ORIGINAL RESEARCH

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIDIABETIC ACTIVITY OF *AVERRHOA CARAMBOLA* LINN. LEAF EXTRACTS

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ABSTRACT

Averrhoa carambola, commonly known as starfruit, belongs to the family *Oxalidaceae.* The present study is attempted to evaluate the anti-diabetic activity of *Averrhoa carambola* leaves and to report the phytochemical constituents and antibacterial activity of three different extracts (Acetone, ethyl Acetate and Hexane) from these leaves. The preliminary screening of *Averrhoa carambola* showed the presence of flavonoids, tannins, alkaloids, glycosides, phenols, terpenoids, saponins, emodols and coumarins. The anti-diabetic activity of *Averrhoa carambola leaves* were evaluated by two assays. *In vitro* glucose uptake assay on cultured L6 cell lines showed that the percentage of glucose uptake increased with increase in concentration of sample. *In vitro* alpha amylase inhibitory assay showed that the percentage of inhibition increased with increase in concentration of sample. These results indicate that the anti-diabetic activity of *Averrhoa carambola* leaves increase with increase in concentration of the sample. This study justifies the traditional uses of this plant in the treatment of diabetes, wound and infections.

KEY WORDS: *Averrhoa carambola,* alpha amylase, anti-diabetic, phytochemical, antibacterial activity, infections

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INTRODUCTION

Averrhoa carambola is a small evergreen tree with a short-trunk or a shrub and belongs to the family Oxalidaceae. It has drooping branches and white wood which turns to reddish. It takes a bushy shape having many branches producing a broad, rounded crown. The soft leaves are compound in nature, medium-green and are spirally arranged around the branches in an alternate fashion[1]. The flowers are lilac or purple-streaked and come up in the axils of leaves at the end of twigs. The attractive fruits are oblong shaped and in measurement they are longitudinally 5 to 6 angled and 6.35-15 cm long and up to 9 cm wide. The fruits are orange-yellow colored having a thin, waxy skin. The fruits are juicy and yellow inside when ripe and have a crisp texture and looked like a star when cut in cross-section. Usually the fruits are oxalic acidic in odor, which varies from strong to mild depending on the plants. Each fruit may contain up to twelve 6-12.5 mm long seeds, and are flat, thin shaped and brown in colour. Some cultivated forms produce seedless fruit[1]. Carambola is widely grown in the provinces of Fukien, Kuangtung and Kuangsi in southern China, Taiwan and India. It is also popular in Philippines and Queensland, Australia and in some parts of the South Pacific islands, particularly in Tahiti, Guam Hawaii and New Caledonia, New Guinea and Netherlands[2]. The plants contain thiamin, riboflavin, niacin, oxalic acid, ascorbic acid, phytofluene, beta-carotine, beta-cryptoflavin, mutatoxanthin, beta-apo-8-carotene, lutein, cryptoxanthin, cryptochrome[3].

The leaves of this plant have been known to be antipruritic, antipyretic and anthelmintic[4]. It has also been reported to be useful for treating scabies,various types of poisoning, pruritus, intermittent fevers and intestinal warms[5]. The fruits are sweet, sour, thermogenic, febrifuge, antipyretic, antiscorbic and tonic[6]. They are also useful in diarrhoea, vomiting, hyperdipsia, haemorrhoids, intermittent fevers, hepatodynia, scabies and various kinds of poisoning and general debility[7,8,9,10]. To judge the traditional use of this medicinal plant, this study was designed to evaluate the possible antidiabetic effect of *Averrhoa carambola* Linn. Our results suggest that the plant possesses significant glucose reducing effect in cells.

MATERIALS AND METHODS

***Collection of plant material*:**

Fresh leaves of *Averrhoa carambola* were collected from Nagercoil, Kanyakumari district during the month of December in the year 2017. The leaves were cleaned and then dried under shade and ground into a fine powder using domestic mixer grinder machine. The fine powder of the plant leaves obtained was stored in an air tight container for further processing and research work.

Preparation of plant extract:

The ground *Averrhoa carambola* (leaves) were transferred into a conical flask and submerged in acetone, ethyl acetate and hexane and incubated for 48 hours. After incubation, the extract was filtered using Whatman no.1 filter paper. The filtrate was then used for further analysis.

