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STUDY OF PHYTOCHEMICAL AND IN-VITRO ANTI-DIABETIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *BUTEA MONOSPERMA*

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ABSTRACT

Diabetes mellitus (DM) is a global health problem and the incidence of DM is increasing at alarming rate all over the world. Many Indian medicinal plants have been reported to possess potential antidiabetic activity and could play important role in the management diabetes. Hydroalcoholic extracts of *Butea monosperma* showed the presence of Flavonoids, Diterpenes, Proteins and Saponins. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.032X + 0.018$, $R^2=0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TFC of hydroalcoholic extract of *Butea monosperma* showed the content values of 0.828respectively. In vitro anti diabetic activity of *Butea monosperma was* determined usingα-amylase inhibition activity. The Percentage inhibition of Acarbose was found to be 37.19µg/ml and Hydroalcoholic extract of *Butea monosperma* percentage inhibition was found to be 127.89µg/ml.

Key words: *Butea monosperma*, Phytochemical Investigation, flavonoids content, In Vitro anti-diabetic activity.

INTRODUCTION:

Diabetes mellitus is an epidemic occurring in adults throughout the world and is the leading cause of kidney failure, heart attack, blindness and lower limb amputation. It is the fourth main cause of death in most developed countries. The prevalence of diabetes is estimated to reach 330 million by the year 2025, according to International Diabetes Federation, with the greatest potential increase being in Africa and Asia. This numerical increase will occur in developing countries. By the year 2025, over 75% of people with diabetes will reside in developing countries, as compared to 62% in 1995 (Eseyin et al., 2010). Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of

investigations (Patel et al., 2012). Many herbs and plants have been described as possessing hypoglycemic activity when taken orally (Rajan et al., 2012). According to the World Health Organization, there are more than 1200 plant species worldwide used in the treatment of diabetes mellitus and substantial number of plant showed effective hypoglycemic activity after laboratory testing (Rajasekar et al., 2010). Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoidsetc. that are frequently implicated as having antidiabetic effect (Malviya et al., 2010). *Buteamonosperma* (BM) commonly known as flame tree, belongs to the subfamily Caesalpinioideae, family Fabaceae (formerly Leguminosae). The plant is commonly called as Palash tree in India. It grows throughout India as well as South Asian peninsula (Shah et al., 2009). It is a medium sized deciduous tree. It grows about 10-15 meters in height. It looks like small bush when the height is 1-2meters due to more branching. Its flower is odourless and looks reddish in the flowering season during springs and leaves are trifoliate. The plant is having numerous medicinal properties like appetizer, laxative, anthelmintic and aphrodisiac etc. The following parts of plants may be used such as flower, gum, seed, leaf, and bark1 (Burli and Khade, 2007; Upadhyay et al., 2011; Gaikwad et al., 2008; Katewa et al., 2010). As per Ayurveda, BM also has the property of reducing Kapha and Vata (Aher et al., 2004). The various parts of BM contains many active constituents e.g. butein, butrin, flavonoid and steroids (flower), glucose, glycosides (roots) tannin (gum), oil, proteinase and polypeptidase (seed) etc. The present article describes the phytocemical and pharmacological activities of different parts of BM. Therefore, the aim of the present study is to determine the antidiabetic activity of hydroalcoholic extract of *Butea monosperma*.

MATERIAL AND METHOD

Plant material

Flower of *Butea monosperma* were collected from local area of Bhopal (M.P.) month of February, 2021.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered flower (Mukherjee, 2007; Parkhe and Bharti, 2019):

Extraction by maceration method

60 gram of powdered flower of *Butea monosperma* were exhaustively extracted with

hydroalcoholic solvent (Methanol: Aqueous: 70:30) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extract (Mukherjee, 2007).

Determination of percentage yield

Calculation of percentage yield

The percentage yield of yield of each extract was calculated by using formula

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Phytochemical screening

Phytochemical examinations were carried out extracts as per the following standard methods.

Thin layer chromatography

Extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) solvent system used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples.

Detection and Calculation of R_f Value

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method [13].10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

***In-vitro* anti-diabetic activity of hydroalcoholic extract**

10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100-500µg/ml were prepared in methanol.100 mg

of dried extract was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. 500 µl of this extract was for the estimation of enzyme inhibition. A total of 500 µl of test samples and standard drug (10-50µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min (Annapandian and Sundaram, 2017). After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

RESULTS AND DISCUSSION

The crude extracts so obtained after maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of hydroalcoholic extracts was found to be 3.9%. The results of qualitative phytochemical analysis of the crude powder of flower of *Buteamonosperma*were shown in Table 1. Hydroalcoholic extracts of *Buteamono sperma*s howed the presence of Flavonoids, Diterpenes, Proteins and Saponins. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.032X + 0.018, R^2=0.998, where X is the quercetin equivalent (QE) and Y is the absorbance. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TFC of hydroalcoholic extract of *Butea monosperma* showed the content values of 0.828respectively Table 2. In vitro anti diabetic activity of *Butea monosperma was* determined usingα-amylase inhibition activity. The Percentage inhibition of Acarbose was found to be 37.19 µg/ml and Hydroalcoholic extract of *Butea monosperma* percentage inhibition was found to be 127.89µg/ml table 5.

