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Investigation on *in-vitro* Antidiabetic and Hepatoprotective activity of the leaves of *Bauhinia phoenicea* Wight & Arn, Fabaceae

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ABSTRACT

Bauhinia phoenicea Wight & Arn. (Syn: Bauhinia benthamii Bedd.) belonging to the family: Fabaceae is an ornamental medicinal climbing shrub endemic to Western Ghats. The plant parts have been used traditionally for treating wounds, diabetes, skin allergies, fungal infections and worm disturbances. The Pharmacognostical and Preliminary phytochemical studies have been reported on the leaves. The plant contains steroids, terpenoids, phenols, tannins, saponins, alkaloids and flavonoids as phytoconstituents. No detailed reports are available on the hepatoprotective and antidiabetic activity of the leaves, hence attempt was undertaken to investigate the hepatoprotective and antidiabetic activity on the leaves of Bauhinia phoenicea by in-vitro methods like MTT assay and α-Amylase inhibition assay respectively. The study revealed that the leaf possess significant antidiabetic and hepatoprotective activities which justifies the therapeutic potential and traditional uses of the plant.

INTRODUCTION

Healing with medicinal plants is holistic and as old as mankind.[1] Enormous number of alkaloids, glycosides, antibiotics, and other phytoconstituents have been isolated from plants, identified and used as curative agents. The Genus Bauhinia is a large and diverse tropical and subtropical genus comprising of approximately 300 species, belonging to the family leguminosae or fabaceae. Most of them possessed bilobed leaves and has been the subject of a number of regional treatments. Species of Bauhinia display a wide range of morphological variations and habit type, being found as trees, shrub, herbs or lianas.[2]

α-amylase is one of the key digestive enzyme which catalyses hydrolysis of starch to maltose in the beginning and at the end convert it to glucose therefore by inhibiting the activity of α-amylase. One can think about the control of diabetes as diabetes is related to increase in glucose level inside body and α-amylase is responsible for the conversion of starch into many simple sugars like dextrin, maltose and glucose.[3] The activity of α-amylase is correlated to the elevated postprandial hyperglycemia therefore control of this enzyme activity becomes significant in the treatment of diabetes.[4]

Oxidative stress becomes a detrimental condition when (Reactive oxygen species) ROS is excess. ROS could damage biological molecules such as proteins, deoxynucleic acid, and lipid membranes, which cause apoptotic or necrotic cell death by disrupting cellular function and integrity [5]. This harmful oxidative stress has been considered as a major cause of cell injuries, which leads to many diseases such as Alzheimer's, cancer, and cardiovascular diseases [6]. ROS generated from mitochondria and other sources causes cell damage through mechanisms involving lipid peroxidation and subsequent liver injury. This also activates many kinases to catalyse degenerative progression in liver tissues.[7] H_2O_2 which occurs as a byproduct of oxidative stress has been implicated in triggering apoptosis in various cell types to evaluate hepatoprotective activity.[8] Herbal products which consist of many compounds have been used by the people and which has become the dependable source of

remedy for various diseases. Hence, the discovery of new potential drugs from medicinal plants with fewer side effects for the treatment of diabetes and liver diseases has to be continued.

Bauhinia phoenicea Wight & Arn is an ornamental medicinal shrub endemic to Western Ghats which is mainly found in the states of Karnataka, Kerala and Tamil Nadu.[9] The plant, known as vallimantharam in Malayalam, Kembu yathaballi in Kannada, Thukarakkali in Tamil, Raktakanchana or Ashmantaka in Sanskit is a large climbing shrub having a height of 40- 45 feet, with alternate, bilobed leaf and scarlet red coloured flowers. *Bauhinia phoenicea,* with common names Crimson mountain ebony or scarlet Bauhinia consists of dark brown, hard, deeply fissured bark and widely spread crown. Traditional claims reveal that the plant is useful for wound healing, diabetes, skin allergies, fungal infections and worm disturbances.[9] The antioxidant, anticancer[10] and antimicrobial[11] activity of *Bauhinia phoenicea* leaves has been reported. Hence this study was designed to carry out investigation on the hepatoprotective and antidiabetic activities of the chloroform extract of the leaves of *Bauhinia phoenicea Wight & Arn.* by MTT assay of H_2O_2 induced peroxidation on $HepG_2$ cells and α-amylase inhibition by starch iodine method respectively.

