ANTI-HYPERGLYCEMIC, A-GLUCOSIDASE INHIBITORY AND ANTIOXIDANT ACTIVITY OF ANTIDESMA SPECIES IN STREPTOZOTOCIN INDUCED DIABETIC RAT

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Abstract

This study aims to evaluate the oral therapeutic efficacy of the methanolic extract of three Antidesma species in type-2 diabetes for achieving normoglycemia and preventing complications such as retinopathy, nephropathy, neuropathy, and microangiopathy. The experiments were conducted in compliance with standard protocols. The methanolic extracts of *Antidesma* species were evaluated for their α-glucosidase inhibitory, antihyperglycemic, and antioxidant effects at different concentrations. The extract of *A. acidum* significantly reduced blood glucose levels at a dose of 50 mg/kg body weight (BW). *A. acidum* exhibited α-glucosidase inhibitory activity against various substrates in the order: starch (87.9%) > PNPG (55.2%) > maltose (51.2%) at a concentration of 200 µg/ml. All Antidesma species demonstrated notable antioxidant activity. The results confirm that *A. acidum* possesses strong antihyperglycemic and α-glucosidase inhibitory activity along with potent antioxidant potential. This study supports the potential of Antidesma species as a dietary supplement for diabetes management and encourages further clinical investigation.

Key words- Anti-Hypoglycemic, alpha glucosidase, *Antidesma sps.*, antidiabetic, antioxidant

1. Introduction

The term diabetes mellitus describes a metabolic disorder of multiple etiologies that is characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The evolution of numerous long-term complications of diabetes mellitus correlate with the severity and duration of hyperglycemia [Thevenod, 2008]. Due to increased protein glycation and the slow accumulation of AGEs in bodily tissues, hyperglycemia plays a significant role in the pathophysiology of diabetes complications. Various pharmacological compounds such as aminoguanidine, pyridoxamine and acarbose have been evaluated for their antiglycative and alpha glucosidase inhibitory abilities. However, despite their inhibitory capacity their side effects are a serious concern for their use in treatment of diabetic patients [Thornalley, 2003]. Compared to these synthetic compounds, natural products are much safer for human consumption. There has been an enormous interest in the development of alternative medicines for diabetes, specifically screening for phytochemicals with the ability to delay or prevent glucose absorption and responsible for the slow increase in blood glucose, as well as inhibition of non enzymatic protein glycation. Recent studies highlight the benefits of use of natural products in the treatment of diabetes, and plant extracts have been evaluated for their glycosidase inhibitory effects.

The species Antidesma acidum, Antidesma ghaesembilla, and Antidesma montanum (family Euphorbiaceae) have been traditionally used for medicinal purposes. This study evaluates the in vitro α-glucosidase inhibitory and antioxidant activities of these species, followed by in vivo assessment of their antihyperglycemic effects in streptozotocin-induced diabetic rats.

2. Materials and methods

2.1. Plant material Extraction

A. acidum, *A. ghaesembilla* and *A. montanum* were collected from different areas of Kolhapur district. The shade dried leaves of plant were chopped and pulverized into a fine powder separately. Plant powder (10%) was soaked in methanol and kept for 24 hour at room temperature in shaking condition. Centrifuge and filter the supernatant through Whatmans filter paper 1. Evaporate the solvents under reduced pressure by using rotary evaporator. This methanolic *A. acidum*, *A. ghaesembilla* and *A. montanum* extracts were used for further study and keep at -4°C until use.

2.2. Animals and Induction of diabetes

Male Wistar rats weighing between 190 and 200 g were kept in a lab setting with unlimited access to food (Amruth, Pune) and water. The Animal Ethical Committee of the Institute and CPCSEA criteria were followed for conducting the experiments. (Registration No. 233/CPCSEA) A single intraperitoneal injection of streptozotocin (70 mg/kg) in 0.1 M citrate buffer (pH 4.5) was used to cause diabetes. Rats that had fasting blood glucose levels more than 200 mg/dl after 14 days were classified as diabetic and included in the study.

