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Original Article

In vitro and *In vivo* Antidiabetic Evaluation of Whole Plant Extracts of *Argyreia imbricata* (Roth) Sant. & Patel

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ABSTRACT

Background: Plants of the genus *Argyreia* have been used in traditional and folk medicines and a variety of pharmacological activities have also been reported. **Objective:** The present study was aimed to evaluate the antidiabetic activity *in vitro* and *in vivo* of the different extracts of the whole plant *Argyreia imbricata*. **Materials and Methods:** The powdered material of the whole plant *Argyreia imbricata* was extracted with solvents such as petroleum ether, chloroform, ethyl acetate and methanol by soxhelation. *In vitro* antidiabetic activity of all the extracts was evaluated by α–amylase and α–glucosidase inhibition assay. Based on the results of *in vitro* evaluation, the extracts selected were subjected to *in vivo* evaluation on the Wistar albino rats with streptozotocin-induced diabetes mellitus. Initially, acute toxicity of the extracts was evaluated and the effective dose (ED_{50}) was fixed. Standard drug Glibenclamide was used for the comparative evaluation. Two doses of test extracts, low dose and high dose were administered to the test animals and their antidiabetic activity was evaluated by means of monitoring the changes in the blood glucose level, hematological and biochemical parameters and histopathology of liver and pancreas of test animals. **Results:** Based on the results of *in vitro* evaluation, the ethyl acetate and methanol extracts were selected for the *in vivo* evaluation. ED_{50} of the test extracts were fixed as 200mg.kg^{-1}. Two doses, 200mg.kg^{-1} and 400mg.kg^{-1} of the test extracts were subjected to evaluation. Both the tested extracts possessed the activity, but the methanol, extracts showed significant activity in all aspects. **Conclusion:** Results of the present study strongly support the antidiabetic activity of tested extracts. Further studies on toxicity, identification isolation of the potential compounds may give useful results for the development of clinically useful chemotherapeutic agents in the future.

Key Words: *Argyreia imbricata* extracts, *In vitro* antidiabetic activity, *In vivo* antidiabetic activity.

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INTRODUCTION

The genus *Argyreia* is one of the larger genera belongs to the family Convolvulaceae consist of about 220 species widely distributed in Asian countries including all over India.[1,2] Many plants of this genus were studied well. For example, *A. speciosa,* one among them studied well in the aspects of phytochemistry, pharmacognosy, and pharmacological activities.[3,4]

Argyreia imbricata, a commonly found plant at an altitude up to 300m mean sea level in south India has not been studied before to our knowledge was selected for our research. It is a dicotyledonous plant, growing well in the rainy season with flowering and fruiting season in the month of August – December. It is a large climber with white-woolly stem; Leaves are 8–12cm, ovate, obtusely acute, rounded or subcordate at base, strigose above, white-tomentose beneath, nerves impressed above; Petiole 3cm long; Cymes terminal; peduncles 5cm long; Bracts and bracteoles small; Flowers few, shortly pedicelled, calyx lobe orbicular; 10×7mm, densely hairy outside, reddish inside, corolla 2cm long, pink. Berry 5×5mm, reddish, densely, hairy outside.[1]

In our previous study[1], the whole plant of *Argyreia imbricata* was collected from Mekkarai, the village area located close to the foothills of the Western Ghats in the Tirunelveli District, Tamil Nadu, India. The collected material was identified and authenticated properly and subjected to extraction, preliminary phytochemical evaluation and spectral characterization. The present study was aimed to evaluate the *in vitro* and *in vivo* antidiabetic activity of the extracts of the whole plant *Argyreia imbricata*.

