



E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2020; 8(1): 58-62
Received: 24-11-2019
Accepted: 28-12-2019

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Comparative evaluation of hypoglycemic activity and phytochemical contents of three Tanzanian medicinal plants

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Abstract

Diabetes is the commonest endocrine disease reported to affect about 10% of the world population. Medicinal plants are widely used for diabetes management because of their easy availability, affordability and less or no side effects when compared to the present synthetic antidiabetic drugs.

This *in vivo* study aimed to compare hypoglycaemic activity of three commonly medicinal plants used traditionally for diabetes management in northern Tanzania *Artemisia afra* Willd. (Aerial parts), *Moringaoleifera* Lam. (Leaves) and *Perseaamericana* Mill. (Leaves). Evaluation for hypoglycaemic activity of 80% aqueous ethanol plant extracts was conducted at a dose of 200 mg/kg body weight, orally in glucose loaded normal white albino mice by using Oral Glucose Tolerance Test (OGTT) method. The statistical analysis of results was carried out by using Student *t*-test followed by one-way Analysis of variance (ANOVA) and Tukey's multiple comparisons at probability value ($p < 0.05$). Phytochemical evaluation focused on the normal reactions of characterization based on precipitation and coloration with standard reagents. At a dose of 200 mg/kg bw.t, the three plants showed significant hypoglycaemic activity by lowering blood glucose level in glucose loaded normal white albino mice at a rate of 23%, 15% and 10% for *M. oleifera*, *P. americana* and *A. afra* four (4) hours administration respectively. Phytochemical evaluation indicated the presence of alkaloids, flavonoids, terpenoids, phenolics, saponins and glycosides. The results indicated that 80% aqueous ethanol extracts of the three plants are capable of managing hyperglycemia in oral glucose loaded normal white albino mice. Thus, confirmed the previously reported hypoglycaemic activities of the plants, besides, the study identified *M. oleifera* as the most potent hypoglycaemic plant among the three plants.

Keywords: Diabetes mellitus, hypoglycaemic activity, medicinal plants, oral glucose tolerance test, phytochemicals

1. Introduction**1.1 Background**

Diabetes Mellitus (DM), generally called Diabetes is the commonest endocrine disorder which is characterized by persistent hyperglycemia due to either defective insulin action, insulin secretion, or the combination of both^[1, 2]. An individual is said to be diabetic if his/her fasting blood glucose level (FBGL) is greater than 7 mmol/L, or has blood glucose of 11.1 mmol/L or more after two hour oral glucose tolerance test (OGTT)^[3].

The prolonged high level of glucose level in blood eventually causes multi-complications such as kidney failure (nephropathy), heart disease (cardiomyopathy), stroke, foot ulcers and damage to the eyes (retinopathy)^[1, 4]. Therefore, these severe multiple complications of diabetes cause poor quality of life and death^[5].

According to world-wide survey, about 10% of the population in the world is affected by diabetes^[5]. The diseases is reported to be the growing health problem in the contemporary world^[6]. For instance, about 100 million people were affected with diabetes in 1980 when compared to about 400 million in 2014^[7] and expected to reach about 600 million in 2045^[8].

In Tanzania, the burden of diabetes is steadily increasing due to many factors such as physical inactivity, obesity, cigarette smoking, alcoholism and aging^[3]. For instance in 2015, about 800,000 cases of diabetes were reported in Tanzania^[4].

Depending on the primary causes, there are three main types of DM, namely, Type I diabetes mellitus (known as Insulin dependent diabetes mellitus or Juvenile diabetes), Type II diabetes (known as Non-insulin-dependent diabetes mellitus or maturity onset diabetes) and gestational diabetes^[9]. Type I diabetes mellitus which accounts to about 5 to 15% of all diabetic patients in the world occurs as a result of insulin deficiency which results from pathologic changes in pancreatic β -cells in the pancreas^[10].

