Pharmacogn. Res., 2024; 16(4):769-783.
https://www.phcogres.com

Original Article

Ethnomedicinal Plants, Associated Indigenous Knowledge and Phytochemical Composition of Extracts with Significant *in vitro* Antidiabetic Activity

Pardon Mugari[1,3], Stephen Nyoni[1], Pamhidzai Dzomba[2,*]

[1]Department of Chemistry, Chinhoyi University of Technology, Chinhoyi, ZIMBABWE.
[2]Department of Chemistry, Faculty of Science and Engineering, Bindura University of Science Education, Bindura, ZIMBABWE.

ABSTRACT

Background: Despite increasing popularity of ethnomedicinal plants in diabetes type 2 management nowadays, indigenous knowledge of their use is often not documented but rather passed on orally from generation to generation. Thus, the present study was designed to collect, analyze, document and generate useful information on ethnomedicinal plants used in management of Diabetes Mellitus Type 2 (DMT2). **Materials and Methods:** Data on ethnomedicinal use was collected using guided interviews, tour guides and information compared with literature search. Model *in vitro* enzyme inhibition assays were employed to investigate antidiabetic activity of the plants ethanol/water (60/40) extracts. Shinoda, Mayer Salkowski test and LC-MS/MS coupled with library search software's was used to determine phytochemical composition of the most active extracts. **Results:** The ethnobotanical survey identified 30 medicinal plant species belonging to 18 plant families of which 14 (46.7)% are newly reported here for their claim as anti DMT2 medicine. Among the 30 plant species reported, 10 were very popular with informant consensus factor of ≥60%. The ethanolic/water (60/40) mixture extract of *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Syzygium cordatum* and *Eureiandra fasciculate* showed the greatest *in vitro* antidiabetic activity, >70%. The present results shows that ethnomedicine plays an important part in the management of DMT2 with *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Syzygium cordatum* and *Eureiandra fasciculate* being the most appraised ethnomedicinal plants. Standard chemical tests and LC-MS/MS analysis showed that the extracts consist of mostly C-glycosylated flavonoids and to lesser extent O-glycosylated flavonoids, alkaloids and phenolic acids. **Conclusion:** The generated results will be important in the prioritization of the plant species for polyherbal drug formulation and further investigation.

Keywords: Eethnomedicines, Antidiabetic activity, *In vitro* enzyme inhibition studies.

Correspondence:
Prof. Pamhidzai Dzomba
Department of Chemistry, Faculty of Science and Engineering, Bindura University of Science Education, Bindura, ZIMBABWE.
Email: pdzomba@gmail.com,
ORCID: 0000-0001-6821-2606

Received: 16-04-2024;
Revised: 04-06-2024;
Accepted: 14-06-2024.

INTRODUCTION

Globally non-communicable diseases such as diabetes, cancers and hypertension are on the increase and becoming a major cause of morbidity and mortality in humans.[1] Among the non-communicable diseases, Diabetes Mellitus Type 2 (DMT2) now accounts for the major cause of morbidity and mortality all over the world.[2] Cost, side effects and treatment failure appears to be the major challenge in management of DMT2.[3,4] Major therapies for DMT2 include, insulin secretagogues, biguanides, insulin sensitizers, alpha glucosidase inhibitors, incretin mimetics, amylin antagonists and sodium-glucose co-transporter-2 inhibitors. Vieira *et al.*,[5] reports that for many patients, available antidiabetic drugs have portrayed low effectiveness in maintaining a long-term glycemic control. Binary drug therapies are often recommended for patients who fail to achieve hypoglycemia with first line oral antidiabetic drug agents as monotherapy. Even though these drugs portray appreciable therapeutic benefits, the conventional dosage forms show differential bioavailability and short half-life, requiring frequent taking causing greater side effects.[6] This leads to therapy ineffectiveness and greater patient non-compliance. Due to the pathological complexity of conventional medicine more and more people are resorting to using ethnobotanicals.