2.3. Experimental design and Oral Glucose Tolerance Test (OGTT)

The animals were randomly divided into four groups with six rats in each group and treated as follows:
Group 1: Normal untreated rats.
Group 2: Streptozotocin induced diabetic control rats.
Group 3: Streptozotocin induced diabetic rats were administered orally with 30mg /kg body weight individual methanolic plant extract.
Group 4: Diabetic animals treated with Glibenclamide orally dose of 5 mg/kg body

Glucose tolerance test was determined with the method of Andrade *et al.* (2005). After plant extract administration, blood sample was collected from tail vein just earlier to 30 min., 60 min. and 120 min. The blood glucose level was measured using Accu-check Glucometer purchased from Roche, India. All the results presented were average values of experiments conducted on a set of six rats.

2.4 In vivo α-glucosidase inhibition activity of *Antidesma acidum (AaL)*, methanolic extract.

Streptozotocin induced diabetic rats was fasted for 16 hr before single administration of methanolic extract. All the experiments conducted on a set of six rats. After the 5 min of 50 µg/kg body weight *AaL* administration, glucose tolerance test was administered by feeding the rats with maltose (2 gm), starch (3 gm) and sucrose (4 gm) per kg of body weight. Blood was withdrawn from tail vein at defined time intervals and blood glucose measured using Accue-check (Accue check, Roche, India). All the results presented in average values of experiments conducted on a set of six rats.

2.5. *In vitro* α-glucosidase inhibition assay

Rat intestinal α-glucosidase was isolated by previously described method [Chougale et. al., 2009]. This isolated enzyme was used in the study to observe inhibitory activity of leaves extract of *A. acidum*. Rat intestinal alpha-glucosidase inhibitory activity of extracts was determined against 25mM maltose, 10mM PNPG and 1% starch as substrate [Patil et. al., 2011]. The percent inhibitory activity of extract was determined by using variable concentrations of plant extracts. (25, 50, 100, 200 and 400 µg/ml)) and IC_{50} value for each extract was calculated. All the analyses were completed in triplicate.

2.6. Antioxidant analysis

i. DPPH radical scavenging activity

DPPH radical scavenging activity of *Antidesma spp.* was evaluated by method Lee *et al*. (2003). The different concentrations of methanolic extract of leaves were allowed to react with 3 ml of DPPH solution. The mixture was shaken and allowed to stand in the dark for 30 min at room temperature. The absorbance of sample was measured on double beam spectrophotometer at 517 nm. Standard curve was prepared by plotting the percent (%) of free radical scavenging activity of ascorbic acid (0.1 mg/ml).

ii. Ferric reducing antioxidant power (FRAP)

Benzie and Strain (1996) method followed to analyze FRAP of plant extract. Different concentrations of plant extract were allowed to react with the FRAP reagent at 37°C for 15 min. The ascorbic acid equivalent antioxidant capacity was calculated by measuring absorbance at 593 nm.

3 Results

3.1. Oral Glucose Tolerance Test (OGTT)

The Antihypergycemic activity of *Antidesma acidum (AaL), A. ghaesembilla (AgL) and A. montanum (AmL) leaves* 30mg/kg body weight oral load of methanolic extract is studied preliminary by OGTT are display in the Fig.1a. The higher glucose level recorded at 30 min and it is decreased up to particular level in 120 min. As shown in figure, blood glucose level of normal control, diabetic control and *A. montanum* treated did not showed much difference, while *AaL* and *AgL* oral dose showed drastic reduction in blood glucose level in two hours. Among that the *AaL extract* show significant reduction in blood glucose level as compare to the other species. Hence AaL extract further evaluated for dose dependence OGTT in diabetic rats.