Qualitative phytochemical screening of the plant extracts:

A small quantity of the sample of leaves extracts of *Averrhoa carambola* was first reconstituted in the solvent used for its extraction (acetone, ethyl acetate and hexane). Different qualitative chemical tests were then performed using standard procedures to investigate the chemical composition of *Averrhoa carambola* extracts[11].

Detection of alkaloids:

The extract was treated with Wagner's reagent. The formation of brown/ reddish precipitate indicates the presence of alkaloids.

Detection of tannins:

Few drops of 1% $FeCl_3$ solution was added to 1ml of solvent extract. The appearance of blue, black, green or blue green precipitate indicates the presence of tannins.

Detection of flavonoids;

The test solution was treated with few drops of 5% $FeCl_3$. The formation of blackish red color indicates the presence of flavonoids.

Detection of phenols:

3ml of distilled water was added to 1ml of solvent extracts and then to this, few drops of neutral 5% $FeCl_3$ solution were added. Formation of dark green color indicates the presence of phenol compounds.

Detection of coumarins:

Few drops of ammonia were added on a filter paper. A drop of extract was added to it. The fluorescence indicates the presence of coumarins.

Detection of terpenoids:

To 1ml of the solvent extract, 2ml of chloroform and 3ml of concentrated H_2SO_4 were added carefully to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Detection of saponins:

1ml of solvent extract and 2ml of distilled water was mixed and shaken vigorously. A stable persistent froth indicates the presence of saponins.

Detection of emodols:

The dry extract was added to 25% ammonia solution. A cherry-red color indicates the presence of emodols.

Detection of phlobatannins:

2ml of aqueous extract was added to 2ml of 1%HCl and the mixture was boiled. Deposition of red precipitate indicates the presence of phlobatannins.

Detection of betacyanin:

2ml of plant extract was added to 1ml of 2N sodium hydroxide and heated for 5minutes at 100ºC. The formation of yellow color indicates the presence of betacyanin.

Detection of quinines:

1ml of concentrated H_2SO_4 was added to 1ml of extract. The formation of red color indicates the presence of quinones.

Detection of anthroquinones:

Small portion of the extract was shook well with 10ml benzene. 5ml of 10% ammonia solution was added to the filtrate and stirred. The production of a pink red or violet color indicates the presence of free anthroquinones.

Detection of cardiac glycosides:

1ml of extract was dissolved in glacial acetic acid containing traces of $FeCl_3$.The tube was held at an angle of 45º; 1ml of concentrated H_2SO_4 was added down the side. The purple ring indicates the presence of cardiac glycosides.

Estimation of anti-diabetic activity of the extract

In vitro glucose uptake assay on cultured L6 cell lines[12]

Maintenance of L6 cell lines:

L6 (rat myoblast cell line) was purchased from NCCS Pune was maintained in Dulbecco's modified eagles media (Sigma Aldrich, USA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5 % CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, EPPENDORF, GERMANY).

Procedure:

The cells were trypsinized (500µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Invitrogen) for 2 minutes and passaged to T flasks in complete aseptic conditions. The cells were then subcultured to a 24 well plate. After attaining 80% confluency, cells were kept in DMEM without glucose for 24 hours. Extracts were added to grown cells at a final volume of 25 µg/mL, 50 µg/mL and 100 µg/mL from the stock solution and incubated for 24 hours in DMEM containing 300mM glucose. An untreated control with high glucose was also maintained. After incubation cells were isolated by spinning at 6000 rpm for 10 minutes. Supernatant was discarded and 200µl of cell lysis buffer (1MTris Hcl, 0.25M EDTA, 2M Nacl, 0.5% Triton) was added. The incubation was done for 30 minutes at 4°C and the glucose uptake was estimated using high sensitivity glucose oxidase kit method. All experiments were repeated in

triplicates and mean average was used for calculations[13,14].

