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Monochoria hastata (L.) Solms Leaf Extract- Evaluation for Its Antioxidant and Anti-Diabetic Activity

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ABSTRACT

The rural area of India still depends upon medicinal plants for management of various diseases. The world has also started to adapt with traditional medical plant knowledge and the market of herbal formulation has increased manifold in recent times. The current study deals with evaluation of antioxidant and anti-diabetic potential of *Monochoria hastata* (L.) Solms leaf extract. *Monochoria hastata* (L.) Solms is a native tree of Assam, India, which is known as *Bhat-meteka* in local language (Assamese). Methanol, chloroform and hexane extract is used to estimate the polyphenol content. The methanolic extract showed highest phenolic content [(83.47 ± 0.18) mg GAE/g of dry material]. The flavonoid content [(62.39 ± 0.57) 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 6.1 ± 0.24 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 7.4 ± 0.18 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 *M. hastata* showed better α-amylase inhibition (19.63 ± 0.45 µg/ml) and α-glucosidase inhibition (IC_{50} of 14.29 ± 0.85 µg/ml) and was comparable to standard drug acarbose (IC_{50} of 17.21 ± 0.24 µg/ml). The results thus provide a newer insight into the use of *M. hastata* leaves for its antioxidant and anti-diabetic property.

INTRODUCTION

India is land of traditional medicines and treatments. The Indian history of treating ailments with medicinal plants can be traced back to centuries. The rural area of India still depends upon medicinal plants for management of various diseases (1). The world has also started to adapt with traditional medical plant knowledge and the market of herbal formulation has increased manifold in recent times. As estimated, the global market share of medical supplies and functional health foods is around 1 trillion and half of the medical supplies on sale are botanical drugs or chemicals obtained from isolation of plant extracts. Tamiflu is a well known antiviral drug which is extracted from star anise, a Chinese herbal medicine (2).

Monochoria hastata (L.) Solms a local tree of Assam, India, which is known as *Bhat-meteka* in local Assamese language. They grow in aquatic land and are also known as arrowleaf pondweed. These are approximately 0.7 - 1.2 meter long. This plant is used as vegetable by the local people, mainly the young shoots. The leaf juice can cure boils when consumed with honey (3). The leaves and roots are also used as digestive and to maintain the uterine tone (4). The previous reports suggest that *M. hastata* (L.) Solms extract poses stigmasterol and it also has gastropathy, hepatopathy and anti lipoxygenase property (5). Following is the taxonomical classification of *M. hastata* (L.) Solms:

Kingdom: Plantae

Family: Pontederiaceae

Genus: *Monochoria*

Species: *M. hastata* (L.) Solms

The irregularities in insulin level can be a reason for diabetes mellitus (DM), which is illustrated by high blood sugar level than the normal in the body thus causing either type I or type II DM and gestational DM (6). As per the report of WHO, low and middle income countries are most affected with this lifestyle disease and the worldwide count for the people suffering from DM is around 422 million people (7). The mortality count is also higher and stands at 1.6 million deaths per year due to DM. DM is a chronic disorder which causes complications like kidney failure, leg amputation due to foot ulcer, diabetic retinopathy, heart attack and strokes (8). Patients suffering from DM basically depend upon insulin treatment

and since the low income people are most affected, therefore a low cost treatment regime is need of the hour. The use of herbal medicine has come as an alternate treatment for the management of DM and researchers are investigating various medicinal plants for a potent anti-diabetic herbal formulation (9). Korean FDA has already approved the use of Ginseng and Banaba extracts to be used for the management of DM (10). The phytoconstituents present in the extracts of plants are facilitating this activity.

Antioxidant therapy for the management of DM in patients is mainly attributed to the capability of antioxidants to neutralize the ROS (reactive oxygen species) produced due to oxidative stress. The oxidative stress results in increased insulin resistance by adipocytokine dysregultion thus insulin regulation in the body gets imbalanced causing DM. The level of biomarkers like protein carbonyl, malondialdehyde and isoprostanes etc in oxidative stress also increases in DM patients. 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 (11, 12). 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. The current study is done to analyze its antioxidant activity and to ascertain its antidiabetic activity.

MATERIALS AND METHODS

Collection of plant and extraction

Fresh leaves of *M. hastate* were collected from Baihata Chariali, Assam, India. The leaves were dried under shade and made into fine powder. Soxhlet apparatus with hot extraction method was used for the extraction process. Dried powdered plant was packed into soxhlet apparatus and the solvents were used according to their increasing polarity i.e hexane, chloroform and methanol. Rotary evaporator (IKA RV 10) was used to dry the extracts under reduced pressure (13). 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. 1853).