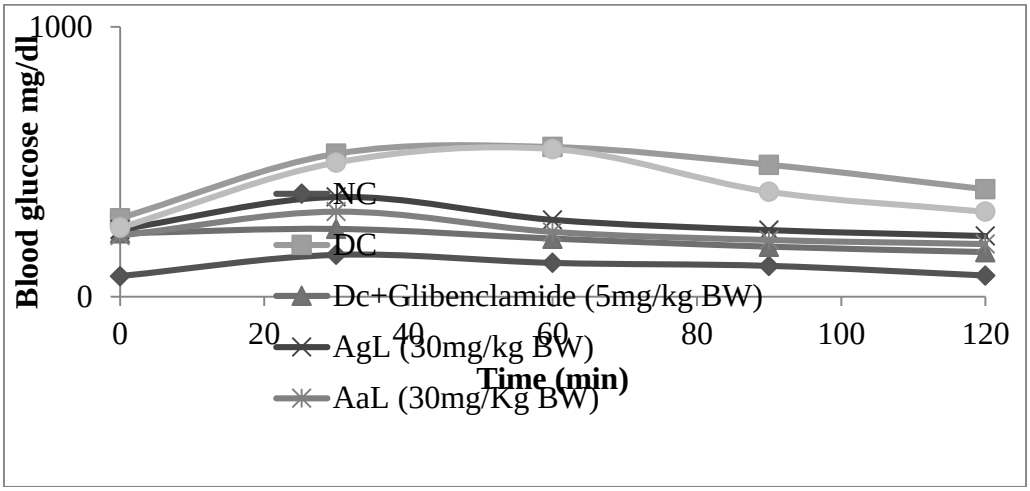

Fig 1a. Oral Glucose Tolerance Test (OGTT) of *Antidesma* species leaves methanolic extract. (NC- Normal control, DC- Diabetic control, Agl- *A. ghaesembilla* leaves, Aal- *A. acidum* leaves, and Aml- *A. montanum* leaves)

Further OGTT perform in various concentrations of *AaL extract* at 500 µg/kg body weight (BW), blood glucose decreased drastically and suddenly (Fig.2b). The *AaL extract* possesses significant hypoglycemic potential as it reversed the fasting blood sugar of diabetic rats to near normalcy. It is also found to be of similar effectiveness to Glibenclamide 5 mg, an existing oral hypoglycemic drug in terms of blood glucose reduction.

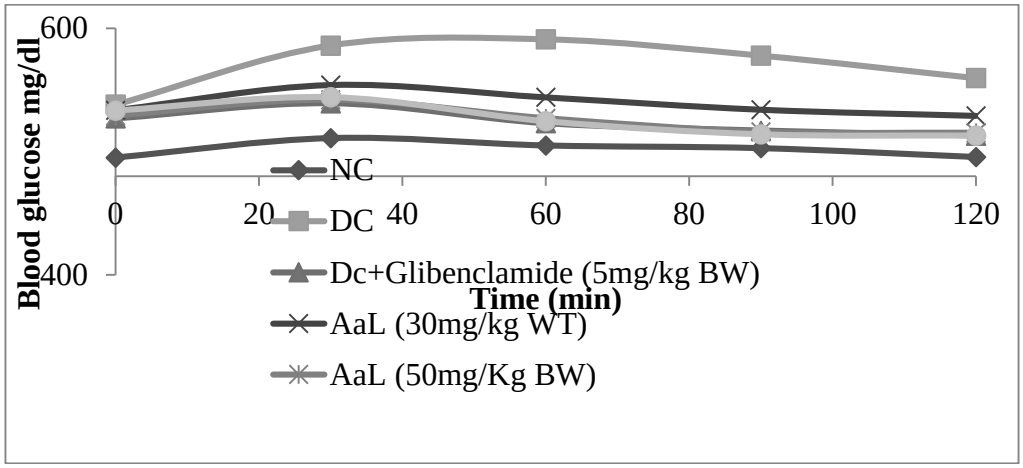

Fig. 1b. Oral Glucose Tolerance Test (OGTT) of different concentrations of *A. acidum* methanolic extract.

3.2 *In vivo* alpha glucosidase inhibition with Maltose and Sucrose

The glucose levels of the diabetic control group were significantly increased as compared to the non-diabetic control group. It can be observed that the methanolic extract shows a similar performance as acarbose the positive control on administration of maltose (Fig 2a). However, it should be noted that while acarbose is used at 60 mg / kg body weight the AaL methanolic extract has been used at a concentration of almost 120 times less i.e. 500 µg/Kg body weight of the experimental animals. There is a significant dampening in the glucose level at 16 min /dl almost to the extent of 150 mg as compared to the diabetic control curve demonstrating the potent action of the inhibitor

