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In Vitro Evaluation of Antioxidant And Anti-Diabetic Potential of Kayea Assamica (King and Prain) Leaf Extract

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ABSTRACT

Kayea assamica (King and Prain) is a native tree of Assam, India, which is known as *Sia-Nahar* in local language (Assamese). The aqueous extract of bark possesses antimalarial activity and methanolic extract of barks possess coumarin derivatives like therapin A, B and C, which are reported to have cytotoxic activity. This research is focused on the antioxidant activity and antidiabetic property of the leaves of *K. assamica.* Methanol, chloroform and hexane extract is used to estimate the polyphenol content. The methanolic extract showed highest phenolic content [(88.03 ± 0.33) mg GAE/g of dry material]. The flavonoid content [(58.44 ± 0.23) mg QE/g of dry material] in the methanolic extract was also highest. The antioxidant capacity was determined by DPPH free radical scavenging assay. The methanol extract was showing comparable IC_{50} value of 5.4 ± 0.54 µg/ml against the standard drug (ascorbic acid). The leaves extracts were tested for capacity to scavenge the ABTS free radicals. The capacity to quench the free radicals was highest in methanolic extract (IC_{50} value of 6.9 ± 0.54 µg/ml). The anti-diabetic capacity was investigated with the assist of α-amylase inhibiting assay and α-glucosidase inhibiting assay. The methanolic extract of leaves of *K. assamica* showed better α-amylase inhibition (17.42 ± 0.25 µg/ml) and α-glucosidase inhibition (IC_{50} of 13.19 ± 0.64 µg/ml) and was comparable to standard drug acarbose (IC_{50} of 19.42 ± 0.86 µg/ml). The result obtained can provide an insight into the utilization of leaves of *K. assamica* as potent antioxidant and anti-diabetic herbal drug.

Keywords: *Kayea Assamica*, DPPH, ABTS, Anti-diabetic, Antioxidant, alpha amylase

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INTRODUCTION

Diabetes Mellitus (DM) is characterized by high blood sugar level than the normal in the body thus causing irregularities in insulin level which attribute the either type I or type II DM and gestational DM. Diabetes mellitus is a chronic disorder which causes complications like kidney failure, leg amputation due to foot ulcer, diabetic retinopathy, heart attack and strokes (1, 2). According to a WHO report, across the globe around 422 million people are affected with DM, and mostly low and middle income countries are affected predominantly. 1.6 million deaths are recorded worldwide due to DM each year (3). People with DM are basically dependent upon affordable treatment which includes insulin too. Various treatment regimes are already in practice but the use of natural products has instigated a newer avenue for the management of DM (4). Ginseng and Banaba extracts amongst others are already approved by Korean FDA for the treatment of DM. The phytoconstituents present in the extracts of plants are facilitating this activity (5).

The occurrence of oxidative cell damage in DM patients increases manifolds than in normal patients due to higher level of ROS (reactive oxygen species). Oxidative stress also inhibits the insulin signals, thus increasing insulin resistance by adipocytokine dysregulation. The level of biomarkers like protein carbonyl, malondialdehyde and isoprostanes etc in oxidative stress also increases in DM patients (6). DM patients felicitate the oxidative stress by various mechanism i.e autoxidation of glucose, increase of advance glycation end-product, activation of protein kinase C and increase in polyol pathway flux. The increased ROS production due to oxidative stress thus contributes to various clinical harmful factors in DM patients like increase level of LDL (low density lipoprotein), beta cell dysfunction, dysfunction of endothelial cells (7). Antioxidants can provide a strategy to prevent the formation of free radical and thus check the oxidative stress and the DM related vascular complication and can be beneficial in management of cardiovascular diseases, maintenance of glucose level and management of dyslipidemia.

Kayea assamica (King and Prain) is a native tree of Assam, India, which is known as *Sia-Nahar* in local language. This tree is tall and blooms from October to May. The fruits are used as fish poison and the aqueous extract of the bark is known to possess antimalarial activity. The pollens are used in fever and dizziness (8). Following is the taxonomical classification:

Kingdom: Plantae

Family: Clusiaceae

Genus: *Kayea*

Species: *K. assamica* (King and Prain)

Synonyms: *Mesua assamica* (King and Prain)

The previous reports suggest that *Kayea assamica* (King and Prain) posess coumarin derivatives like therapin A, B and C in the methanolic extract of barks, which are reported to have cytotoxic activity against human cancer cell lines. The study on antimalarial activity against D6 (choloroquine sensitive) and W2 (choloroquine resistant) clones of *Plasmodium falciparum* has shown positive results (9). The current study is done to analyze its antioxidant activity and to ascertain its antidiabetic activity.

