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Fingerprinting Of Rutin And Invitro Anti- Diabetic Activity Among Dietary Amaranthus L. Species.

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ABSTRACT

Alpha-amylase, (α-amylase) is a protein that hydrolyses the alpha bonds of large, alpha-linked polysaccharides, such as the starch and glycogen, yielding glucose and maltose. It is the major form of amylase found in humans and other mammals. It is also present in seeds containing starch as a food reserve. Amaranthus L. plants has the property to inhibit this starch molecule formation by inhibiting amylase enzyme functioning. Hypoglycaemic activity of vegetables are gaining importance in present scenario. The aim of the study is to detect rutin in Amaranthus L. plants, as rutin possess good antidiabetic property. The methanolic extract of these two plants detected rutin with reference standard rutin Rf value. The alpha amylase inhibitory action showed that there is a dose dependent increase in percentage of inhibition with respect to standard Acarbose. Thus the study highlights the rutin compound and antidiabetic activity in leaves of A.viridis and A.dubius.

Keywords: Amaranthus, Antidiabetic, Polysaccharides, Rutin.

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INTRODUCTION

Lifestyle diseases are increasing day today world due to the appearance of unhygienic food. India considered as diabetic nation in near future, due to the various cases reports across the country. Diabetes is due to inadequate production of insulin or inadequate sensitivity of cells to the action of insulin. The signs and symptoms of both types of diabetes include increased urine output and decreased appetite as well as fatigue. This conditions can be overcome by the utilizing the flora of Indian medicinal plants that are rich in therapeutic molecules. Many ayurvedic formulations are available in order to avoid obesity and related diseases.

The treatment goal of diabetic patients is to maintain near normal levels ofglycemic control, in both fasting and post-prandial conditions. Many natural sourceshave been investigated with respect to suppression of glucose production from thecarbohydrates in the gut or glucose absorption from the intestine [1]. Alpha-amylase catalyses the hydrolysis of alpha-1,4-glycosidic linkages ofstarch, glycogen and various oligosaccharides. Alpha-glucosidase further breaksdown the disaccharides to simple sugars, readily available for intestinal absorption.The inhibition of their activity in the digestive tract of humans is considered to beeffective tool to control diabetes. In addition, these effects may leads to diminishedabsorption of monosaccharides [2]. Therefore, effective andnontoxic inhibitors of alpha-amylase and alpha-glucosidase need to be explored.

In humans, the digestion of starch involves several stages. Initially, partialdigestion by the salivary amylase results in the degradation of polymeric substrates intoshorter oligomers. Later on in the gut these are further hydrolyzed by pancreatic amylases into maltose, maltotriose and small malto-oligosaccharides. The digestiveenzyme (α-amylase) is responsible for hydrolyzing dietary starch (maltose), whichbreaks down into glucose prior to absorption. Inhibition of α-amylase can lead toreduction in post prandial hyperglycemia in diabetic condition [3,4]

In India the distribution of Amaranthus L. is basically distributed over a large area, considered as a weedy plant but many reports showed that Amaranthus L.genus has got medicinal property which is need to be exploited for the modern society. The genus is well known for their medicinal used in ayurveda and A. virdis L. act as a substitute for A. spinosus L.Kandabhasmam prepared from Amaranthus L. which has wound healing and blood purifying property. Also used as an ingredient in formulation like Satavaryadikasayam, Asokaristam, Chandanadithailam (nasal congestion relief)[5].

MATERIALS AND METHOD

Collection of plant

The plants were grown in growbags in garden and the grown up mature twigs were collected and dried for herbarium preparation. The herbarium s sheets were deposited at Kerala Forest Research Institute Peechi, Thrissur, India, for authentification of the plant. The leaves were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40- mesh sieve.

Preparation of Extract

The dried powder of the leaves was extracted by hot extraction method by Soxhlet apparatus, using methanol as solvent. The extract were concentrated by using a rotary evaporator.[6]

Preliminary phytochemical screening

The ethanol extract obtained was subjected for phytochemical screening using standard procedure. The dried extract were dissolved in sufficient amounts of respective solvents and tested for various constituents.[7,8].