Type II diabetes which accounts to about 90 to 95% of all diabetic patients in the world is due to insulin insensitivity where by cells do not respond to insulin properly [11]. Gestational diabetes which occurs in about 5.5 to 8.8% of all pregnancies is a result of combination of insufficient insulin secretion and tissue unresponsiveness to insulin and it occurs during pregnancy though it may improve or disappear after delivery [9].

Although diabetes has been known for the long times, there is no treatment that can cure the disease permanently up to date except determination of the glucose level in the blood every day and manage it by insulin injections and/or oral hypoglycaemic agents [7, 11].

Unfortunately, even the current available treatments to manage the disease such as insulin supplement and various synthetic oral hypoglycaemic agents apart from been not able to cure the disease totally, they are expensive and reported to have some undesirable adverse effects including hypoglycemia, fluid retention, osteoporosis and heart failure after long time application [12, 13].

Hence, there is a great need to develop affordable novel antidiabetic agents having few or no side effects for diabetes treatment. Currently, the tendency is mostly on the use of medicinal plants which are said to have good therapeutic potential and less adverse effects [5, 13]. The therapeutically potentials of medicinal plants are due to the possession of secondary metabolites which play the role. Some of the reported classes of secondary metabolites in plants exerting hypoglycaemic activities are terpenoids, alkaloids, phenolics, flavonoids, saponins, and glycosides [4].

In traditional systems of medicine, more than 400 medicinal plants claimed and documented to have antidiabetic potential. Some of them have been scientifically evaluated and found to possess antidiabetic potential. In addition, some pure compounds having antidiabetic activity have been isolated from these medicinal plants [5].

According to the literature cited in Tanzania, medicinal plants namely *A. afra* Willd. (Aerial parts), *M. oleifera* Lam. (leaves) and *P. americana* Mill. (leaves) are traditionally used for diabetes management [14]. Coincidentally, several scientific reports exist on the hypoglycaemic effects of these medicinal plants when individually evaluated [15-19].

Therefore, the present study focused on comparative evaluation of hypoglycemic activity of the three selected indigenous Tanzanian medicinal plants *A. afra*, *M. oleifera* and *P. Americana*.

Thus, besides conforming hypoglycaemic activities of the selected plants, this study also focused to help identify the most potent hypoglycaemic plant among the three plants.

1.2 Profile of selected medicinal plants

1.2.1 *Artemisia afra*

Artemisia afra Jacq. ex Willd. (family Asteraceae), also known as African wormwood in English is one of the best known and used at large plant in traditional medicine, claimed to possess healing ability against many ailments with inclusion of diabetes [18, 19]. It is an erect, shrubby, and perennial plant that grow up to 2m tall, having leafy and hairy stem. Its leaf shape is narrowly ovate, feathery, and finely divided, growing up to 8 cm long and 4 cm wide, it can also be identified by its aromatic smell [19]. In the Northern regions of Tanzania, the aerial parts of the plant are traditionally used for diabetes treatment [14]. Many experimental studies on the plant as individual reported that it is safe and possess antidiabetic potential [18, 19].

1.2.2 *Moringa oleifera*

Moringa oleifera Lam (family Moringaceae), is a multiuse tree of about 5 to 10 m in height, which is cultivated all over the world [20]. It is a useful medicinal plant in traditional medicine but also reported to possess contents of iron, calcium, vitamins A, C, D & E, phosphorus, zinc, potassium, manganese and protein nutrients [21].

Pharmacologically, the plant was observed to have anti-inflammatory, anti-fungal, anti-diabetic, hepatoprotective, antimicrobial, diuretic, anti-hypertensive, wound healing, anti-ulcerative, anti-cancer and anti-oxidant activities [21, 22].

Traditionally, bark, root, stem, leaves, flowers, pods and seed are used for medicinal and nutrient sources [21]. In the Northern regions of Tanzania, *M. oleifera* leaves are traditionally used for diabetes treatment [14].