MATERIALS AND METHOD

Collection of plant and extraction

Fresh leaves of *K. assamica* were collected from Baihata Chariali, Assam, India. The leaves were shade dried and grinded into fine powder. The dried powder was stored in air-tight container for further use. Dried powdered plant material was extracted using soxhlet apparatus with continuous hot extraction method with increasing polarity i.e hexane, chloroform and methanol. The extracts were dried using rotary evaporator (IKA RV 10) under reduced pressure (10). The leaves were authenticated by Assam Bio-Research Centre, Baihata Chariali, Assam, India under Assam Science Technology and Environment Council, Govt. of Assam, India (Voucher no. 1931).

Estimation of Total phenolic content

The Folin-Ciocalteu reagent method was used to determine the total phenolic content of the leaves of *K. assamica.* 2.5 ml of 10% v/v Folin-Ciocalteu reagent and 2.0 ml of 2% w/v sodium carbonate solution were mixed in a test tube. Then o.5 ml of ethanolic solution of each extract was added in the test tube and shaken. The resultant mixture was then incubated for 15 minutes at 45°C with intermittent shaking. Gallic acid was taken as the standard drug to make the calibration curve. Absorbance was measured at 765 nm using UV Visible spectrophotometer (Shimadzu 1800) (10).

Estimation of total flavonoid content

Flavonoid content of the leaves of *K. assamica* was determined using quercetin as a standard drug. The 0.5 ml of sample solution (1gm/ml) was mixed with 0.1 ml 1 M potassium acetate, 0.1 ml of 10% aluminium nitrate solution and 4.3 ml of 80% ethanol in a test tube. The test tube was stand still for 40 minutes at room temperature. The absorbance of the supernatant liquid was measured in 415nm (10).

DPPH (1,1 -diphenyl-2-picrylhydrazyl) scavenging assay

The scavenging capacity of leaves of *K. assamica* was determined by mixing 1.0 ml of DPPH solution (25mg/L) and 3.0 ml of extract solution in different concentration in test tubes. The test tubes were kept in dark room at room temperature for 30 minutes. The absorbance of each test tubes were measured at 517 nm using UV Visible spectrophotometer (Shimadzu 1800).

The percentage inhibition (I%) of the leaves were calculated by following equation where $A_{control}$ is absorbance of the control solution (containing all of the reagents, except the test sample), and A_{sample} is absorbance of sample solution. Ascorbic acid was taken as the standard drug (11).

$I\% = [(A_{control} - A_{sample})/A_{control}] \times 100$ ..... (1)

ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] scavenging assay

The method described Youn *et al* was used to determine the ABTS radical cation ($ABTS^{+}$) scavenging capacity. Initially, ABTS was dissolved in water to make a 7mM concentration solution. 2.5 mM potassium persulphate solution was added to the above solution to produce ABTS radicals. The resultant final concentration was kept in a dark room at room temperature for 12-16 hours. The final solution is then diluted with ethanol to adjust the absorbance at 734 nm for 0.70. The extract at different concentration was then mixed with 1 ml of diluted ABTS solution and the absorbance was measured at 734 nm after 30 minutes of initial mixing. Ascorbic acid was taken as the standard drug (12). The percentage inhibition was measured using equation 1.

Alpha-Amylase inhibition assay

The method of Vadivelan *et al* was performed for this assay with minor variation. A phosphate buffer (pH 6.9, 0.02M sodium dihydrogen phosphate and 0.006M sodium chloride) was prepared and the plant extract (dissolved in minimum quantity of 10% solution of DMSO) was dissolved in it thus making concentration ranging from 2 to 15 µg/ml. A volume 200 µl of extract was mixed with 200 µl of α-amylase solution and incubated at 30 ˚C for 10 minutes. After incubation, 1% w/v 200 µl solution of starch solution was added in each tube having different concentration of the extract and incubation was done for 3 minutes. Thereafter, 200 µl DNSA (3,5-dinitrosalicylic acid) reagent was added to each test tube to cease the reaction and then boiled at 85-90 ˚C for 10 minutes. After the temperatures of solution in each test tube reduce to room temperature, it was diluted with 5 ml distilled water and the absorbance was measured at 540 nm using UV Visible spectrophotometer. Acarbose was used as the standard drug. Equation 1 was used to determine the percentage inhibition (13).

