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In-vitro and *in-vivo* anti-diabetic activity of various extract of the *Anisomeles malabarica(L)* leaves

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ABSTRACT

Objective: In the present study the ethanolic extracts of *Anisomeles malabarica(L)* (ETAM) leaves were studied for Aldose reductase, alpha (α)- amylase and alpha (α)-glucosidase inhibition using an *in-vitro* and evaluate plasma glucose concentration and lipid profile in streptozotocin-induced diabetic rats in *in-vivo* anti diabetic model**. Methods:** The serial extraction was carried out with a series of solvents: Petroleum ether, ethyl acetate and ethanol with increasing polarity using Soxhlet apparatus. The concentrated and dried extracts were subjected to the antidiabetic activity was assessed by employing standard *in-vitro* and *in-vivo* techniques. **Result:** The result showed ethanolic extract exhibited significant aldose reductase, α-amylase and α-glucosidase inhibitory activities with an all plant extracts respectively and well compared with standard acarbose drug. This knowledge will be useful in finding more potent antidiabetic principle from the natural resources for the clinical development of antidiabetic therapeutics. In *in-vivo* model ethanol extract was found to lower the Fasting blood glucose level significantly ($p < 0.05$) in diabetic rats. *Anisomeles malabarica* (AM) caused a significant ($p < 0.05$) reduction in FBG level. **Conclusion:** The investigation confirms that ethanolic extract exhibited highest antidiabetic activity among all extracts, Additional studies on needed for purification, characterization and structural elucidation of bioactive compounds from ethanolic extract. This study provides scientific evidence that leaves of *Anisomeles malabarica* (AM) have anti-diabetic efficacy. Thus, considering its relative hypoglycemic potency, they may serve as useful therapeutic agents for treating diabetes.

Keywords: *In-vitro* and *in-vivo* antidiabetic, Aldose reductase, α-amylase, α-glucosidase.

1. INTRODUCTION

Diabetes mellitus is an endocrine disorder in which glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic β-cells in insulin dependent diabetes mellitus and inadequate release of insulin from the pancreatic β-cells or insensitivity of target tissues to insulin in non-insulin dependent diabetes mellitus. Aldose reductase, as a key enzyme in the polyol pathway, is reported to catalyze the reduction of glucose to sorbitol. In normal tissue, Aldose reductase has low substrate affinity for glucose, so that the conversion of glucose to sorbitol is little catalyzed. Sorbitol does not readily diffuse across cell membranes and the intra cellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataract, neuropathy and retinopathy. These findings suggest that Aldose reductase inhibitors prevent the conversion of glucose to sorbitol and may have the capacity of preventing and / or treating several diabetic complications[1]. The digestive enzyme (α-amylase) is responsible for hydrolyzing dietary starch to maltose, which breaks down to glucose, prior to absorption. Inhibition of the α-amylase should reduce the unfavorable high postprandial blood glucose peak in diabetics. Intestinal α-Glucosidase inhibitors are reported to be powerful therapeutic agents in carbohydrate metabolic

disorders, especially diabetes mellitus and obesity. Postprandial hyperglycemia and hyperinsulinemia are expected to be diminished by inhibition of poly and oligosaccharide digestion in the intestinal-tract[2]. Several drugs such as biguanides, sulfonylurea and thiazolidenediones are presently available to treat the diabetes mellitus. The use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects[3]. Thus searching for a new class of compounds is essential to overcome diabetic problems ultimately leading to continuous search for alternative drugs[4]. Henceforth, present study was aimed to explore the *Anisomeles malabarica* leaves are evaluating the *in-vitro* and *in-vivo* anti-diabetic activity of the different solvent extracts. *Anisomeles malabarica*, a medicinal plant from the Lamiaceae family, its medicinal values are potential anti-allergic, anti-anaphylactic, anti-bacterial, anticancer, anti-carcinogenic, anti-inflammatory, antiepileptic potential, antifertility, anti-pyretic activity and antispasmodic[5].