Calculation:

$$\text{Total glucose in mg/dL} = \frac{\text{Absorbance of Test}}{\text{Absobance of Standard}} \text{ X } 100$$

$$\text{Glucose uptake (\%)} = \frac{\text{OD of Test} - \text{OD of Control}}{\text{OD of Test}} \text{ X } 100$$

In vitro alpha amylase inhibitory assay:

Different concentrations of samples such as 125µg/mL - 1000µg/mL from a stock concentration of 10mg/mL and make up to 1000µl using 25mM phosphate buffer pH 6.9, containing 25µl of porcine α amylase at a concentration of 0.5 mg/ml were incubated at 25°c for 10 min. After pre incubation, 25µl of 0.5% starch solution in 25mM phosphate buffer pH 6.9 was added. The reaction mixtures were then incubated at 25°c for 10 min. The reaction was stopped with 50µl of 96mM 3, 5 dinitrosalicylic acid colour reagent. The micro plate was then incubated in a boiling water bath for 5 min and cooled to room temperature. Absorbance was measured at 540nm using a microplate reader (Erba, Lisascan)[15].

Calculation:

$$\text{Inhibition (\%)} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

RESULTS AND DISCUSSION

Preliminary phytochemical screening of Averrhoa carambola:

Phytochemical analysis of *Averrhoa carambola* showed the presence of different groups of secondary metabolites like flavonoids, tannins, alkaloids, glycosides, phenols, terpenoids, saponins, emodols and coumarins (Table 1). Of the test extracts, acetone showed positive results for most of the test compounds like flavonoids, alkaloids, coumarins, phenols, tannins, phlobatannins and terpenoids. Phenols, saponins, coumarins, phenols, flavonoids, tannins, emodols and terpenoids were present in ethyl acetate extract. The hexane showed positive results for phenols, saponins, alkaloids and coumarins. Flavonoids and polyphenols are considered as the most powerful secondary metabolite and major bioactive compounds of plants[16]. Present study confirmed that the leaf extract of *Averrhoa carambola* contain flavonoids, although we did not measure the total amount flavonoids. Alkaloids, comprising a large group of nitrogenous compounds interfere with cell division. Hence the presence of alkaloids in *Averrhoa carambola* could account for its use as an antimicrobial agent[17]. Flavonoids are a group of chemicals found in varying amounts in foods and medicinal plants. They have shown to exert potent antioxidant activity against superoxide radicals. Epidemiological studies have indicated that the consumption of flavonoids is inversely related to coronary heart disease mortality[18]. Inhibition of low density lipoprotein (LDL) oxidation has also been attributed to the dietary and supplemental intake of flavonoids and other micronutrients[19,20]. The presence of flavonoids in the plant may be the reason for its therapeutic effects.

Table 1: Qualitative phytochemical analysis of *Averrhoa carambola* extracts

S.No.	Parameters tested	Extracts		
		Acetone	Ethyl Acetate	Hexane
1	Alkaloids	+	-	+
2	Tannins	+	+	-
3	Flavonoids	+	+	-
4	Phenols	+	+	+
5	Coumarins	+	+	+
6	Terpenoids	+	+	-
7	Saponins	-	+	+
8	Emodols	-	+	-

9	Phlobatannins	+	-	-
10	Betacyanin	-	-	-
11	Quinones	-	-	-
12	Anthroquinones	-	-	-
13	Cardiac glycosides	+	+	-

+→ *Positive;* - →*Negative*

Antidiabetic activity of *Averrhoa carambola*

In vitro glucose uptake assay on cultured L6 cell lines:

The results representing the anti-diabetic activity of acetone extract of *Averrhoa carambola* leaves are presented in table 2 and figure 1. The results showed that the percentage of glucose uptake increased with increase in concentration of sample. The increase in percentage of glucose uptake by L6 cell lines shows the increase in anti-diabetic activity of *Averrhoa carambola* leaves proportional to its concentration. The study findings of Gupta et al. (2010) have also clearly demonstrated that the fruits of *Helicteres isora* enhances glucose uptake under *in vitro* conditions by using L6 cell lines[21].

