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PHARMACOLOGICAL EVALUATION OF *FICUS RELIGIOSA* FOR THEIR IN-VITRO HYPOGLYCEMIC ACTIVITY

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ABSTRACT Diabetes mellitus is a metabolic disorder and its management is an important criterion for pharmacotherapy. The medicinal plants play very important role in preventing the progress of the disease. Present study deals with screening of polyherbal extracts using in-vitro techniques for its antidiabetic activity. The plants used in the present study are *Ficus religiosa* which use traditionally for the treatment of various ailments. In the present study different part of *F. religiosa* bark showed highest antioxidant and highest antidiabetic activity. This study indicates the curative benefits of *F. religiosa* in traditional medicinal system.

KEYWORDS : *Ficus religiosa*, antioxidant, Diabetes mellitus

INTRODUCTION

Diabetes is a major metabolic disorder characterized by increases of blood glucose levels resulting from absence of inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action. In fact, diabetes now days a global problem affecting nearly 10 % population all over the world, come to 150 million people. According to the World Health Organization (WHO 2002) report the number cases of diabetes mellitus was 171 million in 2000 which may increase to 360 million in year 2030. As the number of people with diabetes multiplies worldwide, the disease takes an ever increasing proportion of national and international health care budget. Common accepted classification of Diabetes mellitus given by WHO in 1980 and furnished again in 1985 are Type 1 or Insulin-dependent diabetes mellitus (IDDM) and Type 2 or Non-Insulin-dependent diabetes mellitus (NIDDM), Malnutrition related diabetes mellitus (MRDM), and Gastrointestinal diabetes mellitus (GDM).

Salutary plants are best for curing to health of individuals and communities. The mortician's value of these plants lies in some chemical substances that produce a much specific physiological action on the human body and these chemical substances are called as Phytochemical. Phytochemical constituents are the mean source for the establishment of several pharmaceutical industries. The ingredient present in the plants plays an idempotent role in the identification of crude drug. Phytochemical screening is very importunate in identifying the new era of therapeutically and industrially important & tested compound like alkaloids, flavonoids, phenol, saponin, steroids, tannins, terpenoid etc. Now days, the role of antioxidant has been increasingly identified as a critical and specific influence on the biochemistry of living beings. Antioxidant prevents oxidation of other chemical like carbohydrates, lipids, fats, nucleic acid and proteins. It acts as a scavengers the saviour, they prevents cell and tissue damage by preventing as well as retarding the oxidation process. Tissue injury can be cause due to over production of free radicals that harms the blood cells also. Antioxidant capable of removing free radicals and preventing them cause's cell damage by terminating reactive radicals this is done by transferring hydrogen atom from the antioxidant to the reactive radical intermediates.

MATERIAL AND METHODS:

PLANT MATERIALS AND EXTRACTION:

The different plant parts (leaves, bark, fruit, and stem) of *ficus benghalensis* were collected from University campus of MGCGV, Chitrakoot, Satna (M.P. India) washed with water and then washed with methanol and allowed to dry in shade at room temperature. Dried parts of plats grind and passed through 120 no sieve. Take 20gm of sieved powder of *F. Religiosa* then added 100ml (80%) methanol and using cold maturation method for extraction. Extract was filtered through the whatman No.1 filter paper. The filtrate was concentrated under room temperature for dryness.

Phytochemical Screening:-

Phytochemical analysis of different part of *Ficus religiosa* performed by using standarded Procedure.

Quantitative Analysis:

Total poly phenolic content:

Total poly phenolic content of different part of plant extract was measured by using Folin - Ciocaiteu reagent. The 25µl of plant extract diluted with 125µl water followed by addition of 150µl of Folin-ciocalteu (1N) & 25µl of Na_2CO_3 (20% W/V) and incubated at 45 ℃ for 60 min then absorbance was measured by spectro photometrically at 765nm (Bio Tek Synergy H4 multimode micro plate reader Bio Tek instrument, Inc Winoosci, VT, USA). Absorbance was recorded triplicates. Quantification was performed with respect to the standard curve of querecitin (equation). Result was expressed as milligram of querecitin equivalent per ml of extract.