2. Materials and Methods:

2.1. Preparation of different plant extracts

Anisomeles malabarica leaves were collected from the forest of kalakatu, Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India, Palayamkottai, aunthenticated by Chelladurai Botonist. A voucher specimen No (CCRAS-965/2019). Fresh plant leaves were shade dried at room temperature, ground into fine powder and stored in airtight containers. Then extracted (amount 500 g) with solvents of increasing polarity such as petroleum ether, ethyl acetate, and ethanol, for 72 hours with each solvent, by continuous hot extraction using the soxhlet apparatus at a temperature of 60°C[6]. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

2.2. *In-vitro* anti diabetic study

2.2.1. Rat lens Aldose reductase preparation

Crude Aldose reductase was prepared from rat lens. Eyeballs were removed from 9 week old male rats. Animal care and protocols were in accordance with and approved by Institutional Animal Ethics Committee. Lenses were dissected by posterior approach and homogenized in 10 volumes of 100 mM potassium phosphate buffer pH 6.2. The homogenate was centrifuged at 15,000 Xg for 30 min at 4°C and the resulting supernatant was used as the source of Aldose reductase

2.2.2. Determination of aldose reductase activity

The method of Hayman and Kinoshita[7] was used to assay for aldose reductase activity. Enzyme specific activity was calculated as IU/mg protein and this was defined as activity of the enzyme that can produce 1µmol NADP+ from NADPH in 1 min.25. Various concentrations (50-1600 µg/ml) of the ETAM were prepared in triplicate. Exactly 100 µl of concentration prepared was then added to the assay mixture and incubated for 5-10 minutes. The assay mixture was incubated at 37°C and initiated by the addition of NADPH at 37°C. The change in the absorbance at 340 nm due to NADPH oxidation was measured spectrophotometrically. Acarbose was used as standard drug. The inhibition of aldose reductase was calculated using the following Calculations

The percentage inhibition of aldose reductase is calculated as follows:

% Inhibition = Absorbance (control) – Absorbance (test)/ Absorbance (control) X 100

2.2.3. Alpha-amylase inhibitory assay

The Alpha-amylase inhibitory assay of ETAM was evaluated according to a previously described method [8]. In brief, 0.5 ml of extract was mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1 ml of dinitrosalicylic acid color reagent. At this time, the test tubes were placed in a water bath (100 °C for 5 min) and cooled until room temperature was attained. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following Calculations

The percentage inhibition of α-amylase is calculated as follows:

% Inhibition = Absorbance (control) – Absorbance (test)/ Absorbance (control) X 100

2.2.4. α- Glucosidase inhibition bio-assay

To 50 µL of enzyme, add 250 µL of buffer of test extracts ETAM and incubate at 37°C for 30 minutes. Add 500 µL of sucrose solution and

incubate at 37°C for 20 minutes, heat on boiling water bath for 2 minutes to arrest the reaction and cool. Measure glucose concentration by Glucose Oxidase method[9].

2.2.5. Glucose estimation (Glucose oxidase method)

Mix 100 µL of test extracts with 500 µL of glucose reagent (Glucose reagent kit) then incubate at room temperature for 10 minutes. Measure the absorbance at 510 nm.

Calculation

The percentage inhibition of α - glucosidase is calculated as follows:

% Inhibition = Absorbance (control) – Absorbance (test)/ Absorbance (control) X 100

***2*.3. *In-vivo* anti diabetic model**

2.3.1. Acute toxicity study

Acute toxicity study was performed according to OECD - 423 guidelines. Albino rats (n = 6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The ethanolic extract of *Anisomeles malabarica* suspended in normal saline: tween 80 (95:5) which was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. The mortality was observed in 5/6 or 6/6 animals, and then the dose administered was considered as toxic dose. However, the mortality was observed in less than four rats, out of six animals then the same dose was repeated again to confirm the toxic effect. The mortality was not observed, the procedure was then repeated with higher doses such as 50, 300, 1000, and 2000 mg/ kg.