MATERIALS AND METHODS

Plant extract preparation

The whole plant of *Argyreia imbricata* was collected, dried, powdered, and extracted by soxhelation with different solvents such as petroleum ether, chloroform, ethyl acetate and methanol. Each extract was filtered, distilled and the dried extract obtained was preserved for experiments.[1]

In vitro antidiabetic activity

Inhibition of α–amylase activity

Evaluation of α–amylase inhibition activity of *A. imbricata* extracts was done in accordance with the previous literature[5,6] with slight modification. Starch solution (0.5% w/v) used as the substrate in this evaluation was prepared by dissolving potato starch in 20mM phosphate-buffered saline (pH 6.9) and

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kept this mixture in the boiling water bath for 15min. to get a clear solution. The enzyme solution used in the evaluation was prepared by dissolving porcine pancreatic α-amylase in 20mM phosphate-buffered saline (pH 6.9) to obtain the concentration of 1U/ml. The test extracts (10–50µg/ml) and the standard (acarbose) solution (100–1000µg/ml) were prepared in dimethyl sulfoxide (DMSO) solvent. DNS solution was used as the colour reagent ((20ml 96mM 3,5–dinitro salicylic acid, 12gm sodium potassium tartrate in 8ml of 2M NaOH and 12ml de-ionized water).

Three sets, control, positive control, and test were prepared. In case of test sample preparation, a mixture of 1ml each of test extract and enzyme solution was incubated at 25°C for 30min. To this mixture, 1ml starch solution was added and again incubated at 25°C for 20min. The reaction was stopped by adding DNS solution (1ml) and kept in the boiling water bath for 15min. Cooled and the final volume was made with distilled water and the absorbance was measured at 540nm. In case of control, the test extract was replaced with 1ml of DMSO. Acarbose was used as a positive control. Percentage inhibition and half-maximal inhibitory concentration (IC_{50}) scores were calculated. Percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition } = \frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}} \text{ x } 100$$

Abs C – absorbance of the control; Abs S – absorbance of the sample (test extract/standard).

Inhibition of α–glucosidase activity

α–Glucosidase inhibitory activity of *A. imbricata* extracts was evaluated as per the previous literature [6-8] with slight modification. Three sets, control, positive control, and test were prepared in DMSO. Control tube contains DMSO, enzyme and substrate. In positive control, 100µl of Acarbose (0.1–3.2µg/ml) was used instead of test extracts.

100µl of test extracts (25–400µg/ml) in DMSO were pre-incubated at 37°C for 20min with 20µl of α–glucosidase (maltase) enzyme. Then the reaction was started with the addition of 50µl of the substrate solution [p-nitrophenyl glucopyranoside (pNPG) in 20mM phosphate buffer (pH 6.9)] to the mixture and again incubated at 37°C for 20min. and the reaction was stopped by the addition of 50µl of 0.1M Na_2CO_3. The *α*-glucosidase activity was determined by measuring the yellow coloured p-nitrophenol released from pNPG at 405nm. Percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition } = \frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}} \text{ x } 100$$

Abs C – absorbance of the control; Abs S – absorbance of the sample (test extract/standard).

IC_{50} values were determined graphically from the plots of percentage inhibition *Vs* log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

In vivo antidiabetic activity

Plant extract, animal and approval of the study

Based on the results of *in vitro* evaluation, two extracts were selected for *in vivo* evaluation. Healthy, young, adult Wistar albino rats of both sexes weighing about 150–200gm obtained from the Central animal house, Cape Bio Lab & Research Centre, Marthandam, Kanyakumari, Tamil Nadu, India, was used for the experiments. The study was conducted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The institutional animal ethical committee approved the study (CBLRC/IAEC/05/01-2020).

Acute toxicity study

The LD_{50} of the selected extracts was determined as per the guidelines of OECD 423 and in reference with the standard procedure.[9] Required animals were randomly selected, marked facilitating identification, and kept in the standard environmental conditions like ambient temperature (25 ± 1°C), relative humidity (55 ± 5%) and 12h light/dark cycle for five days prior to dosing for adaptation with laboratory conditions. The animals were fed with standard pellet diet and water *ad libitum*. Following the overnight fasting with free access to water, the test extracts were administered in a single dose by gavage using stomach tube. 50mg.kg^{-1} was selected as initial dose and administered to three animals and after 24h, the animals were observed for the number of death. The same procedure was repeated with next doses 300mg.kg^{-1} and 2000mg.kg^{-1}.