Total flavonoid content:

Total flavonoid in the plant extracts, in brief, 100 µl of sample, followed by 100 µl of AlCl3.6H2O in ethanol and 150µl Sodium acetate, solution added. The absorbance at 430nm was taken (BioTeksynergyH4 multi-mode micro plate reader, Bio Tek Instruments, Inc Winooski, VT, USA), after 2.5 h of incubation at 20^0C. Total flavonoid contents were calculated with respect to the standard curve of the flavonoid quercetin dehydrate (equation). Results were expressed as micrograms of quercetin dehydrate equivalents (QE) per ml of the extract.

DPPH Method:-

The assay for free radical DPPH was done by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. In brief, a 96-well microplate, 30 µl of various dilutions (10-100 µg /ml) of methanolic extract 100 µl of tris–HCl buffer (0.1M, pH 7.4) and 150 µl of DPPH solution (0.004% w/v in methanol) were added. The reaction mixture was shaken well. The DPPH decolonization was recorded at 517 nm on a BioTek Synergy H4 hybrid multimode micro plate reader (BioTek instruments, Inc Win-oosci, VT, USA.), after 30 min incubation in dark. The percentage of DPPH scavenging by plant extracts obtained in terms of ascorbic acid equivalent concentration. Quantification was performed with respect to the standard curve of Ascorbic acid equation. Result was expressed as milligram of Ascorbic acid equivalent per ml of extract. Experiment was done in triplicates. DPPH radical's concentration was calculate using the following equation:

DPPH scavenging effect (%) = Ao – A1 / Ao X 100

Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample.

Alpha Glucosidase Inhibition Activity:-

α-glucosidase inhibitory activity was performed following the method of Tripathi et al[5] In brief, Rat-intestinal acetone powder was dissolved in 4 ml of 50 mm ice cold phosphate buffer and sonicated for 6 minutes

at 4°C. After vortexing for 20 minutes, the suspension was centrifuged (10,000 rpm, 4°C, 30 minutes) and the resulting supernatant was used for the assay. A reaction mixture containing 50 µl of phosphate buffer (50 mM; pH 6.8), 20 µl of rat α -glucosidase and 25 µl of sample of varying concentrations was pre-incubated for 5 min at 37°C, and then 25 µl of 3 mM PNPG was added to the mixture as a substrate. After incubation at 37°C for 30 min, enzymatic activity was quantified by measuring the absorbance at 405 nm in a micro titer plate reader (Bio-TEK, USA). Acarbose was used as a positive control and water as negative control. Experiments were done in triplicates. IC_{50} value was quantified using formula (y=0.193x+19.68, R^2=0.965). The percentage of enzyme inhibition by the sample was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 = Absorbance of control
A_1 = Absorbance of sample

The concentration of an inhibitor required to inhibit 50% of enzyme activity under the mentioned assay conditions is defined as the IC_{50} value.

RESULT AND DISCUSSION

Phytochemicals such as phenolics and flavonoidal compounds present in the *ficus religiosa* are well known for its antioxidant and antidiabetic activity. For this reason there are interests in using phenolics and flavonoids rich extracts in the treatment of diabetes and its complications. The quantitative analysis of *F. religiosa*. The total phenol content in plant extract is expressed in term of Cathechol equivalent (the equation of std curve is Y= 0.0318X+ 0.086 R^2=0.9858) were shown in Fig 1 and concentration of total phenolic content were given in table 1. The Flavonoids content in plant extract is expressed in term of qurecetin equivalent (the equation of std curve is Y= 0.0049 X+ 0.0949 R^2=0.9969) were shown in Fig 2 and concentration of Flavonoids content were given in table 1.

