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EXTRACTION, PHYTOCHEMICAL ANALYSIS AND ANTI DIABETIC ACTIVITY OF *ARABOTRYS HEXAPETALUS* ROOTS

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ABSTRACT

Diabetes continues to be a major contributor to cardiovascular disease, lower limb amputation, end-stage renal disease, and blindness. Due to the negative effects of oral hypoglycemic drugs (therapeutic agents) for the treatment of diabetes mellitus, there is currently an increasing interest in herbal therapies. Thus, this study deals with extraction, phytochemical analysis and anti-diabetic activity of *Arabotrys hexapetalus roots*. The roots of plant gathered and subjected to further processing. After qualitative antd quantitative studies the extract anti diabetic effect was checked in STZ induced diabetic rats. Results showed that the % yield of pet. ether and ethanolic extract as 2.3% and 5.8% respectively. The phytochemical screening revealed the presence of carbohydrates, amino acid, and proteins, Flavonoids, Tannins and Phenol, Alkaloids. The total phenol and alkaloid content in ethanolic extract was observed to be 0.756 & 0.632mg/100mg respectively. Further, the body weight and blood glucose level was continuously monitored to check the antidiabetic effect in animal models. It was observed that, in diabetic control the body weight was reduced to 125.2±6.38gm on 21st day. While in Glibenclamide 0.25 mg/kg treated rats the final weight was noted to be 126.2±4.76gm. Further in EEAH (50 mg/kg), EEAH (100 mg/kg) and EEAH (100 mg/kg) treated rats the body weight was measured to be 122.7±3.89gm, 134.7±7.84gm and 137.4±4.88gm respectively. Further, the changes in blood glucose level in all groups was carefully observed. The blood glucose in Glibenclamide (0.25 mg/kg) treated rats was estimated to be 153.8±4.44 mg/dl at 21st day. In case of EEAH (50 mg/kg), EEAH (100 mg/kg) and EEAH (200 mg/kg) treated rats the blood glucose level detected to be 191.3±4.36, 189.2±7.48 mg/dl, 168.66±4.47 mg/dl respectively. On the basis of results of this preliminary study it is concluded that ethanolic extract of *Arabotrys hexapetalus roots* has dose-dependent anti-diabetic activity. The present study shows that the *Arabotrys hexapetalus roots* extract at dose 200 mg/kg has significant anti-diabetic activity.

Keywords: Diabetes, medicinal plants, Phytochemicals, *Arabotrys hexapetalus,* Glibenclamide, EEAH, Streptozotocin

INTRODUCTION

The first written account of diabetes mellitus dates back more than 3,000 years, from an Egyptian manuscript. But the distinction between T1D and T2D was not established until 1936. T2D makes up over 90% of all instances of diabetes, making it the most prevalent kind of the disease.In recent decades, T2D, a chronic metabolic complicated multifactorial disease affecting

multiple organs, has spread over the world like a plague. Peripheral tissue insulin resistance, elevated pancreatic alpha-cell activity, and dysfunctional beta-cells are the hallmarks of type 2 diabetes (Mohajan, 2023; Olokoba *et al*., 2012; Sudore *et al*., 2012)).

Globally, 463 million people suffer with diabetes as of 2019. Approximately 8.8% of adult instances of diabetes mellitus are T2DM, accounting for 90% of cases. An estimated 4.2 million deaths were attributed to diabetes mellitus in 2019. In the US, the cost of diabetes mellitus was approximately 327 billion dollars in 2017. An estimated 727 billion US dollars were spent on health care connected to diabetes mellitus globally in 2017. Of the population, 25–30% are normal diabetics (Afroz *et al*., 2018; Herman, 2017)

The main causes of type 2 diabetes are peripheral tissue insulin resistance and decreased insulin synthesis and secretion by pancreatic beta-cells. Since over 90% of patients had obesity or overweight status at the time of T2D diagnosis, diets that combine high food intake with low energy expenditure are generally considered to be the cause of T2D. Patients with type 2 diabetes can benefit from a number of efficient therapies that lower hyperglycemia. These therapies work by enhancing insulin production or lowering insulin resistance in peripheral tissues. In spite of this, post-diagnosis complications particularly chronic ones are common everywhere in the world. Consequently, diabetes continues to be a major contributor to cardiovascular disease, lower limb amputation, end-stage renal disease, and blindness (Scheen, 2003; Vijan, 2010).