Alpha -Glucosidase inhibition assay

The method described by Bhatia *et al* was used to determine alpha-glucosidase inhibition assay with slight modification. In a test tube, 20 µl (50 µg/ml) alpha-glucosidase was mixed with 5 µl of the plant extract (at different concentrations of 2 to 15 µg/ml). Potassium phosphate buffer (pH 6.8, 60 µl of 67mM) was later added in each test tube and kept for 5 minutes incubation. Thereafter, ρ-nitrophenyl-α-D-glucopyranoside (10 µl of 10 mM) solution was added and further incubated at 37 ˚C for 20 miinutes. Briefly, sodium carbonate (25 µl of 100mM) was

added in each test tube and the absorbance was measured at 405 nm. Acarbose was used as the standard drug. Equation 1 was used to determine the percentage inhibition (14).

Statistical Analysis

All the data were measured in triplicate and then analyzed in analysis of variance (ANOVA) and expressed as mean ± SEM (n=3). Dunnett's multiple comparison tests was used for the data analysis, using SPSS (statistical package for social science). A level of $P<0.05$ was used as the criterion for statistical significance.

RESULTS AND DISCUSSION

Total Phenolic and Flavonoid content

This estimation of total phenolic and flavonoid content was important for the investigation of the capacity of *K. assamica* to scavange the free radicals thus establishing its antioxidant capacity and ability to produce the desired medicinal properties (15). The calibration curve of gallic acid (Y =0.0044 x + 0.031, R_2 = 0.9995) and quercetin (Y = 0.028 8x + 0.005 8, R_2 = 0.999 1) was prepared for the calculation of total phenolic content and total flavonoid content respectively. The phenolic content results were expressed as Gallic acid equivalents in milligram per gram (mg GAE/g) of dried extract. The methanolic extract showed highest phenolic content [(88.03 ± 0.33) mg GAE/g of dry material] than the chloroform extract. The flavonoid content [(58.44 ± 0.23) mg QE/g of dry material] in the methanolic extract was also highest when compared to choloroform extract. The hexane extract showed very limited polyphenolic compound content. It is an established fact that polyphenolic compounds have the ability to neutralize the free radicals and the presence of polyphenols in methanolic extract of *K. assamica* proves that it has antioxidant property and can readily scavenge the free radicals (16). Table 1 and Figure 1 shows the total phenolic content and total flavonoid content of different extracts of *K. assamica.*