2.3.2. Animals

Male wistar rats each weighing 180-220 g was obtained from Aditya Bangalore Institute of Pharmacy Education and Research in Bangalore at Karnataka, India. The guidelines of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) of the Government of India were followed and prior permission was granted from the Institutional animal ethics committee (NO.1611/PO/a/12/CPCSEA). Rodent laboratory chow was access and water *ad libitum*, and rats were maintained on a 12 hour light/dark cycle in a temperature regulated room (20-25^{o}C) during the experimental procedures.

2.3.3. Induction of diabetes

The fasted rats were injected intravenously with 50 mg/kg of STZ. The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and kept on ice prior to use. One week after STZ administration, the rats with Fasting blood glucose (FBG) concentrations of over 150 mg/dl were considered to be diabetic and were used in the experiment[10].

2.3.4. The oral glucose tolerance test (OGTT) in normal and STZ induced diabetic rats

After an overnight fasting, normal and diabetic rats were divided into five groups each with 6 rats in each group. Group I rats received 1 ml of distilled water only. Groups II - IV rats received various extracts of *Anisomeles malabarica* orally at 200 mg/kg respectively. Group V rats received 50 mg/kg standard drug Acarbose. Glucose (3 g/kg) was administered orally to each rat 30 min later. Blood samples (0.5–0.6 ml) were collected from the tail vein in chilled heparinized tubes at -30, 0, 30, 90, 120 and 210 min for the estimation of blood glucose level. After centrifugation (2000 × g), plasma was removed and stored at −20°C. The plasma glucose concentrations were measured by the method of glucose oxidase - peroxides using Span Diagnostic kits.

2.3.5. Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA). The least significant difference test was used for mean comparisons and ($p < 0.05$) was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. Aldose Reductase Inhibition Assay

The results of *in-vitro* antidiabetic effect using Aldose reductase inhibitory assay of the various extracts of *Anisomeles malabarica* and acarbose were shown in Table 1. The ethanol extracts revealed a significant inhibitory action of Aldose reductase enzyme. Pet. ether and Ethyl acetate extracts showed less inhibitory activity; Ethanolic extract showed inhibitory activity of IC 50 value is 258.7 µg/ml. Acarbose is a standard drug used for Aldose reductase inhibitor assay. Acarbose showed inhibitory activity of IC 50 value is 3.1 µg/ml. Aldose reductase is present in all target tissues that develop diabetic complications[11], In our study result revealed that ethnolic extract prevents diabetic complications especially diabetic retinopathy and nephropathy.

3.2. Alpha amylase inhibitory activity

In this study the *in- vitro* alpha amylase inhibitory activities of the PEAM, EAAM and ETAM were investigated. The result of experiment showed that ethanolic extract increased inhibitory

activity against Alpha amylase enzyme shown in Table 2. Pet. ether and Ethyl acetate extracts showed less inhibitory activity, Ethanolic extract showed inhibitory activity of IC 50 value is 239.6 µg/ml. Acarbose is a standard drug used for alpha amylase inhibitor assay. Acarbose showed inhibitory activity of IC 50 value is 2.15 µg/ml. So the plant extracts might be used as starch blockers since it prevents or slows the absorption of starch in to the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugars. In our study, the ethanolic extract showed maximum α- amylase inhibitory activity, which could be attributed to the presence of polyphenols and flavonoids. Because polyphenols are not only capable of reducing oxidative stress but also of inhibiting carbohydrate hydrolyzing enzymes because of their ability to bind with proteins [12].

