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PHYTOCHEMICAL EVALUATION AND IN-VITRO ANTI-DIABETICACTIVITY OF *AVERRHOA BILIMBI* FLOWER

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ABSTRACT

Averrhoa bilimbi is a traditional medicinal plant cultivated in many tropical and subtropical countries of our world. Literature survey on this plant shows that Averrhoa bilimbi is mainly used as a folk medicine in the treatment of many lifestyle disorders like diabetes mellitus, hyperlipidimic, hypertension, and as an antimicrobial agent. The phytochemical screening of Averrhoa bilimbi is carried out. This present research mainly focused on anti-diabetic activity of bilimbi flower. In vitro anti-diabetic activity of ethanolic extract of Averrhoa bilimbi flower was carried out by using alpha amylase inhibition method. The result of our study reveals that the ethanolic extract of the flowers has excellent anti diabetic activity and the active constituent might be useful for the further development in medical field.

KEYWORDS: Averrhoa bilimbi, anti-diabetic activity, alpha amylase inhibition method, in-vitro activity, phytochemical screening.

INTRODUCTION

Medicinal plants are rich and widely accepted source for tradition and modern medicines, phyto pharmaceutical, neutraceuticals, cosmetics etc. In India particular medicinal plants form the backbone of all indigenous system of medicine. A lot of work is carried out using various aspect of medicinal plant at global level.[1] Herbal medicines are those with active ingredients made fromplant parts, such as leaves, roots or flowers.[2] The major use of herbal medicines is for health promotion and therapy for chronic, as opposed to life-threatening, conditions. However, usage oftraditional remedies increases when conventional medicine is effective in the treatment of disease, such as in advanced cancer and in the case of new infectious diseases.[3] According to WHO, plant derived drug constitute the mainstay of

nearly 80% of the population for their primary healthcare. These are either directly extracted from plant or modified through further synthesis. These specific chemical belongs to plant derived compounds called phytochemicals such as the metabolites of primary and secondary metabolism.[4] Medicinal plants are integral component of research development in the pharmaceutical industry. Averrhoa bilimbi is principally cultivated for medicinal purposes in many tropical and subtropical countries of the world.[6] Literature survey about this plant shows that A. bilimbi is mainly used as a folk medicine in the treatment of diabetes mellitus[7], anti-microbial agent[9] and as anti-oxidant agent.[10] Bilimbi (Averrhoa Bilimbi L.) belongs to Oxalidaceae family contained an alkaloid, carbohydrate, phenols, flavonoid, saponin, tannin, triterpenoid, steroid.[11] The useful parts of the Averrhoa bilimbi plant are leaves, flowers, and fruits. The fruit is used for sweets, syrups, and paste for itching.[12]

ANTI-DIABETIC ACTIVITY

Diabetes mellitus is a metabolic disorder characterized by increased in blood sugar level. Insulin hormone made by the pancreas helps glucose from food get into the cells to be used for energy. Anti-diabetic agent is a substance that helps to control the level of glucose (sugar) in the blood.[13] Diabetes is a chronic disease affecting around 2-3% of the population worldwide. Plant materials which are being used as traditional medicine for the treatment of diabetis are considered as one of the good source for a new drug or a lead to make a new drug. One of the therapeutic approaches in Type-2 diabetes is to lower the corresponding postprandial blood glucose values. Alpha-amylase inhibitor plays major role in the management of postprandial hyperglycemia.[15] It inhibits the action of alpha amylase enzyme leading to a reduction in starch hydrolysis to maltose and consequentially lower postprandial hyperglycemia.[16] Various medications are available for the treatment of Type-2 diabetes like biguanides, sulphonylureas, thiazolidinediones etc. But they have also exhibited a number of undesired side effects associated with their uses and thus suggesting other effective alternatives.[17]

MATERIALS AND METHODS

Plant collection

The plant Averrhoa bilimbi [family: Oxalidaceae] was collected from Kasaragod district. The plant material was taxonomically identified by the botanist, Mr Biju. P, Assistant Professor Head of department of Botany, Govt. College Kasaragod. The flower was dried under shade for 7 daysand then powdered with mechanical grinder and stored in an air tight container.

