Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue S-2 Year 2024 Page 406-412

A Study On Evaluation Of Antidiabetic Properties Of Andrographis Paniculata

Suchitra G[1], Keshamma E[2*]

[1]*Associate Professor, Department of Zoology, Government science college (Autonomous), Hassan, Karnataka, India*
[2*]*Associate Professor, Department of Biochemistry, Maharani Cluster University, Palace Road, Bangalore, Karnataka, India*

***Corresponding Author:** Keshamma E*
**Associate Professor, Department of Biochemistry, Maharani Cluster University, Palace Road, Bangalore-560 001 Karnataka, India Email: keshamma.blr76@gmail.com*

Abstract

Diabetes mellitus is a multifactorial, unavoidable metabolic disease that impairs insulin function or secretion, or both, and causes hyperglycemia. Acarbose and voglibose are still used today as an inhibitor of the enzymes that break down carbohydrates, either by themselves or in conjunction with insulin. However, there have been reports of negative side effects from these compounds, including diarrhea, flatulence, abdominal fullness, and liver disorders.In the present study we aimed to screen for antidiabetic activities of methanol extract of *Andrographis paniculata*through *in-vitro* evaluation of inhibition of carbohydrate hydrolyzing enzymes such as, alpha-amylase and alpha- glucosidase. Results depicted that methanolic leaf extract of *A. paniculata* at a concentration range of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml, shown inhibition effect of 41.57%, 53.48%, 62.28%, 73.87%, and 88.34% respectively with an IC_{50} value of 56.24 mg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 25.38 mg/ml. Similarly, in an alpha-glucosidase inhibition assay with methanolic leaf extract of *A. paniculata*at a concentration range of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml, shown inhibition effect of 18.61%, 29.69%, 34.41%, 49.40%, and 61.82% respectively with an IC_{50} value of 32.38 mg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 21.89 mg/ml. Furthermore, the phytochemical analysis revealed that total phenolic quantity was found to be highest (54.44 mgGAE/gextract) in methanolic leaf extract of *A. paniculata* when compared with total flavonoid quantities (0.41 mg QE/g extract). In conclusion, *A. paniculata* possess anti-diabetic properties in methanol extracts. Hence, *A. paniculata*could be explored in development of natural antidiabetic formulations.

Keywords: Andrographis paniculata, Methanol, Leaf extract, Antidiabetic, Alpha-amylase, Alpha-glucosidase

Introduction

Medicinal plants have been integral to man's health and healing since the dawn of human civilization. Despite of significant advancements made in allopathic medicines during the 20th century, both the ancient and contemporary systems of medicine continue to rely heavily on plants as a source of medications. The

majority of the world's populations, who reside in underdeveloped nations, rely on traditional medicine and herbal remedies to meet their primary healthcare needs.[1,2]

Diabetes is an important human ailment afflicting many from various walks of life in different countries. Diabetes mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein. The world health organization has estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030.[3] Allopathic drugs used for the treatment of diabetes have their own side effect and adverse effects like hypoglycaemia, nausea, vomiting, hyponatremia, flatulence, diarrhoea or constipation, alcohol flush, headache, weight gain, lactic acidosis, pernicious anaemia, dyspepsia, dizziness, and joint pain.[4]So instead of allopathic drugs, herbal drugs are a great choice which is having more or less no side effect and adverse effects.[5]

According to reports, up to 8000 plants can be used in the Ayurvedic, Homeopathic, Siddha, Unani, and Tibetan medical systems.[1,2] One of them is Andrographis paniculata commonly known as 'King of Bitter'. It generally grows abundantly in Southeast Asia, Sri Lanka, and India. It can increase in height to a maximum of one meter, with small, hairy white to pink blooms and lanceolate leaves (Figure 1A and 1B). The leaf is the primary therapeutic component, while the entire plant, including the root, is used for a variety of illnesses. In India, it grows in the rainy season, when heat and humidity with lots of sunshine are the ideal climate conditions for the plant. In India, both tribal and traditional medicine systems make considerable use of *A. paniculata* as a home treatment for various illnesses. The most utilized component of *A. paniculata*is the aerial part, and its major bioactive constituents are diterpenoids, flavonoids, and xanthones. However, in Asia and Europe, whole plants and leaves are utilized as folk remedies for a variety of illnesses.[6-9]