Insulin and a range of oral antidiabetic medications, including glinides, biguanides, α-glucosidase inhibitors, and sulfonylureas, are currently the available treatments for diabetes. Products in developing nations are costly and difficult to obtain. Due to the negative effects of oral hypoglycemic drugs (therapeutic agents) for the treatment of diabetes mellitus, there is currently an increasing interest in herbal therapies. Thus, traditional herbal remedies made primarily from plants are utilized, and they are crucial in the treatment of diabetes mellitus (Babiker and Al Dubayee, 2017; .Lee and Lee, 2022)

Herbal medications have become more and more popular as a source of hypoglycemic drugs in recent years. According to estimates by Marles and Farnsworth, over a thousand plant species are utilized as traditional medicine to treat diabetes. The chemical makeup of the plant products utilized as complementary therapies for diabetes has an impact on their biological activities (Raj *et al*., 2016; Alam *et al*., 2022).

These days, a lot of treatments including the use of medicinal plants are advised. Carotenoids, flavonoids, terpenoids, alkaloids, and glycosides are found in most plants, and many of them have anti-diabetic properties. The potential of plants to enhance pancreatic tissue function, either by raising insulin releases or decreasing intestinal glucose absorption, is frequently responsible for the anti-hyperglycemic effects of their treatment. The number of diabetics in the population today is rising, raising worries among the public and medical sector (Singh *et al*., 2014; Rao *et al*., 2010).

The species *A. hexapetalus* is widely found in both southern Asia and southern China. Its fruits and roots are used as traditional Chinese folk medicine to treat scrofula and malaria,

respectively. The flower is bitter and caustic; it helps with headaches, leucoderma, blood and heart disorders, and vomiting. A leaf decoction is administered to treat cholera. Its phytochemical composition includes phenolic chemicals, alkaloids, butyrolactones, anthraquinones, flavonoids, neolignans, terpenoids, leucoanthocyanins, and volatile oils. A. hexapetalus flavonoids, which are rich in phytochemicals, may be altered or utilized as "lead" to create more potent medicinal compounds (Puri, 2020; Quang and Son, 2022).

Numerous studies have examined the in vivo effectiveness, mechanism of action, and adverse effects of antidiabetic plants and their active ingredients using animal models. Owing to the diversity of diabetic disorders in humans, no single animal model can fully capture the characteristics of a specific kind of diabetes in humans. As a result, numerous animal models have been employed, each of which has a unique set of characteristics typical of human diabetic conditions (Hasan *et al*., 2018).

Hypoglycemic activity and the route of action of antidiabetic plant components have been demonstrated in normal nondiabetic animals and animals with impaired glucose tolerance and insulin resistance (but not overt diabetes). It is important to remember that substances that lower blood sugar in animals may not always do the same in humans, and vice versa. Variations in hepatic metabolism, where metabolites are the active substances, could be the cause of this, at least in part. Due to differences in absorption, metabolism, and excretion rates, there can also be significant differences in sensitivity to the same drug between species. Small rodents are the most commonly used animal models because they are less expensive to keep than bigger animals and typically exhibit a faster onset of diabetes, which is consistent with their short lifespan (Eddouks *et al*., 2012; Shevalye *et al*., 2010)

This study deals with extraction, phytochemical analysis and anti -diabetic activity of *Arabotrys hexapetalus* roots.

MATERIALS AND METHODS

Procurement of Plant Material

Root of *Artabotrys hexapetalus* was collected from local market of Bhopal the month of February, 2023. After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant.

Extraction by maceration method

Collected plant namely *Artabotrys hexapetalus* were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant materials were weighed (49gm) and packed in (250 milliliter) air tight glass Bottle. The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant materials were subjected to extraction by ethanol solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using water bath. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated (Abubakar and Haque, 2020).

In vivo anti-diabetic activity

Acute oral toxicity studies

Oral Acute toxicity study was evaluated as per OECD guidelines (425) on Wistar albino rats.

Three animals were selected for maximum tolerable dose (2000mg/kg) of Polyherbal preparation. Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression and mortality after dosing for 24 hours. All observations were systematically recorded with individual records being maintained for each animal. No toxic signs were noticed in animals till day 7. Hence administered dose was considered tolerable (Walum, 1998).

Streptozotocin induced diabetes

Procedure: Diabetes was induced in animals by a single intraperitoneal injection of a freshly prepared Streptozotocin (STZ). STZ solution of 10 mg/ ml was prepared in ice-cold citrate buffer 0.1 M, pH 4.5 kept in ice and was administered at a dose of 60mg/kgbody weight on day 1st. Treatment was given after diabetes induction (day 3rd) for 21 days (Tripathi and Kohli,2013)

Table 1: Animals were divided into five groups containing six animals in each

Group	Dosing and treatment
I	Normal control (Vehicle only, 1ml/100gm)
II	Diabetic control, Streptozotocin60g/kg, i.p.
III	Standard, Diabetic rats were treated with Glibenclamide 0.25 mg/kg once daily for 21 days
IV	Diabetic rats were treated with ethanolic extract of *Arabotrys hexapetalus roots* at 50 mg/kg once daily for 21 days.
V	Diabetic rats were treated with ethanolic extract of *Arabotrys hexapetalus roots* at 100 mg/kg once daily for 21 days.
VI	Diabetic rats were treated with ethanolic extract of *Arabotrys hexapetalus roots* at 200 mg/kg once daily for 21 days.

