

Pharmacognostical Profile and In-vitro Antidiabetic activity of ethanolic extracts of *Dichanthium annulatum* and *Saccharum benghalense*

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ABSTRACT

Diabetes mellitus is a group of metabolic diseases that involve inadequate glucose utilization and excessive glucose production, which leads to hyperglycemia. Before accomplishing in vivo studies, it is crucial to thoroughly investigate the impact of the experimental drugs utilizing in vitro models. Thus, the test chemicals will likely undergo in vitro antidiabetic investigation, such as alpha amylase inhibitory action. *Dichanthium annulatum* and *Saccharum benghalense* belongs to Poaceae family. Poaceae plants have been utilized in folk medicine for a variety of ailments, including hypertension, diabetes, inflammation, anthelmintic, astringent, ulcerative, diuretic, & antioxidant effects. The assay results suggest that the existence of bioactive compounds could be responsible for the versatile medicinal properties of this plant including diabetes, the extract exhibit the IC₅₀ values of alpha amylase inhibitory activity of ethanolic extracts of *Dichanthium annulatum* and *Saccharum benghalense* were 110 µg/mL and 189.655 µg/mL respectively, when compared with Acarbose (IC₅₀ 65.454 µg/mL). The current study proves that the antidiabetic activity of ethanolic extract of *Dichanthium annulatum* and *Saccharum benghalense* leaves by in vitro studies.

Thus, objective of the present study is to investigate the Pharmacognostical screening and in-vitro antidiabetic activity of ethanolic leaves extract of *Dichanthium annulatum*, *Saccharum benghalense* leaves.

Keywords: *Dichanthium annulatum*, *Saccharum benghalense*, Poaceae, Phytochemicals, Cytotoxic effect.

1. INTRODUCTION

Diabetes mellitus, more commonly referred to as diabetes, is a collection of metabolic disorders disrupting the breakdown of carbohydrates in which glucose is produced excessively and not utilized properly, resulting in hyperglycemia [1, 2]. Although there are other types of diabetes, type 1 and type 2 diabetes are the most prominent. Due to the fact beta-pancreatic cells are incapable of adequately adapt, type 2 diabetes is more common, making up 90–95% of all human diabetic episodes. It is also associated by peripheral insulin resistance and relative insulin shortfall [3, 4]. Before beginning in vivo research, it is essential to completely evaluate the experimental drugs' effects implementing in vitro models [5]. Alpha amylase inhibitory activity and other in vitro antidiabetic studies is subsequently intended to be performed on the test compounds [2]. Many of the anti-diabetic pharmaceutical medications on the market today have specific drawbacks and probably serious side effects due to the complex mechanism of diabetes mellitus. As a result, these scenarios have inspired investigators to look for alternative medicinal products from both natural plants and synthetic compounds [6].

Dichanthium annulatum (Poaceae) is a perennial grass and it is also known as marvel grass. Numerous diseases, including as hypertension, diabetes, inflammation, anthelmintic, astringent, ulcerative, diuretic, and antioxidant properties, have been treated with Poaceae plants in traditional medicine [7, 8].

Saccharum benghalense (Poaceae) is synonym for *Tripidium bengalense* that is also known as munj grass that grows in desert regions and along river banks. It had previously been used to following conditions: Vertigo, Fever, Inflammation, Bleeding wounds, Burning sensations, etc [8].

Thus, objective of the present study is to investigate the Pharmacognostical screening and in-vitro antidiabetic activity of ethanolic extract of *Dichanthium annulatum*, *Saccharum benghalense* and *Hippeastrum vittatum* leaves.

2. MATERIALS AND METHODS

Collection and Authentications of Plant:

Fresh leaves of *Dichanthium annulatum* and *Saccharum benghalense* were collected locally from Roorkee, Uttarakhand, India, identified and authenticated by Dr. Sunita Garg (Former Chief Scientist, Head RHMD) and Mr. R.S. Jayasomu (Chief scientist, head RHMD) in CSIR-NIScPR, Delhi. The leaves were washed thoroughly with water to remove dust and dried under the shade at room temperature for 5 days. The dried leaves were ground using kitchen blender to obtain the coarse powder and kept in an air tight container till further use.