Table No. 1: % Yield of *Butea monosperma* (Flower)

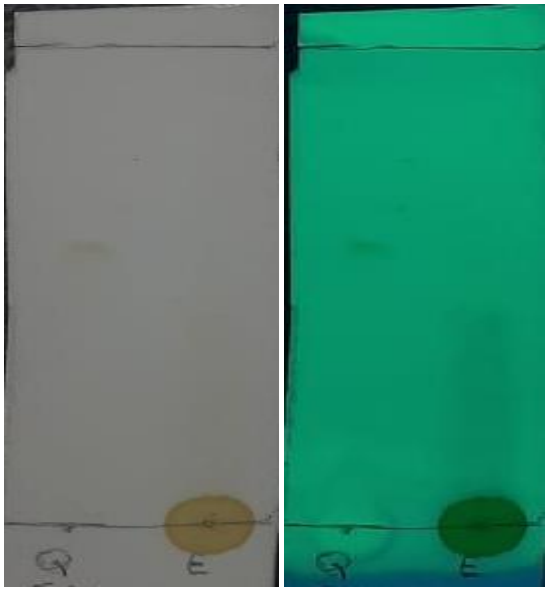
S. No.	Extract	% Yield (W/W)
1.	Hydroalcoholic	3.9

Table No. 2: Result of Phytochemical screening of *Butea monosperma*

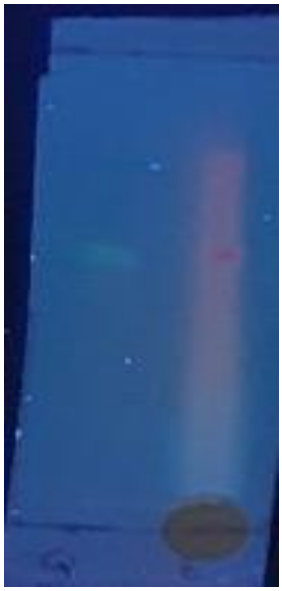
S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Hager's Test:	-ve
2.	**Glycosides** Legal's Test:	-ve
3.	**Flavonoids** Lead acetate Test:	+ve
4.	**Diterpenes** Copper acetate Test:	+ve
5.	**Phenol** Ferric Chloride Test:	-ve
6.	**Proteins** Xanthoproteic Test:	+ve
7.	**Carbohydrate** Fehling's Test:	-ve
8.	**Saponins** Froth Test:	+ve

Table No. 3: Calculation of R f. Value of hydroalcoholic extract of *Butea monosperma*

S.No.	Compound	Rf Value
1.	Quercetin	=0.56
2.	Hydroalcoholic extract Long UV Short UV Normal light	=0.58, 0.74 =0.46 =0



Normal Light **Short UV**



Long UV

Fig. 2: Spot-1= Quercetin, Spot-2=hydroalcoholic extract of *Butea monosperma*

Estimation Total flavonoids content (TFC):

Table No. 4: Preparation of Calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.191
2	10	0.348
3	15	0.514
4	20	0.652
5	25	0.812

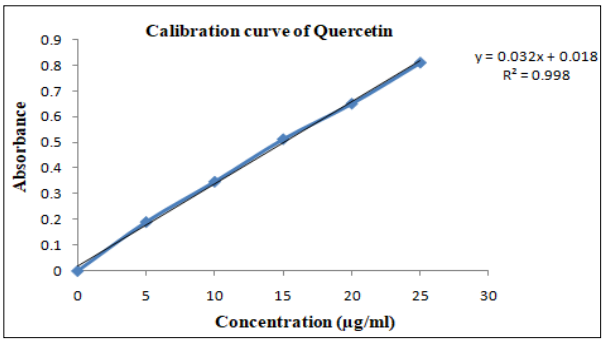


Figure 2: Graph of calibration curve of Quercetin

Table No. 6: Estimation of total flavonoids content of hydroalcoholic extract of *Butea monosperma*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)
1.	**Hydroalcoholic**	0.828

Results of *in vitro*antidiabetic studies

Table 5: Results of *in vitro* antidiabetic studies of Hydroalcoholic extract

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	Hydroalcoholic extract
1.	10	29.32	9.07
2.	20	37.98	14.42
3.	40	49.65	17.16
4.	60	70.89	20.54
5.	80	85.45	35.41
6.	100	86.56	41.78
IC_{50} (µg/ml)		**37.19**	**127.89**

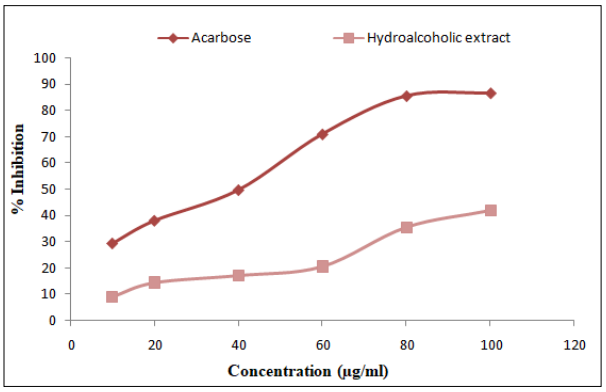


Figure 3: Graph of % Inhibition of acorbose and Hydroalcoholic extract

Conclusion

Management of DM is a global problem. Successful treatment is very important for preventing or at least delaying the onset of long-term complications. Through nature, drugs are available in the form of herbal medicines with very minimal adverse effects when compared to the available synthetic drugs to treat such chronic diseases and disorders. Such herbal drugs as therapeutic agents are a nature's boon when compared to the severe adverse effects of the allopathic medical practice for diabetes. In vitro anti diabetic activity of *Butea monosperma was* determined usingα-amylase inhibition activity. The Percentage inhibition of Acarbose was found to be 37.19µg/ml and Hydroalcoholic extract of *Butea monosperma* percentage inhibition was found to be 127.89µg/ml.

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