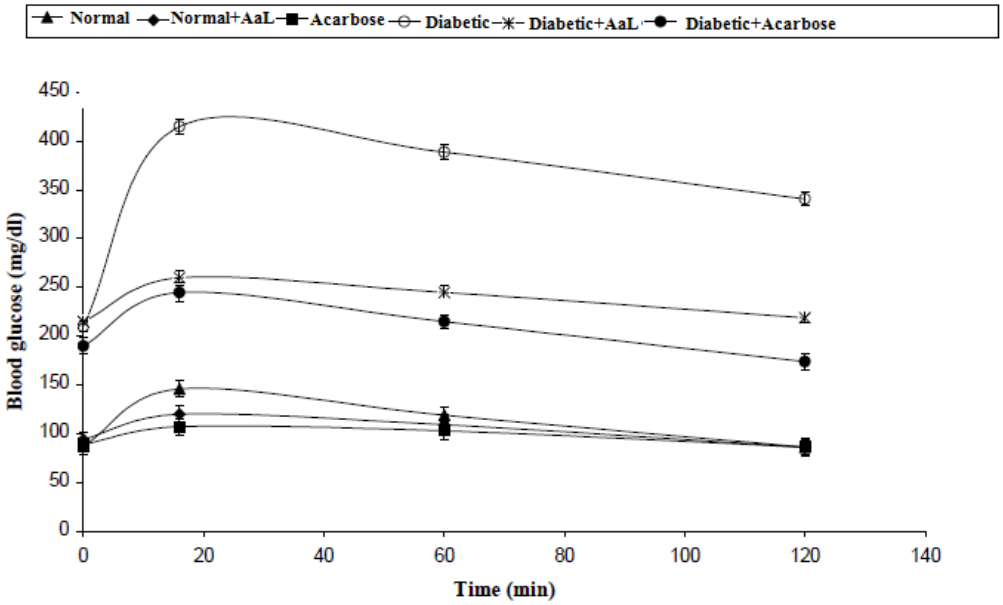

Figure 2a. Variations in levels of blood glucose in diabetic and normal rats after administration of maltose (2 mg/g) in presence of Acarbose 60 mg/Kg, or *AaL* 500 µg/Kg.

Figure 2b depicts the variation in the blood glucose values of diabetic and normal rats after administration of the sucrose with or without the AaL extract (500ug/kg). It can be observed that in case of the diabetic controls, the blood glucose level increased to 415 mg/dl by 16 minutes, it continues to rise to 389 mg/dl in one hour. As against this the diabetic rats with the extract show a decrease up to 295(16 min) to 274(1 hour) mg/dl in the same time period. Acarbose reveals a decrease by about 245 by the 16th minute it shows lesser difference by one hour (215mg/dl). It can be observed from the figure that acarbose is a better inhibitor of sucrose, while methanolic extract of AaL is a better inhibitor of maltase.

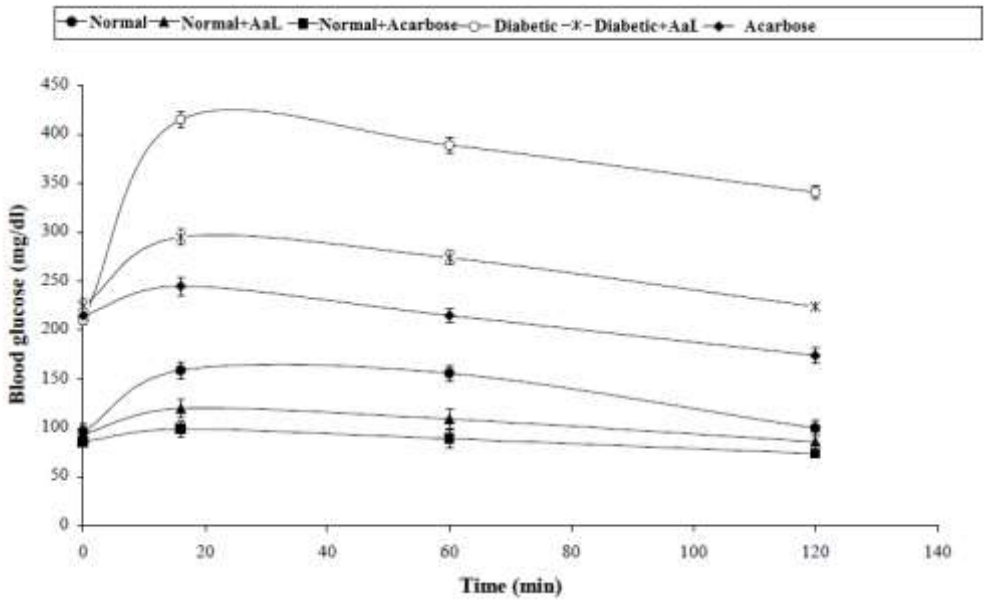

Figure 2b. Variations in levels of blood glucose in diabetic and normal rats after administration of Sucrose (4 mg/g) in presence of Acarbose 60 mg/Kg, or METH 500 µg/Kg.