PHARMACOGNOSTIC STUDY

Macroscopic evaluation of flower

Macroscopic evaluation can be done by means of organs of sense. This refers to the evaluation of flower by colour, odour, size, shape, taste and special features including touch, texture etc. For this purpose authentic specimen of the material under study and the sample of pharmacopoeial quality should be available to serve as a reference.

a) Colour

The untreated samples were examined under diffused sunlight or an artificial light source with a wavelength similar to day light.

b) Size

Size was measured using graduated ruler in millimetres.

c) Odour and taste

Samples were crushed by gentle pressure and examined by repeated inhalation of air over the material.

d) Texture and fracture

The texture was examined by taking small quantity of material and rubbed in between the thumb and forefinger, bent and rupture caused to the sample provided information of the brittleness and appearance of the fractured plane as fibrous, smooth, rough, granular etc.

Determination of moisture content 10g of flower were weighed in a tarred evaporating dish. It was dried at 105^0c with intermittent weighing. The drying and weighing at 1 hour interval was continued until difference between two successive weighing was a constant value. Constant weight was reached when two consecutive weighing after drying for 30 min and cooling in desiccator showed not more than 0.01g difference. The percentage of moisture present in the sample was calculated and tabulated.

Determination of extractive value

The method determines the amount of active constituents in a given amount of plant material when extracted with a solvent. As mentioned in different official books, the extractive values used as a means of evaluating crude drugs which are not readily estimated by other means. For eg: lowering from the prescribed values. This indicates the addition of exhausted wanted material with original drug or incorrect processing of the drug.

Alcohol soluble extractive value

Macerate 2.5g of coarsely powdered air dried flower of Averrhoa bilimbi with 50 ml ethanol in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and then allowed to stand undisturbed for another 18 hours. Filtered rapidly by taking precaution against loss of alcohol. Then 12.5 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, dried at 105^{o}c and weighed. Calculate the percentage w/w ethanol soluble extractive with reference to air dried material.

Water soluble extractive value

Macerated 2.5g of coarsely powdered air dried flower of Averrhoa bilimbi with 50 ml chloroform water at 80oc in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and then allowed to stand for another 18 hrs. Filtered rapidly, then 12.5 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105°c and weighed. Calculated the percentage w/w of water soluble extractive with reference to air dried material.

Determination of Ash valueTotal ash

2gm powder in previously heated crucible. Ignite at 450. Calculate the content of total ash.

Water soluble ash

To crucible containing total ash add 25ml water and boil. Collect insoluble matter in a glass crucible and ignite at 450^{o}C. Crucible and weighed. The weight of residue subtracted from total ash to get water soluble ash.

Acid insoluble ash

Crucible containing total ash adds 25ml HCl. Boil gently and washes with 5ml boiled water and takes the residue in ash less filter paper. Filter paper containing insoluble matter ignited in crucible & calculates the acid insoluble ash.

EXTRACTION OF FLOWER

The powdered flower was extracted by maceration using solvent ethanol. The coarsely powdered crude drug is placed in a stoppered container with the solvent. They are allowed stand the room temperature for a period of at least 3 days with frequent agitation until soluble matter was dissolved. The mixture is then strained. The mart (damp solid material) is pressed and the combine liquid are clarified by filtration after standing.

Phytochemical Screening

For preliminary phytochemical analysis the freshly prepared crude ethanolic extracts of flowers were tested for the presence or absence of phytoconstituents such as reducing sugar, tannins, flavonoids, steroids and alkaloids by using standard phytochemical procedures.

In vitro methods employed in antidiabetic studiesInhibition of alpha-amylase enzyme

A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution. Both control and plant extracts were added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25ºC. Their action was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3,5-dinitro salicylic acid to 3- amino-5- nitro salicylicacid. This reaction is detectable at 540 nm.