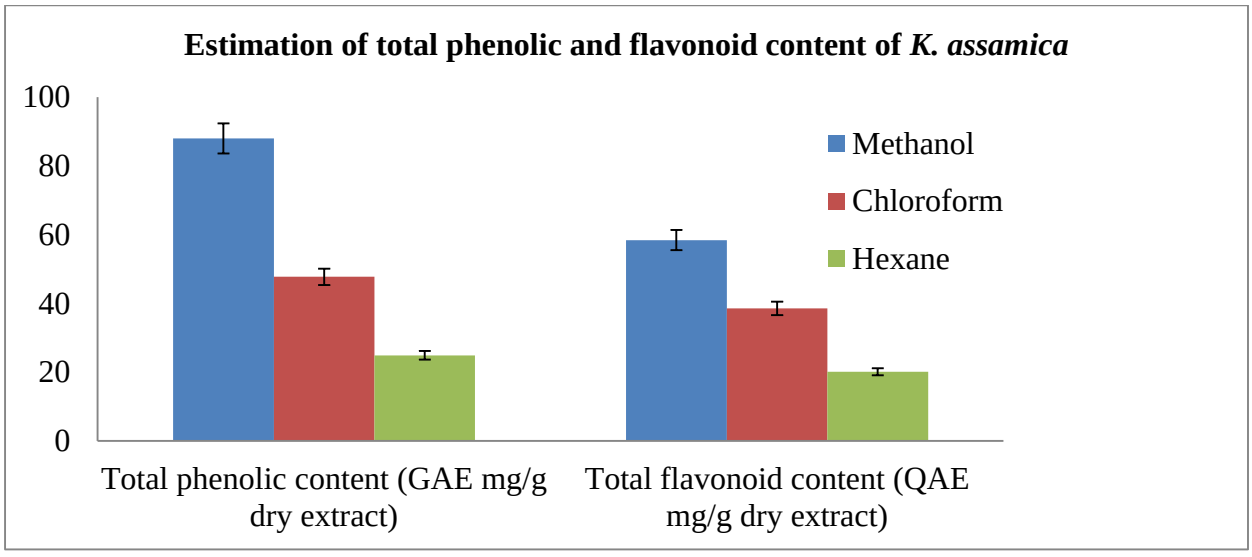

Figure 1: Estimation of total phenolic and flavonoid content of *K. assamica*

Table 1: Estimation of total phenolic and flavonoid content of *K. assamica*

Extracts	Total phenolic content (GAE mg/g dry extract)	Total flavonoid content (QAE mg/g dry extract)
Methanol	88.03 ± 0.33	58.44 ± 0.23
Chloroform	47.76 ± 0.61	38.56 ± 0.44
Hexane	24.89 ± 0.47	20.12 ± 0.59

Each values in the table was calculated by taking average of three experiments and data are given as Mean ± SEM. ($P < 0.05$)

DPPH free radical scavenging activity

The capacity of *K. assamica* to scavenge the free radicals was determined by DPPH free radical scavenging assay. DPPH scavenging capacity was observed for all the extracts. DPPH scavenging activity was seen with a concentration-response relationship. Scavenging capacity was increasing with an increase in concentration. As a positive control, ascorbic acid showed high scavenging activity with IC_{50} (50% inhibitory concentration) of 4.5 ± 0.22 µg/ml. The methanol extract was showing IC_{50} of 5.4 ± 0.54 µg/ml (comparable to standard drug), followed by choloroform with IC_{50} of 18.9 ± 0.65 µg/ml, and hexane extract with IC_{50} of 21.9 ± 0.32 µg/ml. There was a strong scavenging capacity seen in the methanolic extract as compared to choloroform and hexane extracts (Table 2 and Figure 2). The scavenging capacity is observed by the disappearance of purple colour in each test sample having DPPH with estimated with the help of UV-Visible spectrophotometer (at 517 nm). The DPPH free radicals are quenched by the antioxidant constituents of *K. assamica.* This was due to the hydrogen donating ability of the polyphenolic compounds present in the extracts of *K. assamica* leaves (17).

Table 2: Antioxidant activity of *K. assamica*

Extracts/Standard	IC_{50}(µg/ml)	
	DPPH assay	ABTS assay
Methanol	5.4 ± 0.54	6.9 ± 0.54
Chloroform	18.9 ± 0.65	29.47 ± 0.39
Hexane	21.9 ± 0.32	47.61 ± 0.17
Ascorbic acid	4.5 ± 0.22	

Each values in the table was calculated by taking average of three experiments and data are given as Mean ± SEM. ($P < 0.05$)