Physiological Parameters: Body weight of animals was measured using animal weighing balance.

Biochemical Parameters

Samples collection and storage

At the end of the experimental, animals were anaesthetized with intraperitoneal injection of Ketamine (50 mg/Kg i.p.) and blood was collected from retro-orbital puncture in blank (for serum) and EDTA containing ependorff tube (for plasma). The one drop of blood sample swas immediately spread on the marked end of the gluco-strip. After few seconds the gluco-meter was display the blood glucose level. Serum and plasma were obtained by blood centrifugation at 3000rpmfor 15 min. Animals were then sacrificed and pancreas were collected in 10% formalin for histopathology. All biological samples were store at -20^{0}C until analysis.

RESULTS AND DISCUSSION

The elevated blood glucose levels and an inadequate and ineffective insulin secretory response are the hallmarks of diabetes mellitus. Since persistent hyperglycemia is linked to problems, blood glucose regulation and control are essential for averting or treating diabetes complications. In this investigation, rats with STZ-induced diabetes were used to test the antidiabetic potential of *A. hexapetalus* ethanolic extract.

In case of defatting by per ether the extract obtained was observed to be dark brown in colour with % yield of 2.3% with semisolid consistency.

The ethanolic extract was seen to have brown colour, solid consistency and 5.8% yield which is far greater than pet ether. The phytochemical screening revealed the presence of carbohydrates, amino acid, and proteins, Flavonoids, Tannins and Phenol, Alkaloids. The total phenol and alkaloid content in ethanolic extract was observed to be 0.756mg/100mg and 0.632mg/100mg.

In the present investigation, Diabetes was induced in animals by a single intraperitoneal injection of a freshly prepared Streptozotocin. Treatment was given after diabetes induction (day 3rd) for 21 days. Change in Body weight is one of the evaluation criteria in disease progression. Body weight was monitored on initial, day 7, day 14 and day 21. Body weight continuously decline throughout the treatment majorly in diabetic control group animals while in the remaining groups overall no gain no loss was observed.

The predicted manifestations of diabetes in STZ-induced D-group rats were polyphagia, polydipsia, and polyuria; additionally, they failed to gain weight and displayed hyperglycemia, hypoinsulinemia, and hyperlipidemia. Despite polyphagia, the high protein and fat catabolism in diabetic rats allowed for a reduction in body weight.

It was observed that, in diabetic control the body weight was reduced to 125.2±6.38gm on 21st day. While in Glibenclamide 0.25 mg/kg treated rats the final weight was noted to be 126.2±4.76gm. Further in EEAH (50 mg/kg), EEAH (100 mg/kg) and EEAH (100 mg/kg) treated rats the body weight was measured to be 122.7±3.89gm, 134.7±7.84gm and 137.4±4.88gm respectively.

Further, the changes in blood glucose level in all groups was carefully observed. The blood glucose in Glibenclamide (0.25 mg/kg) treated rats was estimated to be 153.8±4.44 mg/dl at 21st day.

In case of EEAH (50 mg/kg), EEAH (100 mg/kg) and EEAH (200 mg/kg) treated rats the blood glucose level detected to be 191.3±4.36, 189.2±7.48 mg/dl, 168.66±4.47 mg/dl respectively.

On the basis of results of this preliminary study it is concluded that ethanolic extract of *Arabotrys hexapetalus roots* has dose-dependent anti-diabetic activity. The present study shows that the *Arabotrys hexapetalus roots* extract at dose 200 mg/kg has significant anti-diabetic activity.

The antihyperglycemic action has been linked to secondary metabolites found in plants, such as alkaloids, polyphenols, flavonoids, saponins, tannins, and terpenoids. The presence of phytochemicals including flavonoids and alkaloids in the powdered leaves of A. hexapetalus may have prevented additional oxidative stress-induced degradation of the functional β-cells that were still intact. As a result, the β-cells keep going and keep making insulin. Additionally, it is asserted that antioxidants like flavonoids may help prevent STZ-induced diabetes by halting the pancreatic oxidative damage and boosting insulin release through the regeneration of pancreatic β-cells.

