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Research Article

Antioxidant and Antidiabetic activity of hydroalcoholic flower extract of *Woodfordia fruticosa* (L.) Kurz

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Abstract

Woodfordia fruticosa (L.) Kurz, (*W. fruticosa*) a member of the Lythraceae family, commonly known as dhaiphul, has been used in traditional medicine to cure common illnesses since ancient times. The most effective part of this plant is the flower which has a high demand in the national as well as the international market of the eastern south part of Asian countries. The present study focuses on exploring the presence of phytochemicals, antioxidants, and antidiabetic potential of hydroalcoholic extract of flower of *W. fruticosa*. Phytochemical test showed the presence of saponin, flavonoid, carbohydrates, alkaloids, tannin, anthraquinone, and triterpenoids in the extract, and it showed an effective result of total phenolic content, total flavonoid Content with the value of 371.96 ± 2.86 mg of gallic acid equivalent per gram of the extract and 113.82 ± 0.178 mg of quercetin equivalent per gram of dry weight. *In vitro* antioxidant assay, for the DPPH assay, the IC_{50} values were found 30.85 ± 0.36 μ g/ml and 22.35 ± 0.54 μ g/ml for the extract of *W. fruticosa* flower and ascorbic acid respectively and for the FRAP Assay, the reducing power assay of plant extract is lesser than standard (Ascorbic acid). *W. fruticosa* also showed significant hyperglycemic activity. In accordance with results, we can conclude that plant consists of a high polyphenolic and flavonoid group which help in antioxidant activity as well as the hydroalcoholic extract shows mild activity against diabetes.

Keywords: *Woodfordia fruticosa*, phytochemical, hydroalcoholic, antioxidants, antidiabetic, DPPH.

INTRODUCTION

Since primitive times, the plant has been used for many purposes in household stuff, dietary supplement as well as medicine. Furthermore, over 80% populations of developing nations usually look upon herbal medicine for their basic health needs according to WHO (World Health Organization) instead of allopathic medication¹. The plant consists of phytochemicals as primary metabolite and secondary metabolite, where the medicinal properties were mainly exhibited by secondary metabolite which contained the compound like phenol, flavonoid, etc, which play important role in defensive nature of plant as well as contain various antioxidants which are helpful for human health by preventing disease². The plants have the potential for anti-diabetic activity because of the presence of secondary metabolites (myricetin, kaempferol, Apigenin, Quercetin, etc), which have activity against those enzymes which are responsible for the disturbance of harmony of glucose level in the body³.

Woodfordia fruticosa (L.) Kurz (Family: Lythraceae) (*W. fruticosa*) is a fully matured plant, mostly available in the high altitude of the subtropical region of the southern part of Asian countries like China, Nepal, India, and in a few places of Africa continent like Comores, Madagascar, and E-Tanzania⁴. The

main phytochemicals found in the *W. fruticosa* are tannins, polyphenolic groups, and flavonoids, and other chemical constituents are saponins, essential oil, carbohydrate etc⁵. According to previous researches *W. fruticosa* flowers extract has anti-inflammatory⁶, gastroprotective⁷, hepatoprotective⁸, antioxidant⁹, Alzheimer's disease¹⁰, and wound healing activity¹¹. The main objective of this work was to explore the phytochemical profile, antioxidant activity, and *in vitro* anti-diabetic properties of the *W. fruticosa* flower.

MATERIAL AND METHODS

Plant materials

The flowers of *W. fruticosa* have been collected from the Sevok, (District: Darjeeling) West Bengal in April and May in 2020. The plant was authenticated (Reference no. CNH/Tech.II/2021/11) by the Botanical Survey of India, Shibpur, Howrah (West Bengal). This plant specimen has been preserved in the laboratory for further reference.

Preparation of the extract

The flowers of the *W. fruticosa* were dried under shade and after the drying; the material is smashed into powder form of mesh 40 with the help of a mechanical grinder. The

resulted powder was subjected to maceration process with hydroalcoholic solvent [Ethanol and water (70:30)]. The hydroalcoholic extract of *W. fruticosa* (HAWF) was filtered and get concentrated by a rotary vacuum evaporator under pressure. For further dryness and storage, it was kept in a desiccator for the experiments to be performed.

Phytochemical Screening

The phytochemical screenings were done with the HAWF for the qualitative analysis of phytochemicals present in the *W. fruticosa* flower. The standard protocol was used for the test for carbohydrates, alkaloids, flavonoids, tannins, triterpenoids, glycosides, and saponins as per ¹²⁻¹³.