MATERIALS AND METHODS

Collection of the Plant Material

The plant material was collected from Karyavattom, Trivandrum District, and Kerala, India in September 2020. The plant was authenticated by Dr. T.S. Swapna, Department of Botany, Karyavattom Campus. A voucher specimen (Voucher No. KUBH 10731) has been deposited for future reference. The leaves were separated from other plant parts, washed, cleaned, dried and powdered.

Reagents

All the reagents used were of analytical grade obtained from Universal chemicals and scientific industries, Kerala

Preparation of Extracts [12]

50g of dried coarse powder of leaves packed in a soxhlet-extractor and extracted successively with solvents like petroleum ether (60-80°C), chloroform, ethyl acetate and methanol. The extraction was continued each times till the solvent in the thimble of extractor become clear. The marc obtained after the methanol extraction was dried and macerated with chloroform water for 24 hrs and filtered to obtain the water extract. All the extracts

Table 1 : Mean absorbance and percentage inhibition of Chloroform extract of *Bauhinia phoenicea* Wight & Arn. and standard Acarbose by Starch Iodine assay.

Sample	Concentration µg/ml	Mean Absorbance	Percentage inhibition
Control	-	0.313 ± 0.0002	-
Chloroform extract	25	0.395 ± 0.001	27.23
	50	0.432 ± 0.0002	38.21
	100	0.514 ± 0.0009	63.901
	200	0.554 ± 0.0001	76.93
Acarbose	25	0.415 ± 0.002	31.63
	50	0.474 ± 0.001	51.95
	100	0.538 ± 0.001	71.73
	200	0.563 ± 0.0008	80.32

Values are expressed in Mean ± SEM, n = 3, The value were compared with control using Dunnets multiple comparison and P value obtained was less than 0.0001, *** = $p < 0.0001$. Therefore, considered as significant

collected were evaporated in a water bath and the dried extracts were stored in a desiccator till required. All the extracts were evaluated for in-vitro anti-oxidant activity. Chloroform extract showed good antioxidant activity hence was selected for carrying out in-vitro antidiabetic and hepatoprotective activity.

Hepatoprotective activity analysis by MTT assay [13,14,15,16,17]

The hepatoprotective activity of the test samples were evaluated using HepG2 cell line. IC_{50} concentration of H_2O_2 (30 µg) was used as a cytotoxic agent. Confluent HepG2 cells were cultured in growth media (DMEM + 10% FBS) at a density of 5×10^4 cells/well in a 96-well tissue culture plate and incubated overnight. Post incubation, cells were treated with varying concentrations of the test sample (25, 50, 100 µL) and incubated for 24 h, thereafter; IC_{50} concentration of H_2O_2 (30 µg) was added and allowed for further 24 h incubation. Post incubation, the treated cells were washed with PBS and incubated with MTT containing growth media. Finally, the medium was removed, and the formazan crystals were dissolved using 150 µl DMSO (100%). The optical density was measured at 570 nm. Untreated cells were kept as control and percentage cell viability in treated cells were calculated.

Antidiabetic activity by α-amylase inhibition by starch iodine method [3,4,18]

The assay mixture was about 120 µl of 0.02M sodium phosphate buffer (pH 6.9), 1.5 ml of α-amylase and plant extracts at a concentration from 25-200 µg/ml (w/v) was incubated at 37°C for 10 min. After that, soluble 1% starch was added at each reaction mixture and incubated at 37 °C for 15 min. Then 60 µl of 1 M HCl was added to the reaction mixture to stop the enzymatic reaction and immediately 300 µl of iodine reagents was added. If any change in colour was noted and at 620 nm the absorbance was read.

Statistical analysis

Statistical analysis was done by using one-way ANOVA followed by Dunnet's test. P values lesser than 0.05 were considered as significant using Graph Pad Prism software version 9.