Physicochemical Parameters:

Total Ash Value:

In a silica crucible that had been previously ignited and weighed, approximate 5 g of powder were accurately weighed and taken. A thin layer of the powder was put on to the crucible's bottom. The powder was gradually burned by increasing the temperature until it was dull red hot and free of carbon. After cooling down the crucible was weighed. To obtain consistent weight, the entire procedure was repeated. The air-dried powder was used for determining the percentage of total ash [9, 10].

Water soluble ash-

25 ml water was added to boil the ash which is obtained as described for the total ash, for 5 min. The matter which was insoluble collected on ash less filter paper and washed with lukewarm water. The insoluble ash was transferred into silica crucible and ignited for 15 min and weighed. To achieve a uniform weight, the entire procedure was repeated. The difference of weight of insoluble matter and weight of total ash was considered as water-soluble ash. The % of water-soluble ash was calculated using the air-dried section as reference [9, 10].

Acid insoluble ash-

The ash obtained as described above was boiled for 5 min with 25 ml of 2N HCl. The insoluble ash was collected on filter paper (ash less) & washed with lukewarm water. The insoluble ash was transferred into a crucible and then ignited and weighed. The procedure was repeated to obtain a uniform weight. The % of acid insoluble ash was calculated with reference to the air-dried drug [9, 10].

Alcoholic extractive value-

A 250 ml stopper of a conical flask with 100 mL of 90% ethanol was filled with 5 g of powdered matter, and the stopper was subsequently replaced. The flask and content was placed in a mechanical shaker for 6hrs and then left to stand for 18hrs time. The mixture was filtered in flask and then 20ml of the filtrate was measured into an evaporating dish, and evaporated to dryness. After drying for approximately three minutes at 105°C in the oven, the residue's constant weight was established [11].

Water extractive value-

The process was similar to the previous one; with the exception that 90% ethanol was substituted by water [11].

Loss on drying

Place 2 to 6 g of the sample material into a weighing bottle which has been precisely weighed, and weigh it perfectly. Finally, dry it for 5-6 hours at 105°C in the oven and cool it in desiccators with silica gel help. When the material is dried to a constant weight, the % of loss on drying is determined [12].

Preparation of extract:

The Dried and powdered leaves of *Dichanthium annulatum* and *Saccharum benghalense* were successively defatted with petroleum ether solvent and then placed in a thimble of Soxhlet apparatus. For 8-10 hours, the extraction was carried out using an ethanol solvent at 40-60°C on heating mantle. After the extraction, the sample extract was filtered and completely dried out. The concentrated dried extracts were collected in air tight container [13].

Quantitative Phytochemical estimation-

The preliminary phytochemical analysis of the extracts carried out using petroleum ether and ethanolic extracts. Detailed phytochemical testing was performed to identify presence or absence of different Phytoconstituents which is present in extracts of *Dichanthium annulatum* and *Saccharum benghalense* by using standard procedures [10, 14, 15, and 16]. The extracts were subjected to following Phytochemical tests:

