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Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI-DIABETIC POTENTIAL OF SELECTED MEDICINAL PLANTS USED TRADITIONALLY FOR DIABETES MANAGEMENT IN TANZANIA

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ABSTRACT

The present study was undertaken to evaluate the anti-diabetic activity of extracts from five selected medicinal plants used traditionally to manage diabetes in Tanzanian namely, *Afzelia quanzensis* (root), *Bridelia duvigneaudii* (root), *Cyphomandra crassifolia* (fruit), *Dioscorea praehensilis* (tuber) and *Ficus fischeri* (stem bark) which were collected in February 2017. The study was conducted from March to August 2017. Phytochemical screening focused on the usual reactions of characterization based on precipitation and coloration with standard reagents. Evaluation for anti-hyperglycaemic activity of 80% aqueous ethanol plant extracts was conducted in normal albino mice by using Oral Glucose Tolerance Test (OGTT). The statistical analysis of results was carried out using Student *t*-test followed by one-way Analysis of variance (ANOVA) and Tukey's multiple comparisons at probability value ($p < 0.05$). Phytochemical screening indicated the presence of terpenoids, phenolics, saponins and glycosides. At a dose of 200 mg/kgbwt; *B. duvigneaudii* root extract exhibited the most significant anti-diabetic activity by lowering blood glucose levels at 0.5, 1, 2 and 3 hours after administration. The results suggested that the 80% aqueous ethanol roots extract of *B. duvigneaudii* is capable of managing hyperglycemia on oral glucose loaded normal albino mice. Thus, this plant may be considered as one of the potential sources for the isolation of new alternative anti hypoglycemic agent (s) for management of diabetes.

Keywords: An Anti-diabetic potential, Anti-hyperglycaemic activity, Phytochemical screening,

1.0 INTRODUCTION

Diabetes Mellitus or commonly known as diabetes is a chronic metabolic disease in which there is an increase in blood glucose levels in the body (hyperglycemia) for a prolonged period due to relative insulin deficiency, resistance, or both[1–3]. Untreated cases of the disease result into long standing complications such as kidney failure, heart disease, stroke, foot ulcers, and damage to the eyes[4].

According to the global statistics of diabetes mellitus, about 382 million people had this disease worldwide in the year 2013, causing death of 1.5–5.1 million people per year in year 2012 and 2013 and

expected to cause death of about 592 million people by year 2030[5]. In 2015, International Diabetes Federation (IDF) reported that, there were more than 822,800 cases of diabetes in Tanzania.

The currently available modern medicines used for diabetes management which include insulin injections and oral hypoglycemic agents have not been able to cure the disease while causing adverse side effects and complications to the patients after prolonged use [3,6,7]. Due to these limitations, anti-diabetic drug discovery has shifted its focus to medicinal plants as alternatives sources of new anti-diabetics[6]. Basing on traditional medicine information, diabetes has been treated with herbs and medicinal plants for a long time[8–10]. However, most of the available ethno-medical information regarding the anti-diabetic potential of these plants is not provided with scientific data. Few of them have undergone scientific and medical evaluation to assess their anti-diabetic efficacy [11,12]. The few scientific evaluated medicinal plants reported to show antidiabetic potential were shown to have useful active phytochemical compounds which include terpenoids, alkaloids, phenolics, flavonoids, saponins, and glycosides[13].

This study therefore aimed to find out phytochemical composition and anti-diabetic potential of *A*. *quanzensis* (root), *B*. *duvigneaudii* (root), *C*. *crassifolia* (fruit), *D*. *praehensilis* (tuber) and *F*. *fischeri* (stem bark) which are traditionally used for management of diabetes in Tanzania[14,15].

2.0 MATERIALS AND METHODS

2.1 Study area

The plants for this study were collected in February 2017 from Tabora and Kagera regions, Tanzania due to the fact that there was an earlier ethnobotanical survey that reported traditional use of these medicinal plants for diabetes management in these two regions [14,15]. The experimental study was undertaken from March to August 2017 at the laboratory of Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Sciences (MUHAS) in Dar es Salaam, Tanzania.

