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Phytochemical Screening and Hypoglycemic Activity of Aqueous, Ethanolic and Methanolic Extracts of *Phyllanthus Amarus* and *Catharanthus Roseus* in Streptozotocin Induced Diabetic Rats

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Abstract – The aim of the present study was to evaluate the hypoglycemic activity of Gymnema sylvestre and Phyllanthus amarus leaf extract in streptozotocin induced diabetic rats. Diabetes mellitus was induced by a single intraperitoneal injection of STZ (50 mg/kg body weight). The experimental set up was formed as follows: Group I–Normal Rats injected with normal saline is considered as vehicle control, Group II–Diabetic control administered with 20% Glucose Soln. for 14 days, Group III–STZ induced diabetic Wistar rats, administered with standard drug (Glibenclamide) 5 mg/kg for 14 days, served as positive control, GROUPS-IV to IX STZ induced diabetic Wistar rats were orally administered with Aqueous, ethanolic and methanolic extracts of Phyllanthus amarus and Catharanthus roseus, at the dose of 300 mg/kg rat body weight respectively. The results of the study indicates that Phyllanthus amarus and Catharanthus roseus extract significantly (P<0.01) reduced the blood sugar level. Though there are many approaches to control diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost. Phyllanthus amarus and Catharanthus roseus are medicinal plants for the potential management of diabetes mellitus. The leaves are used in herbal medicinal preparation. The concerned study reveals the anti-diabetic potential of Phyllanthus amarus and Catharanthus roseus in controlling blood glucose level in Streptozotocin induced Diabetic rats. Bioactive compounds like Lighans and galloatnoids of Phyllanthus amarus and alkaloids such as (catharanthine, vindoline, vindolidine, vindolicine, vindolinine, vinblastine, vincristine, leurosine, and lochnerine) in Catharanthus roseus may be responsible for the significant stimulation of β-cells of pancreas in the production of Insulin. In the current work we have studied the anti-diabetic effect of the aqueous, ethanolic and methanolic extracts of Phyllanthus amarus and Catharanthus roseus on streptozotocin induced diabetic rats. The methanolic and aqueous extracts of Phyllanthus amarus, has significantly reduced the blood glucose levels than the ethanolic extract. Similarly, the ethanolic and methanolic extracts of Catharanthus roseus, has significantly reduced the blood glucose levels than the aqueous extract.

Keywords – Hypoglycemic, Streptozotocin, Catharanthus Roseus, Phyllanthus Amarus, Diabetes Mellitus

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1. INTRODUCTION:

Diabetes is a serious, long-term condition that occurs when the body cannot produce any or enough insulin or cannot effectively use the insulin it produces. According to WHO estimates, 422 million people worldwide are diabetic and 1.6 million deaths were directly caused by diabetes in 2016. Diabetes could become the 7th leading cause of death worldwide by 2030. The increase in the number of deaths is due to a rich diet, obesity and sedentary population [14]. Diabetes is a complex disease both in its physiopathological mechanisms and in the genesis of its complications. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease [41]. Two major classes of diabetes mellitus, IDDM or Type 1or Juvenile onset Diabetes which is caused by destruction of β-cells of pancreatic Islets, and NIDDM or Type 2 or maturity onset diabetes

