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Evaluation of Antioxidant and Anti-Diabetic Potentials of the Ethyl Acetate Leaf Extracts of some Commonly used Medicinal Plants in Ogbomoso, Nigeria

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Abstract:- Medicinal plants are used as alternatives for treating diabetes mellitus and are considered safe when used alone or combined with synthetic drugs to ensure their effectiveness and also reduced the toxic effects of orthodox medicines. Using *in-vitro* antioxidant and anti-diabetic indices, this study evaluated some commonly used medicinal plants for the treatment and management of diabetes mellitus in Ogbomoso.

Four commonly used medicinal plants identified include; *Croton zambezicus* (CZ), *Markhamia tomentose* (MT), *Asphilia africana* (AA), and *Albizia ferruginea* (AF). The ethyl acetate leaf extracts of the plants were prepared using Soxhlet extraction and dried. Total flavonoid content, percentage 1, 1-diphenyl, 2- picrylhydrazyl (DPPH) and hydroxyl radical (OH) scavenging activity, percentage inhibition of lipid peroxidation, as well as alpha amylase and alpha glucosidase activities, were determined using standard international methods.

Total flavonoids content quercetin equivalent (QE) of CZ, MT, AA, and AF obtained, are 170 mg/g QE, 20 mg/g QE, 68 mg/g QE, and 16 mg/g QE respectively in this order CZ >AA>MT>AF at maximum concentrations (400 µg/ml). Percentages of DPPH and OH radical scavenging activities of CZ, MT, AA, and AF are (89.6, 40.7%), (22.83, 35.5%), (16.5, 28.6%), and (31.7, 51.3%) at maximum concentration. The extracts also inhibited lipid peroxidation with these values 56%, 69%, 60% and 72 % at maximum concentration (400 µg/ml). Interestingly, CZ, MT, AA, and AF inhibited alpha amylase and alpha glucosidase activities with these values (21.1, 80.0%), (62.2, 70.9%), (64.2, 63.2%) and (10.5, 85.3%) respectively.

Properties exhibited by these plants are antioxidant and anti-diabetics which provide scientific basis for their usage in traditional medicine for the treatment and management of diabetes mellitus in Ogbomoso.

Keywords:- *Antioxidant, Asphilia Africana, Albizia Ferruginea, Croton Zambezicus, Diabetes Mellitus, Ethyl Acetate Extracts, and Markhamia Tomentose.*

I. INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disorder associated with high glucose level in the blood. It can be classified into three major types which are; type 1, type 2, and gestational diabetes mellitus [1, 2]. Type 1 diabetes mellitus also known as insulin dependent diabetes mellitus is an autoimmune disease characterized with hyperglycemia, which occurs as a result of the destruction of insulin-producing pancreatic β-cells, leading to insulin deficiency [3]. Type 2 Diabetes Mellitus (non-insulin-dependent diabetes mellitus) is a metabolic disorder characterized with insulin resistance and inadequate insulin secretion by pancreatic beta cells. Gestational diabetes mellitus is a form of hyperglycemia that occurs during pregnancy. Diabetes has been linked with oxidative stress which may lead to cellular degeneration making its management very complicated. Diet and lifestyle changes can be used for controlling diabetes mellitus, however, treatment with orthodox drugs have serious side effect such as tissue toxicity and limited availability [4, 5]. Folkloric medicine has pointed to medicinal plants as important sources of bioactive compounds that can be used for management and treatment of diabetes because they are easily obtained, economical, and less toxic [6].

Using herbal alternatives for treatment and management of diabetes has led to low incidence of diabetes among the populace, and the selection of plant materials in a place where herbal therapy is part of the cultural heritage is crucial in drug discovery and preservation of knowledge of herbal therapy [7]. *Croton zambezicus, Markhamia tomentose, Asphilia africana, and Albizia ferruginea* have numerous uses in traditional medicines, the investigation of the antioxidant and anti-diabetic potentials of their leaf extracts may help to validate these claims and their possible potential as template for drug discovery.

II. MATERIALS AND METHODS

- *Materials*

Spectrophotometer, thermostatic water bath, incubator, analytical weighing balance, pH meter, micropipettes, test tube racks, spatula and glass wares.

