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Evaluating the use of root extract of *Abroma augusta* as alpha glucosidase inhibitor for Type-II diabetes

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Abstract: The present study was designed to investigate α-d-glucosidase inhibitory activity of root extract of *Abroma augusta (A.augusta)*. Screening of different extracts (petroleum ether, benzene, chloroform, acetone, ethanol and aqueous extract) of *A.augusta* was carried out in streptozotocin-nicotinamide (STZ-NA) induced Type-II diabetic animals. The most active extract was evaluated for α-d-glucosidase inhibitory activity. α-d-glucosidase from *Saccharomyces cerevisiae* was used as *In-vitro* model to assess α-d-glucosidase inhibitory activity of plant extract. The results showed that petroleum ether extract exhibited the most significant ($p<0.0001$) effect in STZ-NA induced Type-II diabetes and appreciable dose dependent *In-vitro* α-d-glucosidase inhibitory activity with an IC_{50} value of 1150 µg/ml. These results provide that the root extracts of *A.augusta* might be a potential source of antidiabetic agent for the treatment of Type-II diabetes and its α-d-glucosidase inhibitory potential could be one of the possible mechanism for its antidiabetic effect.

Keywords: *Abroma augusta*, Type-II Diabetes mellitus, Alpha glucosidase

Introduction

Diabetes mellitus (DM) is a common disorder of carbohydrate, fat, and protein metabolism in which the circulating glucose concentration is increased and it causes complications such as retinopathy, microangiopathy, neuropathy and nephropathy. This is a chronic incurable condition due to insulin deficiency or lesser action or both that affect 10% of the population[1]. The world prevalence of diabetes among adults is expected to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% i.e., about 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries. However among the two major types of diabetes i.e., Type-I and Type-II, Type-II diabetes mellitus (T2DM) is the commonest form of diabetes constituting 90%- 95% of diabetic population. It is also documented that number of people diagnosed with T2DM globally is estimated to be at 2%-3% of the world population and is rising at a rate of 4%-5% per year [2]. Type-II diabetes is caused by the failure of beta cells to compensate for insulin resistance. This leads to hyperglycaemia, which can in turn exert deleterious effects on β cells. Chances of developing T2DM are increased by obesity and physical inactivity and are augmented further with age. Two most important unmet needs associated with the management of T2DM are the lack of lasting efficacy in reducing hyperglycemia and failure to target primary causes. Different classes of Oral Hypoglycemic Agents (OHA's) with nearly equipotent efficacy are now available targeting the different pathophysiologic factors contributing to T2DM; however, almost all of them are associated with one or the other kind of adverse effect. Several studies have found that certain antidiabetic drugs may carry increased cardiovascular (CV) risks compared to others [3]. One of the therapeutic approach to treat Type-II diabetes is by inhibition of carbohydrate-digesting enzymes. α-Glucosidase inhibitors (AGIs) are among the available glucose-lowering medications [4]. Alpha-glucosidase is a key enzyme in carbohydrate digestion. It catalyzes the hydrolysis of 1,4-α-glucosidic bonds within carbohydrates with release of α-glucose and promotes the increase of blood glucose levels after meal. Alpha-glucosidase inhibitors antagonize the activity of α-glucosidase, thereby delaying intestinal carbohydrate absorption and slowing the sharp rise in blood sugar levels that diabetic patients typically experience after meals [5]. α-glucosidase inhibitors, such as acarbose and miglitol, have been approved for clinical use in the management of Type-II diabetes,

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as well as the treatment of diabetic complications. A main drawback of the currently used α-glucosidase inhibitors, such as acarbose, involves side effects such as abdominal distention, flatulence, meteorism and possible diarrhea [6].

Evidence for the importance of plant extracts in the management of Type-II diabetes is emerging [7]. There has been a search for other effective and safe α-glucosidase inhibitors from natural materials in order to develop a physiological functional food or lead compounds for diabetes treatment [6].