Figure 1A: Showing *A. paniculata*whole plant

Figure 1B: Showing *A. paniculata* plant leaves

*A. paniculata*has been reported to have numerous medicinal properties.[10] Moreover, in Compendium of Medicinal and Aromatic Plants, *A. paniculata*is reported as a leading medicinal plant in treating diabetes, high blood pressure and various other ailments, traditionally.[11] The lack of perfect models for type II diabetes, coupled with financial restrictions on obtaining and maintaining animals, and social restrictions on extensive use of animals in experimentation, indicate that a more practical approach would involve a series of *in-vitro* prescreens before testing a potential new hypoglycaemic agent in animals. The advantages of *in-vitro* models include: (i) Rapid large-scale screening of drug candidates allow promising substances to be identified at an early stage of the drug development process, (ii) The time and costs involved in developing active agents are significantly reduced, and (iii) Cost-intensive and ethically controversial animal experiments can be reduced to a minimum. With these viewpoints, the present study was conducted with the main objective to screen for antidiabetic activities of *A. paniculata*extract by *in-vitro* methodsusingalpha-amylase and alpha-glucosidase inhibition assays.

Materials and Methods

Collection of *A. paniculata*plants

The *A. paniculata* plants were collected from Chikkaballapura district, Karnataka, India. The leaves were separated, and were sprayed with ethanol, and then shade dried at room temperature. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *A. paniculata*were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of methanol. All the extracts were

concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.[12]

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the methanolic leaf extract of *A. paniculata* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[13]The phenolic content of the extract was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water and was expressed in mg gallic acid equivalent (GAE)/g of extract powder (mg GAE/g).

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in methanolic leaf extract of *A. paniculata*.[14]The flavonoid content was determined from extrapolation of calibration curve which was made by preparing quercetin solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg quercetin equivalent (QE)/g of extract powder (mg QE/g).

Alpha-amylase inhibitory assay

The alpha-amylase inhibition assay of methanolic leaf extract of *A. paniculata*was carried out by the method of Miller, (1959).[15]Methanolic leaf extract of *A. paniculata*and standard drug acarbose (20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml) were incubated for 10 minutes at 25°C with 500 µl of 20 mM sodium phosphate buffer (pH 6.8) with 20 µl of amylase (1U/ml). After pre-incubation, each tube was added with 1 ml of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) and incubated for 15 min. One ml DNS was added to arrest the reaction. After that, the tubes were kept in a boiling water bath for 5 min and cooled to room temperature. After that, distilled water (10ml) was added to the reaction mixture, and the absorbance was measured at 540 nm. The test compound was not used in the preparation ofthe control samples. The following formula was used to determine the percent inhibition of alpha-amylase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

Alpha-glucosidase inhibition assay

The alpha-glucosidase inhibition assay of methanolic leaf extract of *A. paniculata*was carried out as described by Matsui et al (1996) with slight modifications.[16]The different concentrations of methanolic leaf extract of *A. paniculata*and standard drug acarbose (20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml) were prepared. Phosphate buffer (1 ml; 100mM, pH 6.8) and 80 µl of test methanolic leaf extract of *A. paniculata*/ acarbose of concentrations (20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml) were added to 20 µl of alpha-glucosidase and incubated at 37°C for 10 minutes. Later, pNPG- 50µl (5mM) was added to the assay mixture to initiate the reaction. Then, the reaction mixture was incubated at room temperature for one hour and arrested the reaction by adding 2.5ml of 0.1 M Na_2CO_3. The absorbance was measured at 400nm to determine the activity of alpha-glucosidase activity.The following formula was used to determine the percent inhibition of alpha-glucosidase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

Results

The results of quantitative estimation of phytochemicals in methanolic leaf extract of *A. paniculata*was represented in Table 1. Results revealed that total phenolic quantity was found to be highest (54.44mg GAE/g extract) in methanolic leaf extract of *A. paniculata*when compared with total flavonoid quantities (0.41mg QE/g extract).