caused by abnormality in gluco-receptor, reduced sensitivity of peripheral tissues to insulin, excess of hyperglycemic hormones like glucagons [25]. Type 2 diabetes is a major public health issue in India, more likely to develop Diabetes at younger ages at lower body mass indices [23] India has the highest number of diabetes cases worldwide (40 million) [41] Another 30 million Indians have pre-diabetes and are at high risk of developing T2DM. T2DM prevention is a priority in Indian populations. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and postprandial blood sugar levels [29]. Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. Type I diabetes (insulin dependent) is caused due to insulin insufficiency because of lack of functional beta cells. Patients suffering from this are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication. Type II diabetes is the more common form of diabetes constituting 70-80% of the diabetic population. Symptoms for both diabetic conditions may include: (i) high levels of sugar in the blood; (ii) unusual thirst; (iii) frequent urination; (iv) extreme hunger and loss of weight; (v) blurred vision; (vi) nausea and vomiting; (vii) extreme weakness and tiredness; (viii) irritability, mood changes etc. Though patho physiology of diabetes remains to be fully understood, experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes [31] and more importantly in the development of diabetic complications [4, 28]. Free radicals are capable of damaging cellular molecules, DNA, proteins and lipids leading to altered cellular functions. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models [26, 30] as well as reducing the severity of diabetic complications [17]. For the development of diabetic complications, the abnormalities produced in lipids and proteins are the major etiologic factors. In diabetic patients, extra-cellular and long lived proteins, such as elastin, laminin, collagen are the major targets of free radicals. These proteins are modified to form glycolproteins due to hyperglycemia. The modification of these proteins present in tissues such as lens, vascular wall and basement membranes are associated with the development of complications of diabetes such as cataracts, micro-angiopathy, therosclerosis and nephropathy [7]. During diabetes, lipoproteins are oxidized by free radicals. There are also multiple abnormalities of lipoprotein metabolism in very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) in diabetes. Lipid peroxidation is enhanced due to increased oxidative stress in diabetic condition. Apart from this, advanced glycation end products (AGEs) are formed by non- enzymatic glycosylation of proteins. AGEs tend to accumulate on long-lived molecules in tissues and generate abnormalities in cell and tissue functions [11, 13]. Although several therapies are in use for treatment, there are certain limitations due to high cost and side effects such as development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc [39]. Based on recent advances and involvement of oxidative stress in complicating diabetes mellitus, efforts are on to find suitable antidiabetic and antioxidant therapy. Medicinal plants are being looked up once again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The treatment of DM is based on oral hypoglycemic agents and insulin. However, DM is also treated in Indian traditional medicine using anti-diabetic medicinal plants [9, 38]. The oral hypoglycemic agents currently used in clinical practice have characteristic profiles of serious side effects [21]. Hence, there is a need to search for newer anti-diabetic agents that retain therapeutic efficacy and are devoid of side effects that could be important sources of such agents. *Phyllanthus amarus* is a monoecious, occasionally dioecious, upright or ascending herb, which grows up to 60 cm high, or occasionally higher, belonging to Phyllanthaceae family, locally known as “Kiru-Nelli”**.** All parts of the plants are used in Ayurvedic medicines because of their medicinal properties. Leaves of the plant are reported to contain lignin, alkaloids, flavonoids and glycosides [11]. *P. amarus* is an important plant of Indian Ayurvedic system of medicine used in the treatment of problems related to stomach, urinogenital tract, liver, kidney and spleen. The whole plant is used in gastropathy, diarrhoea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds. *P. amarus* shows a wide spectrum of pharmacological activities including antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, and diuretic properties [13**]**. *Vinca rosea* (*C. roseus*) Linn. (Apocynaceae) is an herbaceous sub shrub also known as Madagascar periwinkle, *Vinca rosea*, or *Lchnera rosea* worldwide. It is cultivated mainly for its alkaloids, which are having anticancer activities.The two classes of active compounds in Vinca are alkaloids and tannins. *Catharanthus roseus* produces more than 100 monoterpenoids indole alkaloids (TIA) in different organs. The leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs, while roots have antihypertensive, ajmalicine and serpentine. The leaves have been known to contain 150 useful alkaloids among other pharmacologically active compounds. Significant

antihyperglycemic and hypotensive activity of the leaf extracts have been reported in laboratory animals. Leaves and twigs of *Catharanthus roseus* have been reported to have hypoglycaemic activity in streptozotocin induced diabetic rats [23]. As the synthetic drugs used for the treatment of diabetes result in many complications. Hence the use of natural sources such as the leaves of *Phyllanthus amarus* and *Catharanthus roseus* for the treatment of diabetes is safe and non-carcinogenic [5, 27]. Hence the present study was designed to evaluate the anti-diabetic activity of aqueous, ethanolic and methanolic extracts of the *Phyllanthus amarus* and *Catharanthus roseus* leaves against STZ-induced diabetic rats. The effect of the extracts of *Phyllanthus amarus* and *Catharanthus roseus* was compared to Glibenclamide, used as a standard drug against Diabetes Mellitus.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All the chemicals procured for this work were of analytical grade, chloroform, ethanol, methanol, Hydrochloric acid (HCL), Sulphuric acid (H_2SO_4) and α- naphthol were procured from S.D. Fine Chem. Ltd, Mumbai, India.

2.2. Collection of plant materials:

Leaves of *Phyllanthus amarus* and *Catharanthus roseus* collected from Hosahalli and Ayanur Kote villages of Shivamogga District were tentatively identified by Dr. T. Parameshwara Naik a renowned botanist in Shivamogga.