Evaluation of antidiabetic activity

The study was designed in reference with the standard procedure.[10-12]

Treatment protocol

The selected Wistar albino rats were divided into seven groups of six each. Group 1 served as the normal control received diet and water only; Group 2 served as diabetic control received Streptozotocin (STZ) only for the induction of diabetes. Group 3 was taken as standard control received 0.5mg.kg^{-1} of standard drug Glibenclamide. Group 4 was experiment group received 200mg.kg^{-1} of selected extract–1 of *A. imbricata*. Group 5 was treated with 400mg.kg^{-1} of selected extract–1. Group 6 and 7 were treated with 200mg.kg^{-1} and 400 mg.kg^{-1} of selected extract–2 of *A. imbricata* respectively.

Treatment and evaluation

All the animals in the six groups except Group 1 kept free from the diet for 12h prior to inducing diabetes. Diabetes mellitus was induced in all except Group 1 by administering a single dose of Streptozotocin [STZ] (60mg.kg^{-1} i.p). Group 1 (normal control) animals were injected with saline only. 72h after the administration of STZ, the blood glucose level of the treated animals was measured by using electronic glucometer. Animals found with blood sugar level ≥ 180mg/k/dl were considered as diabetic and used for the experiments as per the protocol. The standard drug and the test extracts were administered orally from the 3rd day of Streptozotocin administration and given daily for 28 days. The blood glucose level of the animals was estimated once a week by using electronic glucometer.

At the end of fourth week of treatment, all the rats were sacrificed by cervical dislocation and the blood was collected from each rat by direct cardiac puncture. Serum and plasma were separated by centrifugation at 2500rpm for 10 min. and stored at –20°C for the investigation of different haematological and biochemical parameters. Organs such as the liver and pancreas were removed immediately from the sacrificed animals and kept in 10% formalin solution for histopathological evaluation.

Hematological parameters such as hemoglobin and glycosylated haemoglobin were analyzed by using the equipment, COBAS MICROS OT 18, Roche. Biochemical parameters such as total cholesterol, triglycerides, High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), and Alkaline phosphatase (ALP) were analyzed by using the equipment, COBAS MIRA PLUS – S, Roche.

RESULTS AND DISCUSSION

The *in vitro* antidiabetic activity of petroleum ether, chloroform, ethyl acetate, and methanol extracts of the whole plant *A. imbricata* was evaluated by the inhibition of α–amylase and α–glucosidase activity. The results of α–amylase inhibitory evaluation of test extracts and the standard, acarbose are shown in Table 1. The results clearly indicated that a significant α–amylase inhibition was produced by methanol and ethyl acetate extracts comparing with the standard drug acarbose, particularly, these extracts showed a significant dose-dependent rise in percentage inhibitory activity against α–amylase enzyme comparing with other tested extracts with IC_{50} value 26.81µg/ml and 31.40µg/ml respectively.

The results of α–glucosidase inhibition assay are shown in Table 2. From the results, it was found that the methanol and ethyl acetate extracts showed a significant activity comparing with the standard drug, acarbose. Similar with α–amylase inhibition assay, these extracts showed a significant dose-dependent rise in percentage inhibitory activity against α–glucosidase enzyme comparing with other tested extracts with IC_{50} value 137.27µg/ml and 239.49µg/ml.

Based on the results of *in vitro* evaluation, the ethyl acetate and methanol extracts were selected for the *in vivo* antidiabetic evaluation on diabetes-induced Wistar albino rats. In the acute toxicity evaluation, initially animal death was not observed after the administration of first two doses. But, two out of three animals died within the 24h of administration of third dose 2000mg.kg^{-1}. So, the third dose was concluded as LD_{50} and from this the ED_{50} was fixed as 200mg.kg^{-1} for the selected ethyl acetate (extract–1) and methanol (extract–2) extracts.