Table 1: Quantitative analysis of phytochemicals of methanolic leaf extract of A. paniculata

Phytochemicals	**Methanolic leaf extract of A. paniculata**
Total Phenolics	54.44mg GAE/g extract
Total flavonoids	0.41mg QE/g extract

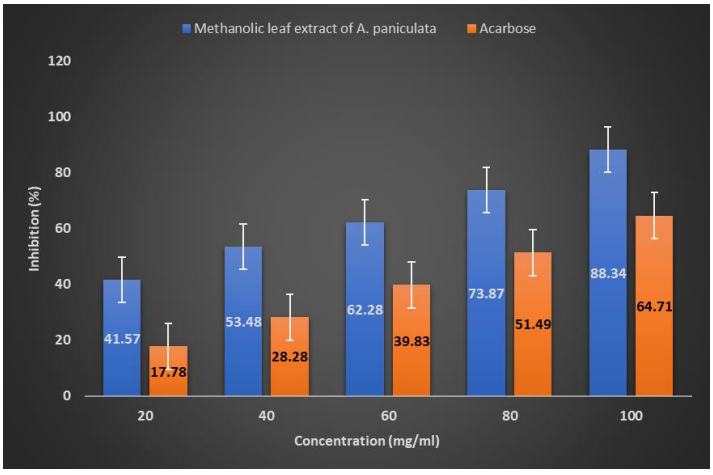
Values are expressed mean; n=3

The results of effect of methanolic leaf extract of *A. paniculata*on alpha-amylase inhibition activity was represented in Table 2 and plotted in Figure 2. Results depicted that methanolic leaf extract of *A. paniculata*at a concentration range of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml, shown inhibition effect of 41.57%, 53.48%, 62.28%, 73.87%, and 88.34% respectively with an IC_{50} value of 56.24 mg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 25.38mg/ml.

Table 2: Effect of methanolic leaf extract of *A. paniculata*on alpha-amylase inhibition activity

Conc. of methanolic leaf extract of *A. paniculata*(mg/ml)	**Inhibition (%)**	**Conc. of Acarbose(µg/ml)**	**Inhibition (%)**
20	41.57± 0.05	20	17.78± 0.13
40	53.48± 0.18	40	28.28± 0.19
60	62.28± 0.16	60	39.83± 0.08
80	73.87± 0.19	80	51.49± 0.12
100	88.34 ± 0.21	100	64.71 ± 0.23
IC_{50} (mg/mL) = 56.24		IC_{50} (mg/mL) =25.38	

Values were expressed Mean ± SD; n=3



Values were expressed Mean ± SD; n=3

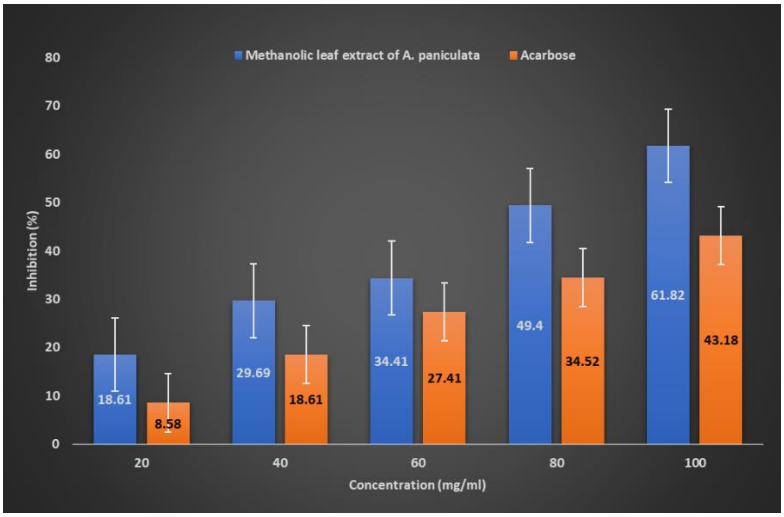
Figure 2: Effect of methanolic leaf extract of *A. paniculata* on alpha-amylase inhibition activity