1.2.3 *Persea americana*

Persea americana Mill (family Lauraceae), also known as Avocado in English, is a tree plant grown mostly in temperate regions and sparsely in tropical regions of the world [23]. In traditional medicine, the plant is used for treatment of anemia, exhaustion, hypercholesterolemia, hypertension, gastritis, gastro duodenal ulcer, renal diseases, diabetes, antipyretic and analgesic purposes [17]. Reports from the Traditional healers as well as from pharmacological assessment revealed that the leaves of the plant has antidiabetic potential [14, 17].

2. Materials and Methods

2.1 Study site

The plants for this study collected in June 2019 from Kilimanjaro and Tanga regions, Tanzania because there was an earlier ethno botanical survey reported traditional use of these plants for diabetes management in these regions of northern Tanzania [14]. Experimental study conducted from July to November 2019 at the laboratory of Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Sciences (MUHAS) in Dares Salaam, Tanzania.

2.2 Selection, collection and authentication of plant materials

Ethnobotanical information about plants selected for this study was obtained by scrutinizing ethnobotanical literature regarding hypoglycaemic plants used for diabetes management in Tanzanian communities [14].

Additionally, literature search was also done to find out the scientific evidences proving the reported hypoglycaemic potentials of the selected plants, that is, *A. afra* [18, 19], *M. oleifera* [15, 16] and *P. americana* [17, 23]. The plant parts were collected and identified by comparison with voucher specimens present in the Herbarium of the ITM-MUHAS in collaboration with specialist botanist from the Department of Botany at the University of Dares Salaam. Voucher herbarium specimens preserved in the Herbarium of the ITM-MUHAS.

2.3 Chemicals and medicine

Chlorpropamide (standard drug) was acquired from COSMOS Pharmaceutical Limited, Nairobi-Kenya. Concentrated sulphuric acid, hydrochloric acid, chloroform, potassium bismuth iodide, ferric chloride, sodium hydroxide and ammonia solution were obtained from MERCK KGaA group, Darmstadt, Germany whereas ethanol was obtained from CARLO ERBA Reagents SAS, Chaussee du Vexin 27100 Val de Reuil-France.

2.4 Preparation and extraction of plant materials

All plant samples were air-dried and crushed into coarse powder by using a milling machine type Y (Hangyu®, China) available at ITM, MUHAS. About 500 gm of each ground plant material was extracted with 80% aqueous ethanol using percolation method at 25-33 °C and after 24 h filtered through whatman number 1 filter paper. The process repeated two times to ensure complete extraction of the plant material.

The extracts were pooled together, the filtrate was evaporated under reduced pressure using rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) at 60 °C. The extracts were further dried by freeze-drying using the Edwards freeze drier (Edwards High Vacuum International Crawley, Sussex, England).

2.5 Test animals

Healthy adult white albino mice of either sex weighing between 20 - 25 gm obtained from the Animal house of ITM, MUHAS were kept in aluminum cages and fed on commercial broiler finisher pellets. The animals were acclimatized to laboratory conditions for 5 days before experiments. Feed and drinking water were provided *ad libitum* during the whole period of the study except during fasting.

2.6 Hypoglycaemic activity evaluation

Hypoglycaemic potential of each extract was evaluated at a dose of 200 mg/kgbw.t via *in vivo* test (in white albino mice) in oral glucose loaded animal model (that is, physiological diabetes induction) by OGTT method as previously described [4, 10, 24].

2.6.1 Oral glucose administration

Test animals acclimatized for 5 days were fasted for 18 hours before the beginning of the experiment, and then orally loaded by gavage with freshly prepared glucose at a dose of 1 gm/kgbw.t 30 minutes after solvent/extract/standard drug administration.