2.2 Selection, collection and authentication of plant materials

Ethnobotanical information about plants selected for this study was obtained through ethnobotanical literature search regarding traditionally claimed antidiabetic medicinal plants without scientific proof. Basing on the above criterion for selection, the plants selected for this study were *C. crassifolia* and *D. praehensilis* collected in Kagera region and *A. quanzensis*, *B. duvigneaudii* and *F. fischeri* collected in Tabora region in Tanzania. The selected plant species were identified by comparison with voucher specimens present in the Herbarium of the University of Dar es Salaam in collaboration with specialist Mr. Haji O. Seleman from the Department of Botany at the University of Dar es Salaam. Voucher herbarium specimens were preserved in the Herbarium of the University of Dar es Salaam with voucher number 5003, 5004, 5005, 5006 and 5007 for *D. praehensilis, C. crassifolia*, *F. fischeri, B. duvigneaudii* and *A. quanzensis* respectively.

2.3 Drugs and chemicals

Chlorpropamide was obtained from COSMOS Pharmaceutical Limited, Nairobi-Kenya. Concentrated sulphuric acid, hydrochloric acid, chloroform, potassium bismuth iodide, ferric chloride, sodium hydroxide, ammonia solution and Benedict's reagent 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 Test animals

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

2.5 Preparation and extraction of plant materials

All plant samples were air-dried and pulverized into coarse powder by using a milling machine type Y (Hangyu®, China) available at ITM, MUHAS. About 400 gm of each ground plant material was extracted with 80% aqueous ethanol using percolation method at 25-33^{o}C and after 24 h filtered through whatman number 1 filter paper. The procedure was repeated two times to ensure exhaustive 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 40 to 50°C. The extracts were further dried by freeze-drying using the Edwards freeze drier (Edwards High Vacuum International Crawley, Sussex, England).

2.6 Phytochemical screening

Qualitative phytochemical analysis for secondary metabolites was carried out for the crude extracts as per standard methods[16,17].

2.6.1 Test for terpenoids

1 gm of each extract was dissolved in 2 ml of chloroform followed by adding carefully 3 ml of concentrated sulphuric acid (H_2SO_4) to form a layer. A red brown colouration on the interface was indicative results for presence of terpenoids.

2.6.2 Test for alkaloids

An amount of 0.5 gm of each extract was dissolved in 5 ml of 1% hydrochloric acid (1% HCl) and warmed on water bath. Then, Dragendorff's Test was done in which filtrates were treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate was indicative results for presence of alkaloids.

2.6.3 Test for phenolics

An amount of 0.1 gm of each extract was heated in 15ml of water (H_2O) for 15min on a water bath and filtering the solution. To 2 ml of filtrate, 2 ml of 5% aqueous ferric chloride was added; formation of blue colour was indicative results for presence of phenols.

2.6.4 Test for flavonoids

Extracts were treated with few drops of sodium hydroxide solution (NaOH). Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid (HCl), was indicative results for presence of flavonoids.

2.6.5 Test for saponins: An amount of 2 gm of each extract was boiled with 20 ml distilled water in a water bath and filteredthen 10 ml filtrates was mixed with 5ml distilled water and shaken vigorously and observe persistence of froth. The frothing was further mixed with 3 drops of olive oil then shaken vigorously and observed for the formation of emulsion to indicate presence of saponins.

2.6.6 Test for glycosides

An amount of 0.5 gm of each extract was stirred with 10 ml of boiled distilled water. This mixture was filtered and 2 ml of the filtrate hydrolyzed with few drops of concentrated hydrochloric acid (HCl) and the solution rendered alkaline with a few drops of ammonia solution. Then, about 5 drops of this solution was added to 2 ml of Benedict's quantitative reagent and boiled. Appearance of reddish brown precipitate was indicative results for presence of glycosides.

2.7 Determination of the anti-diabetic activity

Anti-diabetic potential of each extract was evaluated at a dose of 200 mg/kgbwt via *in vivo* test (using albino mice) in oral glucose loading animal model by OGTT[18,19.]

2.7.1 Oral glucose administration

Test animals acclimatized for 5 days were fasted for 18 hours before the beginning of the experiment and were then orally loaded by gavage with freshly prepared glucose (1 gm/kgbwt) 30 minutes after solvent/extract/chlorpropamide administration.