3.3 In vitro alpha glucosidase inhibition

The *in vitro* α- glucosidase inhibitory activity of methanolic extracts of *A. acidum* leaves is shown in Table 1. Table shows that percent α-glucosidase inhibition increased with increasing concentration of the extracts. Acarbose, commercially used alpha-glucosidase inhibitor was used as a positive control. The IC_{50} value for acarbose was 40 µg/ml. At a concentration of 200 µg/ml, methanolic extract of *A. acidum* leaves showed significant α-glucosidase inhibitory activity. Methanolic extract shows inhibition against different substrates in sequences starch (87.9%) > PNPG (55.2%) > maltose (51.2%).

Table 1 α-glucosidase inhibitory activity of *A. acidum* extract against different substrates.

Concentration (µg)	Starch		Maltose		PNPG		Acarbose	
	% inhibition	IC_{50} µg	% inhibition	IC_{50} µg	% inhibition	IC_{50} µg	% inhibition	IC_{50} µg
25	36.5 ± 0.2		11.9 ±0.2		17.8 ±0.1		19.7 ±0.3	
50	57.6 ± 0.4		24.6 ±0.1		32.0 ±0.4		40.5 ±0.8	
100	78.7 ± 0.1	40	39.0 ±0.5	198	43.1 ±0.1	150	75.9 ±0.6	40
200	89.8 ± 0.6		50.9 ±0.1		53.3 ±0.5		100 ±0.1	
400	89.9 ± 0.1		51.9 ±0.4		54.3 ±0.3		100 ±0.2	

Each value expressed as means ± SD, (n=6), $p<0.05$.

3.4 Antioxidant activity

Figure 3a showing the results of DPPH and FRAP analysis of methanolic extract of *Antidesma* sps. leaves. The extracts of leaves exhibited a notable rise in radical scavenging activity as their concentration increased, and their inhibitory power was found to be comparable to that of ascorbic acid. Methanolic extract of *A. acidum* leaves with 40 µg/ml concentration shows 99.63 ± 0.01 % inhibition of DPPH whereas *A. ghaesembilla* and *A. montanum* shows 98.89 ± 0.01% inhibition.

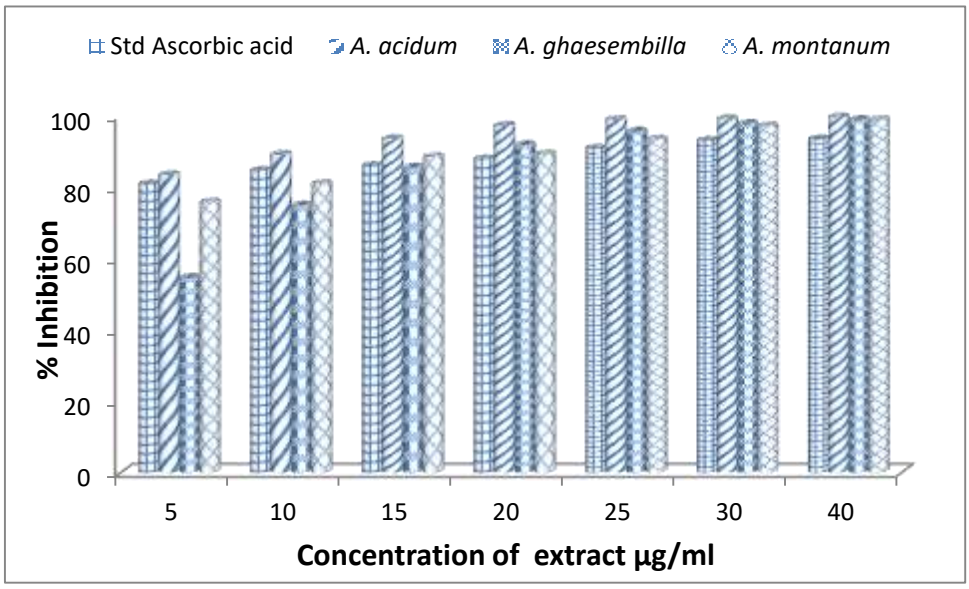

Figure 3a DPPH radical scavenging activity (% inhibition)