2.3. Preparation of plant extract:

The air-dried plant material, leaves of *Phyllanthus amarus* and *Catharanthus roseus* (50-100 g) was washed thoroughly to remove the dirt and impurities, air dried under shade at room temperature and finely grinded with the help of mixer grinder. Extraction of the bioactive compounds was carried out in water, methanol and ethanol. Distilled water was added to the crushed material in a ratio of 1:4 (w/v) and kept at 30^0C for 24 hrs. The organic solvents were added in a ratio of 1:45 (w/v) shaken properly with the residue for 3hrs at their respective boiling temperatures. The extracts were filtered through whatman filter paper and stored at -20^0C for further use.

2.4. Phytochemical screening

The leaf extracts of *Phyllanthus amarus* and *Catharanthus roseus* were analyzed for the presence of alkaloids, glycosides, triterpenoids, steroids, saponins, flavonoids, tannins and carbohydrates according to standard methods [20].

2.4.1. Tests for alkaloid: Test solution was prepared by tritrating 40 to 50 mg extract with dilute acid (10 % acetic acid or 1 to 5 % hydrochloric acid). After filtration, 0.5 to 1 mL filtrate was added with 1 to 2 mL of following reagents.

Mayer's test

Mayer's reagent (Solution I: Dissolve 1.36 g $HgCl_2$ in 60 mL water; Solution II: Dissolve 5 g potassium iodide in 10 mL water. Combine these two solutions and add water up to 100 mL). Test solution (0.5 to 1 mL) was added with 1 to 2 mL of Mayer's reagent and development of white or buff colour precipitates indicates the presence of alkaloid.

2.4.2. Test for flavonoid

Test solution was prepared by dissolving 50 to 100 mg extract in 10 mL methanol/water.

Shinoda test: Test solution (1 to 2 mL) was added with a pinch of magnesium metal powder and a few drops of concentrated hydrochloric acid. Development of orange, pink, red to purple colours indicated the presence of flavones, flavonols or xanthones.

Sulfuric acid test: Test solution (1 to 2 mL) was added with few drops of concentrated sulfuric acid from the side wall of test tubes. Flavones and flavonols dissolve into concentrated H_2SO_4, producing a deep yellow coloured solution. Flavanones give orange to red colour.

2.4.3. Test for Triterpenoids: About 5 ml of extract was mixed in 2 ml of chloroform; 2 ml of acetic anhydride and a few drops of conc. H2SO4 was added. Reddish violet colour indicated the presence of triterpenoids.

2.4.4. Test for Steroids: 10ml of chloroform was mixed with 2ml of extracts and conc. H2SO4 was added to form lower layer. A reddish yellow colour at the interface was an indicative of the presence of steroidal ring.

2.4.5. Test for Saponin: Take 0.1 to 0.2 g of extract and add 10 mL distilled water, and shake vigorously. The appearance of froth that stabilizes for 10 to 15 minutes indicates the presence of saponin.

2.4.6. Test for Glycoside

Salkowaski's test: To the crude extract (about 50 to 100 mg) taken add 2 mL of chloroform, shake well and then add 2 mL of concentrated H_2SO_4 along the side of the test tube. The development of reddish brown colour at the interface indicates the presence of sterol.

2.4.7. Test for Carbohydrates

Molisch's Test: Molisch's reagent (Dissolve 1 g of α- naphthol in 10 mL of methanol or isopropyl alcohol). Test solution (1 to 2 mL) was mixed in a test tube containing 0.5 mL of water, and added with two drops of Molisch's reagent followed by 1 mL of concentrated sulphuric acid from the side of the inclined test tube. Appearance of red brown/violet ring at the interface of acid and aqueous solution indicates the presence of sugars.

2.4.8. Test for Tannins

Ferric Chloride Test : Prepare 5 % solution of ferric chloride in 90 % methanol. Test solution (1 to 2 mL) was added with few drops of ferric chloride solution and development of dark green or deep blue colour indicates the presence of tannins.

2.5. Animal groups and experimental design:

Seventy five Wistar rats were taken initially for this study, which were weighing 150-180g, were kept in separate cages under standard temperature and fed with standard diet, and water. They were divided into nine groups, 1st and 2nd groups included normal (non-diabetic) and diabetic control rats (4 each) that received only distilled water that was free from dissolved salts and colloidal particles which could interfere with the results of the present research and standard diet throughout the trial. Diabetic rats were fed with *Phyllanthus amarus* and *Catharanthus roseus* extracts dissolved at the levels of 100, 200, and 300 mg/kg in distilled water, ethanol, methanol and administered orally as a daily dose for 2 weeks. Out of 75 rats, 4 rats died before grouping and 5 rats were omitted from the study due to mild hyperglycemia (below 150 mg/dL). Remaining 66 rats were divided in to 9 groups, Group-I (4), Group-II (4) and G-III (4) Wistar rats in each group and Group IV to IX had 9 Wistar rats in each group [6].