Calculation of 50% Inhibitory Concentration (IC50)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by,

$$I\ \% = (Ac-As)/Ac\ X\ 100,$$ (Shai et al., 2010).

Where Ac is the absorbance of the control and As is the absorbance of the sample.

RESULTS AND DISCUSSION

The preliminary phytochemical screening tests for the ethanolic extract of Averrhoa bilimbi flower (Table 1) revealed the presence of carbohydrates, alkaloids, flavanoids, tannins, steroidal glycosides, phenols. Any of the secondary metabolites, singly or in combination with others could be responsible for the anti-diabetic activity of the plant. There was a dose-dependent increase in percentage inhibitory activity against alpha- amylase enzyme. At a concentration of 50 ml of plant extract showed 0 percentage inhibiactive 35.66% and for 400 ml plant extract showed inhibition of 74.77%. (Table :2).

Table no. 1: Phytochemical analysis of ethanolic extract of *Averrhoa bilimbi* flower.

SL. No.	Phytochemical Constituents	Name of the Test	Methanolic Extract
1	Alkaloids	Mayer s test Dragondraff test Wagner Test	- + -
2	Carbohydrates	Molish Test Fehling Test Benedicts Test	- - -
3	Tannins	Lead Acetate	-
4	Pseudo tannins	Ferric chloride.	+
5	Chlorogenic acid	Ammonia	+
6	Steroidal Glycosides	Salkowaski	+
7	Anthocyanin	H2So4	-
8	Steroidal Glycosides	Liebermann s Burchard Test	-
9	Saponins glycosides	H2So4	+
10	Flavonoids	Ammonia	-
11	Flavones	Shinoda s Test	+
12	Phenols	Ferric chloride	+
13	Phenols	Borntragers test	-

Table no: 2 In-vitro antidiabetic activity of alpha-amylase method.

SL. NO	SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE (540 nm)	PERCENTAGE INHIBITION (%)
1	Control	-	1.51	-
2	Standard	50	0.579	35.66
		100	0.452	49.77
		200	0.391	56.55
		300	0.308	65.77
		400	0.227	74.77
3	Ethanolic extract	50	0.997	35.38
		100	0.953	38.23
		200	0.928	39.85
		300	0.894	42.06
		400	0.789	52.38

From the alpha amylase inhibitory activity study, it was evident that, there was a dose-dependent increase in percentage inhibitory activity against alpha- amylase enzyme. At a concentration of 50 ml of plant extract showed 0 percentage inhibiactive 35.66% and for 400 ml plant extract showed inhibition of 74.77%.

CONCLUSION

Averrhoa bilimbi is a traditional medicinal plant cultivated in many tropical and subtropical

countries of the world. Literature survey report shows that number of studies was done on anti-diabetic activity of *A. bilimbi* using different plant parts. But no more anti-diabetic studies were done on flower of *A.bilimbi*. So, the present study focused on the anti-diabetic activity of flower of *A.bilimbi* using alpha amylase inhibition method. Initially extraction was done by maceration. Maceration is a perfect method to get the highest yield while using the flower. The physicochemical evaluations like moisture content, extractive value, and ash value are determined. The alcohol soluble extractive value of the extract is found to be more than that of water soluble extractive value. Preliminary phytochemical studies were carried out on *A.bilimbi* flower extract, the results revealed the presence of flavonoids, triterpenoids, glycosides, saponins, and alkaloids etc. From the preliminary phytochemical screening it was confirmed that the flavonoid is present in the *A.bilimbi* flower. Also the survey of literature revealed that, the presence of flavonoid content, which is responsible for the anti-diabetic activity. The invitro anti-diabetic activity of extract of *A.bilimbi* flower was determined by using alpha amylase inhibition assay. Hence, it is concluded that ethanolic extract of *A.bilimbi* flower shows significant anti-diabetic activity as compared to standard drug. Based on invitro study results, it might be useful as a lead molecule for pharmaceutical industries, so the current work requires further modification to get better pharmacological action.

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