Herbal medicinal plants' ability to improve glucose uptake in diabetic animal models by boosting insulin production and sensitivity in peripheral tissues as well as by inhibiting glucose transporter proteins in cell membranes through the action of flavonoid glycosides, may account for their antihyperglycemic mechanism.

Table 2: % Yield of crude extracts

Extracts	Colour	Consistency	Yield (% w/w)
Artabotrys hexapetalus			
Pet ether	Dark brown	Semisolid	2.3%
Ethanol	Brown	Solid	5.8%

Table 3: Qualitative phytochemical tests for *Artabotrys hexapetalus* extract

Phytoconstituents	Results of phytochemical tests
i) Primary Metabolites	
Carbohydrates	**Present**
Amino acids	**Present**
Proteins	**Present**
Resins	Absent
ii) Secondary Metabolites	
Steroids	Absent
Glycosides	Absent
Flavonoids	**Present**
Tannins and Phenol	**Present**
Alkaloids	**Present**

Table 4: Estimation of total phenolic and alkaloid content of *Artabotrys hexapetalus*

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.756	0.632

Table 5: Effect of ethanolic extract of *Arabotrys hexapetalus* roots on body weight of diabetic rats

Groups	Treatment	Body weight (gm) (mean±SEM)			
		Initial	Day 7	Day 14	Day 21
I	Normal Control	128.2±5.36	128.7±5.66	126.4±5.77	128.1±5.38
II	Diabetic control	133.1±6.22	130.2±6.59	127.3±6.58	125.2±6.38
III	Standard (Glibenclamide 0.25 mg/kg)	133.3±4.28	127.6±4.58	126.7±4.63	126.2±4.76
IV	EEAH(50 mg/kg)	130.6±3.24	128.3±3.52	126.1±3.44	122.7±3.89
V	EEAH (100 mg/kg)	132.9±7.56	129.4±7.22	133.5±7.74	134.7±7.84
VI	EEAH (100 mg/kg)	136.4±4.24	133.5±4.35	138.7±4.74	137.4±4.88

Table 6: Effect of ethanolic extract of *Arabotrys hexapetalus* roots on blood glucose level in diabetic rats

Groups	Treatment	Blood Glucose Level (mg/dl)			
		Day 3	Day 7	Day 14	Day 21
I	Normal Control (NC)	83.4±4.37	85.3±2.54	87.1±3.41	86.2±4.58
II	Diabetic control	168.5±3.54^{a***}	172.2±3.29^{a***}	186.7±3.62 ^{a***}	197.7±3.71 ^{a***}
III	Standard (Glibenclamide 0.25 mg/kg)	151.6±3.55^{a***}	145.0±2.29 ^{a***}, b***	148.5±2.17 ^{a***}, b***	153.8±4.44 ^{a***}, b***
IV	EEAH (50 mg/kg)	149.4±4.38^{a***}	163.2±3.67 ^{a***}, b***, c***	174.2±4.78 ^{a***}, b***, c***	191.3±4.36 ^{a***}, b***, c***
V	EEAH (100 mg/kg)	146.8±5.54 ^{a***}	153.6±±3.24 a***, b***, c***	169.5±5.83 ^{a***}, b***, c***	189.2±7.48 ^{a***}, b***, c***
VI	EEAH (200 mg/kg)	156.5±4.45^{a***}	151.41±3.98 a***, b***, c***	166.51±2.65^{a***} , b***, c***	168.66±4.47^{a***} , b***, c***

Values are mean ± SEM from a group of six animals. *p<0.05, **p<0.01 and***p<0.001

a- Significance difference as compare to normal control group

b- Significance difference as compare to Diabetic control group

c- Significance difference as compare to standard treated group

CONCLUSION

According to our research, *A. hexapetalus* root ethanolic extract had a hypoglycemic effect in diabetic rats produced with STZ. The inclusion of phenolic and flavonoid chemicals in *A. hexapetalus* may have contributed to its antidiabetic benefits by mitigating the harm that STZ had caused to the pancreas. The anti-hyperglycemic properties of *A. hexapetalus* root powder are linked to elevated levels of insulin sensitivity and plasma insulin concentration. This study demonstrates the potential of *A. hexapetalus* as a natural oral medication with hypolipidemic and antihyperglycemic properties. The precise mechanism underlying *A. hexapetalus* root antihyperglycemic activity requires more thorough biochemical and pharmacological research. The function of the corresponding preparations as hypoglycemic agents in the management of diabetes may be revealed by further research employing the purified active principle from the ethanolic root extracts of *A. hexapetalus*.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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