According to National Center for Complementary and Integrative Health (NCCIH) USA, about 30% adults and 12% children opt for alternative treatment that were discovered out of the main stream conventional medicine with approximately USD 30.2 billion out of pocket expenditure.[7] Patients often turn to alternative therapies in the effort to find low cost, safe, natural and more effective medicines. The major disappointment in the use of alternative

DOI: 10.5530/pres.16.4.89

medicines is their lack of standardization of active components and limited knowledge about them. Due to the fact that diabetes is a complex condition, it also requires multimodal treatment regimens of which ethnomedicines may provide added benefits due to presence of diverse phytocompounds. Ethnomedicines have been observed to consist of a number of phytochemicals like flavonoids, triterpenes and alkaloids that display antidiabetic activity with similar mode of actions like current therapies.[8-10]

Investigating ethnomedicines is crucial because local medicines and indigenous knowledge systems play an important role in societies be it Chinese, Indian or African.[11] Over the years this has resulted in development of new drugs with different mode of action, like the case of artemisinin an antimalarial drug was developed from Chinese traditional medicine.[12] This shows the importance of studying and documenting traditional medicines. Like in most African countries, Cigaro resettlement area in Chegutu district is endowed with many botanicals with potential to be utilized as complementary and alternative medicine however most have not been documented and studied under scientifically controlled experiments especially traditional medicine for DMT2.[13] Therefore, this study is important because it aims to contribute to the harnessing of indigenous knowledge systems and medicines in treatment of DMT2. It is also vital because it seeks to provide more knowledge through *in vitro* test and LC-MS/MS profiling of active phytocompounds. This will enable prioritization of the plant species for future studies on polyherbal complementary and alternative medicine formulations.

MATERIALS AND METHODS

Ethnobotanical survey was conducted in Chegutu district in Cigaro, a communal resettlement area using guided interviews during February to June 2023. The interview guide was designed to solicit responses for, name of the plant, location, history of use, history of effectiveness, history of toxicity, parts of plant used, preparation, dosage used and route of administration. After this, a comprehensive literature review to collect detailed information about the medicinal plants used for diabetes type 2 and associated knowledge was conducted. Dimethyl sulphoxide (DSMO), enzymes, α-amylase, α-glucosidase, Protein Tyrosine Phosphatase 1B (PTP 1B), Dipeptidyl peptidase-4 (DPP IV), starch and 3, 5, Di-Nitro Salicylic acid (DNS) were purchased from Sigma-Aldrich, German. Sodium carbonate, 2-chloro-4-nitrophenol-α-D-maltotrioside, p-nitrophenol phosphate, p-nitro-phenyl-α-glucopyranoside, glypro-p-nitroanilidine, TrisHCl buffer, di-sodium hydrogen phosphate, sodium dihydrogen phosphate, standards, acarbose, sitagliptin and ursolic acid were purchased from Merck, South Africa.

Ethnobotanical study data collection process

Initially the study involved travelling to the area of study and interacting with the community health officers and herbalist to get generalized information about the area and the activities. The data collection was then carried out with the assistance of the community health workers, herbalist, local persons and sampling assistants. All key informants were identified initially and those who agreed to participate in the study, filled in consent forms. They were then interviewed, using a structured interview guide. The prepared interview guide was translated into local language (Shona) and then back into English to check its suitability for purpose and consistency. A pre-test run of the interview guide was performed and based on the results, it was revised and administered. The data on indigenous knowledge about the perception and explanation of DMT2, methods of diagnosis, medicinal plants used for the treatment of DMT2, plant parts used and preparation approaches were collected. Additionally, the data collectors observed the treatment practices and the plants used by traditional healers. At the end of the interview, the medicinal plants were collected from the forest with the help of the herbalist and community health workers. The collected plant samples were authenticated using taxonomist at National herbarium and relevant literature by using taxonomic keys and images. The botanical names along with their authority citation and plant family names were further checked with websites including, www.tpl.org, www.eflora.com and www.ipni.org. Voucher specimens were then deposited at the Hillbright Science Education College herbarium for future reference with voucher specimen numbers 2023/01-30DMT2.

Literature survey study

Comprehensive literature search was conducted on the plants that were claimed to be used for the treatment of DMT2 in Chegutu using different search engines including Google scholar, PubMed, Science finder and Scopus. Keywords such as plant name, plants name and DMT2 treatment and herbal medicine for DMT2 were used. Information obtained from the search was compared with the ethnomedicinal claims and value of the plants.

Selection Criteria

Journal articles were selected for the study. Publications were systematically reviewed by searching using year of publication starting with year 2023. Selection of documents was conducted using the following inclusion criteria: (1) at least one part of the plant used in the treatment of DMT2 mentioned; (2) bioassay and phytochemical characterization conducted. The first author carried out an independent study to select the articles into specific folders which were identified by species name. The gathered information was then accuracy checked independently by the other two authors. The details of medicinal plants were extracted from each study using an abstraction form with headings including scientific family and name, local names, plant parts used, methods of preparation, specific uses, bioactivity assays, clinical assays and phytochemical characterization.