Estimation of total phenolic content

Total phenolic content of the leaves of *M. hastata* was determined by the Folin-Ciocalteu reagent method. 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 0.5 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. UV Visible spectrophotometer (Shimadzu 1800) was used to measure the absorbance at 765 nm (13).

Estimation of total flavonoid content

The total flavonoid content of the leaves of *M. hastate* 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 standstill for 40 minutes at room temperature. The absorbance of the supernatant liquid was measured in 415nm (13).

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

The scavenging capacity of leaves of *M. hastata* was determined by mixing 1.0 ml of DPPH solution (25 mg/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 quenching of DPPH radicals by the extracts will be evaluated. The absorbance of each test tube was 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 (14).

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

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

ABTS scavenging capacity was determined by the method described by Youn *et al*. 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 (15). The percentage inhibition was measured using equation 1.

Alpha-amylase inhibition assay

The *in-vitro* anti-diabetic activity of *M. hastata* was determined by the 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 concentrations 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 (16).

Alpha-glucosidase inhibition assay

The *in-vitro* anti-diabetic activity of *M. hastata* was also determined by 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 minutes. 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 (17).

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

The antioxidant activity of *M. hastata* can be established by the estimation of total phenolic and flavonoid content in the *M. hastata* extracts which shows the capacity of the extracts to scavenge the free radicals produced due to oxidative stress. 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 = 0.9991) 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 [(83.47 ± 0.18) mg GAE/g of dry material] than the choloroform extract. The flavonoid content [(62.39 ± 0.57) mg QE/g of dry material] in the methanolic extract was also highest when compared to chloroform extract. The polyphenolic compound content in the hexane extract was nominal. 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 *M. hastata* proves that it has antioxidant property and can readily scavenge the free radicals (18). Table No.1 and Figure No.1 shows the total phenolic content and total flavonoid content of different extracts of *M. hastata*.

Table No. 1: Estimation of total phenolic and flavonoid content of *M. hastata*

Extracts	Total phenolic content (GAE mg/g dry extract)	Total flavonoid content (QAE mg/g dry extract)
Methanol	83.47 ± 0.18	62.39 ± 0.57
Chloroform	45.63 ± 0.26	32.87 ± 0.45
Hexane	26.69 ± 0.41	19.79 ± 0.59

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

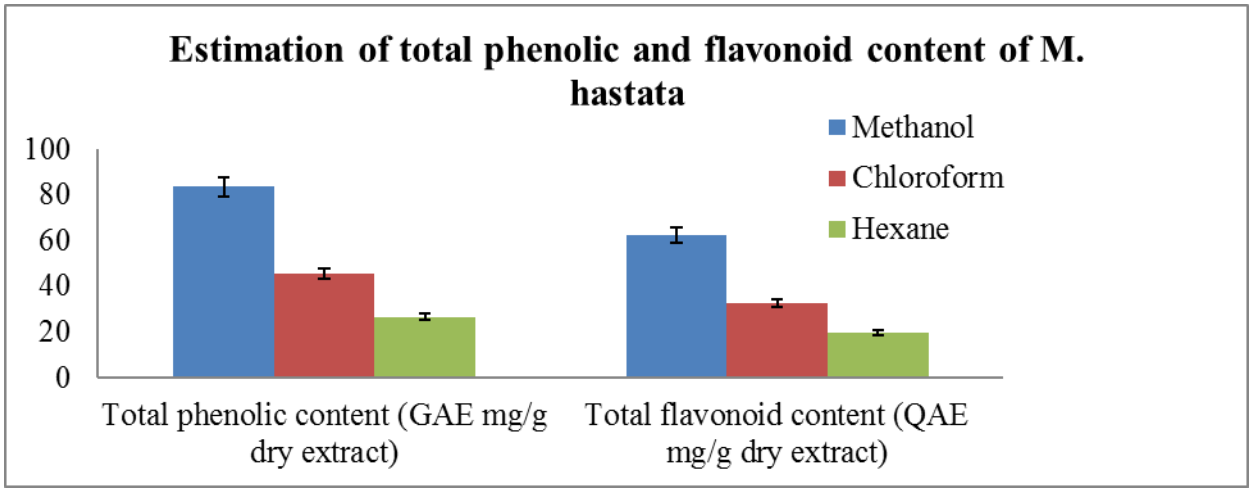

Figure No. 1: Estimation of total phenolic and flavonoid content of *M. hastata*

DPPH free radical scavenging activity

DPPH scavenging capacity was examined for all the extracts. The capacity of *M. hastata*to scavenge the free radicals was determined to evaluate the antioxidant property. 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 5.5 ± 0.51 µg/ml. The methanol extract was showing IC_{50} of 6.1 ± 0.24 µg/ml (the result is comparable to standard drug), followed by choloroform with IC_{50} of 19.6 ± 0.41 µg/ml, and hexane extract with IC_{50} of 35.7 ± 0.22 µg/ml. 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). There was a strong scavenging capacity seen in the methanolic extract as compared to choloroform and hexane extracts (Table No. 2 and Figure No. 2). The DPPH free radicals are quenched by the antioxidant constituents of *M. hastata*. This was due to the hydrogen donating ability of the polyphenolic compounds present in the extracts of *M. hastata leaves* (19).