In the *in vivo* evaluation, blood glucose level, various hematological and biochemical parameters, histopathology of the liver and pancreas of experimental animals were analyzed. The results of blood glucose level analysis are shown in Table 3. The results showed that there were no significant changes in the glucose level of normal control animals in the observation days. But it was observed that the glucose level was increased gradually without any changes in the diabetic control animals. The standard control animals received Glibenclamide showed a marked reduction in the blood glucose level at the 28th-day observation. It was found that on the 28th day of observation, both the tested extracts (animals of Group 4–7) showed a significant reduction in the blood glucose level of experimental animals, importantly, the ethyl acetate extract (animals of Group 4 & 5) showed more significant results comparing with the methanol extract (animals of Group 6 & 7).

Analysis of changes in glycosylated hemoglobin revealed a significant rise in its level in diabetic control (Group 2) animals compared with normal control (Group 1) animals. Also, it was observed that there were no significant changes in the glycosylated hemoglobin level of animals of Group 3 to 7 comparing with normal control animals. Importantly, the animals received methanol extract 400mg.kg^{-1} (Group 7) showed a very significant score compared with other treatment groups. From the results, it was found that the level of enzymes such as SGOT, SGPT, and ALP in the diabetic control animals was elevated compared with normal control animals. Treatment of animals with standard drug Glibenclamide (Group 3) showed reversal of their level similar to that of normal control animals. The results clearly indicated the positive effect of tested extracts on the level of these enzymes. Both ethyl acetate and methanol extracts showed a significant reversal analyzed enzymes level in the experiment animals (Group 4–7) towards normal in comparison with standard control animals, particularly, the high dose of methanol extract showed more significant results (Table 4).

Analysis of changes in the level of total cholesterol, triglycerides, HDL, LDL, and VLDL in the serum of experimental animals showed significant results (Table 5). All these lipid profiles markedly differed abnormally in diabetic control animals comparing with normal control one. But in the treatment group animals, these scores were reversed to normal. Comparing with normal and standard control, the methanol extract-treated animals showed a significant effect than ethyl acetate extract-treated animals.

In the histopathology analysis, normal hepatocyte morphology was found in the liver of normal control animals. In diabetic control, the hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, Kupffer cell proliferation, hepatocyte necrosis and mononuclear infiltrate were identified. But, the standard control showed mild hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis. All other low dose and the high

Table 1: α–amylase inhibitory activity of test extracts and standard drug (acarbose).

Concentration (µg/ml)		% inhibition of α–amylase activity				
		Standard	Test extracts			
Standard	Test	Acarbose	Pet. ether	Chloroform	Ethyl acetate	Methanol
100	10	9.32 ± 0.57	3.85 ± 0.23	4.27 ± 0.76	6.91 ± 0.28	7.90 ± 0.72
200	20	41.50 ± 1.00	24.70 ± 0.81	27.56 ± 0.93	33.60 ± 0.42	36.28 ± 1.22
400	30	62.63 ± 1.85	43.21 ± 0.54	47.80 ± 0.66	50.07 ± 0.77	52.57 ± 1.60
800	40	71.70 ± 2.60	55.32 ± 0.27	58.70 ± 0.11	64.78 ± 0.19	68.72 ± 2.47
1000	50	89.42 ± 2.60	69.08 ± 0.33	74.58 ± 1.17	79.60 ± 1.83	82.55 ± 2.63
IC_{50}		152.67	36.18	33.52	31.40	26.81

Table 2: Effect of test extracts and standard drug on α–glucosidase inhibition assay.

Concentration (µg/ml)		% inhibition of α–glucosidase activity				
		Standard	Test extracts			
Standard	Test	Acarbose	Pet. ether	Chloroform	Ethyl acetate	Methanol
0.1	25	17.42 ± 0.65	1.53 ± 0.84	3.08 ± 0.62	3.98 ± 0.80	4.24 ± 0.24
0.2	50	39.57 ± 1.45	08.61 ± 0.18	11.65 ± 0.53	14.48 ± 0.35	16.50 ± 0.65
0.4	100	62.88 ± 2.95	22.43 ± 0.69	26.20 ± 0.32	31.51 ± 0.70	35.53 ± 1.21
0.8	200	76.20 ± 2.72	52.23 ± 0.21	54.71 ± 0.13	66.70 ± 0.28	70.22 ± 1.60
1.6	400	91.19 ± 2.69	71.07 ± 0.37	78.80 ± 0.37	83.51 ± 0.19	88.21 ± 2.81
IC_{50}		0.290	281.41	253.80	239.49	137.27

Table 3: Effect of selected extracts on blood glucose level of experimental animals.