The results of effect of methanolic leaf extract of *A. paniculata* on alpha-glucosidase inhibition activity was represented in Table 3 and plotted in Figure 3. Results depicted that methanolic leaf extract of *A. paniculata*at a concentration range of 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml, shown inhibition effect of 18.61%, 29.69%, 34.41%, 49.40%, and 61.82% respectively with an IC_{50} value of 32.38mg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 21.89mg/ml.

Table 3: Effect of methanolic leaf extract of *A. paniculata*on alpha-glucosidase inhibition activity

Conc. of methanolic leaf extract of *A. paniculata* (mg/ml)	**Inhibition (%)**	**Conc. of Acarbose (µg/ml)**	**Inhibition (%)**
20	18.61 ± 0.09	20	8.58 ± 0.06
40	29.69 ± 0.22	40	18.61 ± 0.19
60	34.41 ± 0.13	60	27.41 ± 0.28
80	49.40 ± 0.34	80	34.52 ± 0.21
100	61.82 ± 0.27	100	43.18 ± 0.19
IC_{50} (mg/mL) = 32.58		IC_{50} (mg/mL) = 21.89	

Values were expressed Mean ± SD; n=3



Values were expressed Mean ± SD; n=3

Figure 3: Effect of methanolic leaf extract of *A. paniculata* on alpha-glucosidase inhibition activity

Discussion

In Ayurvedic medicine, there are numerous herbs which have been used historically for treating a large variety of ailments. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. *A. paniculata* is a plant that has been effectively used in traditional Asian medicines for centuries.[17,18] Diabetes is an important human ailment afflicting many from various walks of life in different countries. A more practical approach would involve a series of *in-vitro* prescreens before testing a potential new hypoglycaemic agent in animals. This is because there are no perfect models for type II diabetes, and there are social and financial constraints on the extensive use of animals in experimentation.Furthermore, alpha-amylase and alpha-glucosidase inhibitors have become a new treatment strategy to combat diabetes mellitus.[19] Therefore, in the present study we aimed to screen for antidiabetic activities of methanol extracts of *A. paniculata*leaf parts by *in-vitro* methods usingalpha-amylase and alpha-glucosidase inhibition assays.

Alpha-amylase and alpha-glucosidase are the enzymes responsible for digestion of carbohydrates and increasing the postprandial glucose levels in diabetic patients. Inhibiting their activity could help in controlling postprandial hyperglycemia.[20]Our study results revealed that that total phenolic quantity was found to be highest (54.44 mg GAE/g extract) in methanolic leaf extract of *A. paniculata* when compared with total flavonoid quantities (0.41 mg QE/g extract). The methanolic leaf extract of *A. paniculata*exhibited IC_{50} value of 56.24mg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 25.38mg/ml in on alpha-amylase inhibition assay. Similarly, the methanolic leaf extract of *A. paniculata*exhibited an IC_{50} value of 32.38mg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 21.89mg/ml in alpha-glucosidase inhibition activity.