Determination of Total Phenolic Content (TPC)

A stock solution of standard and extract was prepared in a concentration of 1mg/1ml in distilled water from which 0.4 ml of sample/standard was withdrawn into the test tube and then, 0.4 ml Folin-ciocalteu reagent and 4 ml distilled water was added to samples, which was incubated for 5 minutes in the dark place. After 5 min, 4 ml 7% Sodium carbonate solution was added in each tube, and volume makeup was done up to 10 ml by distilled water, which was further incubated in a dark place for 90 minutes at room temperature. Measurement of the absorbance was taken at 730 nm using distilled water as blank and gallic acid as standard. A Calibration curve was made with gallic acid concentrations in the range of (10, 50, 100, 200, 400, and 800 µg/ml). Total phenolic content was stated in mg gallic acid equivalents (GAE) per gram of dry weight¹⁴.

Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric assay was performed to determine the total flavonoid content of the extract. Stock solution of standard and HAWF was prepared in a concentration of 1mg/1ml in methanol from which 0.2 ml of 10 % (w/v) solution in methanol was added in each sample/standard. Thereafter, 0.2 ml of 1 M Potassium acetate solution was added and then, 5.6 ml of distilled water was added to volume makeup which will incubate for 30 min at room temperature. Measurement of the absorbance was taken at 415 nm using, Methanol as blank and Quercetin as standard. A calibration curve was made with quercetin concentration in the range of (10, 50, 100, 200, and 400 µg/ml). The total flavonoid content was expressed as quercetin equivalents per gram of dry weight¹⁵.

Determination of *In Vitro* Antioxidant Activity

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of HAWF was measured by using DPPH (1, 1-diphenyl-2-picryl-hydrazil). Stock was prepared in 1 mg/1ml Methanol for extract and standard. Dilution was done with (10, 25, 50, 125, and 200 µg/mL) in which 1ml DPPH (concentration 0.3mM) solution was added in each sample which was incubated for 30 min in dark at room temperature. After that, Measurement of the absorbance at 517 nm was taken by spectrophotometer, where methanol was blank and 1 ml DPPH solution as a control. The scavenging activity of DPPH was calculated with the formula:

$$\% \text{DPPH} \text{ scavenging activity or } (\% \text{ inhibition}) = \left[1 - \frac{\text{Abs}(s)}{\text{Abs}(c)} \right] \times 100$$

Where Abs(c) is the absorbance of the control and Abs(s) is the absorbance of standard/sample.

The IC₅₀ (inhibitory concentration) is calculated by plotting the percentage of inhibition against concentration (µg/ml)¹⁶.

Ferric Reducing Antioxidant Power (FRAP)

The reducing power assay of the HAWF was calculated by using potassium ferricyanide. Stock of 1mg/ml, extract, and standard (Ascorbic acid) was prepared in methanol. Dilution of stock was done with methanol in the concentration of 100-300 µg/ml, in which 2.5 ml of Phosphate buffer (pH-6.6, 0.2M) and 2.5 ml of 1% potassium ferricyanide was added. Shake the above solution properly and incubate it in the water bath at 50°C for 25 minutes. After incubation, the solution was allowed to cool down and then, 2.5 ml of 10% Trichloroacetic acid was added to the respective solution which will be placed in a vortex for well mixing. From the above mixture, 2.5 ml of solution was pipette out and diluted with 2.5 ml of distilled water respectively. At last 0.5 ml of 0.1%, Ferric chloride was added and then mixed. The absorbance of the solution was measured at 700nm. The blank solution contains everything except standard/extrac¹⁷.

In vitro Antidiabetic activity

α-amylase Inhibition Assay

For the determination of α-amylase inhibition assay of *W. fruticosa* initially, a stock solution of 1mg/ml, extract or acarbose was prepared in sodium phosphate buffer (0.02 M and pH 6.9, which was maintained by 0.006 M NaCl solution). The dilution of stock was done in the concentration of 100-1000 µg/ml from which 500 µl was withdrawn from each dilution tube mixed with 500 µl of α-amylase solution (13U/ml) and thus, the mixture of the tube was kept for incubation at 37°C for 30 minutes. After this, 500 µl of 1% starch solution was added to each tube and incubated at 37°C for 10 minutes. 1 ml DNSA solution was added to each tube and thus, the tube was set down for boiling at water bath for 10 min to stop the reaction. After the tubes get cooled down, 10 ml distilled water was added to the tube, and absorbance were measured at 540 nm using Phosphate buffer as blank and 100% enzyme activity solution (except sample/ standard, everything was added) as control. The percentage of inhibition for α-amylase activity was calculated by the below equation:

$$\% \text{ inhibition} = \left[1 - \frac{\text{Abs}(s)}{\text{Abs}(c)} \right] \times 100$$

Where Abs(c) is the absorbance of the control and Abs(s) is the absorbance of standard/sample. IC₅₀ represents sample concentration (µg/ml) required to decrease the activity of biological function of compound¹⁸⁻¹⁹.