Animal group	Blood glucose level (mg/dl)				
	Day of observation				
	0	7	14	21	28
G_1	102.33 ± 6.83	115.50 ± 1.89	110.66 ± 1.11	113.00 ± 1.65	107.83 ± 1.62
G_2	248.00 ± 7.01	265.50 ± 2.70	276.16 ± 2.08	295.66 ± 1.24	320.50 ± 1.74
G_3	346.66 ± 10.96***	118.50 ± 1.53***	174.83 ± 3.58***	115.16 ± 1.17***	114.16 ± 1.93***
G_4	350.16 ± 5.76***	138.16 ± 1.21***	173.83 ± 2.64***	129.16 ± 1.64***	123.66 ± 1.76***
G_5	321.33 ± 6.24***	138.16 ± 2.90***	137.33 ± 2.08***	139.66 ± 1.73***	128.83 ± 1.87***
G_6	365.16 ± 5.94***	143.50 ± 2.05***	145.83 ± 1.70***	139.00 ± 1.63***	130.33 ± 2.17***
G_7	334.50 ± 7.14***	231.16 ± 2.63^{ns}	293.00 ± 2.63***	166.00 ± 1.46***	133.50 ± 1.28***

G_1–Normal control; G_2–Diabetic control; G_3–Standard control; G_4–Group (Ethyl acetate extract 200mg.kg^{-1}); G_5–Group (Ethyl acetate extract 400mg.kg^{-1}); G_6–Group (Methanol extract 200mg.kg^{-1}); G_7–Group (Methanol extract 400mg.kg^{-1}); All values are expressed as mean ± SEM for 6 animals in each group; ***p<0.001significance between normal control Vs diabetic control and treated groups.

Table 4: Effect of selected extracts on haemoglobin and liver enzymes of experimental animals.

Animal group	Haemoglobin	Glycosylated haemoglobin	SGOT	SGPT	ALP
G_1	12.63 ± 1.56	4.76 ± 0.37	25.99 ± 3.18	51.50 ± 8.26	130.83 ± 7.06
G_2	10.86 ± 0.22	12.66 ± 0.78	59.68 ± 6.71	63.60 ± 5.82	203.21 ± 9.36
G_3	12.77 ± 0.38**	5.36 ± 0.39**	34.85 ± 2.10	56.71 ± 3.69	137.65 ± 5.12
G_4	11.78 ± 0.44^{ns}	6.64 ± 0.43**	41.33 ± 2.36	60.60 ± 2.42	145.31 ± 2.16
G_5	12.73 ± 0.26**	6.13 ± 0.66**	38.13 ± 3.61**	59.50 ± 2.57**	141.08 ± 2.05**
G_6	12.93 ± 0.48**	5.68 ± 0.36*	37.25 ± 1.25***	61.76 ± 3.51***	142.41 ± 3.17***
G_7	13.48 ± 0.41***	3.68 ± 0.42**	35.23 ± 2.34***	58.51 ± 2.42***	138.23 ± 2.10***

G_1–Normal control; G_2–Diabetic control; G_3–Standard control; G_4–Group (Ethyl acetate extract 200mg.kg^{-1}); G_5–Group (Ethyl acetate extract 400mg.kg^{-1}); G_6–Group (Methanol extract 200mg.kg^{-1}); G_7–Group (Methanol extract 400mg.kg^{-1}); All values are expressed as mean ± SEM for 6 animals in each group; ns–not significance; **p<0.01, ***p<0.001significance between normal control Vs diabetic control and treated groups.

Table 5: Effect of selected extracts on lipid profile of experimental animals.