Sterptozotocin-nicotinamide is a method currently used to induce diabetes in animals that resemble non obese T2DM in man. The induction of diabetic rats with STZ increases the production of free radicals that damage the pancreatic DNA and thus affects insulin secretion. This is achieved by depleting nicotinamide (NAD) which is a substrate of poly ADP ribose synthetase, an enzyme which involved in DNA repair. Pre-treatment of experimental animals with NAD allows minor damage to pancreatic beta cell [8].

Abroma augusta Linn (Sterculiaceae), commonly known as Ulatkambal (Bengali and Hindi) is a large spreading bushy shrub with fibrous bark and irritant hairs, widely distributed (native or cultivated) throughout the hotter part of India- U.P, Sikkim (3000 ft.), Khasia hills (4000 ft.) and Assam. The fresh viscid sap of the root bark is considered to be a valuable emmenagogue and uterine tonic. It is also used in dysmenorrhoea [9]. Some preliminary studies reported antidiabetic activity of aqueous extract of dried root bark of *Abroma augusta* L. diabetic rats [10, 11]. Nahar et al. reported that the methanolic extract of leaves of *Abroma augusta* L. significantly reduced the blood glucose level in alloxan-induced diabetic rats when administered at a dose of 300 mg/kg/da. [12]. The aim of present investigation is to screen the effect of various extracts of roots of *Abroma augusta* in Type-II diabetic animals and to evaluate the α-d-glucosidase glucosidase inhibitory potential of most active extract to contribute to the understanding of its mechanism of action in Type-II diabetes.

Animals

Albino Wistar rats of either sex, weighing (150-200 g), were obtained from Animal House, Shri Guru Ram Rai Institute of Technology and Science, Dehradun (Uttarakhand). The animals were kept under standard conditions of 12:12 h light and dark cycle in polypropylene cages and fed with standard laboratory diet and water *ad libitum*. The animals were acclimatized to laboratory condition for seven days before commencement of experiment. The study was approved by Institutional Animal Ethical Committee (IAEC), Shri Guru Ram Rai Institute of Technology and Science, Dehradun (Uttarakhand). (Regd. No. 264/CPCSEA)

Chemicals

Streptozotocin was purchased from Sigma-Aldrich (St Louis, MO, USA). Standard drug, glimepiride was obtained as a gift sample from Ranbaxy lab, Poanta Sahib, (H.P.), India. 4-Nitrophenyl-α-d-glucopyranoside, α-d-glucosidase from *Saccharomyces cerevisiae* (Sigma–Aldrich, Milwaukee, USA), acarbose was gifted by Biocon, Ltd., Bangalore, India. All the chemicals used in the experiment were of analytical grade and obtained from Himedia laboratories Pvt. Ltd. Mumbai, India.

Preparation of plant extract

Fresh root parts of *A.augusta* were collected from local herbal garden of Dehradun (Uttarakhand). The plant was taxonomically identified and authenticated at the Forest Research Institute (FRI), Dehradun. A voucher specimen (No. 157029) was deposited in the Botany division of FRI, Dehradun. Fresh root parts of *A. augusta* were washed and shade dried than coarsely powdered in a grinder. Powder dried plant were extracted with solvents of increasing polarity (petroleum ether, benzene, chloroform, acetone and Ethanol) by the method of continuous hot extraction. Aqueous extract was prepared by the process of maceration. Each extract were concentrated, dried *in vacuo* and the residue stored in a desiccators for further use.

Experimental induction of diabetes [13].

Type-2 diabetes mellitus or Non-insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single intraperitoneal injection of STZ (40 mg/kg b.w.), 15 min later; the rats were

given the intraperitoneal administration of nicotinamide (NAD) (110 mg/kg b.w.). STZ was dissolved in citrate buffer (pH 4.5) and NAD was dissolved in normal physiological saline. During the first 24 h of diabetes induction, STZ-treated animals were allowed to drink 5% glucose solution to overcome drug-induced hypoglycemia. Hyperglycemia was confirmed by the elevated blood glucose levels determined at 72 hrs, then on day 7 and 14 after injection. Animals showing fasting blood glucose higher than 230 mg/dl were considered as diabetic and used for the further study.

Acute toxicity test [14]

Acute oral toxicity study for the test extracts of the plant was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity. Healthy albino Wistar rats (200–250 g) were used for this study. Animals were fasted (food but not water was withheld overnight) prior to dosing. The fasted body weight of each animal was determined, and the dose was calculated according to the body weight.

