

Physicochemical, antidiarrheal and antidiabetic potential of super food (*Moringa oleifera* Lam.)

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Abstract: There is a long history of natural products for the treatment of infections and diseases. The objective of present study was to investigate the organoleptic, microscopic, physico-chemical, phytochemical, antidiarrheal and antidiabetic potential of leaf, flowering bud and stem bark of *Moringa oleifera* L. Macroscopic, microscopic, physico-chemical parameters and phytochemical screening were carried out. Diarrhea was induced with castor oil (10ml/kg), verapamil (3, 10 and 30mg/kg) were used as standard antidiarrheal drug and extract of *Moringa oleifera* at (100, 300 and 1000mg/kg) was used for treatment. Alpha glucosidase inhibitory assay was carried out by using acarbose (0.5mM) and extracts (5.0 mg/ml). Diabetes was induced by alloxan (150mg/kg), while glibenclamide (10mg/kg) was used as standard drug, and extracts (at the doses of 500mg/kg) were used to determine the antidiabetic activity. Results showed the presence of primary and secondary metabolites, treatment at the dose of 1.0g/kg of leaf, flowering bud and stem bark showed 94 ±2.527, 85.42±5.460 and 84.58±6.138% protection respectively whereas verapamil (10mg/kg) showed 94.84±3.27% protection. Alpha glucosidase inhibition of stem bark (0.5mg/ml) was 95.43±1.47 and flowering bud 94.78±1.25 whereas acarbose (5mM) inhibition was 92.23±0.14%. Stem bark and flowering bud extract (500mg/kg) decreases the blood glucose level from 388.5±35.83 to 226.3±47.10 and 322.5±48.35 to 173.8±29.5 respectively whereas glibenclamide (10 mg/kg) decreases the blood glucose level from 320.7±22.9 to 146.3±17.7 and increases the body weight of the experimental animal. It was concluded from the results that stem bark has strong antidiabetic potential while leaves of the plant have promising antidiarrheal effect.

Keywords: *Moringa oleifera*, standardization, anti-diarrheal, alpha glucosidase.

INTRODUCTION

Natural products particularly plants are being used as alternative medicine and their active constituents are used for the source of food, means of transportation, fertilizers, Clothing, treatment of infectious diseases and dart poison but still many species of plants kingdom are needs to be examined thoroughly for their possible pharmacological values (Abbas *et al.*, 2016, Salim *et al.*, 2008). Use of medicinal plants is growing day by day for alleviating diseases due to fewer side effects, as new life threatening diseases are emerging and existing drugs are not working against these emerging diseases (Manik *et al.*, 2013). Human being are better treated with herbal medicines as compared to pure isolated compound (Yuan *et al.*, 2016). Medicinal substances obtained from the plants are used in medicine in many forms either as single purified drug or in advanced extract form in combination with other

ingredients (Rashid *et al.*, 2021). Due to unhygienic livelihood condition, peoples of African and South-East Asian regions are very prone to several common diseases like diarrhea in which the normal bowel movement and frequency of the stool changed because of infections by various types of bacteria, virus, and parasites. Some drugs are used for the treatment of diarrhea, but these drugs show adverse effects and microorganisms are tend to develop resistance towards them (Tadesse *et al.*, 2017). The use of herbal drugs in the treatment of diarrhea is a common practice in many African and South-East Asian countries (Akuodor *et al.*, 2011).

Diabetes mellitus is a common health problem worldwide with rapid increase in prevalence having impact on health, quality of life, and on health care system. World Health Organization (WHO) has emphasized the assessment of traditional plant for the treatments of diabetes as they are less toxic, effective, less side effects and good choice for oral therapy (Sundaram *et al.*, 2020; Khan *et al.*, 2020).

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Therefore, the search for more effective and safe agents from natural sources especially from plant origin has become important area of dynamic research. At present, around 25% of total available drugs are isolated from plants and there are numerous evidence available about the use of medicinal plants for their pharmacological and biochemical properties (Rahman *et al.*, 2015).