Animal group	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
G_1	168.81 ± 4.56	69.95 ± 3.48	55.55 ± 1.52	50.64 ± 4.11	13.99 ± 0.69
G_2	238.96 ± 4.65	187.58 ± 5.90	22.40 ± 1.32	132.17 ± 0.89	67.51 ± 0.48
G_3	160.45 ± 4.19***	91.22 ± 10.68***	50.47 ± 1.80***	69.91 ± 0.74***	28.64 ± 1.68***
G_4	218.89 ± 4.86^{ns}	135.81 ± 13.74^{ns}	32.40 ± 1.11^{ns}	97.32 ± 1.44^{ns}	55.73 ± 1.81^{ns}
G_5	205.98 ± 5.56**	109.91 ± 16.60**	36.42 ± 3.52**	89.85 ± 1.95**	42.18 ± 4.62**
G_6	210.07 ± 5.74**	104.38 ± 7.04**	33.43 ± 1.90**	93.90 ± 1.42**	48.36 ± 1.42**
G_7	185.32 ± 2.79***	97.32 ± 9.75***	46.23 ± 1.11***	78.21 ± 1.38***	34.88 ± 1.94**

G_1–Normal control; G_2–Diabetic control; G_3–Standard control; G_4–Group (Ethyl acetate extract 200mg.kg^{-1}); G_5–Group (Ethyl acetate extract 400mg.kg^{-1}); G_6–Group (Methanol extract 200mg.kg^{-1}); G_7–Group (Methanol extract 400mg.kg^{-1}); All values are expressed as mean ± SEM for 6 animals in each group; ns–not significance; **p<0.01, ***p<0.001significance between normal control Vs diabetic control and treated groups.

dose of test extract-treated groups sections exhibited moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less Kupffer cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

The small pores system in the liver sinusoidal endothelial cells is the site where the transfer of nutrients between blood and liver take place. In diabetic control, the liver sinusoidal was identified as congested because of the accumulation of high glucose concentration in blood. Section of the entire test extracts treated group exhibited moderate hepatic congestion due to the reduction in the blood glucose level. In diabetic control, it was identified that small droplets of lipids are retained as pericentre globular micro steatosis which may due to hyperglycemia that impaired the normal process of synthesis and elimination of triglycerides. In standard control and the test extracts treated group section showed a minimal pericentre globular micro steatosis that pointed out that the impairment in the triglycerides synthesis and elimination was balanced.

The Kupffer cells are the scavenger cells of the liver. In hyperglycemia, the liver cells become inflamed and more Kupffer cells were proliferated to phagocyte the inflammatory cells. It was found that in the diabetic control, the Kupffer cell proliferation was increased because the diabetic condition leads to elevated level of lipopolysaccharides whereas the standard control and test extracts treated group showed the Kupffer cell proliferation is moderate which clearly indicated that the treatment increases the insulin sensitivity thereby decreasing the level of lipopolysaccharides leads to reduction in the kupffer cell proliferation and the impairment of β- cell function gets improved.

Histopathological analysis of the pancreas revealed a normal histological architecture in the normal control group animals. In diabetic control, the pancreatic β cell degeneration was found which may due to the administration of STZ. But, it was found that the extracts treated groups significantly regenerate the pancreatic β cells. All these results strongly supported the antidiabetic nature of the tested extracts.

CONCLUSION

In the present study, the powdered material of the whole plant *Argyreia imbricata* was subjected to soxhelation with solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate, and methanol.

Among these four extracts, two, the methanol and ethyl acetate extracts showed significant activity in the *in vitro* antidiabetic evaluation by α–amylase and α–glucosidase inhibition assay. Based on these results, these two extracts were selected for the *in vivo* antidiabetic evaluation on Wistar albino rats with streptozotocin-induced diabetes mellitus. In this evaluation also, the selected methanol and ethyl acetate extracts exhibited the antidiabetic activity, particularly, the methanol extract showed significant activity which suggested that some compounds from these extracts appeared to be promising for the treatment of diabetes mellitus. Of course, further studies of these extracts need in the aspects of toxicity, identification and isolation of the potential compounds. Our future studies in this direction may give useful results for the development of clinically useful chemotherapeutic agents.