Limit test at 2000 mg/kg

The drug was administered in the dose of 2000 mg/kg body weight orally to one animal. A total of six animals were tested. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 days. No animal died. Therefore, the LD_{50} is greater than 2000 mg/kg.

An investigation with $1/20^{th}$, $1/10^{th}$, and $1/5^{th}$ of 2000 mg/ kg, i.e. 100, 200, and 400 mg was done in pre-screening. For all the extracts, 250 mg/kg (slightly more than 200 mg/kg) was found to be effective against diabetes but with different efficacy, hence this dose was used for all the extracts in final screening.

Experimental design

Animal were divided into nine groups having six rats in each group and all groups of animal were received treatment for 21 days. Group- 1: Normal control, Group-2: Diabetic control, Group-3: Diabetic animal + glimepiride (10 mg/kg); Group-4: Diabetic animal + petroleum ether extract of *A.augusta* (AA); Group-5: Diabetic animal + benzene extract of AA; Group-6: Diabetic animal + chloroform extract of AA; Group-7: Diabetic animal + acetone extract of AA; Group-8: Diabetic animal + ethanolic extract of AA; Group-9: Diabetic animal + aqueous extract of AA.

Collection of blood sample

Blood was collected from retro-orbital plexuses and serum samples were analyzed for blood glucose and lipid profile. Fasting blood glucose measurements was done on 1^{st}, 7^{th}, 14^{th}, 21^{st} day of the study by GOD-POD method.

In-vitro α-d-glucosidase inhibitory activity of plant extract

α-D-Glucosidase (from S. cerevisiae) inhibitory activity:

The α-d-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenyl α-d-glucopyranoside (4-NPGP) according to S. Bisht et al., with slight modification [15]. The assay media contained 0.1 M phosphate buffer of pH 6.9, 1 mM 4-NPGP (3.8 ml) as a substrate, 1 U/ml α-d-glucosidase from *S. cerevisiae* (4 µl) and plant extract (4 µl) in the range of 500-2000 µg/ml and standard (acarbose, 4 µl) drug in range of 1–100 mM. The reaction was initiated by addition of 4-NPGP at 37 ◦C for 20 min and terminated by addition of 1 M sodium carbonate (400 µl). Enzyme activity was quantified by measuring absorbance at 405 nm in triplicate. One unit of α-d -glucosidase activity was defined as amount of enzyme liberating 4-nitrophenyl (1.0 µM) per min. The α-glucosidase inhibitory activity (%) was calculated as follows:

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{extract}}{Abs_{control}} \times 100$$

The IC_{50} value was defined as the concentration of α-d-glucosidase inhibitor that inhibits 50% of α-d-glucosidase activity and were calculated by graphical method.

Statistical Analysis

Blood glucose levels of different groups were analyzed by using two way ANOVA followed by tukey's multiple comparison as post-hoc test. The limit of statistical significance was set at $P<0.05$. All

the results were expressed as the mean ± Standard error mean (SEM).

Results

Acute toxicity studies

Acute toxicity studies conducted revealed that the administration of all the root extract (up to a dose of 2000 mg/kg) of *A.augusta* did not produce significant changes in behavior of the animals. No death was observed up to the dose of 2000 mg/kg b.w. The rats were physically active. These effects were observed during the experimental period (14 days). The results showed that in single dose the plant extracts had no adverse effect, indicating that the medium lethal dose (LD_{50}) could be greater than 2000 mg/kg body weight in rats. In acute toxicity study, no toxic symptoms were observed up to dose of 2 g/kg body weight. All animals behaved normally. No neurological or behavioral effects could be noted. No mortality was found up to 14 days study.