Moringa oleifera Lam. is a perennial softwood medicinal plant belongs to family Moringaceae, consists of 13 species, widely distributed in northern India, Pakistan and Nepal (Nasir *et al.*, 2016, Urva *et al.*, 2017, Vasanth *et al.*, 2014), commonly known as "Miracle Tree, Horseradish tree, Mother's Best Friend, Radish tree, Drumstick tree in English, Sohanjna in local language of Pakistan, used for centuries as traditional medicine as well as for industrial purpose (Raja *et al.*, 2016). It contains considerable quantity of cytokinins, auxins, phenolic, Ca, K, Zn and iron (Fe) (Nasir *et al.*, 2016), sitosterol, caffeoylquinic acid, quercetin, kaempferol (Vasanth *et al.*, 2014), methionine, cystine, tryptophan, lysine, β -carotene, protein, ascorbic acid, benzyl isothiocyanate flavonoids and carotenoids (Urva *et al.*, 2017), used for cardiovascular diseases, regulate blood sugar and cholesterol level (Vasanth *et al.*, 2014), treated arthritis, rheumatism, hypertension (Urva *et al.*, 2017), having anti-gout, antiseptic, hypo-cholesterolemic, immune modulating property, used as anti-proliferative agent, fresh vegetable, livestock fodder, green manure, biogas and bio pesticide (Adeniyi *et al.*, 2021) (Padayachee and Baijnath, 2020).

The aim of present research work is to carry out the standardization, anti-diarrheal (*in-vivo*) activity, α -glucosidase enzyme inhibition and anti-diabetic activity of flowering buds, leaves and stem bark.

MATERIALS AND METHODS

Collection and extraction of plant material

Moringa oleifera L. was collected from Dera Ismail Khan (Pakistan) in the month of February 2016, identified by Prof. Dr. Qazi Najm-us-Saqib (CIIT Abbottabad) with voucher specimen No 210/2016 CIIT and specimens of plant were deposited in COMSATS Herbarium for future reference. The fresh leaves, flowering buds and stem bark of *Moringa oleifera* L. were cleaned and washed to eradicate foreign material and dust particles. The plant parts were dried in shade and grinded to coarse powder with the help of pestle and mortar. 500.00 g of each plant part was extracted with methanol three times successively for seven days at room temperature then squeezed with muslin cloth and filtered. It was evaporated by using Vacuum Rotary Evaporator (Rotavapor R-200, Buchi) under reduced pressure (0.07 MPA) at 40°C. Dried extract of 18.0 g leaf, 15.0 g flowering bud and 20.0 g stem bark were then stored in light resistant well closed container (Chemat *et al.*, 2020).

Macro-morphological and physico-chemical evaluation

The *Moringa oleifera* L. (leaf, flowering buds and stem bark) were subjected to macroscopic studies and analysis of physicochemical constants of these parts of plants were performed according to the standards guidelines (Barbhuiya *et al.*, 2021, Dash *et al.*, 2021) (Dev *et al.*, 2021).

Microscopic evaluation

Fresh *Moringa oleifera* L. leaf and stem bark sections were cut with the help of sharp blade manually. Several permanent and temporary mounts of the microscopic sections of the leaf and bark specimen made and were examined under microscope (Binocular Zoom Light Microscope, Model AxL, LABO, Germany) and photographs of the microscopic sections were taken with the help of digital camera (Fatima *et al.*, 2020).

Phytochemical screening

The qualitative phytochemical tests were carried out for the identification of diversified phyto-constituent present in the powdered crude drug by using the standard protocol (Olayinka *et al.*, 2021).

Preparation of animals

Laboratory bred normal male Balb^c albino mice weighing 22-30g were used. The animals were maintained under standardized animal housing conditions (room temperature with light/dark cycle of 12hrs.) with indefinite approach to water and standard diet during the study period. Experiments on animals were performed according to the institutional guidelines. The experimental protocol was approved by Institutional Animal Ethics Committee of COMSAT Institute of Information Technology Abbotabad wide letter No IAEC/ COMSAT/ 1622/19.

Anti-diarrheal activity

Anti-diarrheal potential of the crude methanolic extract of leaf, flowering bud and stem bark was investigated by castor oil induced diarrhea in mice according to the standard method described by (Shoba *et al.*, 2001) with some modifications. The experimental animals Balb^c albino mice fasted for 18 hrs, were initially screened with 10ml/kg orally of castor oil and the animals showing diarrhea were selected for the experiment. The animals were divided into fourteen groups, each containing six animals. Group I (control group) and was given normal saline (10ml/kg). Group II (negative control) and was given castor oil (10ml/kg). Group III, IV and V which served as positive control were administered verapamil at the dose of 3, 10 and 30mg/kg respectively. The test groups (Group VI, VII, VIII) received the extracts of the leaves, (Group IX, X, XI) flowering buds and (Group XII, XIII, XIV) stem bark at the dose of 100, 300 and 1000 mg/kg respectively. The mice were placed into cages and the floor of the cage was lined with blotting paper and

floor lining was changed every hour. During an observation period of 4 h, the total numbers of diarrheic faeces excreted by the animals were recorded. The activity of each group was expressed as percent protection (%) of diarrhea and was calculated as follows:

% protection of defecation calculated based on number of dry feces in each cage comparison to wet. i.e., $[(A-B)/A] \times 100$ (Shoba and Thomas, 2001) (Mapesa *et al.*, 2021). Where A indicated mean number of defecations caused by castor oil; B indicated mean number of defecations caused by drug or extract.