Table 1: Preliminary phytochemical tests for plant extracts

Phytoconstituents	Test	Test	Observation
Carbohydrate	Molisch Test	1ml extract + 10ml H ₂ O + 2 drops alcoholic anaphthol solution + 2ml H ₂ SO ₄ (conc.)	Purple ring at the junction
	Fehling's Test	1ml extract + 1ml of Fehling's solution A and B + Heat	Brick red precipitate
	Benedict's Test	1ml extract + 1ml of Benedict's reagent + Heat	Green, yellow or red colour appear depending on the amount of sugar
	Barfoed's Test	1ml extract + 1ml of Barfoed's reagent + Heat	Red colour
Alkaloids	Mayer's Test	2ml extract + few drops of mayer's reagent	Creamy precipitate
	Hager's Test	2ml extract + few drops of hager's reagent	Yellow precipitate
	Wagner's Test	2ml extract + few drops of wagner's reagent	Reddish-brown precipitate
Flavonoids	Lead acetate Test	1ml extract + few drops of lead acetate solution	Yellow precipitate
	Alkaline reagent test	1ml extract + few drops of sod. hydroxide	Intense yellow colour appear which becomes less on addition of few drops of dil. Acid.
Glycosides	Borntrager's Test		
	Legal Test	1ml extract + pyridine + 1ml sodium nitropruside + 10% NaOH	Pink to red colour
	Keller-Killani Test	2ml extract + 3ml glacial acetic acid + 1 drop of 5% ferric chloride add 0.5ml H ₂ SO ₄ (conc.)	Blue colour in the acetic acid layer
Protein and Amino acids	Biuret's Test	1ml extract + 10% NaOH + 0.7% CuSO ₄ + heat	Violet or pink colour indicate presence of
	Ninhydrin Test	3ml extract + 5% ninhydrin solution + heat	Blur colour
Saponins	Froth Test	1ml extract + 20 ml DW Shake for 13 min.	Foam formation
Terpenoids and Steroids	Salkowski's Test	Extract dissolved in 5ml of Chloroform. Above solution + H ₂ SO ₄ (conc.) + allowed to stand for 5 min.	Lower layer turning into golden yellow colour

	Libermann-Burchard's Test	1ml of extract treated with chloroform & few drop of acetic anhydride. Above solution + H ₂ SO ₄ (conc.) + allowed to stand for 5 min.	Formation of brown ring at the junction of the two layer and upper layer turning green
Tannins and Phenolic Compounds	Ferric chloride Test	Filtrate + few drop of ferric chloride	Blackish precipitate
	Lead acetate Test	1ml extract + few drop lead acetate	Reddish brown precipitate
	Gelatin Test	Filtrate + 1% gelatin	White precipitate

Spectrophotometric Quantification of Total Phenolic Content: -

Folin-Ciocalteu Assay was used to measure the Total Phenolic Content. In s different test tube, 2.5 ml of Folin-Ciocalteu Phenol reagent mixed with Dichanthium annulatum and Saccharum benghalense ethanolic extracts (0.2 mL from stock solution). After 5 min, the mixture was mixed with 10 ml of a 7.5% Na₂CO₃ and 13 ml of de-ionized distilled water. The mixture was kept in the dark for 90 min time at 25°C, after which the absorbance was measured at 760 nm. The calibration curve, which was obtained by making gallic acid solution (20 - 100µg/ml), was extrapolated to calculated the TPC. The estimation of the phenolic compounds was carried out in triplicate form. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried material [17].

Spectrophotometric Quantification of Total Flavonoid Content: -

Aluminium chloride method was used to measure the Total Flavonoid Content. In a 10 ml test tube, 0.5 ml of Dichanthium annulatum and Saccharum benghalense ethanolic extract 0.15 ml of NaNO₂ (5%) & 0.15 ml of AlCl₃.6H₂O (10%) was mixed in separate test tube. After 5 min add 1 ml of 4 % NaOH. After properly mixing well, the absorbance at 510nm was measured against the reagent blank. The standard curve for total flavonoids was created using rutin standard solution (20 to 100µg/ml) under the same procedure previously described. The total flavonoids were expressed as mg of rutin equivalents per gram of dried fraction [17,18].

In-vitro anti diabetic activity

By α-Amylase Activity

The plant extracts and Standard drug Acarbose of different concentration such as 100, 200, 300, 400 and 500 µg/ml were prepared in phosphate buffer solution having 6.8 pH and dissolved with 0.25 ml of α-amylase solution and blended well. The sample was incubated at 37°C temperature for 5 min. add 0.5 ml of starch solution and incubate it for 3 min at 37°C. Then DNSA reagent was added and boiled at 100°C temperature for 5 min to terminate the reaction. The reaction mixture was cooled to room temperature and the absorbance was measured at 540 nm in spectrophotometer. Control solution represents 100% enzymatic activity and was conducted in similar way by replacing extract with vehicle. Additionally, in order to compensate for any possible intrinsic absorbance generated by the test samples, a blank assay was carried out utilizing the test samples in their appropriate concentrations without the enzyme [19]. Using the formula below the inhibitory activity of the extracts and the standard drug Acarbose was calculated in comparison with the negative control (100% enzyme activity):