2.7.2 Experimental design

At the beginning of the experiment body weight to the nearest gram and fasting blood glucose levels of the animals were determined.

Seven groups of white albino mice, six albino mice (n = 6) in each received the following treatment schedule:

Group I: Negative control (distilled water, 5 ml/kgbwt orally)

Group II: Positive control (chlorpropamide, 100 mg/kgbwt orally)

Group III: 80% aqueous ethanol *Bridelia duvigneaudii* roots extract (200 mg/kgbwt orally)

Group IV: 80% aqueous ethanol *Dioscorea praehensilis* tubers extract (200 mg/kgbwt orally)

Group V: 80% aqueous ethanol *Ficus fischeri* stem barks extract (200 mg/kgbwt orally)

Group VI: 80% aqueous ethanol *Afzelia quanzensis* roots extract (200 mg/kgbwt orally)

Group VII: 80% aqueous ethanol *Cyphomandra crassifolia* fruits extract (200 mg/kgbwt orally)

2.7.3 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 glucose meter known as ACCU-CHEK® Active (Roche Diabetes care GmbH, Mannheim – Germany)[20,21].

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). The 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 approval

During the study the following issues were taken into consideration: Few mice were kept in each cage to enable mice to express their normal behavior. Clean water and appropriate feed was given and mice were kept at the temperature of 22^{o}C (±3^{o}C) and the relative humidity was 55 %. An ethical clearance of the protocol for this study was given by the Director of Research and Publications of MUHAS.

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical analysis

The results in the Table 1 represent the phytochemical analysis of 80% aqueous ethanol extracts from the selected Tanzanian medicinal plants. According to these results, the phytochemical analysis showed the presence of terpenoids (three plants), phenolics (all plants), saponins (all plants) and glycosides (two plants).

Previous studies reported that medicinal plants contain phytochemical compounds responsible for anti-diabetic activity which include terpenoids, alkaloids, phenolics, flavonoids, saponins, and glycosides[13]. Phytochemical screening revealed the presence of terpenoids, phenolics, saponins and glycoside in *B. duvigneaudii*. These constituents may in part be responsible for the observed significant activity of *B. duvigneaudii* roots extract either singly or in synergy with one another.

3.2 Anti-diabetic potential

At a dose of 200 mg/kgbwt, *B. duvigneaudii* roots extract revealed its anti-diabetic potential by lowering blood glucose on glucose loaded normal albino mice statistically different from that of untreated group ($p < 0.05$) at 0.5, 1, 2 and 3 hrs after administration (Table 2). The extracts of the other plants (*D. praehensilis*, *F. fischeri*, *A. quanzensis* and *C. crassifolia*) did not show significant reduction in blood glucose levels after administration ($p < 0.05$) as shown in Table 2.

Evaluation of anti-diabetic potential of 80% aqueous ethanol extracts of the selected Tanzania medicinal plants was carried out in oral glucose loaded white albino mice. Loading oral glucose in animals is reported to cause physiological induced diabetes mellitus because the blood glucose level of the animal is momentarily increased without damage to the pancreas [22]. The 80% aqueous ethanol extract of *B. duvigneaudii* roots was observed to demonstrate significant anti-hyperglycaemic activity in oral glucose loaded white albino mice when compared to other medicinal plants selected for this study.

Chlorprpamide is a synthetic anti-diabetic drug belonging to the group of sulphonylureas anti-diabetic drugs which cause hypoglycemia by stimulating insulin secretion from the pancreas[20]. The observed reduction in blood glucose level of the oral glucose loaded mice by chlorprpamide in this study shows a reverse state of diabetes. In this study, treatment with the 80 % aqueous ethanol roots extract of *B. duvigneaudii* caused significant decrease in blood glucose level of treated oral glucose loaded mice compared to untreated oral glucose loaded mice which is a reverse diabetic state characteristic.