Anti- α -glucosidase activity

α -glucosidase inhibitory effect of leaf, flowering bud and stem bark at concentration of 5mg/mL were carried out in comparison to standard drug acarbose at concentration of 0.5mM in triplicates and IC_{50} (μ g/mL) were found out by using the standard protocol (Bljajić *et al.*, 2021).

Induction of diabetes

Animals were fasted overnight to make them susceptible to develop diabetes then alloxan monohydrate (150 mg/kg of body weight) dissolved in 0.9% sodium chloride, and was administered intraperitoneal to the male Swiss albino mice. After 72 hr mice with diabetes mellitus was indicated by hyperglycemia with blood glucose range of 200 to 400 mg/dl (Solikhah *et al.*, 2020).

Experimental design

In vivo antidiabetic activity was performed by (Solikhah *et al.*, 2020) (Jain and Sanjay, 2010) model with slight modification. Animals were divided into seven groups of six mice each. Standard pellet diet and water were provided to the animals. Group I (Normal group) receiving only vehicle, Group II (Diabetic control group), Group III is the diabetic group receiving single dose of glibenclamide at the dose of (10 mg/kg of body weight) orally daily for 15 days, Group IV and VI is normal mice group receiving a single dose of bark and bud extract at the dose of (500 mg/kg of body weight) daily for 15 days orally. Group V and VII are Diabetic groups receiving a single dose of bark and bud extract at the dose of (500 mg/kg body weight) orally daily for 15 days. Blood glucose levels were measured by using blood glucose test strip NIPRO monitoring system before the treatment and on the 3rd, 6th, 9th, 12th and 15th day on fasting mice after collecting the blood by amputation of the tail tip under mild anesthesia. Body weights of all experimental mice were recorded at the start and on the 0th, 7th and 15th day of the experiment (Solikhah *et al.*, 2020).

STATISTICAL ANALYSIS

Values were recorded as Mean \pm standard error of the mean. Statistical difference between the means was determined as $P < 0.05$ (one-way ANOVA, Dunnett's t-test, Graph pad prism software version 8.0.1(244)).

RESULTS

Results of macroscopic evaluation of leaf, flowering bud and stem bark are presented in table 1, whereas physicochemical parameters of leaf, flowering bud and stem bark powder are shown in table 2. Preliminary phytochemical screening for the presence of primary and secondary metabolites in leaf, flowering bud and bark powder are shown in table 3. Table 4 showed the Anti-diarrheal potential of leaf, bud and bark whereas alpha glucosidase inhibitory assay is presented in table 5 and effect of bark and bud methanolic extract effect on blood glucose level is shown in table 6. Transverse section of stem bark is shown in fig. 1 while fig. 2 shows the transverse section of leaf. Effect of stem bark and flowering bud on blood glucose level of diabetic mice is shown in fig. 3 and 4 respectively. Fig. 5 showed the effect of different extract on body weight of diabetic rats.

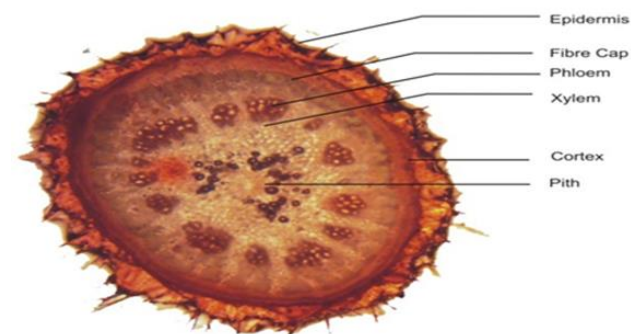


Fig. 1: Transverse Section of stem bark of *Moringa oleifera* L.