Effect A. augusta root extracts in STZ-NA induced T2DM

The study demonstrated the fasting plasma glucose (FPG) values, before and after treatment for 21 days in normal, diabetic untreated and diabetic treated with standard drug glimepiride (10 mg/kg b.w.) and petroleum ether extract, benzene extract, chloroform extract, acetone extract, ethanol extract and aqueous extract (250 mg/kg b.w.) The fasting glucose (FPG) values remained more or less the same in normal group i.e. 92.83± 4.045 before and 112.0±3.416 mg/dl after 21 days. However after treatment with 10 mg/kg b.w. of glimepiride and 250 mg/kg b.w. of petroleum ether extract, benzene extract, chloroform extract, acetone extract, ethanol extract and aqueous extract of *A. augusta* showed high initial FPG values (213.5±9.946 and 210.50±10.23, 271.00±7.78, 270.70± 6.908, 273.90± 7.32, 268.18± 6.87 and 265.59± 7.773) which came back close to normal level (106.83±4.324 and 109.50±5.59, 259.96±6.99, 260.83± 6.356, 259.64± 6.356, 198.43± 3.129 and 192.47± 9.98 respectively) after 21 days. Petroleum ether extract of *A.augusta* demonstrated most significant ($p<0.0001$) antidiabetic effect as it started to exhibit significant effect ($p< 0.0001$) from day 7 of treatment. However, aqueous and ethanol extracts started to show significant effect ($p<0.001$ and 0.01 respectively) from day 14 of treatment (Table 1).

Table 1: Effect of treatment for 21 days with root extracts of *A. augusta* on fasting plasma glucose level in type -2 diabetic animals

	Fasting Plasma Glucose (mg/dl) mean ± SEM			
Groups	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Normal control	92.83± 4.045	95.67±4.364	105.83±3.911	112.0±3.416
Diabetic control	231.66± 8.724^{a*}	291 ± 5.426 ^{a*}	298.33 ± 8.720 ^{a*}	305 ± 13.784 ^{a*}
Diabetic + Glimepride	213.5±9.946	182.67±7.632 ^{b*}	145.67±6.702 ^{b*}	106.83±4.324 ^{b*}
Diabetic +Pet. Ether extract	210.50±10.23	185.20±7.35 ^{b*}	140.11±7.98 ^{b*}	109.50±5.59 ^{b*}
Diabetic +Benzene extract	271.00±7.78	268.89±6.21	263.34±6.67 ^{b‡}	259.96±6.99 $^{b≠}$
Diabetic + Chloroform extract	270.70± 6.908	267.87±6.79	262.77± 5.367 ^{b‡}	260.83± 6.356 ^{b†}
Diabetic +Acetone extract	273.90± 7.32	268.59 ±7.256	262.20± 8.934 ^{b‡}	259.64± 6.356 $^{b≠}$
Diabetic + Ethanolic extract	268.18± 6.87	263.53± 5.98	259.98± 6.952 ^{b†}	198.43± 3.129 ^{b*}
Diabetic + Aqueous extract	265.59± 7.773	259.56± 8.65	251.61± 12.28 $^{b≠}$	192.47± 9.98 ^{b*}

Fasting blood glucose values (mg/dl) are the mean ± SEM of six rats; ^{a} significant compared with normal control group and ^{b} significant compared with diabetic control group, *= $p<0.0001$, $^{≠}$ = $p< 0.001$, †= $p< 0.01$, ‡= $p<0.05$

α-D-Glucosidase inhibitory activity of acarbose and petroleum ether extract and A.augusta:

α-d-glucosidase inhibitory activity (IC_{50}) of acarbose was found to be 4 mM and the maximum percentage inhibition exhibited by acarbose was found to be at 25 mM (75.63%) **(Figure 1)**. Percentage inhibition demonstrated by various concentrations (500, 1000, 1500 and 2000 µg/ml) of plant extract were found to be 31.398%, 44.187%, 58.259%, 64.817% **(Figure 2)**. The results indicated the dose dependent inhibition of alpha glucosidase enzyme by the extract with an IC_{50} value of 1150 µg/ml. The *In-vitro* alpha glucosidase activity confirmed the results observed in animal model.