These groups are as follows:

GROUP-I : Normal (Rats injected with normal saline is considered as vehicle control).

GROUP-II: Diabetic control (was administered with 20% Glucose Soln. for 14 days)

GROUP-III: STZ induced diabetic Wistar rats were administered with standard drug

(Glibenclamide) 5 mg/kg for 14 days, served as positive control.

GROUPS-IV to IX: STZ induced diabetic Wistar rats were administered with Aqueous, ethanolic and methanolic extracts of *Phyllanthus amarus* and *Catharanthus roseus* at the dose of 300 mg/kg rat body weight respectively.

On day 14 all animals were deprived of food, and water was given ad libitum. On day 15 blood glucose levels were measured using Glucometer (Accu-chek) by tail pinching method. Data was analyzed using one way ANOVA followed by suitable post-hoc test. All the values are reported as Mean ± SEM. Statistical significance was set at pH 0.05. Gloves, face mask was used in addition to protective clothing's and slippers to ensure adequate personal health and safety and to avoid inhalation and skin contact with the test items.

2.6. Acute toxicity studies

Following the guidelines of OECD (Organization for Economic Co-operation and Development), the acute oral toxicity [33], was performed on the adult Wistar rats. The rats were kept fasting for overnight providing only water, after which the extracts (aqueous, ethanol, and methanol) were administered orally at the dose level of 5 mg/kg. The animals were observed continuously for 3 hours for general behaviour, neurological and autonomic profiles and then every 30 minutes for next 3 hours and finally for mortality after 24 hours till 7 days. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 100, 200, and 300 mg/kg body weight.

2.7. Experimental induction of diabetes

Diabetes was induced in the adult Wistar rats by injecting intra-peritoneally with a single dose of 50 mg/kg streptozotocin after dissolving it in freshly prepared ice-cold citrate buffer (0.1M, pH 4.5) after 18 hrs of fasting. After the injection, they had free access to feed and water and were given 20% glucose solution for 24 hrs to prevent initial drug induced hypoglycemic shock or mortality [34]. The normal control rats received only distilled water and standard diet. Development of diabetes mellitus in the rats were confirmed by testing fasting blood glucose level (FBGL) using Accu-chek Glucometer, after 48 hrs of STZ injection. The rats with FBSL higher than 200 mg/dL were considered diabetic and were selected for the study [3] [Table-1].

2.8. Drug Administration: The group-I control rats received normal saline and fed on normal diet. The group-II was diabetic control. The group-III rats received a standard anti-diabetic drug Glibenclamide of 5 mg/kg orally dissolved in normal saline solution. The *Phyllanthus amarus* and *Catharanthus roseus* leaf extract at the dose of 100, 200, and 300 mg/kg body weight was given orally to the groups IV to IX respectively. Then blood glucose levels were estimated on every 1st, 7th, and 15th day.

3. RESULTS

3.1. Phytochemical Screening of *Phyllanthus amarus* and *Catharanthus roseus* leaf extracts:

The phytochemical analysis of aqueous, methanolic, and ethanolic leaf extracts of *Phyllanthus amarus* powder revealed the presence of alkaloids, flavonoids, triterpenoids, steroids, saponins, glycosides, carbohydrates and tannins. Alkaloids were present in all, but abundantly in aqueous extract. Flavanoids were present in all but moderately in methanolic extracts. Triterpenoids were moderately present only in the aqueous and present in ethanolic extracts. Steroids were moderately present methanolic, and just present in other extracts. Saponins were present abundantly in aqueous, moderately in methanolic and just present in ethanolic extracts. Glycosides were moderately present in aqueous and methanolic extracts. Carbohydrates were present only in the aqueous and methanolic extracts. Tannins were moderately present in aqueous and methanolic extracts than ethanolic extracts. The phytochemical analysis of aqueous, methanolic, and ethanolic leaf extracts of *Catharanthus roseus* powder revealed the presence of alkaloids, flavonoids, triterpenoids, steroids, saponins, glycosides, carbohydrates and tannins. Alkaloids were abundantly present in the ethanolic extract, moderately in methanolic extract, merely present in aqueous extract. Flavanoids were abundantly present in ethanolic and methanolic extract but absent in aqueous extract. Triterpenoids were moderately present in ethanolic and methanolic extracts, absent in aqueous extract. Steroids were present only in the ethanolic extracts. Saponins were abundantly present in the ethanolic and methanolic extracts, just present in the aqueous extract. Glycosides were merely present in all the three extracts. Carbohydrates were present only in the ethanolic and methanolic extracts. Tannins were abundantly present in the ethanolic, moderately present in methanolic and just present in the aqueous extracts (Table-1).