In concurrence with our study findings various studies reported in literature have demonstrated antidiabetic activities of *A. paniculata*. Husen and Nallapan, reported that aqueous extract (50 mg/kg) of *A. paniculata* produced a significant reduction (52.9%) in blood glucose level in streptozocin-induced hyperglycaemic rats. Freeze dried material of *A. paniculata* (6.25 mg/kg body weight) produced a more significant reduction (61.81%) in blood glucose level. Furthermore, the results of their study showed that the aqueous extract of *A. paniculata*did not produce significant reduction in the blood glucose level in normoglycemic rats.[21]

Hossain et al., demonstrated that aqueous and ethanolic extracts of *A. paniculata*showed significant hypoglycemic activity than chloroform and petroleum ether.[22] The ethanolic extract of aerial parts of *A. paniculata*has significantly decreased fasting serum glucose level in streptozotocin-diabetic rats compared with the control but inactive in normal rats.[23] Decoction of aerial parts of *A. paniculata* plant has been reported significant antidiabetic effect in alloxan-induced diabetic rats.[24] Similarly, aqueous extract of *A. paniculata* also exhibited anti-diabetic potential.[25] The anti-diabetic potential of *A. paniculata* plant due to the presence of diterpenoid lactones or may be a synergistic effect of flavonoids.[26]

Some of the parameters used to determine the activity of *A. paniculata*as anti-diabetic are as follows: (i) Preprandial and postprandial blood glucose levels,[27] (ii) Expression of GLUT-4 protein in muscle tissues,[28,29]

(iii) Hypoglycemic activity of glibenklamide,[28] and (iv) HOMA-IR index (homeostatic model assessment-insulin resistance).[30]

Conclusion

The outcome of this study indicates that *A. paniculata*possess both anti-diabetic properties in methanol extracts. Hence, *A. paniculata*is useful in treatment and prevention of diabetes without any side effects and with the natural properties of the plant. Therefore, *A. paniculata*could be explored in development of natural antidiabetic formulations.

References

1. Khajuria AK, Manhas RK, Kumar H, Bisht NS. Ethnobotanical study of traditionally used medicinal plants of Pauri district of Uttarakhand, India. Journal of Ethnopharmacology. 2021; 276:114204.
2. Joshi RK, Satyal P, Setzer WN. Himalayan aromatic medicinal plants: a review of their ethnopharmacology, volatile phytochemistry, and biological activities. Medicines. 2016;3(1):6.
3. Sarah W, Gojka R, Anders G, Richard S, Hilary K. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care. 2004;27(5):1047-53.
4. Tabish SA. Is diabetes becoming the biggest epidemic of the twenty-first century?. International Journal of health sciences. 2007;1(2):5-8.
5. Manikandan R, Anand AV, Muthumani GD. Phytochemical and in vitro anti-diabetic activity of methanolic extract of *Psidium guajava* leaves. International journal of current microbiology and applied sciences. 2013;2(2):15-9.
6. Joy PP, Thomas J, Samuel M, Skaria Baby P. Impact of quebracho tannins supplementation on productive and reproductive efficiency of dairy cows. Med Plants. 1998;449-632.
7. Sharma A, Lal K, Handa SS. Standardization of the Indian crude drug Kalmegh by high pressure liquid chromatographic determination of andrographolide. Phytochemical analysis. 1992;3(3):129-31.
8. Akbar S. Andrographis paniculata: a review of pharmacological activities and clinical effects. Alternative Medicine Review. 2011;16(1):66-77.
9. Therasa SA, Sobiya G, Parimala SM. Leaves of *Andrographis Paniculata* Is an Antioxidant and Anticancer Agent. Asian Journal of Pharmaceutical and Clinical Research. 2020;13(8):213-7.
10. Govindarajan M, Sivakumar R. Adulticidal and repellent properties of indigenous plant extracts against Culex quinquefasciatus and *Aedes aegypti* (Diptera: Culicidae). Parasitology research. 2012;110:1607-20.
11. Sukhdev SH, Dev D, Rakesh KV. Compendium of medicinal and aromatic plants. New Delhi: ASIA. 2006;2:50-148.
12. Shi Y, Burn P. Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. Nature reviews Drug discovery. 2004;3(8):695-710.
13. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidative substrates by means of Folin-Ciocalteau reagent, Packer L. Methods in Enzymology. 1999; 299:152-78.
14. Ordonez AAL, Gomez JD, Vattuone MA, Lsla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chemistry. 2006;97(3):452-8.
15. Miller GL. Use of dinitro salicylic acid reagent for determination of reducing sugar. Analytical chemistry. 1959;31: 426-428.
16. Matsui T, Yoshimoto C, Osajima K, Oki T, Osajima Y. In vitro survey of α-glucosidase inhibitory food components. Bioscience, biotechnology, and biochemistry. 1996;60(12):2019-22.
17. Jayakumar T, Hsieh CY, Lee JJ, Sheu JR. Experimental and clinical pharmacology of *Andrographis paniculata* and its major bioactive phytoconstituent andrographolide. Evidence-Based Complementary and Alternative Medicine. 2013:846740.
18. Md. Sanower H, Zannat U, Abubakar S, and K. M. Hafizur R. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A Review of Ethnobotany, Phytochemistry, and Pharmacology. Scientific World Journal. 2014:1 -28.
19. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. Bioscience, biotechnology, and Biochemistry. 1993;57(1):123-4.
20. Poovitha S, Parani M. In vitro and in vivo α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC complementary and alternative medicine. 2016;16:1-8.