$$\text{Inhibition (\%)} = [(Abs_C - Abs_T) / Abs_C] \times 100$$

3. RESULTS:

Percentage yield of crude extracts

70-80 gm plants leaves were used. After performing Soxlet extraction the % yield of leaves extract of Dichanthium annulatum and Saccharum benghalense in petroleum ether and ethanol obtained are shown in Table 2.

Table 2: Amount and % yield of extracts.

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	Dichanthium	Petroleum	Dark yellow to	80.00 gm	1.187	1.48

	annulatum	Ether	brown			
2.	Dichanthium annulatum	Ethanol	Brown	78.00 gm	8.689	11.13
3.	Saccharum benghalense	Petroleum Ether	Dark yellow to brown	70.00 gm	0.645	0.92
4.	Saccharum benghalense	Ethanol	Dark Brown	68.00 gm	3.690	5.42

Physico-chemical parameters

Physicochemical parameter of *Dichanthium annulatum* and *Saccharum benghalense* (leaves) were presented in Table 3.

Table 3: Physico-chemical parameters of *Dichanthium annulatum* and *Saccharum benghalense*:

S.No	Tests	<i>Dichanthium annulatum</i>	<i>Saccharum benghalense</i>
1.	Total ash value (% w/w)	6.21	4.96
2.	Water soluble ash (% w/w)	3.49	1.98
3.	Acid insoluble ash (% w/w)	0.98	0.42
4.	Water extractive value (% w/w)	1.01	0.94
5.	Alcoholic extractive value (% w/w)	1.55	1.24
6.	Loss on drying (% w/w)	2.00	1.60

Preliminary phytochemical screening:

Phytochemical parameters of ethanolic leaves extracts of *Dichanthium annulatum* and *Saccharum benghalense* were presented in Table 4.

Table 4: Preliminary Phytochemical studies of *Dichanthium annulatum* and *Saccharum benghalense*:

S.No	Phytochemical tests	Reagents used	<i>Dichanthium annulatum</i>		<i>Saccharum benghalense</i>	
			Pet. ether	Ethanol	Pet. ether	Ethanol
	Alkaloids	Mayer's Test	+	+	+	+
		Hager's Test	+	+	+	+
		Wagner's Test	+	+	+	+
	Terpenoids	Salkowski Test	+	+	+	+
		Libermann-Burchard's Test	-	+	-	+
	Carbohydrates	Molisch's Test	+	+	+	+
		Fehling's Test	-	+	-	+
		Benedict's Test	+	+	+	+
		Barfoed's test	-	+	-	+
	Flavonoids	Lead Acetate Test	-	+	+	+

		Alkaline Reagent Test	-	+	-	+
	Tannins and Phenolic Compounds	FeCl ₃ Test	-	+	-	+
		Lead Acetate Test	+	+	+	+
		Gelatine Test	+	+	+	+
	Saponins	Froth Test	+	+	+	+
	Protein	Ninhydrin Test	+	-	-	+
		Biuret's Test	-	+	-	-
	Glycosides	Legal's Test	-	+	-	+
		Keller Killani Test	-	+	+	+
		Borntrager's Test	-	-	+	-

+: Detected; -: Not detected.

Total Phenolic contents:

The determination of the total phenolic content of ethanolic leaves extracts of *Dichanthium annulatum* and *Saccharum benghalense* were expressed as mg of gallic acid equivalents and per mg/gram dry weight of sample and presented in Table 5. The TPCs were calculated using the following linear regression equation obtained from the standard plot of gallic acid: $y=0.005x+0.002$, $R^2=0.998$ (Figure:1) Where y is absorbance and x is the amount of gallic acid in μg .