2.6.2 Experimental design

At the start of the experiment, body weight (in gram) and fasting blood glucose levels of the animals were determined. Five groups of white albino mice (n = 6) of either sexes were formed randomly in each received the following treatment schedule:

Group I: Negative control (1% Carboxymethyl cellulose, 1% CMC 5 ml/kg orally)

Group II: Positive control (Chlorpropamide, 100 mg/kg orally)

Group III: 80% aqueous ethanol *A. afra* aerial parts extract (200 mg/kg orally)

Group IV: 80% aqueous ethanol *M. oleifera* leaves extract (200 mg/kg orally)

Group V: 80% aqueous ethanol *P. Americana* leaves extract (200 mg/kg orally)

2.6.3 Blood glucose determination

Blood glucose level in blood collected from each mouse by partial tail amputation procedure from the tail vein was measured after glucose loading at 0.5, 1, 2, 3 and 4 hours by commercially available glucose kit based on a glucose

oxidase enzymatic assay and determined by a Glucometer called ACCU-CHEK® Active (Roche Diabetes care GmbH, Mannheim –Germany) as previously described [4, 5, 25, 26].

The percentage reduction/lowering of blood glucose level of each plant extract was determined after 4 hours using the equation (1) as previously described [7].

Percent lowering of blood glucose

$$\text{Level} = (1 - \text{WE}/\text{WC}) \times 100 \dots \dots \dots (1)$$

Whereas,

WE = the mean blood glucose level in various extracts (group III to V) administered mice at 4 hours and WC = the mean blood glucose level in control group (group I) administered mice at 4 hours.

2.7 Phytochemical evaluation

Qualitative phytochemical analysis of classes of secondary metabolites reported to have hypoglycaemic activity [4] was carried out for the crude extracts as per standard methods namely, alkaloids (Dragendorff's test), terpenoids (Salkowski's test), phenolics (ferric chloride test), flavonoids (alkaline reagent test), saponins (foam test), and glycosides (Keller Killiani test) previously described [27, 28].

2.8 Data and statistical analysis

The results of blood glucose levels were expressed as mean ± Standard Error of the Mean (SEM) with sample size (n = 6). Statistical analysis of results was carried out using Student *t*-test followed by one-way Analysis of variance (ANOVA) and Tukey's multiple comparisons probability value (*p* < 0.05).

2.9 Ethical consideration

Key ethical issues consideration in this study towards adherence to animal welfares include; keeping few mice in each cage to enable expression of their normal behavior, giving animals clean and safe water as well as appropriate food as previously described [4]. Therefore, the study obtained review from the MUHAS Ethical Review Committee and commenced after receiving ethical approval of the Director of Research and Publications of MUHAS.

3. Results and Discussions

3.1 Hypoglycaemic activity evaluation

In this study, all plant extracts exhibited significant reduction in blood glucose levels in glucose loaded normal white albino mice after administration statistically different from that of untreated group.

At a dose of 200 mg/kgbw.t, both 80% aqueous ethanol leaves extracts of *M. oleifera* and *P. americana* exhibited hypoglycaemic potential by lowering blood glucose of glucose loaded normal white albino mice statistically different from that of untreated group (*p* < 0.05) at 1, 2 and 3 hours after administration (Table 1).

On the other hand, at a dose of 200 mg/kgbw.t, 80% aqueous ethanol extract of *A. afra* aerial parts demonstrated its hypoglycaemic potential in glucose loaded normal white albino mice statistically different from that of untreated group (*p* < 0.05) at 2 and 3 hours after administration as shown in Table 1.

Table 1: Mean blood glucose level of white albino mice (mmol/L) recorded at 0, 0.5, 1, 2, 3 and 4 hours after oral administration of solvent/standard drug/extracts

Time (h)	Mean blood glucose level of mice (mmol/L) (<i>p</i> -value)				
	NC (5 ml/kg)	PC (100 mg/kg)	MO (200 mg/kg)	PA (200 mg/kg)	AA(200 mg/kg)
0	5.72±0.18	6.47±0.23 (0.09)	5.25±0.14 (0.07)	6.22±0.16 (0.41)	5.9±0.34 (0.64)
0.5	9.7±0.79	6.93±0.59 (0.02)*	8.1±0.37 (0.09)	9.32±0.42 (0.68)	8.23±0.48 (0.14)
1	7.78±0.66	4.75±0.59 (0.01)*	5.25±0.29 (0.01)*	5.82±0.29 (0.02)*	6.27±0.33 (0.07)
2	6.15±0.39	4.33±0.17(0.003)*	4.98±0.22 (0.02)*	4.97±0.21 (0.02)*	4.98±0.35 (0.02)*
3	6.72±0.57	3.78±0.19 (0.001)*	4.72±0.13 (0.01)*	4.97±0.22 (0.01)*	4.92±0.23 (0.01)*
4	5.7±0.65	3.78±0.19(0.03)*	4.4±0.17 (0.08)	4.82±0.28 (0.24)	5.1±0.26 (0.41)