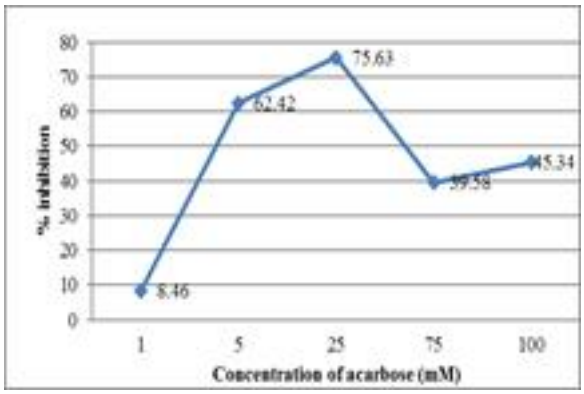

Figure 1: α-D glucosidase inhibitory activity of acarbose

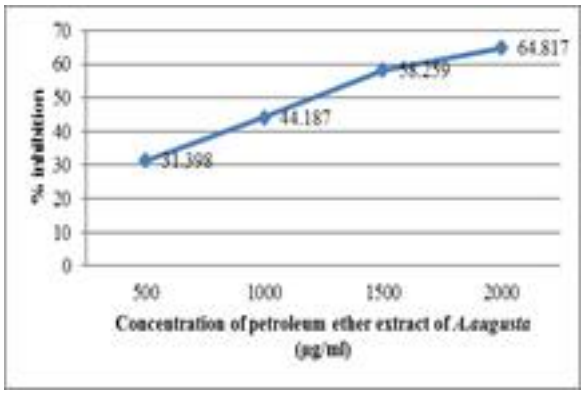

Figure 2: α-D-glucosidase inhibitory activity of petroleum ether extract of *A.augusta*

Discussion

Various pharmacological approaches have been used to improve diabetes via different modes of action including stimulation of insulin release, inhibition of gluconeogenesis, increasing the number of glucose transporters and reduction of glucose absorption from the intestine. Inhibition of carbohydrate metabolizing enzyme such as α-d-glucosidase is one of the important antidiabetic therapy to impair glucose absorption. Inhibitors of α-d-glucosidase delay carbohydrate digestion and prolongs the overall time for carbohydrate digestion, resulting in a decrease in the rate of glucose absorption thus causes reduction in postprandial hyperglycemia. It has been established that reduction of postprandial hyperglycemia contributes to decrease in hemoglobin A1C (HbA1C) in diabetic patients, consequently, reduces the appearance of chronic vascular complications [16,17]. The retardation and delay of carbohydrate absorption with a plant-based α-glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus and borderline patients [18].

The aim of the present investigation was to establish the α-d-glucosidase inhibitory potential of *A. augusta.* Initially we screened various extracts of root of *A.augusta* for their effect on STZ-NA induced Type-II diabetes. The results indicated that among all the extracts the most significant effect ($p<0.0001$) was exhibited by petroleum ether extract as it started to show the best significant effect from day 7 of treatment with root extract *A. augusta.* Further the most active extract (petroleum ether extract) was examined for their α-d-glucosidase inhibitory activity. Petroleum ether extract demonstrated appreciable α-d-glucosidase inhibitory activity with an IC_{50} value of 1150 µg/ml. The results of the study indicated that the reduction of blood glucose level in Type-II diabetic animals could be due to the inhibition of intestinal glucosidase(s).

Furthermore nature of some compounds present in the root extracts (steroids such as stigmasterol and *β*-sitosterol)[19] are in accordance with those reported by Kumar S., et al., as being effective in STZ-NA induced Type-II diabetes and potential inhibitors of α-d-glucosidase(s) [20].

Conclusion

The results of the study provide scientific support for the use of *A.augusta* in the traditional system of medicine for the treatment of Type-II diabetes. The study further supports the traditional use of plants based on their inhibitory activity of glucose absorption in the gut.

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References

1. Kumar S, Sharma S, Vasudeva N, Ranga V, *In vivo* anti-hyperglycemic and antioxidant potentials of ethanolic extract from *Tecomella undulata*, Diabetol Metab Syndr, 2012, 4, 33.

2. Kumar R, Pate DK, Prasad SK, Sairam K, Hemalatha S, Antidiabetic activity of alcoholic leaves extract of *Alangium lamarckii* Thwaites on streptozotocin-nicotinamide induced type 2 diabetic rats, Asian Pacific Journal of Tropical Medicine, 2011, 904-909.