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➢ *Reagents and Chemicals*

Ethyl acetate, methanol, ferric chloride, hydrochloric acid, quercetin, ascorbic acid, alpha -glycosidase, alpha-amylase, 3,5-dintrosalicylic acid, sodium hydrogen phosphate, potassium hydrogen phosphate, starch, acetic acid, ascorbic acid, sodium lauryl sulfate, ferrous sulfate, 2-deoxy ribose, ethylene diamine tetracetic acid, 4-nitrophenyl B-D-glucopyranoside, sulphuric acid, DPPH reagent, thiobarbituric Acid, thiochloroacetic acid and sodium carbonate. All reagents used were of pure analytical grades.

➢ *Collection of Plant Materials and their Identification.*

Fresh leaves of *Croton zambezicus*, *Markhamia tomentose*, *Asphilia africana*, and *Albizia ferruginea* were collected from Dananu Orire Local government area, Ogbomoso and authenticated by Taxonomist at the Botany unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, with voucher numbers LHO 700, LHO 746, LHO 743, and LHO 745 deposited.

➢ *Preparation of Ethyl Acetate Extracts.*

Freshly harvested leaves were sorted and air dried in the laboratory at room temperature and further powdered. About 400 grams of the powdered leaves were extracted in 2500 ml of ethyl acetate for 72 hours, the filtrates were separated from the residue and subjected to Soxlet extractions and dried [8]. Ethyl acetate leaf extracts obtained were used for the determination of total flavonoid content, DPPH and hydroxyl radical scavenging activities, inhibition of lipid peroxidation, as well as alpha amylase and alpha glucosidase activities.

➢ *Total Flavonoid Content and DPPH Radical Scavenging Assay*

Total flavonoids content were determined using the aluminum chloride colorimetric method [9]. The calibration curve was made by preparing quercetin solutions at different concentrations in methanol. The DPPH radical scavenging assay was determined using a method described by Mensor, et al [10].

➢ *Hydroxyl Radical Scavenging Assay and Inhibition of Lipid Peroxidation.*

Hydroxyl radical scavenging assay was based on the degradation of 2-deoxyribose and the amount of degradation was quantified using a spectrophotometer [11]. Inhibition of lipid peroxidation was based on the principle that ferrous ions (Fe^{2+}) from ferrous sulfate can initiate lipid peroxidation in an oxygenated environment [12].

➢ *Inhibition of Alpha amylase and Alpha Glucosidase Activities.*

Alpha amylase inhibition was centered on assessing a substance or compound's capacity to hinder the enzyme's activity, inhibition of alpha-amylase activity was determined using inhibition method described by Apostolidis and Lee [13]. Alpha glucosidase inhibition assay was based on the use of chromogenic substrate, which will be hydrolyzed by alpha-glucosidase producing a yellow compound that can be measured spectrophotometrically [14].

III. RESULTS

Table 1 Total Flavonoid Content of Ethyl Acetate Leaf Extracts of *Croton Zambezicus, Markhamia Tomentose, Asphilia Africana and Albizia Ferruginea* (Mg/G) Quercetin Equivalent (Qe).

Concentration (µg/ml)	***Croton zambezicus* (CZ) extract**	***Markhamia tomentosa* (MT) extract**	***Aspillia africana* (AA) extract**	***Albizia ferruginea* (AF)extract**
50	10	5	9	7
100	17	10	10	8
150	20	10	12	10
200	23	12	16	11
250	35	14	44	16
300	38	15	46	7
350	132	15	49	6
400	170	20	68	7

Table 2 Percentage 1, 1-Diphenyl, 2- Picrylhydrazyl Radical Scavenging Activity of Ethyl Acetate Leaf Extracts of Croton *Zambezicus, Markhamia Tomentose, Asphilia Africana and Albizia Ferrugine. (%)*

Concentration (µg/ml)	***Croton zambezicus* (CZ) extract**	***Markhamia tomentose* (MT) extract**	***Aspillia Africana* (AA) extract**	***Albizia ferruginea* (AF) extract**
50	89.60	6.46	1.48	0.40
100	88.55	10.40	5.42	6.22
150	87.96	10.71	8.58	11.67
200	85.09	12.55	9.32	13.80
250	83.48	15.26	12.34	22.03
300	82.36	17.23	12.87	25.67
350	80.63	22.34	13.41	27.44
400	83.54	22.83	16.52	31.73