Table: 2 *In vitro* glucose uptake assay on cultured L6 cell lines

Concentration of sample (µg/mL)	Absorbance	Glucose (mg/dl)	Glucose uptake (%)
Control	0.0964	56.74	0
Sample :			
25	0.1188	69.92	18.85
50	0.1385	81.52	30.40
100	0.1566	92.17	38.44

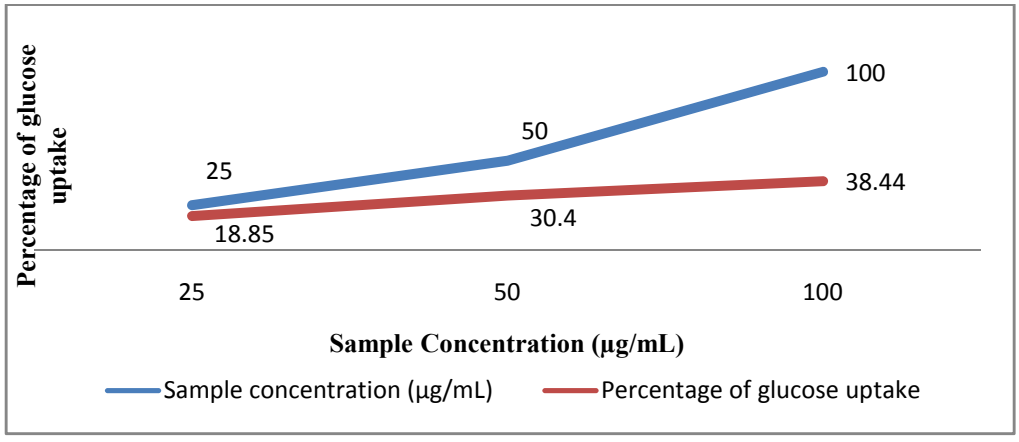

Figure: 1 *In vitro* glucose uptake assay on cultured L6 cell lines

In vitro alpha amylase inhibitory assay

The results showing the anti-diabetic e□ects of *Averrhoa carambola* leaves and almost the same e□ect of standard drug Acarbose are tabulated in table 3 and figures2a and 2b. The results showed that the percentage of inhibition increased with increase in concentration of sample. The increase in percentage of inhibition shows the increase in anti-diabetic activity of *Averrhoa carambola* leaves proportional to its concentration.

Table: 3 *In vitro* alpha amylase inhibitory assay

Concentration (µg/mL)		OD at 540nm	Percentage inhibition
Standard	**Control**	0.123	0
	125	0.0543	55.85
	250	0.0539	58.61
	500	0.0492	60
	1000	0.0322	73.82
Sample	**Control**	0.9034	0
	125	0.6823	24.47
	250	0.6272	30.57
	500	0.5669	37.25
	1000	0.4912	45.63