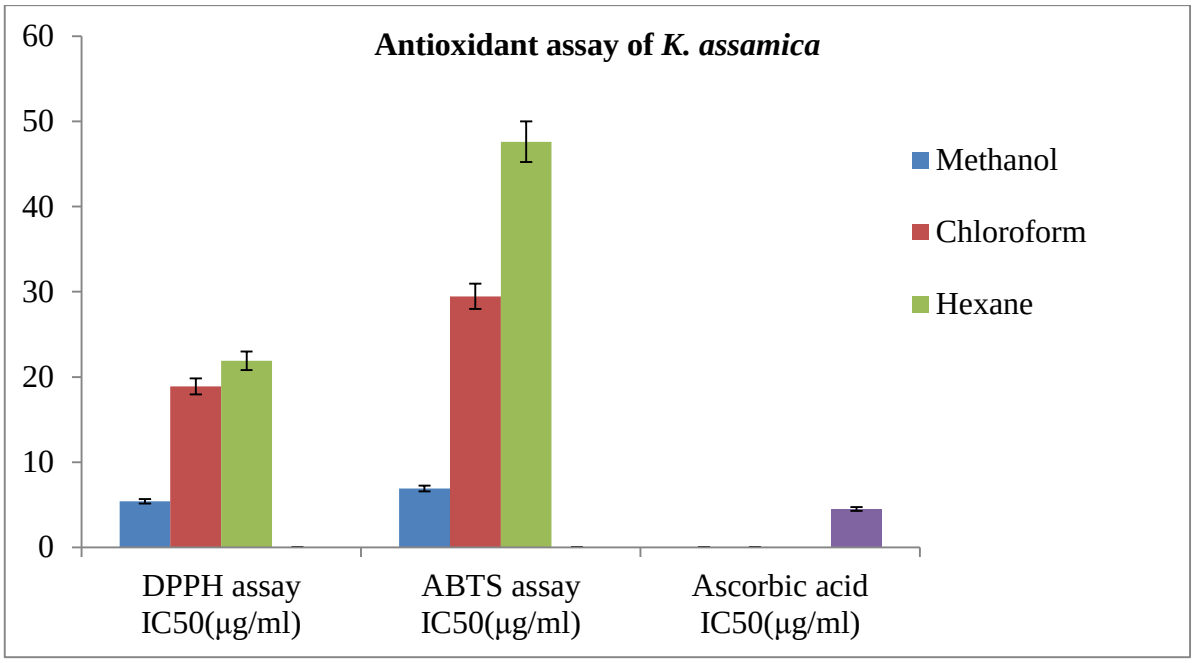

Figure 2: Antioxidant activity of *K. assamica*

ABTS free radical scavenging activity

The all leaves extracts of *K. assamica* were tested for capacity to scavenge the ABTS free radicals. The capacity to quench the free radicals was highest in methanolic extract. The methanolic extract was showing IC_{50} value of 6.9 ± 0.54 µg/ml. The scavenging capacity of methanolic extract was comparable to the standard drug. The chloroform and hexane extract were showing limited scavenging capacity with IC_{50} value of 29.47 ± 0.39 µg/ml and 47.61 ± 0.17 µg/ml respectively (Table 2 and Figure 2). The results were satisfactory and thus further confirm the presence of antioxidant phytoconstituents in *K. assamica* (18).

Alpha-Amylase inhibition assay

The anti-diabetic capacity of *K. assamica* is investigated with the assist of α-amylase inhibiting assay model. The α-amylase inhibitors are also known as starch blockers. The starch and oligosaccharides are converted into maltose, maltriose and simple sugars for the absorption in the body and thus distress the diabetic patients, but the α-amylase inhibitors delay the absorption by blocking the hydrolysis of 1,4-glycosidic linkage. This mechanism suggests that α-amylase inhibition provides a relationship to the anti-diabetic activity (19). All the three extracts of the plant leaves of *K. assamica* were investigated and the results were satisfactory. The dose-dependent increase is evident in the percentage inhibition capacity of *K. assamica.* The standard drug taken was acarbose and it showed IC_{50} of 19.42 ± 0.86 µg/ml. The methanolic extract of *K. assamica* showed IC_{50} of 17.42 ± 0.25 µg/ml. The chloroform and hexane extract showed IC_{50} of 45.37 ± 0.36 µg/ml and 91.89 ± 0.52 µg/ml respectively (Table 3 and Figure 3). The results clearly establish that methanolic extract showed similar scavenging capacity in comparison to the standard drug. The scavenging capacity of choloroform and

hexane extract was not satisfactory as evident from the results. The presence of polar compounds in the methanolic extract may attribute to the anti-diabetic property of *K. assamica.*