HPTLC Fingerprinting Analysis

The complete CAMAG TLC equipment consists of a fully automatic sample Linomat V sample applicator, a developing chamber. Finally, a Camag TLC scanner is available allowing densitometric evaluation

of chromatograms and CATS 4 software for interpretation of data. About 10 mg of methanolic extract of A. viridis and A. dubiuswas taken and dissolved in respective solvents and the volume was made up to 10 ml in a standard flask (1000µg/ml). Standard (0.1 mg) was taken and dissolved in methanol. This was transferred into a standard flask and the volume was made up to 100 ml to prepare 100 µg/ml solution. Silica gel 60 F254 and HPTLC aluminium sheets were used as adsorbent (stationary phase). The extracts were applied point-wise from 1000 µg/ml sample solution, 10 µl of the sample was applied on HPTLC aluminium sheets as different tracks in the form of 6 mm wide bands by using a Camag semi-automatic Linomat 5 spotter at a distance of 12 mm. Nitrogen gas was also supplied for simultaneous drying of bands and then using drier for completely drying of bands. HPTLC of different extracts was performed by using the mobile phase: Ethyl acetate: Acetic acid: Formic acid: Water (10:1.1:1.1:2.6). To saturate the chamber, 10 ml mobile phase was placed in each flat-bottomed Camag twin trough TLC chamber, 30 min before the development of the PTLC plate. The chamber was sealed with parafilm and covered with a steel lid. The developed plates were then dried and scanned using a TLC scanner 3 with Wincats software under 364 nm.[9] All plates were visualized directly after drying and a fingerprint profile was photo documented using a CamagReproster – 3 under 254 nm and 366 nm in UV and visible light. The digital images of the chromatograms were evaluated with the programme CAMAG Video Scan. The captured image was subjected to a visual inspection on the computer screen. The differences found, are specified by the HPTLC system in which the difference is detected and by the Rfvalue (and colour) of a compound in the system.

In vitro anti-diabetic activity

α-amylase assay:

α-amylase was dissolved in phosphate buffer saline (PBS- 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of sample solutions (0.25 mL) were mixed with α-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:

Inhibition (%) = [(Abs1 − Abs2)/Abs1] × 100 where, Abs1=sample and Abs2 = control [8]

RESULT AND DISCUSSION

The preliminary phytochemical screening using respective reagents showed the presence of secondary metabolites (Alkaloids, flavonoids, phenols, triterpenoids and glycosides) as shown in table 1. Previous reports showed that Amaranth contain secondary metabolites like phenols and flavonoids that make it a therapeutic important plant [10].These secondary metabolite has property to protect from harmful micro-organism. Each metabolite have specific role in plant metabolism and can act as free radical scavenging agents.

Table 1: Phytochemical screening of A. viridis and A.dubius plants

S.No.	Name of test	A. viridis	A. dubius
1.	Wagner's Test	+	+
2.	Fe Cl_3 test for phenols	+	+
3.	Shinoda for flavonoids	+	+
4.	Salkowski test for triterpenoid and steroids	+	-
5.	Baljet's Test for glycosides	+	+

+- Presence;- Absence

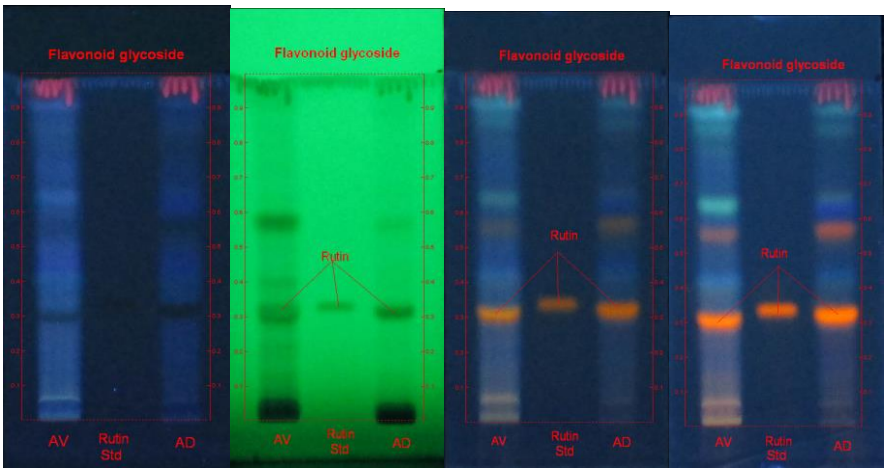
Hptlc studies help to detect secondary metabolite in medicinal plants. It's a simple and cost effective tool to authenticate plant species among themselves. In the present study rutin standard were detected for A. viridis and A. dubius with Rf 0.39. By using chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the concentration of the chemically characteristic