DPPH is a stable free radical at room temperature which when accepts an electron or hydrogen radical becomes a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. On reaction with antioxidant or free radical there is decrease in absorbance of DPPH radical because of scavenging of the radical by hydrogen donation. There is change in color from purple to yellow which is visually noticeable. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity. Data showed the DPPH radical scavenging activity of FRFE and the standard ascorbic acid. In this study DPPH radical scavenging activity of *F. religiosa* plants was compared with standard ascorbic acid. The graphical representation of data is shown in figure 3 representing graph of % inhibition of DPHH assay ascorbic acid. Table 3 shows the IC50 value of selected plants and ascorbic acid.

This study α-glucosidase activity of *F. religiosa* plants was compare with standard acarbose. The graphical representation of data is shown in figure 4 representing graph of % inhibition of α-glucosidase. Table shows the IC50 value of selected plants and acorbose used as standard. α-glucosidase are very important in many biological processes, including breakdown of edible carbohydrate. α-glucosidase is one among the number of glucosidases located in the brush border surface membrane of intestinal cells, and is a key enzyme of carbohydrate digestion. The inhibition of α-glucosidase by *F. religiosa* bark can be attributed to the presence of flavonoids and phenolic glycosidase having potential antioxidant activity. The bark of *F. religiosa* show strong antioxidant and antidiabetic activity IC_{50} is 48 and 83.72µg/ml. On the basis of previous review the bark of *F. religiosa* contain various phyto constituent to control blood sugar level.

Table 1: Total polyphenolic and total flavonois content of different part of *Ficus religiosa*

S. N.	Plant name	Total polyphenol (µg/mg)	Flavonoids (µg/mg)
1	Peepal leaf	0.215	162.46
2	Peepal bark	1.51	43.48
3	Peepal stem	2.23	92.67
4	Peepal fruit	4.31	121.85

Table 1: DPPH scavenging activity and α glucosidase activity of different part of *Ficus religiosa*

S.N.	Plant name	DPPH activity (IC_{50}) µg/mg	α glucosidase activity (IC_{50}) µg/mg
1	Peepal leaf	72.01	84.94
2	Peepal bark	48.02	83.74
3	Peepal stem	92.11	87.95
4	Peepal fruit	55.10	93.17

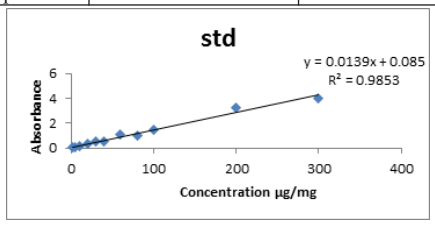

Fig1: Standard graph of cethachol for total phenol

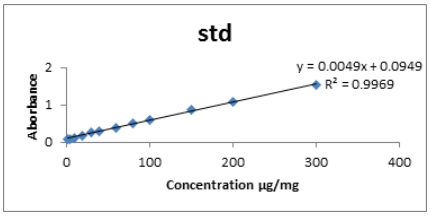

Fig 2: Standarderd graph of qurecetin for Flavonoid

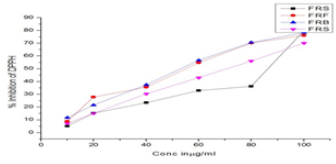

Fig-3: Standarded graph of *F. Religiosa* for DPPH activity

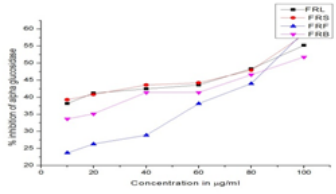

Fig 4: Standarded graph of different part of *Ficus religiosa* for DPPH activity

CONCLUSION:

The present study concludes that the F. religiosa is important medicinal plant to cure various disease because its contain variety of Phytochemical in different part of selected plants. The overall result obtain by present study we observed that the bark part of *Ficus religiosa* shows strong antioxidant and andtidiabetic activity it mean phyto constituents found in bark part of *Ficus religiosa* responsible for antidiabetic activity in diabetic patients.

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