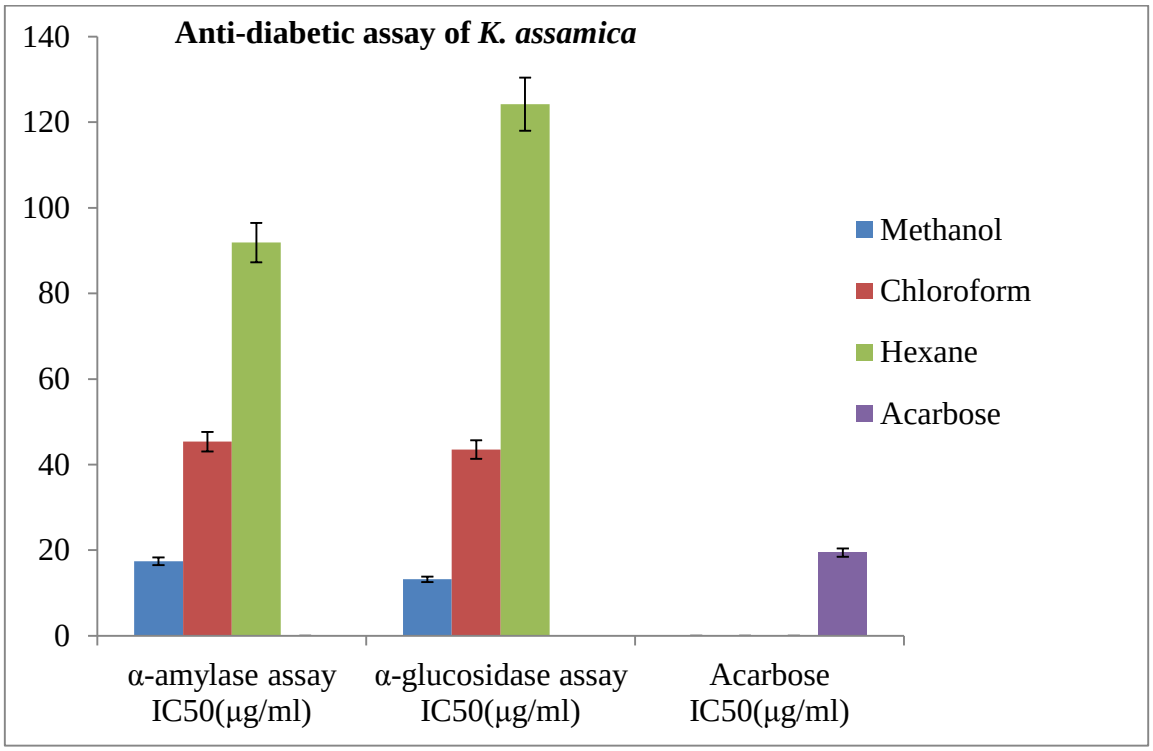

Figure 3: Anti-diabetic activity of *K. assamica*

Table 3: Anti-diabetic activity of *K. assamica*

Extracts/Standards	**IC_{50}(µg/ml)**	
	α-amylase assay	**α-glucosidase assay**
Methanol	17.42 ± 0.25	13.19 ± 0.64
Chloroform	45.37 ± 0.36	43.52 ± 0.27
Hexane	91.89 ± 0.52	124.24 ± 0.78
Acarbose	19.42 ± 0.86	

Each values in the table was calculated by taking average of three experiments and data are given as Mean ± SEM. ($P < 0.05$)

Alpha -Glucosidase inhibition assay

The inhibitory capacity against α-glucosidase was determined with the help of a substrate known as ρ-nitrophenyl-α-D-glucopyranoside. The results were compared with the standard drug acarbose. The α-glucosidase enzyme hydrolyses dietary carbohydrates and produces glucose for the uptake in humans. The inhibition of α-glucosidase thus inhibits the glucose absorption and helps in decrease of post prandial hyperglycemia. The α-glucosidase reacts with ρ-nitrophenyl-α-D-glucopyranoside to give ρ-nitrophenol. The resultant colour of the solution is yellow, and the enzyme activity is measured based on the absorbance exhibited by the yellow colour of ρ-nitrophenol (20). The methanolic extract of *K. assamica* was able to inhibit the α-glucosidase enzyme as evident by the results (IC_{50} of 13.19 ± 0.64 µg/ml). The chloroform and hexane extract of *K. assamica* showed IC_{50} value of 43.52 ± 0.27 µg/ml and 124.24 ± 0.78

µg/ml respectively (Table 3 and Figure 3). The anti-diabetic property of methanolic extract of *K. assamica* can be ascribed by the presence of carbohydrates in the extract.

CONCLUSION

In this study, the estimation of antioxidant capacity with higher phenolic content and higher flavonoid content ascertained that leaves of *K. assamica* can be a significant source of herbal antioxidant. Furthermore, the assessment of antioxidant property by DPPH radical scavenging assay and ABTS radical scavenging assay relates to the capability of leaves of *K. assamica* to scavenge the free radicals. The fact that antioxidant property can be useful in treatment of ROS-related disease like DM, α-amylase inhibiting assay and α-glucosidase inhibiting assay model was performed and methanolic extract of leaves of *K. assamica* showed good results. The selected plant thus can be proposed for developing a potent herbal anti-diabetic drug. However, further in vitro and in vivo studies need to be performed to develop medicinal preparations or nutraceuticals.