3.3. Alpha Glucosidase Inhibition Assay

The results of *in-vitro* antidiabetic activity using alpha glucosidase inhibitory assay of PEAM, EAAM, ETAM and acarbose were shown in Table 3. The extract revealed a significant inhibitory action of -glucosidase enzyme. Pet. ether and Ethyl acetate extracts showed less inhibitory activity, Ethanolic extract showed inhibitory activity of IC 50 value is 328.2 µg/ml. Acarbose is a standard drug used for alpha glucosidase inhibitor assay. Acarbose showed inhibitory activity of IC 50 value is 3.978 µg/ml. Thus the inhibition of the activity of alpha glucosidase by ETAM would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation[13].

3.4. *In-vivo* anti diabetic model

3.4.1. Acute toxicity study

Acute toxicity study showed that various extracts of *Anisomeles malabarica* did not produce any toxic symptoms when administered orally to rats. The lethal dose (LD-50 value) was of 2000 mg/kg body weight.

3.4.2. Effect of different leaf extracts of *Anisomeles malabarica* on FBG of normal and diabetic rats

Various extracts of *Anisomeles malabarica* (200 mg/kg) were evaluated in normal and diabetic rats along with the standard drug Acarbose (50 mg/kg). In normal and diabetic rats, among all the extracts, ETAM reduced plasma glucose concentration significantly ($p < 0.05$) as like Acarbose which was summarized in table 4 and 5. Administration of streptozotocin caused rapid destruction of pancreatic cells in rats, which led to impaired glucose-stimulated insulin release and insulin resistance, both of which are marked feature of diabetes. The blood glucose-lowering effect of plant extracts is generally dependent upon the degree of pancreatic β cell destruction and useful in moderate streptozotocin-induced diabetes[14]. Among all the extracts tested, the ethanol extract produced significant reduction in the blood glucose level comparable to that Acarbose treatment. At present, the exact Mechanism of action of ETAM is not yet known and will be the subject of further studies.

Table - 1: Aldose Reductase Inhibition Assay

	Conc (µg/ml)	OD @405 nm	% Inhibition	IC 50(µg/ml)
Control	**0**	**0.669**	**0.00**	**0**
Acarbose	0.3125	0.515	23.02	3.1
	0.625	0.457	31.69	
	1.25	0.373	44.25	
	2.5	0.299	55.31	
	5	0.169	74.74	
	10	0.109	83.71	
Pet. Ether Extract	50	0.628	6.13	IC50 was not calculated due to lesser inhibition
	100	0.650	2.83	
	200	0.593	11.36	
	400	0.574	14.20	
	800	0.585	12.56	
	1600	0.518	22.57	
	50	0.634	5.23	IC50 was not calculated due to lesser inhibition
	100	0.606	9.42	

Ethyl Acetate Extract	200	0.596	10.91	
	400	0.584	12.71	
	800	0.508	24.07	
	1600	0.455	31.99	
Ethanol Extract	50	0.506	12.02	258.7
	100	0.405	35.09	
	200	0.552	21.63	
	400	0.543	24.07	
	800	0.427	36.80	
	1600	0.319	45.78	

Table - 2: Alpha amylase inhibitory assay

	Conc. µg/ml	**OD at 590nm**	**% Inhibition**	**IC50 µg/ml**
Control	**0**	**0.71**	**0.00**	**0**
Acarbose	0.3125	0.65	8.99	2.15
	0.625	0.58	18.82	
	1.25	0.50	30.48	
	2.5	0.44	37.64	
	5	0.31	55.90	
	10	0.27	62.08	
Pet. Ether Extract	50	0.65	8.58	IC 50 as not calculated
	100	0.60	15.43	
	200	0.53	25.87	
	400	0.48	32.28	
	800	0.37	47.51	
	1600	0.25	80.46	
Ethyl Acetate Extract	50	0.70	1.69	IC 50 as not calculated
	100	0.68	5.11	
	200	0.63	10.96	
	400	0.61	14.04	
	800	0.63	12.50	
	1600	0.52	17.12	
Ethanol Extract	50	0.74	14.36	239.6
	100	0.81	34.54	
	200	0.66	50.18	
	400	0.51	61.34	
	800	0.40	62.83	
	1600	0.33	78.54	