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CONFLICTS OF INTERESTS

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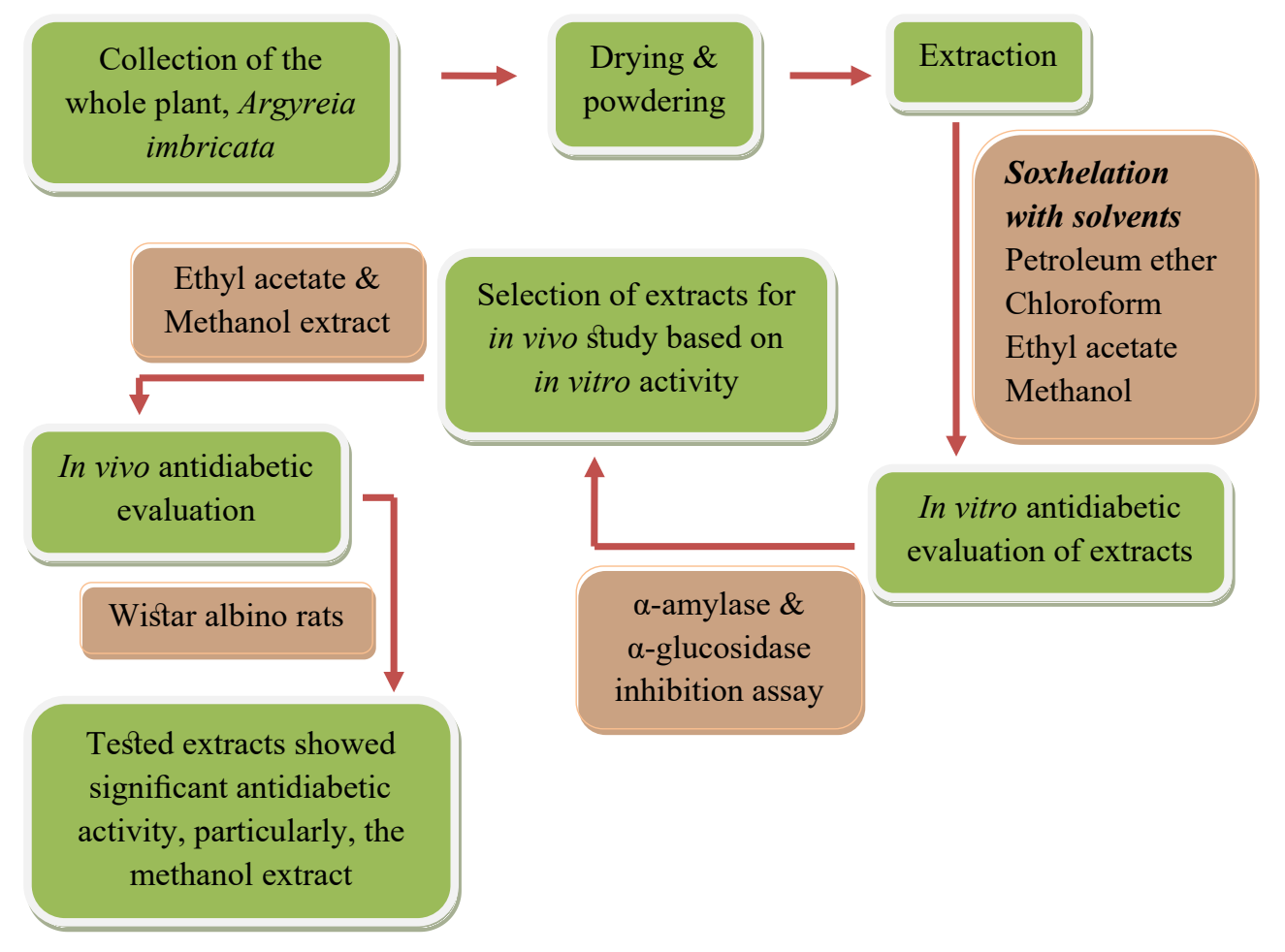
GRAPHICAL ABSTRACT



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