Table-1. Phyto-chemical screening of *Phyllanthus amarus* and *Catharanthus roseus* leaf extract

PLANT SPECIES	FAMILY	PART USED	EXTRACT	PHYTOCHEMICALS PRESENT							
				Alkaloids	Flavanoids	Triterpenoids	Steroids	Saponins	Glycoside	Carbohydrates	Tannins
Phyllanthus amarus	Phyllanthaceae	Leaves	Water	+++	+	++	+	+++	++	+	++
			Methanol	+	++	-	++	++	++	-	++
			Ethanol	+	+	+	+	+	+	+	+
Catharanthus roseus	Apocynaceae	Leaves	Water	+	-	-	-	+	+	-	+
			Methanol	++	+++	++	-	+++	+	+	++
			Ethanol	+++	+++	++	+	+++	+	+	+++

'+++' = present in high quantity, '++' = present in moderate quantity, '+' = present, '-' = absent

3.2. Acute toxicity studies

This study showed no mortality up to the dose of 500 mg/kg body weight. So, the extracts were safe for long term administration.

3.3. Anti-diabetic activity

The blood glucose levels measured in normal and experimental rats in initial and at the 1st 7th and 15th days of treatment are given in (Table-2). Streptozotocin-induced diabetic rats showed significant increase in the levels on blood glucose as compared to normal rats. Oral administration of aqueous, ethanolic and methanolic extracts (300 mg/kg) showed significant decrease ($p<0.05$) in blood glucose level. The oral administration of methanolic and aqueous extracts (300 mg/kg) showed significant decrease ($p<0.05$) in blood glucose level than the ethanolic extract in *Phyllanthus amarus*. Oral administration of methanolic and aqueous extracts (300 mg/kg) showed significant decrease ($p<0.05$) in blood glucose level than the ethanolic extract in *Catharanthus roseus*. The standard drug, Glibenclamide decreased blood glucose level in 15 days treatment.

Table: 2. Effect of aqueous, ethanolic and methanolic extracts of *Phyllanthus amarus* and *Catharanthus roseus*, on blood glucose levels in Streptozotocin induced diabetic Wistar rats.

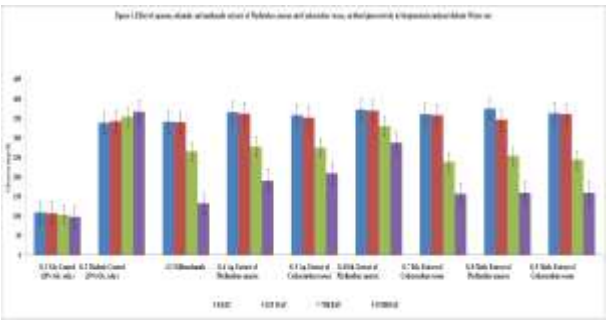
Group & Treatment	Dose (mg/kg rat b.wt.)	Blood Glucose (mg/dL)			
		Pre-treatment 0 Day	Post-treatment 1st Day	Post-treatment 7th Day	Post-treatment 15th Day
G-1 Vehicle Control (20% Glucose soln.)	10 mL/kg	109.50 ± 3.64	107.66 ± 2.44	103.55 ± 3.48	98.33 ± 2.66
G-2 Diabetic Control (20% Glucose soln.)	10 mL/kg	338.66 ± 24.61*	342.66 ± 21.60*	351.60 ± 24.60*	367.5 ± 21.93*
G-3 Glibenclamide	5	341.60 ± 19.97*	339.66 ± 16.17*	266.66 ± 18.17*	132.66 ± 6.38
G-4 Aqueous Extract of		365.30 ± 12.17*	362.18 ± 15.17*	277.40 ± 29.10*	190.22 ± 8.28.*
G-5 Ethanolic Extract of *Phyllanthus amarus*	300	371.66 ± 14.56*	370.08 ± 12.04*	329.30 ± 12.34*	287.83 ± 19.28*
G-6 Methanolic Extract of *Phyllanthus amarus*	300	374.80 ± 8.64*	346.30 ± 19.22*	254.53 ± 28.36*	160.15 ± 8.45*
G-7 Aqueous Extract of *Catharanthus roseus*	300	358.28 ± 12.19*	352.16 ± 13.14*	274.40 ± 20.08*	210.22 ± 8.28.*
G-8 Ethanolic Extract of *Catharanthus roseus*	300	360.34 ± 18.60*	358.18 ± 16.64*	239.28 ± 11.30*	156.63 ± 18.18*
G-9 Methanolic Extract of *Catharanthus roseus*	300	362.80 ± 8.64*	360.30 ± 19.22*	244.43 ± 18.26*	159.65 ± 11.36*