ABTS free radical scavenging activity

The all leaves extracts of *M. hastata* 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 7.4 ± 0.18 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 32.63 ± 0.47µg/ml and 57.54 ± 0.31µg/ml respectively (Table No.2 and Figure No.2). The results were satisfactory and thus further confirm the presence of antioxidant phytoconstituents in *M. hastata* (20).

Table No. 2: Antioxidant activity of *M. hastata*

Extracts/Standard	IC_{50} (µg/ml)	
	DPPH assay	ABTS assay
Methanol	6.1 ± 0.24	7.4 ± 0.18
Chloroform	19.6 ± 0.41	32.63 ± 0.47
Hexane	35.7 ± 0.22	57.54 ± 0.31
Ascorbic acid	5.5 ± 0.51	

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

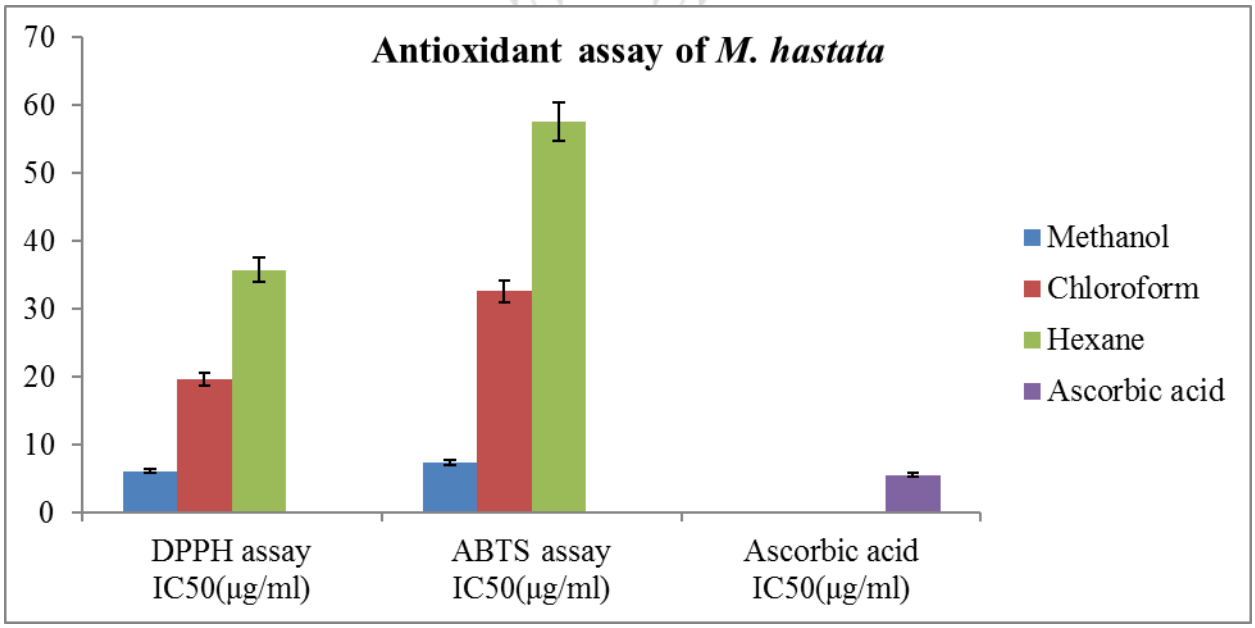

Figure No. 2: Antioxidant activity of *M. hastata*

Alpha-amylase inhibition assay

Alpha-Amylase inhibition assay model was used to evaluate the anti-diabetic capacity of *M. hastata*. All three extracts of the plant leaves of *M. hastata* were investigated and the results

were satisfactory. The dose-dependent increase is evident in the percentage inhibition capacity of *M. hastata*. Acarbose was used as the standard drug which showed IC_{50} value of 17.21 ± 0.24 µg/ml. The methanolic extract of *M. hastata* showed IC_{50} of 19.63 ± 0.45 µg/ml. The chloroform and hexane extract showed IC_{50} of 56.28 ± 0.69 µg/ml and 97.24 ± 0.33 µg/ml respectively (Table No.3 and Figure No.3). The results clearly establish that methanolic extract showed similar scavenging capacity in comparison to the standard drug. The scavenging capacity of chloroform and hexane extract was not satisfactory as evident from the results. 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 (21). The presence of polar compounds in the methanolic extract may attribute to the anti-diabetic property of *M. hastata.*