Key: AA: *Artemisia afra*, MO: *Moringaoleifera*, PA: *Perseaamericana*, NC: Negative control, PC: Positive control and *: *p* < 0.05 statistically different from negative control animals

On determination of percentage reduction/lowering of blood glucose level of each plant extract at a dose of 200 mg/kg bw.t, the three plants showed a significant hypoglycaemic activity by lowering blood glucose level in tested animals at a rate of 23%, 15% and 10% for *M. oleifera*, *P. americana* and *A. afra* 4 hours administration respectively as shown in Table 2. The results indicate that, 80% aqueous ethanol leaves extract of *M. oleifera* exhibits the strongest hypoglycaemic activity in glucose loaded normal white albino mice when compared to both 80% aqueous ethanol *P. americana* leaves extract and 80% aqueous ethanol *A. afra* aerial parts extract.

Previous studies reported that, phytochemicals in a complex mixture of phytochemicals tend to interact resulting into either potentiating the effect of bioactive phytochemicals or interfering with their activity, the interaction which causes interference of bioactivity of phytochemicals in their complex mixture tends to lower or loss of activity [4].

Therefore, this may be among of the reasons for the strongest observed hypoglycaemic activity of 80% aqueous ethanol leaves extract of *M. Oleifera* when compared to both 80% aqueous ethanolleaves extract of *P. americana* and 80% aqueous ethanolalerial parts extract of *A. afra*.

Table 2: Percentage reduction of blood glucose level in mice exerted by standard drug/plant extracts 4 hours after administration

Standard drug/extract administered	Percentage reduction of blood glucose level in mice exerted by standard drug/extracts 4 hours after administration
Chlorpropamide (100 mg/ kg)	34%
<i>M. oleifera</i> (200 mg/ kg)	23%
<i>P. americana</i> (200 mg/ kg)	15%
<i>A. afra</i> (200 mg/ kg)	10%

3.2 Phytochemical evaluation

The results of phytochemical evaluation of 80% aqueous ethanol extracts from the selected plants indicated the presence of alkaloids (all three plants), flavonoids (all three plants), terpenoids (all three plants), phenolics (all three plants), saponins (two plants) and glycosides (two plants) as shown in Table 3.

Previous studies indicated that alkaloids, flavonoids, terpenoids, phenolics, saponins and glycosides in plants exert hypoglycaemic activity [29]. Therefore, the observed significant hypoglycaemic activities from the studied plants may have been attributed by the presence of these phytochemical acting either singly or in combination.

Table 3: Phytochemical group of compounds present in the extracts of the plant materials

Plant species (plant part)	Classes of secondary metabolites tested					
	Alkaloids	Cardiac glycosides	Flavonoids	Phenolics	Saponins	Terpenoids
<i>M. oleifera</i> (leaves)	+	+	+	+	+	+
<i>P. americana</i> (leaves)	+	+	+	+	+	+
<i>A. afra</i> (aerial parts)	+	-	+	+	-	+

Key: +: Present and -: Absent of a particular class of secondary metabolite

4. Conclusion

This study indicates that the 80% aqueous ethanol of the plant extracts contain bioactive compounds responsible for hypoglycaemic activity. Furthermore, the results indicated the plant extracts are capable of managing hyperglycemia on oral glucose loaded normal white albino mice. Thus, confirming hypoglycaemic activities of the selected plants previously reported. Additionally, the results also identified the most potent hypoglycaemic plant among the three selected plants.

5. Acknowledgement

The authors enthusiastically acknowledge to the Swedish International Development Cooperation Agency (SIDA) for funding this study.

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