α-glucosidase Inhibition assay

For the determination of the α-glucosidase inhibitory activity of plant *W. fruticosa* initially, the stock solution of 1mg/ml, of extract or acarbose was prepared in sodium phosphate buffer (100mM, pH 6.8) from which dilution was made from 100-500 µg/ml. 100 µl from each dilution tube was withdrawn in which 40 µl α-glycosidase (1U/ml) solution was added and then kept for incubation at 37 °C for 20 minutes. After preincubation, 70 µl 5mM PNPG (para-nitrophenyl- beta glucopyranoside) which was made with buffer was added and again incubation was done at 37 °C for 60 minutes. 2.5 ml 0.1 M Na₂CO₃ solution was added in each tube to stop the reaction and thus, absorbance was measured at 400 nm using Phosphate buffer as blank and 100% enzyme activity solution (except sample/ standard, everything was added) as control. The percentage of inhibition for α-glucosidase activity was calculated by the below equation:

$$\% \text{ inhibition} = \left[1 - \frac{\text{Abs}(s)}{\text{Abs}(c)} \right] \times 100$$

Where $\text{Abs}(c)$ is the absorbance of the control and $\text{Abs}(s)$ is the absorbance of standard/sample. IC_{50} represents sample concentration ($\mu\text{g/ml}$) required to decrease the activity of biological function of compound²⁰⁻²¹.

Statistical analysis

The experiment was conducted by taking each concentration in a triplicate. All values were presented as mean \pm standard deviation. Data were statistically calculated by utilizing one-way ANOVA followed by Dunnett's t-test.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical analysis showed the presence of several chemical groups like flavonoids, alkaloids, carbohydrates, tannins, glycosides, triterpenoids, and saponins in HAWF.

Determination of total phenolic content

The total phenolic content of HAWF was assessed by utilizing Folin-Ciocalteu method where this reagent is formed by the mixture of two compounds is phosphotungstic acid and phosphomolybdic acid which oxidize to blue color in presence of phenol group; the blue color is due to the reduction of acid into tungsten and molybdenum. Gallic acid was used as standard and hence, a calibration curve (Figure 1) of the standard was established. The phenolic compound content was determined as gallic acid equivalents using the following linear equation based on the calibration curve: $A = 0.0022C - 0.0143$, $R^2 = 0.9988$. A is the absorbance, and C is gallic acid equivalents (μg). The total phenolic content of the extract was expressed as mg of gallic acid equivalent per gram of dry weight of the extract. The level of total polyphenolic compounds was 671.96 ± 5.6 of gallic acid equivalent per gram of the extract.

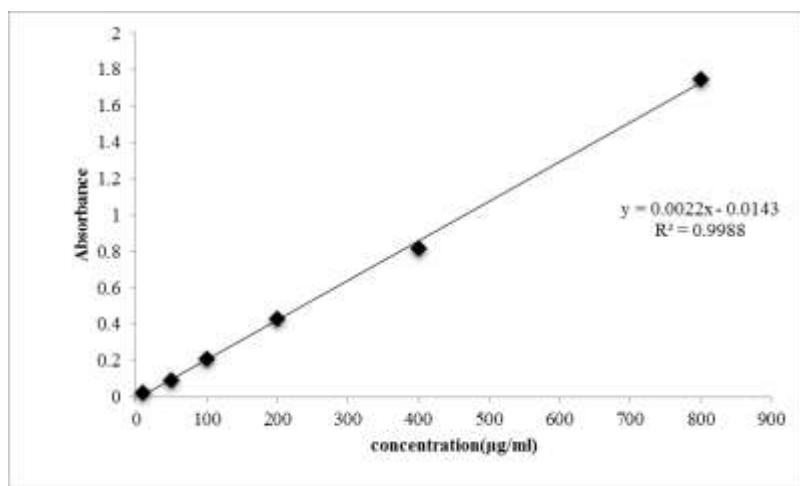


Figure 1: Calibration curve of gallic acid

Determination of total flavonoid content

The total flavonoid content of HAWF was assessed by utilizing the aluminum chloride colorimetric method, where this reagent forms a complex with flavones and flavonol which can be easily detected at 415nm in a UV spectrophotometer. Quercetin was used as a standard whose