constituents is not exactly the same for different samples of drug. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic component of the herbal drug.[11][12].Rutin is a flavonoid glycoside that has got therapeutic activity in treating cancer, diabetics and other related disorders. The detectioninfluences the nature and concentration of the mobile phase components on retention [13]. In most instances a linear correlation was found between the Rf value and concentration of stronger solvent in the mobile phase, which allows the calculation of the optimum eluent composition. Thus in the present study proper elution of components were visualized in 254 nm and 366 nm respectively with Rf value as 0.39. The area percentage seen for A. viridis as 66.20 and A. dubiuswith 69.04 as shown in table 2.On spraying with poly ethylene glycol an orange colour band was detected for all two species along with the tstandardrutin as shown in fig.1.

Table 2: HPTLC profiles of Rutin with respect to Amaranthus species.

S.No.	Samples	Rf values	Area percentage
1.	Rutin	0.39	100
2.	A. viridis(AV)	0.39	66.20
3.	A. dubius(AD)	0.39	69.04

Figure1: HPTLC Chromatogram of Methanolic extract of AmaranthusL.and standard Rutin



UV 366 nm UV 254 nmUV 366 nm after dipping in solution A UV 366 nm after dipping in solution B

Amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and thereby lower postprandial glucose levels. Slowing the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes. In the present study alpha amylase inhibitory activity of rutinwas compared with the two dietary Amaranth showing its antidiabetic activity. From HPTLC studies rutin concentration in two Amaranth species are in detectable quantity, which has direct relation to the alpha amylase inhibition function. Table 3 & Fig.2 shows the inhibitory percentage of enzyme along with standard Acarbose and Rutin. Appreciable percentage of inhibition was shown by rutin with 79.5% followed by A. viridis (78.2%)andA. dubius (75.01%). Thus the methanolic extract had the potential to amylase inhibition activity. Many in vivo studies were done for other species of Amaranthus L. proving its Antihyperglycemic and hypolipidemic property in experimental models [14].

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Table 3: Comparison of anti-diabetic activity of Amaranthus plants with Rutin standard

S.No.	Conc.µg/ml	A.viridis	A.dubius	Rutin	Acarbose
1.	50	31.53	28.12	25.82	41.01
2.	100	48.3	47.1	40.11	54.71
3.	250	55.9	52.18	55.3	73.10
4.	500	63.14	68.4	61.1	88.34
5.	1000	78.2	75.1	79.5	94.02

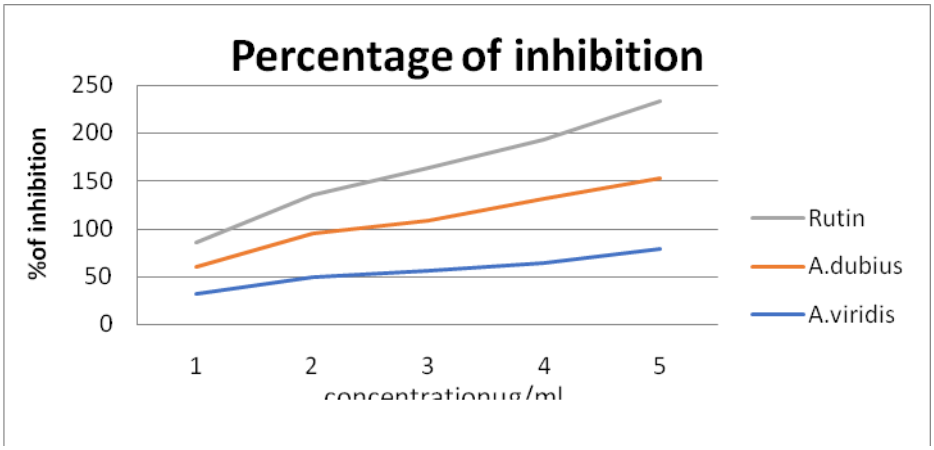

Fig 2: Comparison of Antidiabetic activity

CONCLUSION

Medicinal plants had got phytochemicals that can counteract on the molecular receptors that change metabolic functions of human body. Diabetics are a cause of disease that directly affects metabolic and physiological functioning of normal human body. Plants have got the potency to interfere with these molecules to make it normal functioning. The methanolic extract of leaves of two Amaranth plants along with Rutin played a great role in inhibiting the amylase enzyme. Further isolation of active compounds need to done in order to find out the correct mechanism of action.

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