The ferric reducing antioxidant power (FRAP) of extract of leaves was represented in the Figure 3b. It was discovered that as extract concentration increased, so did its capacity to convert Fe3+ to Fe2+ ions. At 0.5 mg/ml of extract, the FRAP activity of *A. montanum* is 0.418 ± 0.051 mg AAE, *A. ghaesembilla* is 0.525 ± 0.004 mg AAE, and *A. acidum* leaves exhibit 0.513 ± 0.005 mg AAE.

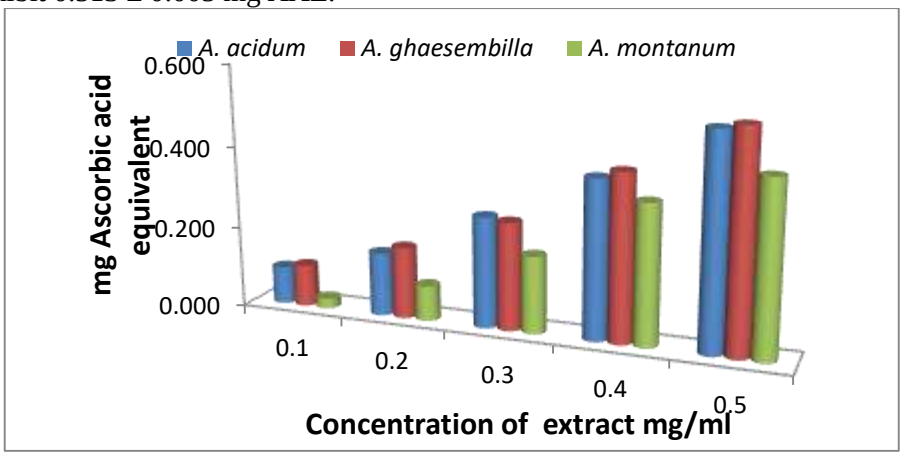

Figure 3b Ferric reducing antioxidant power (FRAP)

Preliminary OGTT and multi substrate OGTT evaluation suggested that the plant possesses good α-glucosidase inhibitory action which may contribute to its anti-hyperglycaemic effect. Glibenclamide is one of the oral antidiabetic drugs belonging to group of sulfonylurea, famous for their hypoglycaemic activity. In present study the plant distillate reduces blood glucose level by similar mechanism as Glibenclamide. Oral glucose tolerance test (OGTT) is one of the standard methods used to determine diabetes mellitus. Glibenclamide, *A. acidum* and *A. ghaesembilla* leaves extract enhanced the glucose tolerating efficiency of the rat as compared to the normal controls. While, this effect compared in both leaves extract with Glibenclamide, *A. acidum* showed comparable hypoglycaemic activity. To evaluate OGTT of natural and synthetic products is performed in experimental animal models after induction of diabetes by several methods. To induce non-insulin-dependent diabetes in animals, streptozotocin is commonly used which produces moderate hyperglycaemia having clinical symptoms similar to type 2 diabetes (Srinivasan and Ramarao, 2007). Streptozotocin causes alkylation of pancreatic deoxyribonucleic acid by entering to the β-cell via glucose transporter 2 and induces activation of poly (ADP ribosylation) that causes depletion of cellular nicotinamide adenine dinucleotide (NAD+) and adenosine triphosphate. As a result, the leaves extract after oral administration shows a very considerable reduction in blood glucose level in streptozotocin induced diabetic rat (Bantie and Gebeyehu, 2015).

