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PHYTOCHEMICAL SCREENING AND EVALUTION OF ANTI DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF*MANGIFERA INDICA LINN* ROOT BARK

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ABSTRACT:

India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine.Diabetes mellitus is one of the most common chronic disease, in which elevated levels of glucose in blood. There is no.of drugs used to treat it, even though there is need to develop new drug from plant source which are harmless. *Mangifera indica Linn* (Mango) is referred to as king of fruits, is commonly used in ayurvedic medicine.Mostly leaves, stem bark, root bark, flowers, fruits, seeds are used to treat wide range of pharmacological activities in vitro and in vivo,like anti-oxidant, anti- inflammatory, antibacterial, antifungal, analgesic, Hepato protective,anti plasmodial, neuro protective,immunomodulatory, antihyperlipidimic and anti - diabetic activity etc. Especially the root bark of *Mangifera indicaLinn*are having different pharmacological activities likeanti-oxidant, anthelmintic,anti-allergic etc.It was noticed that there was no report on anti- diabetic activity of root bark. So, presentresearch paper is aimed to carry out phytochemical screening and Anti-diabetic activity of methanolic extract of*Mangifera-indica Linn*root bark. The methanolic extract of root bark containsflavonoids, Glycosides, steroids, saponins,alkaloids, phenlos, amino acids, carbohydrates, which was confirmed by I.R Spectrum. The methanolic extract of root bark was evaluated for its anti-diabetic activity by α- amylase enzyme inhibition method.It shows significant anti diabetic activity by inhibiting α-amylase activity. As the concentration of drug increases the inhibition effect also increases.The results are compared with standard drug like Acarbose.

KEY WORDS: Root Bark, Anti- diabetic Mangifera –indica, Methanolic extract.

INTRODUCTION:

In modern world, the synthetic drugs are readily available and more effective in curing numerous diseases. Even though there are some people who still preferring folk medicines, because of their less harmful effects due to non-toxic in nature and easily available at reasonable costs. Natural products have a unique chemical diversity which results in their biological activities.Therefore, researchers are improving their attention to folk medicines,looking for new lead discoveries for develop better drugs[1,17].

Diabetes mellitus is one of the most common chronic disease, in which elevated levels of glucose in blood was observed.It is classified into Type-I (insulin dependent) and Type –II (non-insulin dependent). To treat Type-II Diabetes, synthetic drugs are available, even

though there is need to develop a new drug from natural source which is harmless[23].

Mangifera indica Linn (MIL) is a species of *Mangifera* commonly known as mango, Aam. Mango is one of most popular of all tropical fruits. It is native tropical Asia and has been cultivated in Indian subcontinent and is now found in many tropical countries. Various parts of plants are used as dentifrice, antiseptic astringent, stomachic,laxative, dysentery, anaemia, asthma, bronchitis, cough hypertension, insomnia, rheumatism, haemorrhage, piles, anti-diabeticactivity[17,23].

Root bark are having different types of phytochemicals like flavonoids, poly phenolic compounds, alkaloids, saponins, glycosides,triterpenoids. Mainly contain Mangeferin, proto catechuic acid,Catechu in [27,33,40].

Materials:

Methanol,Acarbosetablets,alpha-amylaseenzyme,Methanol,3,5-dinitrosalicylicacid, Alchol, Starch reagent, Phosphate Buffer solution, Pottasium dihydrogen phosphate, Disodium hydro phosphate,Hydro chloric acid, Distilled water.

INSTRUMENTS:

1.Weighing balance (DS852G,ESSAE).

2.Rota vapouriser (HEI DOLPH,No-1-517-61000-00-0).

3.FT-IR (ECO-ATR, 1-191-9913).

4. U.V spectrophotometer (model no.117).

METHOD:

Preparation of methanolic extract of Root bark of *Mangifera indica Linn*:

The root of MIL was taken out cleaned wiped out the root bark and separated out. Root bark are kept for shade drying in room for 20-25 days. The dried root bark was chopped into pieces, powdered into fine particles and weighed. The dried powder was macerated for 7-10 days in methanolic solution. The methanolic extract was collected by filtration and extract was concentrated by using rota-vapour. The collected concentrated extract was air dried and stored in cool for further use.