3. Kalra S, Emerging Role of Dipeptidyl Peptidase-IV (DPP-4) Inhibitor vildagliptin in the management of type 2 diabetes, JAPI, 2011, 59, 237-245.

4. Adolfo AC, Jaime BJ, Ren´e C´ardenas-V´azquez, Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes, J Ethnopharmacol, 2008, 116, 27–32.

5. Wu C, Shen J, He P, Chen Y, Li L, Zhang L, et. al., The α-glucosidase inhibiting isoflavones isolated from *Belamcanda chinensis* leaf extract, Rec Nat Prod, 2012, 6(2), 110-120.

6. Dong L, Jing Man N, Preliminary study of an α-glucosidase inhibitor from the roots and stems of *Polygonatum sibiricum* Red, Asian Journal of Traditional Medicine, 2008, 3(5), 179-185.

7. Pierre W, Jeugo A, Gildas H, Ulrich MC, Modeste WN, et al., Hypoglycemic and hypolipidemic effects of *Bersama engleriana* leaves in nicotinamide/streptozotocin-induced type 2 diabetic rats, BMC Complem Altern M, 2012, 12, 264.

8. Oyedemi SO, Bradley G, Afolayan AJ, Beneficial effect of aqueous stem bark extracts of *Strychnos henningsii* gilg in streptozotocin-nicotinamide type-2 diabetic wistar rats, Int J Pharmacol, 2011, 7(7), 773-781.

9. KR Kirtikar, BD Basu. Indian Medicinal Plants. Sudhindra Nath Basu, Bahadur Ganj, Allahabad; 1918. p. 210-211.

10. Eshrat MH, Effect of *Coccinia indica* (L.) and *Abroma augusta* (L.) on glycemia, lipid profile and on indicators of end organ damage in streptozotocin induced diabetic rats, Indian J Clin Biochem, 2003, 18(2), 54-63.

11. Ali Hussain HEM, Hypoglycemic, hypolipidemic and antioxidant properties of combination of *Curcumin* from *Curcuma longa*, Linn, and partially purified product from *Abroma augusta*, Linn. in streptozotocin induced diabetes, Indian J Clin Biochem, 2002, 17(2), 33–43.

12. Nahar L, Ripa FA, Zulfiker AHM, Rokonuzzaman M, Haque M, Islam KMS, Comparative study of anti-diabetic effect of *Abroma augusta* and *Syzygium cumuni* on alloxan-induced diabetic rat, Agr Biol J N Am, 2010, 1(6), 1268–1272.

13. Ananda PK, Kumarappan CT, Christudas S, Kalaichelvan VK, Effect of Biophytum sensitivum on streptozotocin and nicotinamide-induced diabetic rats, Asian Pacific Journal of Tropical Biomedicine, 2012, 31-35.

14. OECD guidelines for the testing of chemicals (Acute oral toxicity – up and down procedure). Available from: http://www.oecd.org

15. Bisht S, Ravi Kant, Kumar V, α-D-Glucosidase inhibitory activity of polysaccharide isolated from *Acacia tortilis* gum exudate, Int J Biol Macromol, 2013, 59, 214–220.

16. Misbah H, Aziz AA, Aminudin N. Antidiabetic and antioxidant properties of *Ficus deltoidea* fruit extracts and fractions, BMC Complem Altern M, 2013, 13:118.

17. Adisakwattana S, Chanathong B, α-glucosidase inhibitory activity and lipid-lowering mechanisms of *Moringa oleifera* leaf extract, Eur Rev Med Pharmacol Sci, 2011, 15, 803-808.

18. Subramanian R, Asmawi MZ, Sadikun A, *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide, Acta Biochim Pol, 2008, 55(2), 391–398.

19. Gupta B, Nayak S, Solank S, Abroma *augusta* Linn: A review. *Der Pharmacia Sinica,* 2011, 2(4), 253-261.

20. Kumar S, Kumar V, Prakas O. Enzymes inhibition and antidiabetic effect of isolated constituents from *Dillenia indica,* BioMed Res Int, 2013, 382063.

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