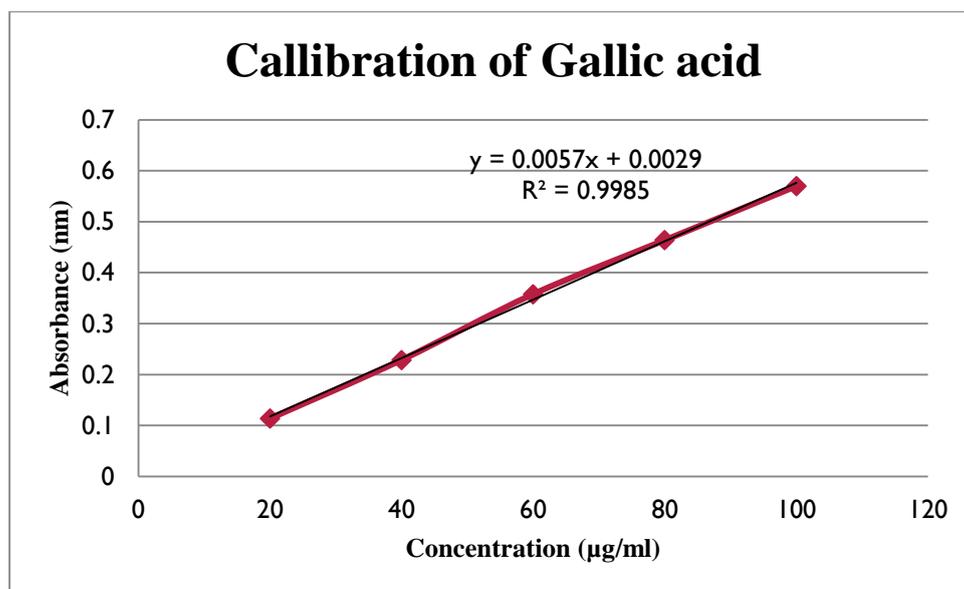


Figure 1: Graph represent standard curve of Gallic acid

Table 5: Total Phenolic content of *Dichanthium annulatum* and *Saccharum benghalense*

Total Phenolic Content (mg/g equivalent to Gallic acid)		
Extracts	<i>Dichanthium annulatum</i>	<i>Saccharum benghalense</i>
Absorbance Mean \pm SD	0.3056 \pm 0.002	0.3864 \pm 0.003
TPC	60.72	76.88

Total Flavonoids contents:

The total flavonoids content of the ethanolic leaves extracts of *Dichanthium annulatum*, *Hippeastrum vittatum* and *Saccharum benghalense* were expressed as % of Rutin equivalent per mg/gm dry weight of sample and presented in Table 6. The TFCs were calculated using the following linear regression equation obtained from the standard plot of Rutin: $y=0.003x+0.005$, $R^2=0.996$ (Figure:2) Where y is absorbance and x is the amount of rutin in μg .