Phytochemical screening:

The methanolic extract was tested for the following chemical constituents like proteins, carbohydrates, Amino acids, Alkaloids, Glycosides, terpenoids, steroids, saponins, and phenols by using different types of chemical tests, the results arepresented in the table no.1,their presencewas conformed by I.R-spectrum,which are tabulated in table no.2.

Evaluation of Anti-diabetic activity: -α-Amylase enzyme inhibition method:

Theanti-diabetic activity of methanolic extract of root bark of MIL was performed by using in

vitro method i.e. Inhibition of alpha amylase enzyme[15].

The hydrolysis of starch by amylase as a function of temperature. The reducing sugar produced from the hydrolysis is determined by 3,5 – di nitro salicylic colorimetric assay. 1ml of α-amylase (0.5 mg/ml) solution was transferred into all 10ml volumetric flasks (6) and the sample at various concentrations (100-500µg/ml) was added. To this 1% of starch solution and 100 µl of 0.2mm of phosphate buffer (pH-6.9) was added. The reaction was allowed to be carried out at 37ºC for 5min and terminated by addition of 2 ml of 3,5-dinitrosalicylic acid reagent. The reaction mixture was heated for 15 min at 100ºC and diluted with 10 ml of distilled water in ice bath. The α-amylase activity was determined by measuring colour intensity at 540 nm in spectrophotometer. The absorbance values were reported in table no-4 and the percentage inhibition was calculated and reported in table by using the following formulae.

% inhibitory activity = (Ac-As)/Ac ×100

Where, Ac -Absorbance of the control As-Absorbance of the sample.

RESULTS:

Phytochemical studies show the presence of compounds which were presented in Table-1,where "+" sign indicating positive result and "-"sign indicating negative result. The presence of these phytochemicals was confirmed by I.R-spectrum (Table-2).

The anti-diabetic activity was determined by measuring the absorbance of test solutions at 540nm, as the concentration of drug increases the absorbance of test solution decreases (Table-3). Calibration graph was plotted by taking concentration on X-axis and absorbance on Y-axis (Fig-1). From the absorbance values, calculated the % inhibition and IC_{50} value mentioned in table-4. Calibration graph was plotted by taking % inhibitionon X-axis and absorbance on Y-axis (Fig-2).

DISCUSSION:

The phytochemical screening and I.R results shows the presence of Flavonoids, Glycosides, Steroids, Saponins, Alkaloids and Phenols in methanolic extract.

From the results it was noted that the methanolic extract of root bark MIL shown significant effect on α-amylase activity. From the calibration graph, it shows that as the concentration of extract increases the activity of α-amylase enzyme decreased. From this we are reporting

that the methanolic extract of root bark MIL havinganti-diabetic activity, which is also dose dependent. This was also supported by percentage inhibition values along with IC_{50} value.The result showed that the Methanolic extract of root bark of *MIL* has excellent Anti-diabetic activity.

CONCLUSION:

The methanolic extract of *Mangifera indicaLinn*root barkhaving different types of phytochemicals. The Anti-diabetic activity of methanolic extract of *Mangifera indicaLinn*root bark was evaluated by in vitro method (α-amylase inhibition activity) and it exhibited significant activity when comparing with standard. So, it can be used to treat diabetes. However, we want to continue further investigation for isolation of chemical constituents and other biological activities of the extract.

ABBREVIATIONS

MIL -Mangifera indica Linn

IC_{50}- Minimum inhibition concentration

I.R- Infrared spectroscopy

TABLES

Table-1 Results of phytochemical screening of Methanolic extract of root bark.

Phytoconstituents	Methanolic extract
Steroids	++
Flavonoids	+ ++
Glycosides	+ -
Phenols	+++
Saponins	+
Alkaloids	+
Amino acids	+
Carbohydrates	+
Proteins	-

Table- 2- IR Bands of Methanolic extract of of root bark MIL

S.No	Functional group	IR- Range	IR-band
01.	Amine (N-H)	3300-3500	3393.73
02.	Alcohol(O-H)	3600-3640	3634.60
03.	Alkane (C-H)	2850-2970	2888.96
04.	Aromatic (C=C)	1600-1680	1645.69
05.	Aromatic ring	1450-1600	1495.50
06.	Amines(C-N)	1020-1360	1196.09
07.	Aldehyde or ketone (-co-)	1550-1850	1758.06

Table-3: Computed values absorbencies of Methanolic extract of root bark of MIL.