Alpha-glucosidase inhibition assay

The *in-vitro* anti-diabetic property was also investigated using 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 methanolic extract of *M. hastata* was able to inhibit the α-glucosidase enzyme as evident by the results showing IC_{50} value of 14.29 ± 0.85 µg/ml. The chloroform and hexane extract of *M. hastata* showed IC_{50} value of 51.28 ± 0.43 µg/ml and 93.36 ± 0.37 µg/ml respectively (Table No.3 and Figure No.3). 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 postprandial 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 (22). The anti-diabetic property of methanolic extract of *M. hastata* can be ascribed by the presence of carbohydrates in the extract.

Table No. 3: Anti-diabetic activity of *M. hastata*

Extracts/Standards	IC_{50} (µg/ml)	
	α-amylase assay	α-glucosidase assay
Methanol	19.63 ± 0.45	14.29 ± 0.85
Chloroform	56.28 ± 0.69	51.28 ± 0.43
Hexane	97.24 ± 0.33	93.36 ± 0.37
Acarbose	17.21 ± 0.24	

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

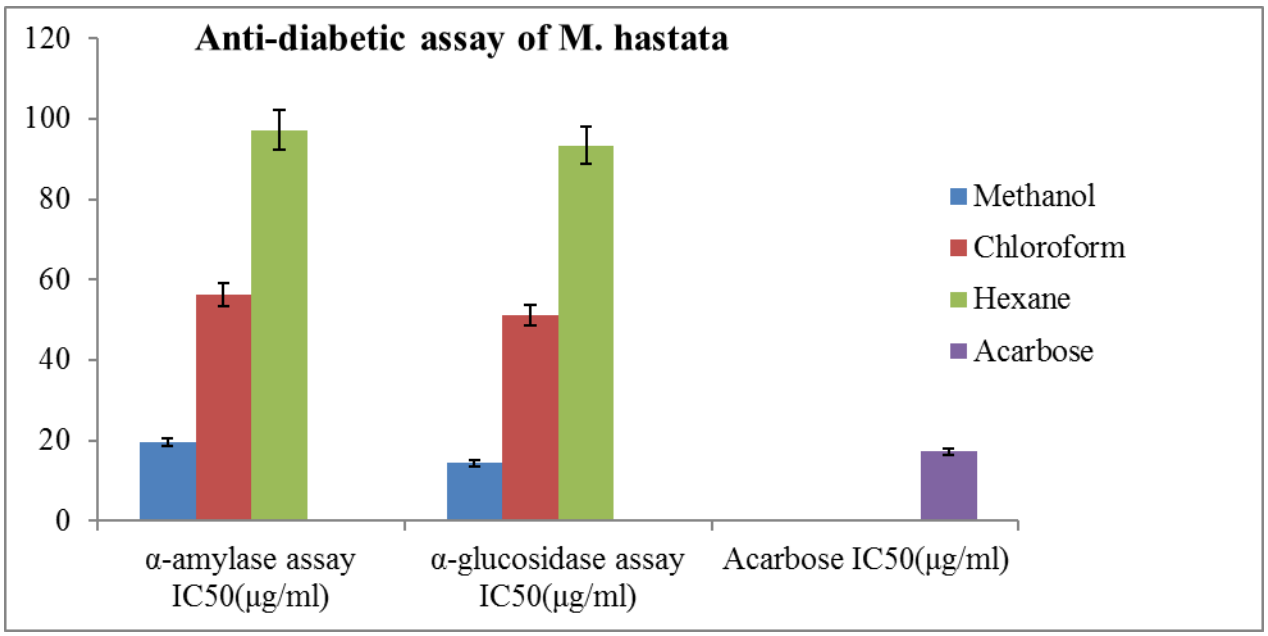

Figure No. 3: Anti-diabetic activity of *M. hastata*

CONCLUSION

The current study on *M. hastata* was done to evaluate its antioxidant property and anti-diabetic property. The antioxidant capacity was estimated and higher phenolic content and higher flavonoid content ascertained that leaves of *M. hastata* can be a significant source of herbal antioxidant. The DPPH radical scavenging assay and ABTS radical scavenging assay was done to estimate the antioxidant property and the capability of leaves of *M. hastata* to quench the DPPH radical and ABTS radical establish that the leaves of *M. hastata* have the capacity to scavenge the free radicals and might have antioxidant property. 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 *M. hastata* showed good results. With consideration of the all the results obtained thus propose that leaves of *M. hasata* can be a potent candidate to act as anti-diabetic herbal plant but further *in-vitro* and *in-vivo* studies are needed to be executed for incorporating *M. hasata* in herbal formulations or nutraceuticals.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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