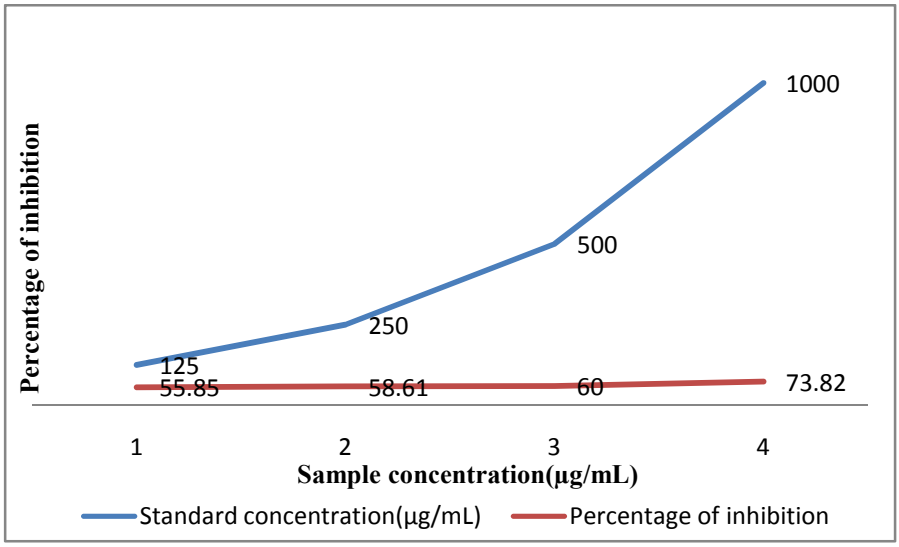

Figure: 2a *In vitro* alpha amylase inhibitory assay for standard drug, acarbose

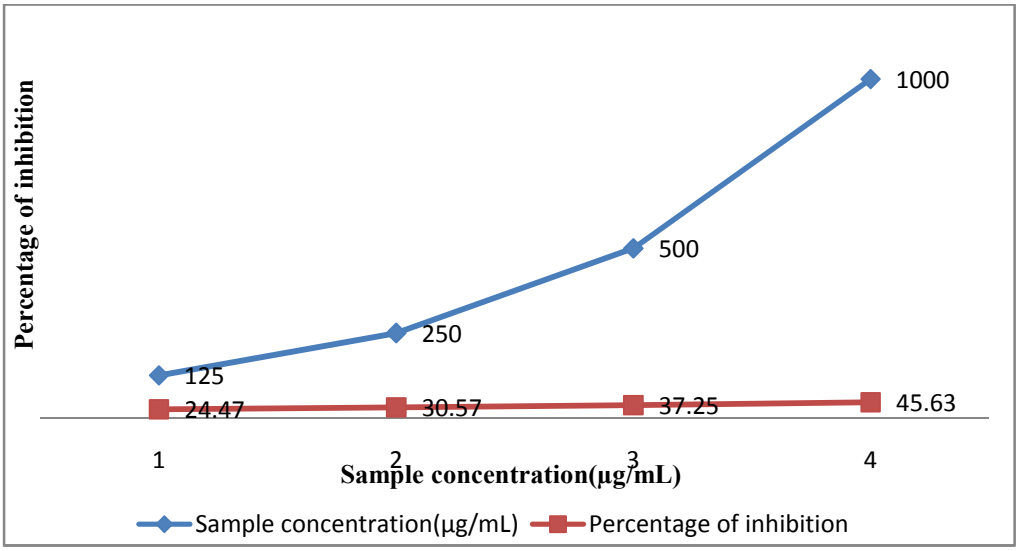

Figure: 2b *In vitro* alpha amylase inhibitory assay for acetone extract of leaves of *Averrhoa carambola*

The α-amylase inhibitory studies performed demonstrated that acetone extract of *Averrhoa carambola* leaves had significant inhibitory potentials. The inhibition percentage of acetone extracts is almost similar to that of acarbose, a widely used and marketed anti-diabetic drug. These α-amylase inhibitors are also called as starch blockers as they prevent or slows the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugars[22]. The α amylase inhibitory activity in methanol extract is most likely to be due to polar compounds and is worth investigating further and isolating pure active compounds. The most commonly involved active constituents in antidiabetic activity are flavonoid, tannins, phenolics and alkaloids. Some hypotheses relate to their effects on the activity of pancreatic ß cells (synthesis and release) or the increase of the insulin sensitivity or the insulin-like activity of the plant extracts. All of these actions may be responsible for the reduction or abolition of diabetic complications[23]. The extract may influence in a positive manner the pancreatic secretion of insulin, or the extract may increase the glucose uptake[24]. It is also possible that the extract may inhibit glucose absorption in gut, thus reducing the presence of glucose in serum[25].

CONCLUSION

According to the results, the acetone leaf extracts of *Averrhoa carambola* exhibited remarkable α-amylase inhibitory activity. Phytoconstituents present in the acetone extract might be responsible for the observed activity.Hence leaves of *Averrhoa carambola* has the potential to be used in ayurvedic decoctions in controlling and treatment of Type II diabetes mellitus. Furthermore, this study has opened opportunities for future research in searching for novel effective drugs for diabetics. These studies justify the traditional use of this plant in the treatment of diabetes and wound. However, further study is necessary for elucidating the active principles. The recent study supports the rational use of this plant as a folklore medicine which could be the source of pharmacologically active compounds.

AUTHOR'S CONTRIBUTION

Ms. Sona Rajashree carried out the study design and analysis of the plant extract. Ms. Sangeetha VS coordinated the work and and helped to draft the final manuscript. Both the authors read and approved the final manuscript.

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