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Table 3 Percentage Hydroxyl Radicals Scavenging Activity of Ethyl Acetate Leaf Extracts of Croton *Zambezicus, Markhamia Tomentose, Asphilia Africana and Albizia Ferruginea.(%)*

Concentration (µg/ml)	***Croton zambezicus* (CZ) extract**	***Markhamia tomentosa* (MT) extract**	***Aspillia africana* (AA) extract**	***Albizia ferruginea* (AF) extract**
50	8.3	17.6	16.2	10.0
100	9.1	21.2	21.4	32.2
150	13.8	21.4	22.2	43.9
200	18.6	24.8	26.1	45.7
250	29.2	27.3	26.5	46.1
300	39.9	28.0	28.6	47.0
350	40.7	31.5	21.8	50.4
400	40.7	35.1	-5.1	51.3

Table 4 Percentage Inhibition of Lipid Peroxidation of Ethyl Acetate Leaf Extracts of Croton Zambezicus, Markhamia Tomentose, Asphilia Africana, and Albizia Ferruginea.(%)

Concentration (µg/ml)	***Croton zambezicus* (CZ) extract**	***Markhamia Tomentosa* (MT) extract**	***Aspillia Africana* (AA) extract**	***Albizia Ferruginea* (AF) extract**
50	20	1	15	14
100	36	4	32	36
150	38	10	37	38
200	42	14	40	45
250	46	15	52	49
300	49	16	53	55
350	54	40	51	72
400	56	69	60	69

Table 5 Percentage Inhibition of Alpha Amylase Activity of Ethyl Acetate Leaf Extracts of *Croton Zambezicus, Markhamia Tomentose, Asphilia Africana and Albizia Ferruginea.* (%)

Concentration (µg/ml)	***Croton zambezicus* (CZ) extract**	***Markhamia tomentose* (MT) extract**	***Aspillia Africana* (AA) extract**	***Albizia ferruginea* (AF) extract**
50	2.0	14.4	22.4	-2.0
100	2.8	18.9	40.3	2.7
150	5.6	22.5	39.4	4.0
200	8.7	24.3	45.8	7.5
250	9.2	25.7	51.9	8.7
300	11.9	28.7	56.3	8.9
350	16.9	29.8	61.2	9.5
400	20.1	62.2	64.2	10.5

Table 6 Percentage Inhibition of Alpha-Glucosidase Activity of Ethyl Acetate Leaf Extracts of Croton *Zambezicus, Markhamia Tomentose, Asphilia Africana and Albizia Ferruginea.(%)*

Concentration (µg/ml)	***Croton zambezicus* (CZ) extract**	***Markhamia tomentose* (MT) extract**	***Aspillia Africana* (AA)extract**	***Albizia ferruginea* (AF) extract**
50	80.0	70.9	63.2	85.3
100	72.6	60.9	57.2	78.1
150	71.0	58.2	51.7	75.5
200	59.0	53.2	46.3	71.0
250	48.4	44.5	29.9	43.8
300	39.5	38.5	25.9	30.8
350	22.6	32.1	20.4	16.5
400	21.0	23.4	10.9	7.1

IV. DISCUSSION

The used of herbal therapy for treatment and management of diabetes mellitus has been of great interest, because there have been several reports of successful treatment of different disease conditions with herbal therapy, and it is usually opted for due to its cost-effectiveness, easy access, and potency [6]. Studies showed that two-thirds of people suffering from diabetes were frequently using medicinal plants to control their blood glucose levels and improve their health [15, 16].

Flavonoids is a group of naturally occurring phenolic compounds produced in plant, they helps in regulating cellular

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activity and also act as free radicals scavengers. Total flavonoid content expressed in quercetin equivalent (Table 1) of ethyl acetate leaf extracts of *Croton zambezicus* (MT), *Markhamia tomentose* (MT), *Asphilia Africana* (AA), *and Albizia ferruginea* (AF) increases in a concentration dependent manner with maximum concentration at 400 µg/ml (170 mg/g QE), 400 µg/ml (20mg/g QE), 400 µg/ml (68 mg/g) QE, and 250 µg/ml (16 mg/g QE), respectively. This result is an evidence of the presence bioactive compounds which have antioxidant properties [17-19].