Previous studies revealed that, medicinal plant have different mechanisms of anti-diabetic action through which their effects are exhibited that might include promoting regeneration of β-cells of Islets of Langerhans in the pancreas, enhancement of insulin release and activity on the cells, decrease glucose uptake at the duodenal cellular level and other aspects of small intestine and the presence of high level of fiber in plants which interferes with carbohydrate absorption[23]. The 80 % aqueous ethanol roots extract of *B. duvigneaudii* may have acted through one of the above mechanisms. Phytochemical compounds like terpenoids, phenolics, saponins and glycosides present in this extract have been reported to exert anti-

diabetic activity[13] through one of the mechanisms mentioned above. However, some of the medicinal plants for this study were observed to posses two (*A. quanzensis* and *C. crassifolia*), three (*F. fischeri*) or four (*D. praehensilis*) of the phytochemicals but they did not exhibit anti-diabetic activity. It is reported that phytochemicals within the complex mixture of phytochemicals tend to interact resulting into either potentiating the effect of bioactive phytochemicals or interfering with their activity[24]. Therefore, the interaction which causes interference of bioactivity of phytochemicals in their complex mixture may have occurred for the plants which did not exhibit anti-diabetic activity for this study.

4.0 CONCLUSION

This study indicates that the 80 % aqueous ethanol roots extract of *B. duvigneaudii* contains bioactive compounds with anti-diabetic potential. Therefore, this study serves as a first step in revealing anti-diabetic potential of *B. duvigneaudii* before isolation and identification of pure bioactive compound(s) responsible for anti-hyperglycaemic activity for development and formulation of anti-diabetic agent(s).

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6.0 Tables

Table 1: Phytochemical group of compounds present in the extracts of the plant materials

Plant species (part used)	Classes of secondary metabolites tested					
	Terpenoids	Alkaloids	Phenolics	Flavonoids	Saponins	Glycosides
B. duvigneaudii (roots)	+	-	+	-	+	+
D. praehensilis (tubers)	+	-	+	-	+	+
F. fischeri (stem barks)	+	-	+	-	+	-
A. quanzensis (roots)	-	-	+	-	+	-
C. crassifolia (fruits)	-	-	+	-	+	-

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

Table 2: Mean blood glucose level of mice (mmol/L) recorded at 0, 0.5, 1, 2, 3 and 4hr after oral administration of various extracts at a single dose of 200 mg/kgbwt

T (h)	Mean blood glucose level of mice (mmol/L) (*p*-value)						
	NC (5 ml/kgbwt)	PC (100 mg/kgbwt)	BDR	DPT	FFSB	AQR	CCF
0	4.18±0.33	3.53±0.26 (0.09)	3.8±0.25 (0.6)	3.02±0.19 (0.12)	3.73±0.34 (0.78)	3.83±0.44 (0.66)	3.93±0.17 (0.34)
0.5	6.78±0.32	5.72±0.19 (0.01)	5.9±0.66 (0.031)	7.55±0.76 (0.38)	6.73±0.51 (0.088)	6.95±0.48 (0.12)	8.93±0.46 (0.72)
1	5.2±0.30	3.33±0.16 (0.002)	4.5±0.34 (0.039)	5.23±0.64 (0.23)	5.83±0.40 (0.46)	5.65±0.35 (0.34)	6.2±0.39 (0.72)
2	4.53±0.17	2.67±0.25 (0.01)	3.4±0.34 (0.042)	3.57±0.27 (0.50)	4.15±0.28 (0.69)	4.22±0.30 (0.62)	4.7±0.21 (0.74)
3	3.93±0.21	2.28±0.23 (0.02)	2.7±0.40 (0.049)	2.95±0.11 (0.25)	3.53±0.33 (0.74)	3.68±0.31 (0.84)	3.88±0.23 (0.87)
4	3.36±0.16	1.85±0.19 (0.04)	2.1±0.37 (0.37)	2.68±0.17 (0.40)	2.75±0.21 (0.89)	3.37±0.34 (0.97)	3.58±0.26 (0.98)

Key: BDR: *Bridelia duvigneaudii* roots, DPT: *Dioscorea praehensilis* tubers, FFSB: *Ficus fischeri* stem barks, AQR: *Afzelia quanzensis* roots, CCF: *Cyphomandra crassifolia* fruits, NC: Negative control, PC: Positive control

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