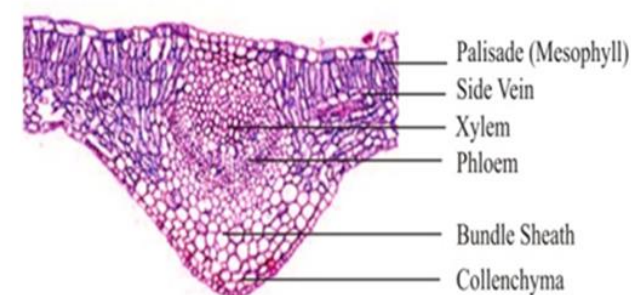


Fig. 2: Transverse section of *Moringa oleifera* L. leaf

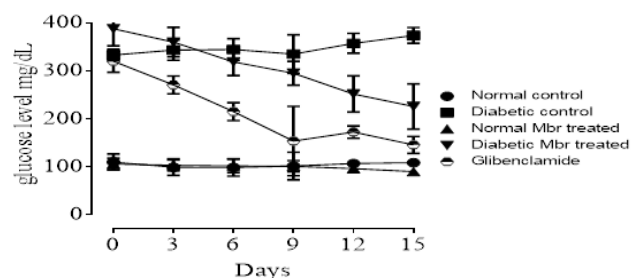


Fig. 3: Effect of stem bark on blood glucose level of diabetic mice.

Table 1: Macro-morphological study of leaf, bud and bark of *Moringa oleifera* L.

Sr. No	Character	Observation		
		Leaf	Bud	Bark
1.	Color	Upper green /lower light green	Yellowish petal	Grey/ Dark green
2.	Odor	Characteristic	Pleasant	characteristic
3.	Taste	Sour	Bitterent	None
4.	Length	2-2.5cm	1-1.5 cm	varies
5.	Broad/Thickness	-	2cm	2-4 cm
6.	Shape	Elliptical /Obovate	Campanulate	Irregular
7.	Texture	Herbaceous	Bisexual	Corcky light in weight
8.	Fracture	-	-	Deeply fissured
9.	Inner Surface	-	-	Smooth/cream color
10.	Outer Surface	-	-	Rough

Table 2: Physicochemical parameters of leaf, bud and bark powder of *Moringa oleifera* L.

Sr. No	Parameters	Leaf Powder	Bud Powder	Bark Powder
1.	Moisture constant (LOD) (% w/w)	28±0.12	16±0.23	6.5 ± 0.22%
2.	Total ash (% w/w)	16.6±0.2	11±0.31	9.2 ± 0.15%
3.	Acid insoluble ash (% w/w)	8±0.11	7.4±0.11	1.5 ± 0.21%
4.	Water soluble ash (% w/w)	4±0.05	3.7±0.04	2.5 ± 0.11%
5.	Alcohol soluble extractive values (% w/w)	25±0.4	21±0.3	9.4 ± 0.21%

Table 3: Preliminary phytochemical analysis of leaf, bud and bark powder of *Moringa oleifera* L.

Sr. No	Test	Leaf	Buds	Bark
1.	Carbohydrates	++	++	+
2.	Proteins	++	++	-
3.	Glycosides	++	+	++
4.	Flavonoids	+	++	-
5.	Volatile oil	+	+	-
6.	Tannins	-	+	++
7.	Saponin	+	+	-
8.	Alkaloids	++	++	-
9.	Terpenoids	++	+	-
10.	Steroids	++	++	++

*Where +: Present in low concentration, ++: Present in high concentration, -: Absent

Table 4: Anti-diarrheal activity of leaf, bud and bark of *Moringa oleifera* L.