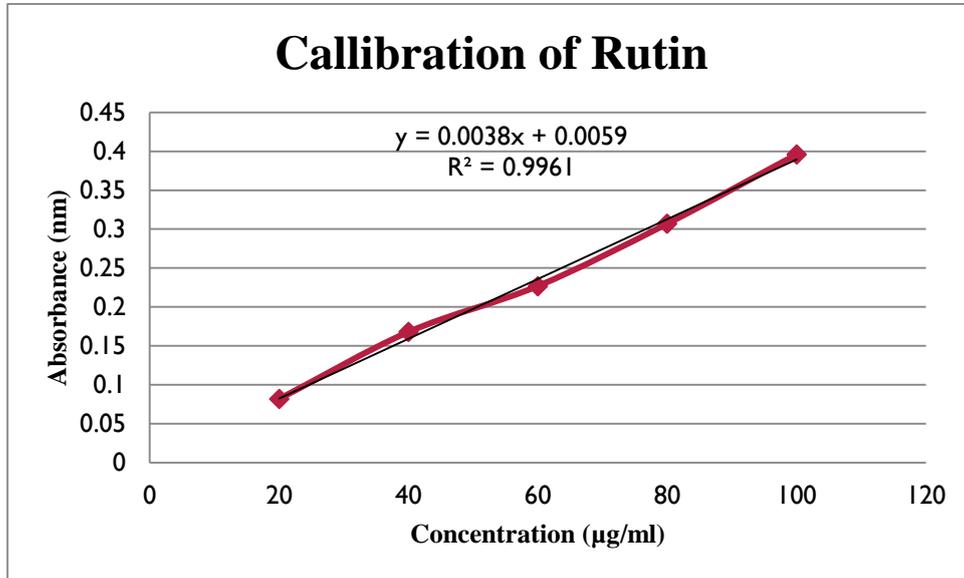


Figure 2: Graph represent standard curve of Rutin

Table 6: Total Flavonoid content of *Dichanthium annulatum* and *Saccharum benghalense*

Total Phenolic Content (mg/g equivalent to Rutin)		
Extracts	<i>Dichanthium annulatum</i>	<i>Saccharum benghalense</i>
Absorbance Mean \pm SD	0.1713 \pm 0.002	0.2108 \pm 0.004
TPC	55.43	68.60

In vitro α -Amylase Inhibitory Activities:

The result was given in table 7 and figure 3. In this investigation, the in-vitro anti diabetic activity of ethanolic leaves extracts of *Dichanthium annulatum* and *Saccharum benghalense* extract was evaluated. α -amylase activity of *Dichanthium annulatum* and *Saccharum benghalense* ethanol extract exhibited percent inhibition 65.006 and 68.807% its IC_{50} value were found to be 110.00 and 189.655 $\mu\text{g/ml}$. Acarbose was used as a reference compound which exhibited percent inhibition 75.622% and showed IC_{50} value of 65.454 $\mu\text{g/ml}$. All extracts showed concentration-dependent in vitro α -amylase inhibitory activities, with the highest percentage inhibition exhibited by the *Saccharum benghalense* extract.

Table 7: In-vitro anti-diabetic activity of ethanolic leaves extract of *Dichanthium annulatum* and *Saccharum benghalense*

Concentration ($\mu\text{g/ml}$)	% Inhibition (Acarbose)	% Inhibition (Ethanolic extract of <i>Dichanthium annulatum</i>)	% Inhibition (Ethanolic extract of <i>Saccharum benghalense</i>)
100	52.686	48.361	43.643
200	57.142	55.045	52.031

300	63.040	58.322	57.929
400	66.710	61.598	59.895
500	75.622	65.006	68.807
IC50	65.454	110.00	189.655

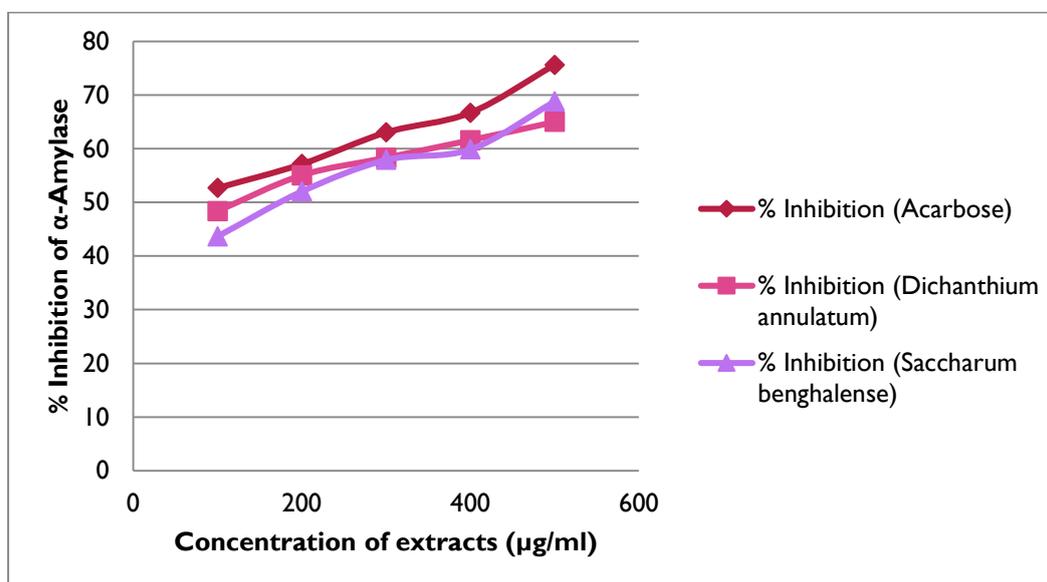


Figure 3: Effects of ethanolic leaves extract of *Dichanthium annulatum* and *Saccharum benghalense* at varying concentration on alpha amylase activity as compared to Acarbose.