Hydroalcoholic extract of *Emblica officinalis* Gaertn. significantly reduces the serum glucose level (Patel *et al*. 2013). Hypoglycemic activity of methanolic extract of *Antidesma ghaesembilla* Gaertn. leaves showed decreased mean glucose level 56.65% that is 132.75 ± 24.17 mg/dl, when administration of the crude extract at doses of 400 mg/kg BW for 21 days (Gargantiel and Ysrael, 2014). Present study reported that the *A. ghaesembilla* leaves distillate (30 mg/kg BW) reduces the blood glucose level up to 225 ± 3.01 mg/dl. Whereas 2000 mg/kg ethyl acetate extract of *Mallotus repandus* (Willd.) Muell. reduces blood glucose level up to 6.960 ± 0.254 mmol/L (Hasan *et al.* 2014). As compared to that ethanolic extract of *Tragia tennifolia* Benth. (53 mg kg^{-1}) reduces the 45% blood glucose level (Koffuor *et al.* 2011). Chromatographic fraction of *Jatropha gossypifolia* L. leaves extract in ethyl acetate shows hypoglycaemic activity (Granados *et al.* 2015). Methanolic extract of *Acalypha wilkesiana* Mull. Arg. root 400 mg/kg reduces 74.06% blood glucose level than the 200 mg/kg (67.81%) dose (Odoh *et al.* 2014). Whereas the *A. acidum* leaves distillate screened with different concentration shows that at 50 mg/kg BW reduces the blood glucose level up to 176 ± 3.04 mg/dl, is significant than the all these plants. Due to the shown efficacy of the leaves distillate of *A. acidum and A. ghaesembilla*, further studies on the plant's potential medical value is highly warranted.

Results show the effect of increasing concentration of the methanolic extract on alpha glucosidase inhibition. The extract has the potential to inhibit the enzyme almost linearly up to 200 µg/ml. Further increase in the concentration of the methanolic extract did not demonstrate any more inhibition. The intestinal α-glucosidase is a complex possessing both the sucrase and maltase enzyme. It has been observed earlier that some of the α-glucosidase inhibitors show preference for either of these activities, for example acarbose is better inhibitor of sucrose than maltase. In order to study the methanolic extract mechanism different substrates were studied. It can be seen that methanolic extract shows strong inhibitory action against 1% starch which was followed by PNPG and then maltose.

The 500 µl methanolic extract of *Macaranga tanarius* (L.) Mull. Arg. Leaves showed strong inhibitory activity against sucrase (67%) and maltase (62%) (Gunawan- Puteri and Kawabata, 2010). Present study revealed that 400 µg methanolic extract of *A. acidum* leaves shows activity by using maltose as substrate (52.2 ± 0.2%). The α-glucosidase inhibitory activity of the methanolic extract of *A. acidum* leaves was evaluated using p-nitrophenyl-α-D-glucopyranoside as a substrate and this was compared with acarbose. Extract shows IC_{50} values 200 µg/ml. Highest α-glucosidase inhibitory activity was found in fractions of *Antidesma bunius* L. ethyl acetate extract of stem bark and methanolic extract of leaves with IC_{50} values 5.73 and 8.04 ppm (Elya *et al.* 2012). Similar kinds of results in ethyl acetate extract were noted in *Antidesma celebicum* Miq. stem bark and leaves having IC_{50} values 8.06 and 57.60 µg/ml (Elya *et al.* 2013). Bothon *et al.* (2012) reported the ethanolic extract of *Bridelia ferruginea* Benth. stem bark shows α-glucosidase activity IC_{50} values 1.4 ± 0.04 µg / mL was higher than the acarbose. Aqueous ethanolic extract (80%) of *Antidesma bunius* (Linn.) Spreng. leaves exhibited α-glucosidase inhibitory activity IC_{50} values 34.77 ± 1.66 µg mL-1 (Lawag *et al.* 2012). Elya *et al.*, (2012a) reported the α-glucosidase inhibitory activity of ethanolic extract of some plants belonging to Euphorbiaceae with IC_{50} value, *Antidesma bunius* (L.) Spreng Folium 7.94 µg/ml, *Antidesma bunius* (L.) Spreng. Cortex 3.90 µg/ml, *Antidesma celebicum* Miq. Cortex 3.93 µg/ml, *Antidesma celebicum* Folium 2.34 µg/ml, *Antidesma montanum* (Blume) Folium 2.83 µg/ml, *Antidesma neurocarpum* Miq. Folium 4.22 µg/ml, *Blumeodendron toksbrai* (Blume.) Kurz. Cortex 22.82 µg/ml, *Blumeodendron toksbrai* (Blume.) Kurz. Folium 64.78 µg/ml, *Croton argyratus* Blume. Folium 366.07 µg/ml, *Cephalomappa malloticarpa* J. J. Sm. Cortex 12.22 µg/ml, *Cephalomappa malloticarpa* J. J. Sm. Folium 2.66 µg/ml, *Galearia filiformis* Blume. Folium 21.54 µg/ml, *Sumbaviopsis albicans* (Blume) J. J. Sm. Cortex 42.66 µg/ml, *Sumbaviopsis albicans* (Blume) J. J. Sm. Folium 43.40 µg/ml and *Suregada glomerulata* (Blume) Baill. Folium 57.46 µg/ml. Utami *et al.* (2015) analyzed the *Antidesma celebicum* leaves extract in ethyl acetate, fraction shows higher α-glucosidase inhibitory activity (IC50 value 57.60 µg/ml) than the other solvent extracts. However, it is lower than the above mentioned results. Arial part and flowers of *Euphorbia hebecarpa* Boiss. shows α- glucosidase inhibition in methanolic extract 9 ± 1% and in aqueous 4 ± 1% (Gholamhoseinian *et al.*, 2008). Screening of *Jatropha curcas* L. leaves exhibit α-glucosidase inhibitory activity (IC50 value 29.67 ppm) (Munim *et al.*, 2013).