Values are expressed as mean ± SEM; n=9 *p= 0.05 statistically significant Vehicle control versus Diabetic control/ Treatment groups (G4-G9) #p 0.05= statistically significant Vehicle control versus Diabetic control/ Treated groups (G4-G9)

3.4. Discussion

The phytochemical analysis of *Phyllanthus amarus* and *Catharanthus roseus* leaves revealed the presence of alkaloids, flavonoids, triterpenoids, steroids, saponins, glycosides, carbohydrates and

tannins (Table-1). The results showed that *Phyllanthus amarus* and *Catharanthus roseus* leaves extract caused a significant (P<0.05) reduction in the blood glucose levels in diabetic rats. The methanolic and aqueous extract showed a significant reduction in blood glucose level than the ethanolic extracts of *Phyllanthus amarus*. Similarly methanolic and aqueous extracts (300 mg/kg) showed significant decrease (p<0.05) in blood glucose level than the ethanolic extracts in diabetic rats. But, the anti-Diabetic activity may be due to the presence of compounds like lighans, alkaloids, flavonoids, galloatnoids and glycosides in *Phyllanthus amarus* and alkaloids such as (catharanthine, vindoline, vindolidine, vindolicine, vindolinine, vinblastine, vincristine, leurosine, and lochnerine) in *Catharanthus roseus*. This was almost similar to results obtained with reference drug glibenclamide (341.60- 132.66 mg/dL). These results confirmed the use of *Phyllanthus amarus* and *Catharanthus roseus* leaves in the Indian traditional practice as an anti-diabetic agent [2]. The bioactive compounds such as lighans, alkaloids, flavonoids, galloatnoids and glycosides in *Phyllanthus amarus* and alkaloids such as (catharanthine, vindoline, vindolidine, vindolicine, vindolinine, vinblastine, vincristine, leurosine, and lochnerine) in *Catharanthus roseus* may be responsible to bring about a decrease in blood glucose level by potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from β-cells of the islets of Langerhans. Number of plants has been used traditionally in treatment of diabetes and some have been proven scientifically to have hypoglycemic activity. These plant extract contain compound like polysaccharides, flavonoids, terpenoids and tannins, steroid, polypeptides and alkaloids, responsible for the antidiabetic activity. Higher blood glucose levels are expected in streptozotocin induced diabetic rats, since streptozotocin causes a massive reduction in insulin release, by the destruction of the β-cells of the islets of Langerhans and inducing hyperglycemia [35]. A number of other plants have been reported to exert hypoglycemic activity through insulin release-stimulatory effects [19, 40, 32]. However, the possible mechanism by which the drug brings about its hypoglycemic actions may be by competitively inhibiting the glucose receptors at intestinal level, either by increasing the pancreatic secretions of Insulin from the cells of Islets of Langerhans or its release from bound insulin. The antidiabetic activity was found to be significant and dose dependent. All the doses showed maximum reduction of blood glucose level on the 7th day. Out of three doses 100, 200, and 300 mg/kg. The dose 300 mg/kg showed more significant reduction of blood glucose levels when compared with that of standard. (Table-2) The aim of the present study was to evaluate the antidiabetic effect of aqueous, ethanolic and methanolic extracts of *Phyllanthus amarus* and *Catharanthus roseus* against streptozotocin-induced diabetic rats (Figure-1). The standard drug, Glibenclamide has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic β-cells [43]. The possible mechanism by which leaves brings about a decrease in blood glucose level may be by potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from β-cells of the islets of Langerhans. But, the *Phyllanthus amarus* and *Catharanthus roseus* leaves which were collected from Hosahalli and Ayanur Kote villages in Shivamogga district of Karnataka state, India have anti-diabetic properties. These results confirmed the use of *Phyllanthus amarus* and *Catharanthus roseus* leaves in traditional system of medicine to treat diabetes in India. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypoglycemic effect of *Phyllanthus amarus* and *Catharanthus roseus* leaves.



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