calibration curve (Figure 2) was established. Linear equation based on the calibration curve is: $A = 0.0034C + 0.06$, $R^2 = 0.9974$. A is the absorbance, and C is quercetin equivalents ($\mu\text{g/ml}$). The total flavonoid content of the extract was found to be 113.82 ± 0.178 mg/g of quercetin equivalent per gram of dry weight.

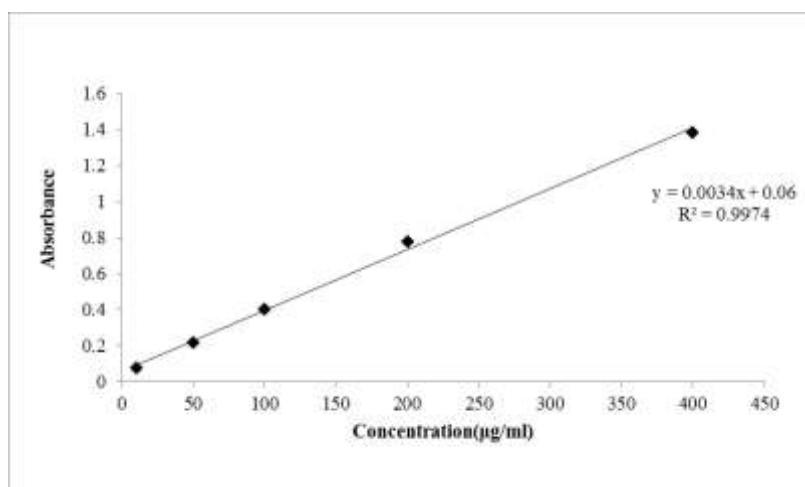


Figure 2: Calibration curve of quercetin.

Determination of *In Vitro* Antioxidant Activity

DPPH Free Radical Scavenging Activity

This assay helps in the detection of the reduction of DPPH, where DPPH is reduced to DPPH₂ due to the presence of an antioxidant compound present in the extract, and because of this, the decolorization occurs, which can be easily detected

in absorbance at 517 nm. Figure 3 represents the graph between the percentage of inhibition and concentration, which shows a constant increment in scavenging property of plant extract and reference standard i.e. Ascorbic acid. The IC₅₀ values were found to be $30.85 \pm 0.36 \mu\text{g/ml}$ and $22.35 \pm 0.54 \mu\text{g/ml}$ for HAWF and ascorbic acid respectively.

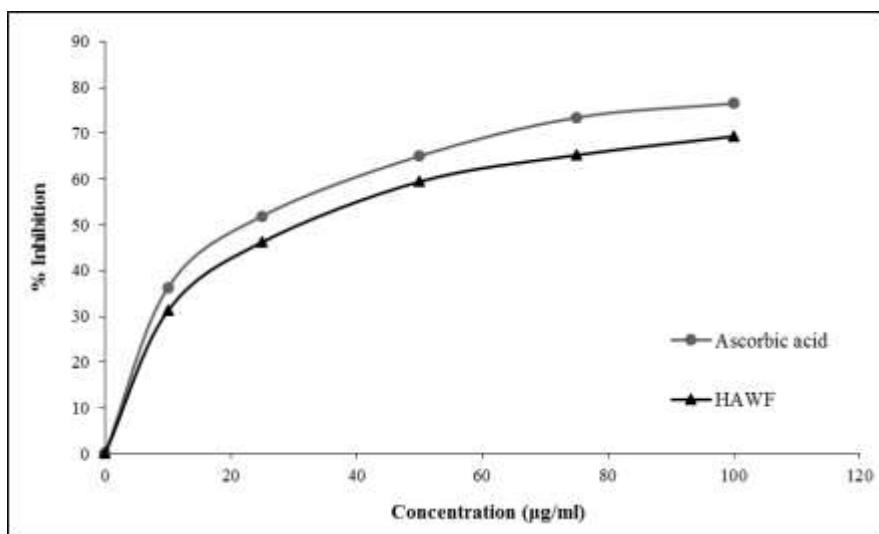


Figure 3: DPPH radical scavenging activity of hydroalcoholic extract of *W. fruticosa* and ascorbic acid.