21. Husen R, Pihie AH, Nallappan M. Screening for antihyperglycaemic activity in several local herbs of Malaysia. Journal of Ethnopharmacology. 2004;95(2-3):205-8.
22. Hossain MA, Roy BK, Ahmed K, Chowdhury AS, Rashid MA. Antidiabetic activity of *Andrographis paniculata*. Dhaka University Journal of Pharmaceutical Sciences. 2007;6(1):15-20.
23. Zhang XF, Tan BH. Anti-diabetic property of ethanolic extract of *Andrographis paniculata* in streptozotocin-diabetic rats. Acta Pharmacologica Sinica. 2000;21(12):1157-64.
24. Reyes BA, Bautista ND, Tanquilut NC, Anunciado RV, Leung AB, Sanchez GC, Magtoto RL, Castronuevo P, Tsukamura H, Maeda KI. Anti-diabetic potentials of Momordica charantia and *Andrographis paniculata* and their effects on estrous cyclicity of alloxan-induced diabetic rats. Journal of ethnopharmacology. 2006;105(1-2):196-200.
25. Akhtar MT, Bin Mohd Sarib MS, Ismail IS, Abas F, Ismail A, Lajis NH, Shaari K. Anti-diabetic activity and metabolic changes induced by *Andrographis paniculata* plant extract in obese diabetic rats. Molecules. 2016;21(8):1026.
26. Zhang Z, Jiang J, Yu P, Zeng X, Larrick JW, Wang Y. Hypoglycemic and beta cell protective effects of andrographolide analogue for diabetes treatment. Journal of translational medicine. 2009; 7:1-3.
27. Nugroho AE, Andrie M, Warditiani NK, Siswanto E, Pramono S, Lukitaningsih E. Antidiabetic and antihiperlipidemic effect of *Andrographis paniculata* (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats. Indian journal of pharmacology. 2012;44(3):377-81.
28. Syamsul ES, Nugroho AE, Pramono S. Aktivitas Antidiabetes Kombinasi Ekstrak Terpurifikasi Herba Sambiloto (*Andrographis paniculata* (Burn. F.) NESS.) dan Metformin pada Tikus DM Tipe 2 Resisten Insulin. Majalah Obat Tradisional. 2011;16(3):124-31.
29. Zhang Z, Jiang J, Yu P, Zeng X, Larrick JW, Wang Y. Hypoglycemic and beta cell protective effects of andrographolide analogue for diabetes treatment. Journal of translational medicine. 2009;7:1-3.
30. Adriawan IR, Andrie M, Susilowati R, Pramono S, Nugroho AE. Evaluasi Efek Anti-Diabetes Melitus Ekstrak Terpurifikasi *Andrographis paniculata* (Burm. f.) Nees dan Andrografolid Dengan Parameter Indeks HOMA-IR. Trad. Med. J. 2014;19(1):19-23.