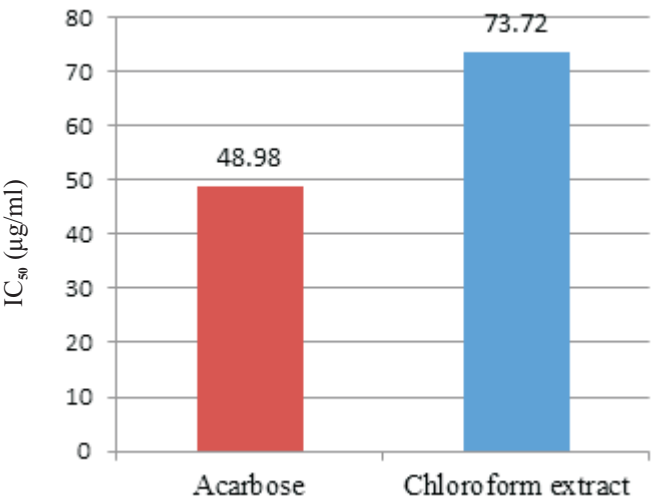
RESULTS

The percentage inhibition of α-amylase enzyme was found to increase with increase in concentration of chloroform extract of leaves of *Bauhinia phoenicea* Wight & Arn. The values are tabulated in Table no.1. IC_{50} values of standard Acarbose and chloroform extracts were compared and is depicted in Graph no. 1.

The protective effect of the chloroform extract on cell death induced by oxidation of H_2O_2 was evaluated. The IC_{50} of H_2O_2 was investigated by inducing oxidation on HepG2 cells whereby percentage cell viability decreased with increasing concentration of H_2O_2. The values are depicted in Table no. 2 and IC_{50} was found to be 30.08 µM .

IC_{50} of H_20_2 was found to be 30.08 µM. The cell viability of HepG2 cells was found to increase with increase in concentration of the leaf extract i.e, in a dose dependant manner and the obtained values have been tabulated in Table no.3.

Values are expressed in mean ± SEM, n=3. The value were compared with control using Dunnets multiple comparison and P value obtained was less than 0.05,**= p <0.05. Therefore, considered as significant.



Graph 1 : Comparative IC_{50} (µg/ml) value Chloroform extract of Bauhinia phoenicea Wight & Arn. and standard Acarbose

Table 2 : MTT result of Hydrogen Peroxide.

Concentration (µM)	Percentage cell viability
6.25	82.15
12.5	73.52
25	61.36
50	47.15
100	36.59

DISCUSSION

Plant drugs are of great value in the field of treatment and cure of diseases. The preliminary phytochemical evaluation of chloroform extract of the leaves of *Bauhinia phoenicea* revealed the presence of large number of secondary metabolites such as flavonoids, saponins and steroids. These secondary metabolites might be responsible for traditional claims and therapeutic activity of this plant. From different literatures, it was reported that the plant has antioxidant, antimicrobial and anticancer activity.

The percentage inhibition of alpha amylase enzyme was found to increase with increase in concentration of chloroform leaf extract. The activity of α-amylase is correlated to the elevated postprandial hyperglycemic conditions. Inhibition of α-amylase enzyme decreases the conversion of carbohydrates into glucose in the blood thereby reducing the chance for hyperglycemia. Thus the chloroform leaf extract of *Bauhinia phoenicea* Wight & Arn inhibited α-amylase enzyme thereby substantiating its antidiabetic activity and confirming the traditional claim of the plant

Table 3 : Percentage cell viability when treated with chloroform extract of Bauhinia phoenicea Wight & Arn.