Oxidative stress is an imbalance between free radicals and antioxidant which can cause damages to organ and tissue resulting to diseases conditions such as diabetes mellitus. DPPH radicals scavenging assay is used for rapid screening of the antioxidant properties of plants [20]. The percentage DPPH radical scavenging activity of the ethyl acetate leaf extracts of *Croton zambezicus* (MT), *Markhamia tomentose* (MT), *Asphilia Africana* (AA), *and Albizia ferruginea* (AF) (Table 2). They scavenged the radicals in a concentration-dependent manner with maximum concentrations of 50 µg/ml (89.6%), 400 µg/ml (22.83%), 400 µg/ml (16.5%), and 400 µg/ml (31.7%) respectively. Properties exhibited by the plant extracts are due to presence of phytochemicals which are known scavengers of free radicals [21-23].

Hydroxyl radicals produced in biological systems are known to be toxic and the most reactive species which has been recognized as extremely damaging [24]. Hydroxyl radical scavenging activity is one of the best indicators of the antioxidant potential of natural products, including plant extracts and their constituents [25]. In this study, ethyl acetate leaf extracts of *Croton zambezicus* (CZ), *Markhamia tomentose* (MT), *Asphilia Africana* (AA), *and Albizia ferruginea* (AF) showed concentration-dependent increase in hydroxyl radicals scavenging activity (Table 3), with maximum activities at 400 µg/ml (40.7%), 400 µg/ml (35.5%), 300 µg/ml (28.6%), and 400 µg/ml (51.3%) respectively. Result obtained is an indication that the bioactive component of the leaf extract has the ability to donate proton or hydrogen atoms to stabilize free radicals which may be useful in the management of diabetes associated oxidative stress [26, 27].

Lipid peroxidation is a process induced by free radicals which can leads to oxidative deterioration of polyunsaturated lipids causing cell membrane lipid disruption and damages [28]. In this study, ethyl acetate leaf extracts of *Croton zambezicus* (MT), *Markhamia tomentose* (MT), *Asphilia Africana* (AA), *and Albizia ferruginea* (AF) inhibited lipid peroxidation in a concentration-dependent manner (Table 4), with maximum inhibition at 400 µg/ml by 56%, 69%, 60%, 72% respectively. Properties exhibited by the extracts are indications of the presence of phenolic compounds [22, 29, 30].

Alpha-amylase is a digestive enzyme that catalyzes the initial steps in the hydrolysis of starch into smaller oligosaccharides such as maltose, maltriose, and other simple sugars. In diabetes conditions, rapid metabolism of carbohydrates may lead to elevated postprandial hyperglycemia which could lead to complications if not managed. The inhibition of alpha-amylase is one of the most important therapeutic targets for delaying or preventing the absorption of starch into the body resulting to reduced postprandial hyperglycemia [31, 32]. In this study, ethyl acetate leaf extracts of *Croton zambezicus* (MT), *Markhamia tomentose*(MT), *Asphilia Africana* (AA), and *Albizia ferruginea* (AF) inhibited alpha-amylase activity in a concentration dependent manner with maximum inhibition of 21.1%, 62.2 %, 64.2 % and 10.5% respectively at 400 µg/ml (Table 5). Properties shown by the leaf extracts are due to the presence of bioactive compounds such as flavonoids [33, 34].

Alpha-glucosidase is an enzyme that catalyses the final step in the hydrolysis of starch. Inhibition of α-glucosidase delays the digestion and absorption of carbohydrates and the postprandial blood glucose is maintained at a lower level leading to a decreased insulin demand. However, alpha-glucosidase is thus regarded as an important therapeutic target for the treatment and management of diabetes mellitus [35, 36]. In this study ethyl acetate leaf extracts of *Croton zambezicus* (MT), *Markhamia tomentose* (MT), *Asphilia Africana* (AA), and *Albizia ferruginea* (AF) inhibited alpha-glycosidase activity in a concentration-dependent manner with a maximum inhibition of 80.0%, 70.9%, 63.2%, and 85.3% at 50 µg/ml respectively (Table 6). The result obtained is an indication that the bioactive component of the leaf extracts has the potentials to inhibit alpha-glycosidase and may be responsible for its local usage.

V. CONCLUSION

This study revealed that certain bioactive compounds with antioxidant and anti-diabetic properties are present in the selected commonly used medicinal plants in Ogbomoso especially flavonoids , which gives scientific support for the local use of thessse plant extracts for treatment and management of diabetes mellitus and the extracts may also be explored as templates in drug discovery and design.

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