Ferric Reducing Antioxidant Power (FRAP)

In this experiment the extract/standard changes color from pale yellow/white to greenish-blue or dark blue depending on the concentration. The color change is due to the presence of an antioxidant group present in the plant which reduces ferricyanide (Fe^{2+}) to ferrocyanide (Fe^{3+}) and when the ferric chloride was added, the ferric-ferrous complex was formed

which will be measured at 700nm. The reducing power assay of plant extract/standard increases with concentration, Figure 4 represents the reducing power assay of both standard and extract, from this figure it is concluded that the reducing power assay of HAWF is lesser than standard (Ascorbic acid) but it contains the compound which is responsible for antioxidant activity.

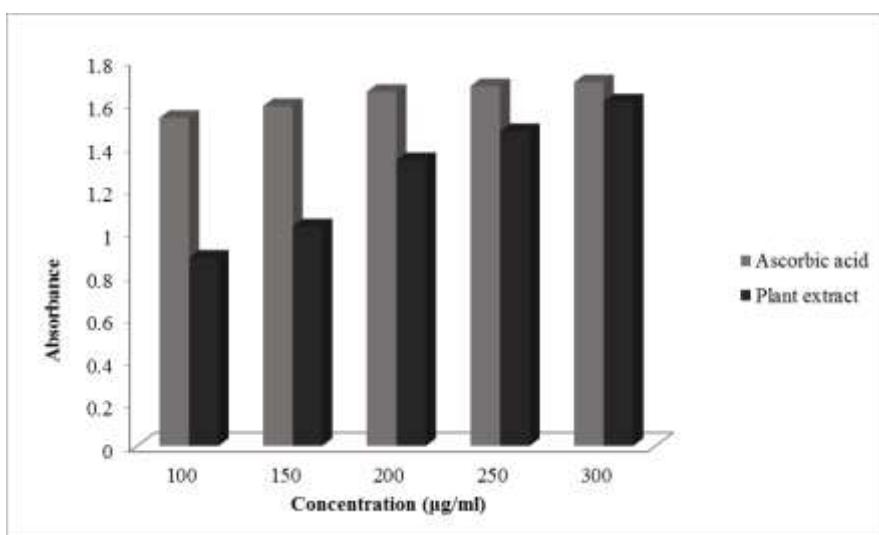


Figure 4. Reducing power assay of HAWF and ascorbic acid at different concentrations.

In vitro Antidiabetic activity

α-amylase Inhibition Assay

The α-amylase breakdown the starch and responsible for the production of glucose, hence when this enzyme activity is controlled it helps to overcome the postprandial hyperglycemia. The α-amylase inhibitory activity was

measured in HAWF and acarbose, from that it was observed that acarbose has higher activity than the extract with the increase in concentration. The anti-α-amylase activity of HAWF and acarbose is presented in Figure 5. HAWF showed significant ($p < 0.01$) anti-α-amylase activity ($\text{IC}_{50} = 64.27 \pm 1.47 \mu\text{g/ml}$) compared to standard inhibitor acarbose ($\text{IC}_{50} = 58.56 \pm 1.23$).