Table - 3: Alpha Glucosidase Inhibition Assay

	Conc (µg/ml)	OD @ 405nm	% Inhibition	IC50 (µg/ml)
Control	0	0.996	0.00	0.00

Acarbose	0.3125	0.864	13.25	3.978
	0.625	0.748	24.90	
	1.25	0.665	33.23	
	2.5	0.504	49.40	
	5	0.474	52.41	
	10	0.197	80.22	
Pet. Ether Extract	50	0.849	14.76	IC 50 as not calculated
	100	0.839	15.76	
	200	0.834	16.27	
	400	0.827	16.97	
	800	0.745	25.20	
	1600	0.713	28.41	
Ethyl Acetate Extract	50	0.781	21.59	IC 50 as not calculated
	100	0.77	22.69	
	200	0.746	25.10	
	400	0.724	26.31	
	800	0.642	36.54	
	1600	0.686	41.19	
Ethanol Extract	50	0.899	31.28	328.2
	100	0.941	35.18	
	200	0.889	28.73	
	400	0.587	39.71	
	800	0.951	57.04	
	1600	0.894	60.98	

Table - 4: Effect of different *Anisomeles malabarica* leaf extracts on plasma glucose concentration in normal rats

Treatments	**Time (min) before and after glucose administration**					
	-30	**0**	**30**	**90**	**150**	**210**
Normal animals	72.3±2.3	79.6±2.1	183.2±2.6	146.4±2.8	95.8±7.8	84.2±6.4
Petroleum ether (200mg/kg)	79.5±1.2	72.5±6.3	172.3±4.4	111.3±1.4	104.5±3.2	92.3±2.2
Ethyl acetate (200mg/kg)	75.2±1.7	69.4±2.5	133.3±2.8	112.4±4.2	101.4±2.3	91.5±2.1
Ethanol (200mg/kg)	72.4±2.8	71.4±1.6	120.2±4.9	97.1±1.5	93.6±1.3*	82.5±1.8*
Acarbose (50mg/kg)	75.5±3.1	79.8±2.3	121.1±2.2	94.4±1.2	83.9±1.1*	75.3±3.4*

Data are expressed as mean±SEM. n = 6 rats per group. *$p < 0.05$, compared to normal control group.

Table-5: Effect of different *Anisomeles malabarica* leaf extracts on plasma glucose concentration in STZ induced diabetic rats

Treatments	**Time (min) before and after glucose administration**					
	-30	**0**	**30**	**90**	**150**	**210**

Diabetic animals	82.1±1.3	89.6±3.6	293.2±1.6	266.4±2.3	255.8±1.3	241.2±0.4
Petroleum ether (200mg/kg)	89.5±1.8	85.5±2.3	282.3±2.4	251.3±1.1	244.5±1.2	232.3±2.1
Ethyl acetate (200mg/kg)	86.2±1.1	89.4±1.2	283.3±2.1	252.4±2.2	241.4±2.1	229.5±1.1
Ethanol (200mg/kg)	82.1±2.4	84.4±2.5	250.2±1.9	237.1±1.8	213.6±1.3*	184.5±0.2*
Acarbose (50mg/kg)	85.7±1.1	89.7±1.3	231.1±3.2	224.4±1.9	204.9±1.1*	171.3±3.3*

Data are expressed as mean±SEM. n = 6 rats per group. *$p < 0.05$, compared to normal control group.

4. CONCLUSION

To investigate the *in-vitro* and *in-vivo* antidiabetic activities of the ethanol extract of *Anisomeles malabarica* has been analysed. As a result, we found that the extract of ETAM inhibitory activity against aldose reductase, α-amylase and α- glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus. Although the *in-vivo* antidiabetic effects of ETAM results indicate that has potential as a crude drug and a dietary health supplement. The plant showed significant anti diabetic activity, so the compound isolation, purification and characterization which are responsible for inhibiting activity, have to be done for the usage of antidiabetic agent.

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