Sample	Doses	No of Dry feces in 4 hrs	No of wet feces in 4 hrs	Total no of feces in 4 hrs	Protection n %	Protection % Mean ±S.D.
Normal saline	10 ml/kg	3,6,4,6,7,3=29	0,0,0,0,1=1	3,6,4,6,7,4=30	96.6	94.45±5.55
Castor Oil	10 ml/kg	0,0,1,0,0,0 = 1	3,4,3,3,4,3 = 22	3,4,4,3,5,4 = 23	0.95	5.5±5.5
Leaves	100 mg/kg	5,4,6,3,7,5= 30	2,3,1,2,0,1= 09	7,7,7,5,7,6=39	77	76.27±6.724
	300 mg/kg	8,6,6,9,8,5=42	1,2,1,0,2,3=09	9,8,7,9,10,8=51	82	82.223±5.22
	1000 mg/kg	10,9,11,9,8,7=54	0,1,0,0,1,1=03	10,10,11,10,9,8= 57	94	94 ±2.527
Buds	100 mg/kg	3,2,4,5,2,3=19	2,3,1,2,3,4=15	5,5,5,7,5,7=34	56	55.71±7.104
	300 mg/kg	5,3,4,2,4,3=21	2,1,3,2,4,2=14	7,4,7,4,8,5=35	60	60.59±4.326
	1000 mg/kg	7,4,5,5,6,4=31	1,2,0,1,2,0=06	8,6,5,6,8,4=37	83	85.42±5.460
Bark	100 mg/kg	2,3,4,4,1,3=17	3,1,1,2,2,1=10	5,4,5,6,3,4=27	62	61.67±8.143
	300 mg/kg	3,4,3,5,2,4=21	1,2,1,1,1,1=07	4,6,4,6,3,5=28	75	73.61±2.562
	1000 mg/kg	4,5,3,2,7,4=25	1,0,2,0,1,1=05	5,5,5,2,8,5=30	83	84.58±6.138
Verapamil	3 mg/kg	6,6,5,7,6,6=36	3,2,3,2,2,2=14	9,8,8,9,8,8=50	72	71.99±2.4
	10 mg/kg	5,4,6,5,5,5=30	0,0,1,0,1,0=2	5,4,7,5,6,5=32	94	94.84±3.27
	30 mg/kg	3,4,5,6,5,5=27	0,1,0,0,0,0=1	3,5,5,6,5,5=28	96	96.67±3.33

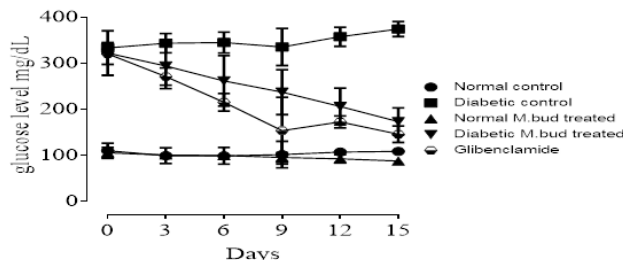
Table 5: α -glucosidase inhibition activity of leaf, bark and bud of *Moringa oleifera* L.

S. No	Sample	% Inhibition	IC ₅₀ (μ g/mL)
1	Leaf (0.5 mg/ml)	12.34	-
2	Bark (0.5 mg/ml)	95.43 \pm 1.47	95.38 \pm 0.57
3	Bud (0.5 mg/ml)	94.78 \pm 1.25	43.28 \pm 0.68
4	Acarbose (0.5mM)	92.23 \pm 0.14%	38.25 \pm 0.12

Table 6: Effect of bark and flowering bud on blood glucose level *Moringa oleifera* L.

Groups	Zero day	3 rd day	6 th day	9 th day	12 th day	15 th day	% variance
Normal	110.5 \pm 16.4	99.83 \pm 17.2	99.33 \pm 18.1	102.2 \pm 28.8	107.5 \pm 3.5	109.2 \pm 2.9	43.61%
Diabetic	333.7 \pm 12.0	344.0 \pm 20.9	345.3 \pm 22.7	335.8 \pm 40.3	358.0 \pm 20.7	374.7 \pm 16.3**	4.42%
N+ Br treated	106.5 \pm 12.06	103.7 \pm 11.48	102.5 \pm 13.96	101.7 \pm 11.64	96.83 \pm 6.494	90.67 \pm 7.257	5.67%
D+ Br treated	388.5 \pm 35.83	360.8 \pm 30.73	319.3 \pm 28.54	295.8 \pm 24.98	252.5 \pm 37.78	226.3 \pm 47.10	20.22%
N + bud treated	106.2 \pm 11.23	101.2 \pm 8.08	100.2 \pm 8.704	95.83 \pm 10.23	93.00 \pm 9.121	88.17 \pm 5.70**	6.60%
D + bud treated	322.5 \pm 48.35	294.3 \pm 49.25	262.0 \pm 54.88	237.7 \pm 48.76	206.5 \pm 40.01	173.8 \pm 29.5**	22.11%
D + Glb	320.7 \pm 22.9	271.5 \pm 18.7*	215.7 \pm 18.8*	154.2 \pm 72.5*	173.0 \pm 13.0*	146.3 \pm 17.7*	32.80%