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REFERENCE

1. Craig ME, Jeferies C, Dabelea D, Balde N, Seth A, Donaghue KC. Defnition, epidemiology, and classification of diabetes in children and adolescents. Pediatric Diabetes. 2014; 15(20): 4–17.
2. Wilkinson MJ, Zadourian A, Taub PR. Heart Failure and Diabetes Mellitus: Defining the Problem and Exploring the Interrelationship. Am J Cardiol. 2019; 124: 3-11.
3. Dall TM, Yang W, Gillespie K, Mocarski M, Byrne E, Cintina I, Beronja K, Semilla AP, Iacobucci W, Hogan PF. The Economic Burden of Elevated Blood Glucose Levels in 2017: Diagnosed and Undiagnosed Diabetes, Gestational Diabetes Mellitus, and Pre diabetes. Diabetes Care. 2019; 42(9): 1661-1668.
4. Bang H, Kwak JH, Ahn HY, Shin DY, Lee JH. Korean Red Ginseng Improves Glucose Control in Subjects with Impaired Fasting Glucose, Impaired Glucose Tolerance, or Newly Diagnosed Type 2 Diabetes Mellitus. J Med Food. 2014; 17(1): 128–134.
5. Park C, Lee JS. Mini Review: Natural ingredients for diabetes which are approved by Korean FDA. Biom Res. 2013; 24 (1): 164-169.
6. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiol Rev. 2009; 89: 27–71.

7. Chokki M, Cudălbeanu M, Zongo C, Dah-Nouvlessounon D, Ghinea IO, Furdui B, Raclea R, Savadogo A, Baba-Moussa L, Avamescu SM, Dinica RM, Baba-Moussa F. Exploring Antioxidant and Enzymes (A-Amylase and B-Glucosidase) Inhibitory Activity of Morinda lucida and Momordica charantia Leaves from Benin. Foods. 2020; 9(4): 1-21
8. Gogoi B. Anti-Cancerous Potentiality of Coumarins of Mesua assamica (King and Prain) Kosterm. – An Endemic Plant of Assam. Int J Pharmacog Phytochem Res. 2018; 9(7): 939-942.
9. Lee KH, Chai HB, Tamez PA, Pezzuto JM, Cordell GA, Win KK, Tin-Wa M. Biologically active alkylated coumarins from Kayea assamica. Phytochemistry. 2003; 64(2): 535-41.
10. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants Basel). 2019; 8(4): 96-108.
11. Dudonné S, Vitrac X, Coutiere P, Woillez M, Mérillon JM. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J Agric Food Chem. 2009; 57: 1768–1774.
12. Youn JS, Kim YJ, Na HJ, Jung HR, Song CK, Kang SY, Kim JY. Antioxidant activity and contents of leaf extracts obtained from Dendropanax morbifera LEV are dependent on the collecting season and extraction conditions. Food Sci Biotechnol. 2019; 28(1): 201–207.
13. Vadivelan R, Krishnan RG, Kannan R. Antidiabetic potential of Asparagus racemosus Willd leaf extracts through inhibition of α-amylase and α-glucosidase. J Tradit Complement Med. 2019 Jan; 9(1): 1–4.
14. Bhatia A, Singh B, Arora R, Arora S. In vitro evaluation of the α-glucosidase inhibitory potential of methanolic extracts of traditionally used antidiabetic plants. BMC Complement Altern Med. 2019; 19: 74-88
15. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat Res-Fund Mol Mutagen. 2005 ;579: 200–213.
16. Yang H, Shin Y. Antioxidant compounds and activities of edible roses (Rosa hybrida spp.) from different cultivars grown in Korea. Appl Biol Chem. 2017; 60: 129–136.
17. Rahman MM, Islam MB, Biswas M, Khurshid Alam AH. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC Res Notes. 2015; 8: 621.

18. Tai A, Ohno A, Ito H. Isolation and Characterization of the 2,2′-Azinobis(3 ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Cation-Scavenging Reaction Products of Arbutin. J Agric Food Chem. 2016; 64: 7285–7290.
19. Ranilla LG, Kwon YI, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresour Technol. 2010; 101: 4676–4689.
20. Kwon YI, Vattem DA, Shetty K. Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. J Asian Pac J Clin Nutr. 2006; 15: 107–118.