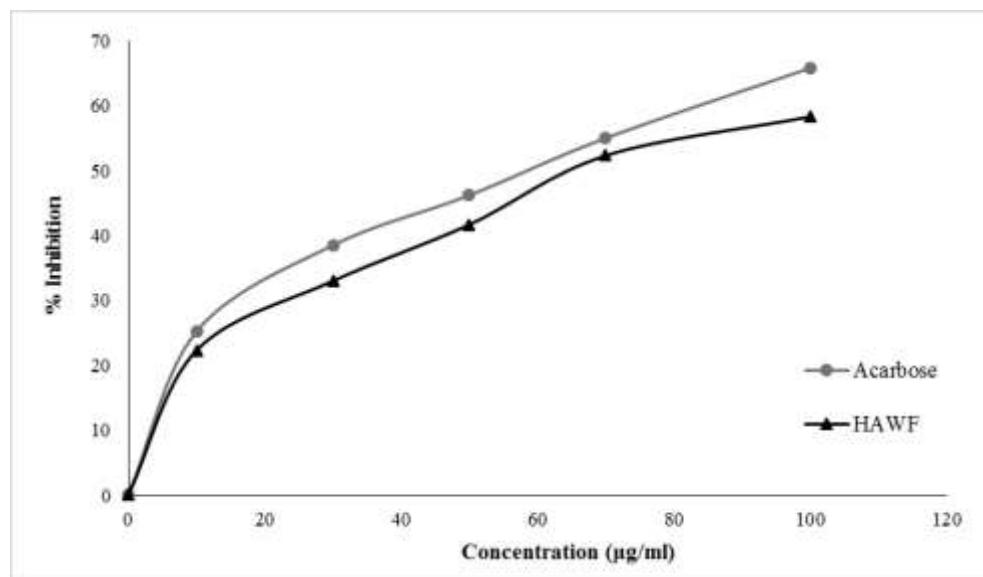


Figure 5. α -amylase inhibitory effect of hydroalcoholic extract of *W. fruticosa*.

α -glucosidase Inhibition assay

The plant contains various flavonoid groups which are considered to be responsible for inhibition of α -glucosidase activity, the glucosidase was mainly responsible for the breakdown of starch and is considered to be responsible for type 2 diabetes. Hence the HAWF was examined for this α -glucosidase activity which was found that the activity of α -

glucosidase has been reduced more as compared to the reference compound i.e acarbose with the increase in its extract concentration. The α -glucosidase inhibitory activity of HAWF and acarbose is presented in Figure 6. The α -glucosidase inhibitory activity of HAWF ($IC_{50} = 71.53 \pm 1.37 \mu\text{g/ml}$) was significant ($p < 0.01$) compared to standard inhibitor acarbose ($IC_{50} = 51.96 \pm 0.87$).

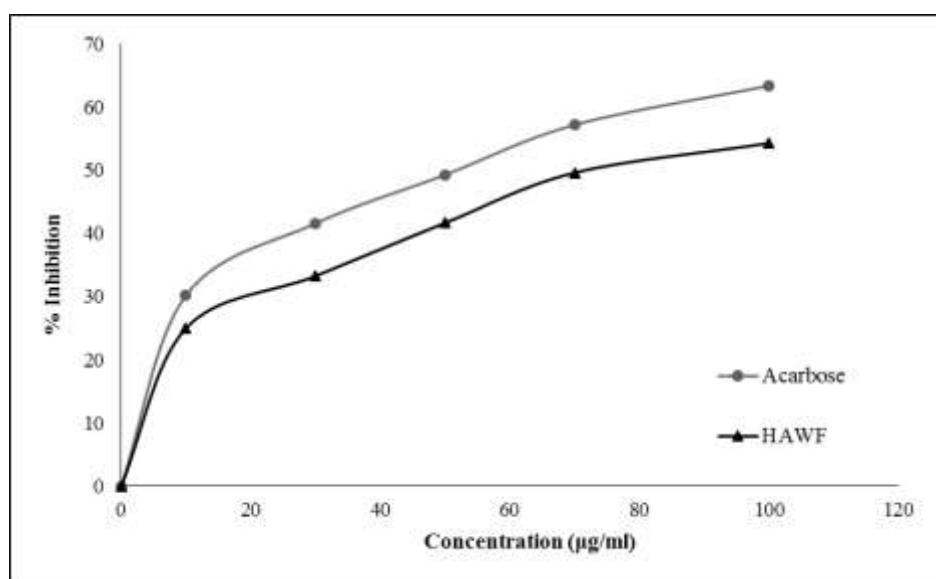


Figure 6. α -glucosidase inhibitory effect results of hydroalcoholic extract of *W. fruticosa*.

CONCLUSION

Phytochemical investigation *W. fruticosa* flower confirmed the presence of flavonoid, alkaloid, carbohydrate, tannin, glycoside, and triterpenoid compounds. In this experiment, it was found there is the presence of flavonoid groups and phenolic group of compounds in the flower of *W. fruticosa*. The presence of these compounds are responsible for the entrapment of free radical and act as a natural antioxidant as well as have antidiabetic activity of the flower of *W. fruticosa*. This evaluation helps in the investigation of compounds that may help combat many harmful diseases.

HAWF showed potent antioxidant activity by inhibiting DPPH. The reducing power of the extract increased with the increasing amount of sample. In addition, the extract was found to contain a significant amount of total phenols and flavonoids, which play a major role as antioxidants. It also showed effective antidiabetic activity by inhibiting α -amylase and α -glucosidase. Thus the α -amylase and α -glucosidase inhibitory activity may be due to the presence of polyphenolics in the extract which have been identified earlier.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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