*Where N= Normal, D= Diabetes, Glb = Glibenclamide, Br= Stem bark

**Fig. 4:** Effect of flowering bud on blood glucose level of diabetic mice.

DISCUSSION

Anatomical characters vary from plant to plant and also influenced by environmental factors. These anatomical characters play a significant role in the classification of those plants into species, genera and families, whose become anatomically and morphologically so close and their commonsensical place in the taxonomic system is indefinite (Gilani *et al.*, 1992). Moreover, variations in investigative features of medicinal plants significantly influenced the pharmacological properties, potency and chemical nature of constituents (Sadraei *et al.*, 2003). Due to these reasons, histological studies of specialized cell provide significant information during the studies of biological activities. Standardization is an important tool for measure purity, quality and for identification of sample. Macroscopy and microscopy is the simplest method to identify the correct source of material. Phytochemical screening showed the presence of carbohydrates, proteins, glycosides, flavonoids, alkaloids, terpenoids, steroids in leaves and flowering bud whereas glycosides, tannins and steroids are present in the stem bark of plant.

Castor oil induces diarrhea due to ricinolic acid which is the hydrolytic product of constituents of castor oil

(Chakrabarty *et al.*, 2021). This ricinolic acid produces changes water and electrolyte transport which results in the production of strong contractions of distal and transverse colon. Due to these reasons strong antidiarrheal agent may inhibit the bowel contraction to produce antidiarrheal effect. In the present study, the administration of normal saline showed insignificant increase in the number of wet droppings with a significant percent protection i.e. 94.45 \pm 5.55 (Group I) and castor oil (Group II) showed an insignificant percent protection i.e. 5.5 \pm 5.5. Thus, the normal control group (saline-treated) did not showed diarrhea in any of the animals, while all mice in the castor oil (-ve control) treated group showed diarrhea. The antidiarrheal results showed that the methanolic extracts of the leaves, flowering buds and stem bark of *Moringa oleifera* L. at doses of 100, 300 and 1000mg/kg of body weight, decreases the number of wet feces with a respective increase in percent protection in a dose dependent manner. The antidiarrheal property of the extracts of *Moringa oleifera* L. was determined by its protective effect against the castor oil-induced diarrhea in mice. The percent protection exhibited by the leaves extract was 77% at the dose of 100mg/kg of body weight whereas verapamil (standard drug) showed 72% at the dose of 3 mg/kg of body weight which indicate that the leaves extract has a significant antidiarrheal activity which is greater than verapamil. The methanolic extract of the stem bark showed significant antidiarrheal activity with a % protection of 62, 75 and 83% whereas flowering bud extract showed % protection of 56, 60 and 83% at the dose of 100, 300 and 1000mg/kg of body weight.

In our study the extracts exhibited a dose-dependent protective effect against diarrhea, which is in accordance with the expectation, as calcium antagonists are reported to possess antidiarrheal property (Oliveira *et al.*, 2021). Since the methanolic extract of Leaves successfully inhibited the castor oil-induced diarrhea, it can be

assumed that the antidiarrheal action was exerted by anti-secretory mechanism. This was also evident from the reduction of total number of wet feces in the test groups during the experiment.

Presently used synthetic enzyme inhibitors cause gastrointestinal side effects, therefore natural alpha amylase and glucosidase inhibitors from the dietary plants can be used as an effective therapy for treating post prandial hyperglycemia with minimal side effects. Type 2 diabetes mellitus can be treated by the use of oral alpha glucosidase inhibitors as they decrease the rate of carbohydrate digestion (Bhat *et al.*, 2008). The inhibitory activity of the methanolic extracts of stem bark and flowering bud on α -glucosidase was investigated and the results showed that the buds and bark extract was found to be highly effective against this enzyme.

Oral administration of *Moringa oleifera* L. methanolic extract of flowering bud (500 mg/kg of body weight) exhibited substantial ($P < 0.05$) decrease in blood glucose (322.5 ± 48.35 to 173.8 ± 29.53 mg/dl) and increase in body weight of diabetic mice (27.17 ± 2.041 to 31.83 ± 2.639) compared with untreated diabetic mice (32 ± 1.8 to 25 ± 3.8). This was almost similar to results obtained with standard drug Glibenclamide at the dose of 10mg/kg of the body weight (320.7 ± 22.9 to 146.3 ± 17.7 mg/dl). The stem bark extract also showed significant reduction in the blood glucose level from day 0 to day 15 with an improvement in the body weight in diabetic mice (24.67 ± 0.8165 to 28.83 ± 2.927). The anti-diabetic effect of the bark, however, was lesser than that of the bud and Glibenclamide.

CONCLUSION

It is concluded from the present research work that leaves possess strong antidiarrheal effect while flowering bud and stem bark have strong antidiabetic potential.

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