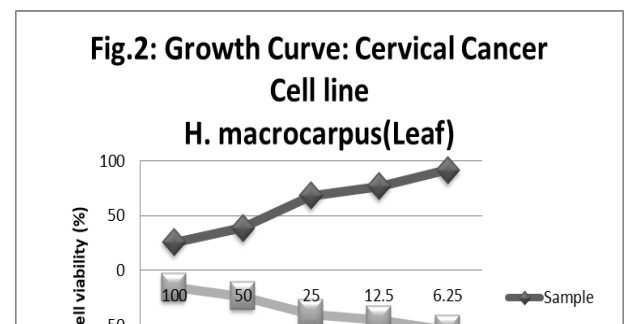
### Anticancerous activities of *Hydnocarpus macrocarpus*

#### Anti-cancerous activity

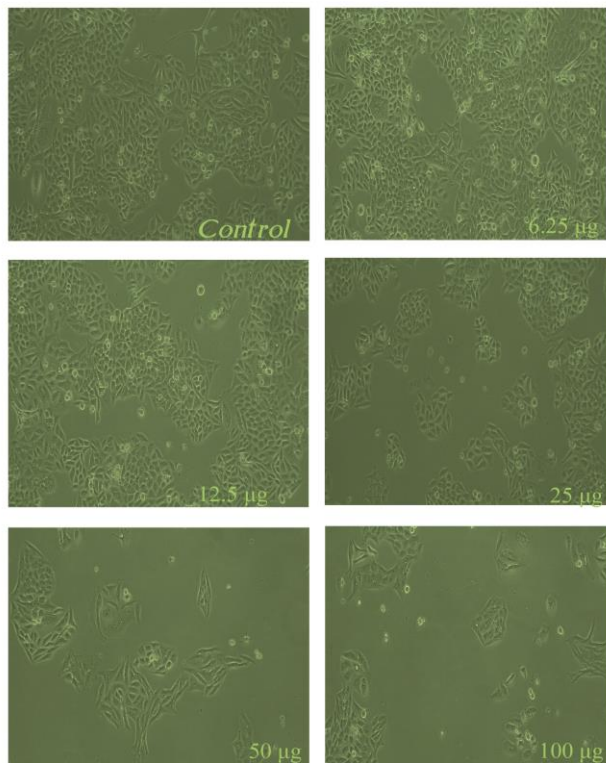
The anti-cancerous studies of cervical cancer cell line of acetone extract of leaf showed optimum level of inhibitory activity. The results for cell growth inhibition by the extract against cervical cancer cell lines for various concentrations are shown in Table 2. As the concentration increases there is an increase in the cell growth inhibition by decrease in the cell viability. It showed 25.6% of control growth at 100 µg concentration (Table 2, Fig. 2, & Plate 1) and 91.46% of control growth at 6.25µg concentration. The cell line studies are shown by the MTT assay method.

**Table 2** Anticancerous Activities of *Hydnocarpus macrocarpus*

S. No	Cervical cancer cell line		
	% of control growth		
	Concentration (µg/ml)	<i>H. macrocarpus</i> (Leaf)	Standard
1	100	25.6	-15.8
2	50	39.02	-23.5
3	25	68.29	-39.9
4	12.5	76.83	-45.5
5	6.25	91.46	-54.2



Plate

Anticancerous activity of *Hydnocarpus macrocarpus* leaf extract against cervical cancer cell line

## Conclusion

From the above anticancerous and antidiabetic results, it is concluded that the acetone leaf extract of the *H. macrocarpus* in antidiabetic test showed moderate activity, than the standard. And anticancerous result showed more effective inhibition in the lower concentration (6.25µg) and it may be concluded that *H. macrocarpus* optimum level of potential and therapeutic values.

## Acknowledgment

The Author is thankful to Dr. V Anand Gideon, Research Guide, Associate Professor and Head of The Department of Botany, Bishop Heber College (Autonomous) Tiruchirappali. He is also indebted to Director and Head Dr. S. John Britto S. J. The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous) for providing necessary facilities.

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