S.No	Concentration of extract(in µg)	Absorbance			Average Absorbance
		Day 1	Day2	Day 3	
01	100	0.659	0.575	0.601	0.611
02	200	0.582	0.484	0.526	0.530
03	300	0.491	0.301	0.401	0.397
04	400	0.301	0.277	0.299	0.292
05	500	0.158	0.117	0.132	0.135

Table-4: Computed values % inhibition of α –amylase by the Methanolic extract of root bark of MIL.

S. No	Average Absorbance	% inhibition	IC_{50} VALUE
01	0.611	61.1%	210
02	0.530	53.0%	
03	0.397	39.7%	
04	0.292	29.2%	
05	0.135	13.5%	

FIGURES

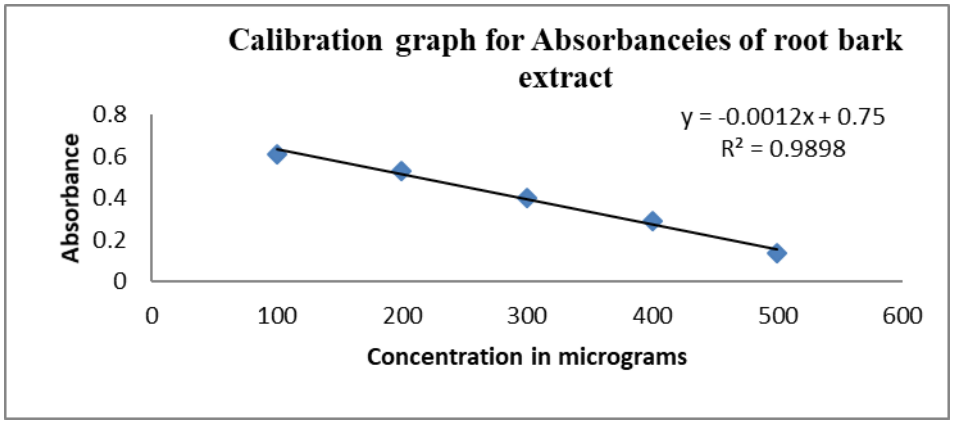

Fig-1 -Calibration graph for absorbance of Methanolic extract of root bark of MIL

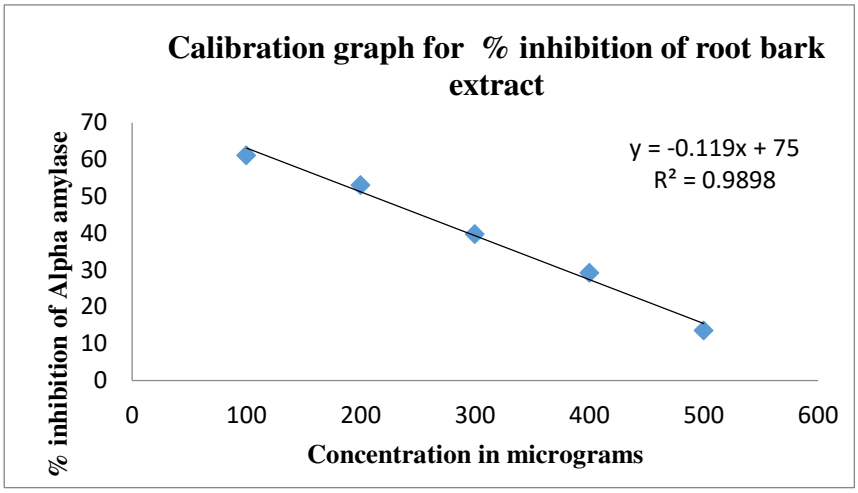

Fig-2 -Calibration graph for % inhibition of Methanolic extract of root bark of MIL.

UNITS AND SYMBOLS

ml – millilitre

µl – microliter

µg –microgram

mg –milligram

% - percent

^{0}C – degree Celsius

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