α-Glucosidase inhibitors are able to reduce the sudden postprandial spikes in glucose levels which are damaging. Long term usage has shown benefits in cardiovascular outcomes. So here, *A. acidum* has firstly reported as alpha glucosidase inhibitory activity, which has potential to generate good lead molecules in control of diabetes. Since the time of immemorial, oral traditional medicinal plants have been under to treat patients with diabetes specially type II. In many countries of the world, was mentioned a good number of plants used for care diabetes and time of them have been evaluated experimentally and the active principle were isolated. The species of *Antidesma*, particularly *A. acidum* is showed good results. However, searching for new antidiabetic drugs form natural plants is still attractive because they contain substances, which make alternative and safe effect on diabetes.

A sample with high antioxidant activity has a low IC50 value. According to Gargantiel and Ysrael (2014), the IC50 value of DPPH was 89.7 ppm, and it was discovered that the extract of *A. ghaesembilla* leaves increased in decreasing power ability as concentration grew. Picot et al. (2014b) observed that the methanolic and aqueous extract of *A. madagascariensis* Lam leaves showed 296.33 ± 9.48% and 135.76 ± 3.04% inhibition of DPPH and 21.08 ± 0.77 mM mg -1 and 11.33 ± 0.26 mM mg -1 FRAP activity.

Mallotus philippensis Muell. leaves methanol, chloroform and aqueous extracts exhibit 60.1 ± 2.5 mg/g dry weight and 59.6 ± 3.2 mg/g dry weight reducing power; leaves also have 70.7 ± 3.7 mg/g dry weight and 39.9 ± 3.4 mg/g dry weight total antioxidant capacity; and 65 ± 4.0% and 68.0 ± 2.5% DPPH activity (Akhtar et al., 2015).

According to Keerthana et al. (2014), *Euphorbia heterophylla* L. exhibits an IC50 value of 141.11 ± 4.23µg/ml for antioxidant activities and a reducing power of 258.08 ± 3.21µg/ml for scavenging assays. The DPPH radical scavenging activity of several sections of *Macaranga tanarius* (L.) Mull. Arg. was investigated by Kumazawa et al. (2014). The leaves of the male and female plants showed 85.22 ± 2.44% and 74.65 ± 0.30% inhibition, respectively.

Conclusion

The present investigation could support to the evidence that *Antidesma acidum* and *A. ghaesembilla* have potential to decrease blood glucose level. These studies therefore could provide the biochemical rationale for the benefit of *Antidesma* based dietary supplement and the basis for further clinical study. It is suggested that leaves with high blood glucose lowering effect may be used for the development of pharmaceutical food to control the blood glucose level of diabetic patients.

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