4. DISCUSSION:

Abnormal absorption of glucose causes type-II diabetes mellitus [20]. Diabetes is not a single disease; it is a group of heterogeneous syndrome such as heart attack, stroke and peripheral vascular disease and many more [21]. Increasing cost of medicines is very important factors which motivate the researchers towards herbal medicaments with no side effects [22].

The present study is to investigate the Pharmacognostical screening and in-vitro antidiabetic activity of ethanolic leaves extract of *Dichanthium annulatum*, *Saccharum benghalense* leaves.

Plants leaves used were 70-80 gm. After performing extraction of *Dichanthium annulatum* and *Saccharum benghalense*, the percentage yield (Table 2) of leave extracts in petroleum ether solvent were found to be 1.48 % (1.187 gm) and 0.92 % (0.645 gm) respectively. The percentage yield (Table 2) of leave extract of *Dichanthium annulatum* and *Saccharum benghalense* in ethanolic extracts were found to be 11.13 % (8.689 gm) and 5.42 % (3.690 gm) respectively.

The present finding revealed that physicochemical evaluation (Table 3) of *Dichanthium annulatum* and *Saccharum benghalense* (leaves) were found to be Total ash value (6.21 & 4.96), Water soluble ash (3.49 & 1.98), Acid insoluble ash (0.98 & 0.42), Water extractive value (1.01 & 0.94), Alcoholic extractive value (1.55 & 1.24) and Loss on drying (2.00 & 1.60).

The present finding of Phytochemical screening of the ethanolic extracts of *Dichanthium annulatum* and *Saccharum benghalense* (Table 4) confirmed phytochemical constituents like carbohydrates, alkaloids, flavonoids, terpenoids, steroid, glycosides, protein & amino acid, tannins, phenolic and saponins which could be responsible for the medicinal properties of both plant.

The Phenolic and flavonoid are the secondary metabolites of plants. They show different functions of plants [23]. In our study, the determination of the total phenolic content (Table 5) of ethanolic extracts of *Dichanthium annulatum* and *Saccharum benghalense* were expressed as mg gallic acid equivalents and per mg/gram dry weight of sample and the total flavonoids content (Table 6) of the ethanolic extracts of *Dichanthium annulatum* and *Saccharum benghalense* were expressed as percentage of Rutin equivalent per mg/gm dry weight of sample. TPC of ethanolic extracts of *Dichanthium annulatum* and *Saccharum benghalense* showed the content values of 60.72 and 76.88 mg/gm respectively. The total flavonoids content of ethanolic extracts of *Dichanthium annulatum* and *Saccharum benghalense* showed the content values of 55.43 and 68.60 mg/gm respectively.

The present finding reveals that α -amylase activity of *Dichanthium annulatum* and *Saccharum benghalense* ethanol extract exhibited percent inhibition 65.006 and 68.807% its IC_{50} value were found to be 110.00 and 189.655 μ g/ml. Acarbose was used as a reference compound which exhibited percent inhibition 75.622% and showed IC_{50} value of 65.454 μ g/ml. All extracts showed concentration-dependent in vitro α -amylase inhibitory activities, with the highest percentage inhibition exhibited by the *Saccharum benghalense* extract.

5. CONCLUSION:

In this present study we evaluated Pharmacognosics and in vitro alpha amylase activity of ethanolic leaves extract of *Dichanthium annulatum* and *Saccharum benghalense*. The plants showed significant inhibition activity, so further the in-vivo antidiabetic activity has to be done for the usage of activity.

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