Concentration	Absorbance	Percentage Cell Viability
Control	0.415	50
$_2O_2$ (30 µM)+ 25µg/ml chloroform extract	0.422	55.82
H_2O_2 (30 µM)+ 50 µg/ml chloroform extract	0.490	64.81**
H_2O_2 (30 µM)+ 100 µg/ml chloroform extract	0.583	77.16**

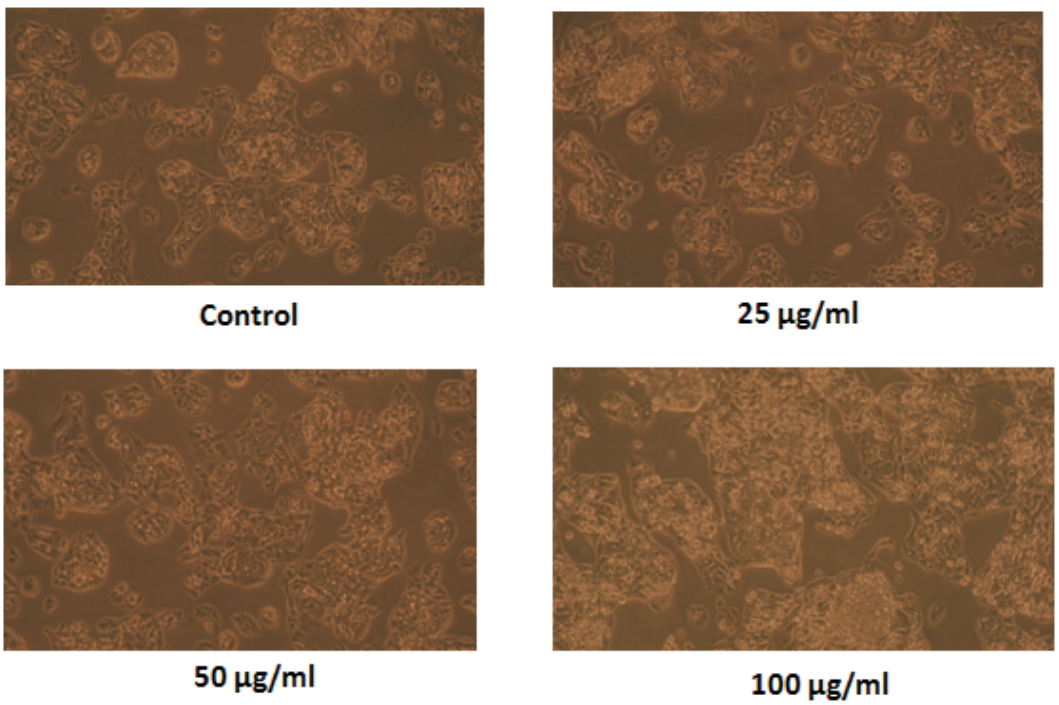


Fig 1 : Percentage cell viability during evaluation of Hepatoprotective activity of chloroform extract of *Bauhinia phoenicea* Wight & Arn. at different concentrations

HepG2 cells (Human liver carcinoma cells) have been exploited as a model to study the hepatoprotective effect of the test compound in this study. H_2O_2 is also widely used as an inducer of oxidative stress in in-vitro models, which could lead to cell death[19]. In addition, it was reported that the cell damage effect induced by H_2O_2 could be attenuated by treating with antioxidants [20]. Therefore, the protective effect of the test compounds on cell death induced by H_2O_2 was investigated by HepG2 cell model. The percentage cell viability of HepG2 cells was found to increase with increase in concentration of the leaf extract in a dose dependant manner. This may be due to the protective effect induced over the H_2O_2 treated HepG2 cells, by the flavonoids or antioxidant principles present in the chloroform extract of *Bauhinia phoenicea*.

CONCLUSION

Diabetes and liver damage are among the major health problems worldwide which may even lead to death. Continuous research for effective natural medicine with lesser side effects is hence a requirement of today's world. The present study showed that the chloroform leaf extract of Bauhinia phoenicea Wight & Arn possess significant antidiabetic and hepatoprotective activity in a dose dependant manner. The results of this study directs further researches to evaluate the therapeutic potentialities of the phytoconstituents present in the leaf of Bauhinia phoenicea Wight & Arn. Further *in-vitro* and *in-vivo* studies are to be conducted for the substantiation of the traditional claims of the drug thereby changing this plant into a future promising drug.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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