Preliminary *in vitro* antidiabetic activity assays

This was determined through enzyme inhibition assays using four different enzymes, protein tyrosine phosphatase 1B, (insulin action model), dipeptidyl peptidase-4, (insulin secretion model), alpha glucosidase, (carbohydrates metabolism model in the duodenum) and alpha amylase (carbohydrates metabolism model in the mouth and stomach). Plant extracts were prepared by macerating 10 g of powdered material (0.75 micron) in (60/40) ethanol/water solvent mixture for 24 hr on an orbital shaker. The contents were filtered using filter papers and evaporated to dryness under vacuum.

Alpha-amylase inhibition assay

Alpha-amylase inhibition activity of leaf, root and stem bark composite extracts were determined based on the UV spectrophotometric assay using acarbose as the standard compound.[14] The extracts were dissolved in DMSO to give concentrations of 200 mg/mL each. The enzyme solution was prepared by mixing α-amylase in 100 mL of 40 mM phosphate buffer, pH 6.9. The contents were measured by mixing 50 mL of extract/acarbose, 20 mL of α-amylase solution and 1 mL of 2-chloro-4-nitrophenol-α-D-maltotrioside. The mixture was incubated at 37°C for 5 min. The absorbance was measured at 405 nm spectrophotometrically (UV-vis Thermo Scientific Genesys 10S spectrophotometer with visionlite software). Similarly, a negative control experiment was carried out without the extracts or acarbose. Percentage inhibition was calculated by the following equation

Where %I is percentage inhibition, A_C= absorbance of control experiment (without inhibitor) and A_S=is absorbance of experiment with standard inhibitor or with extracts.

Alpha-glucosidase inhibition assay

The α-Glucosidase inhibition experiments were conducted following a slightly adjusted method by Shai *et al.*[15] The enzyme solution was made by melting α-Glucosidase in 50 µL of phosphate buffer (100 mM, 6.9 pH). Nearly, 20 µL of extract/acarbose at 1,000 µg/mL was added to 10 µL of enzyme solution and incubated for 15 min at 37°C. Then, 20 µL of 5-mM p-nitro-phenyl-α-glucopyranoside was added as a substrate and incubated again at 37°C for 20 min. The reaction was stopped by adding 50 µL of 0.1M Na_2CO_3. The absorbance of the released p-nitrophenol was measured at 405 nm using UV-vis Thermo Scientific Genesys 10S spectrophotometer. The inhibition % was calculated by the equation:

Where %I is percentage inhibition, A_C= absorbance of control experiment (without inhibitor) and A_S=is absorbance of experiment with standard inhibitor or with extracts.

Dipeptidyl peptidase IV inhibition assay

Composite stem, roots barks and leaves, (60/40) ethanol/water extracts DPPIV inhibitory activity was screened following a method reported by Bharti *et al.*[16] with minor modifications using a total well volume of 100 µL. Crude extracts or sitagliptin (standard inhibitor of DPPIV) were diluted to various concentration in the range 0-100 µg/mL using 50 mM Tris HCl buffer at pH 7.5 to a final volume of 35 µL. Absorbance of the solutions were recorded at 405 nm followed by adding 15 µL of 0.05 U/mL of DPPIV enzyme. The mixture was pre-incubated for 10 min at 37°C to allow maximum contact of enzyme with inhibitor followed by addition of 50 µL of 0.2 mM Glypro-p-nitroanilidine diluted in TrisHCl buffer. The resultant mixture was incubated at 37°C for 30 min. The reaction was terminated by adding 25 µL of 25% glacial acetic acid. The absorbance results were compared with results of a control experiment (without inhibitor). The percentage inhibition was computed using the equation;

Where %I is percentage inhibition, A_C= absorbance of control experiment (without inhibitor) and A_S=is absorbance of experiment with standard inhibitor or with extracts.

PTP1B inhibition assay

PTP 1B inhibition assay was carried out according to a slightly modified method reported by Muhammad *et al.*[17] The analyses were carried out in the reaction mixtures (100 µL) total volume, composed of extracts in the concentration range 0-100 µg/mL, p-nitrophenol phosphate (2 mM) in Bis-Tris buffer (50 mM, pH 7.2) and PTP 1B (10 mM). The mixtures were incubated at 37°C for 30 min followed by termination by adding 20 µL of 10M NaOH. The amount of p-nitrophenol produced was determined by measuring the increase in absorbance at 405 nm using a UV-vis spectrophotometer, p-Nitrophenol which were formed non-enzymatically were also determined spectrophotometrically at 405 nm without PTP 1B. Similar experiments were repeated with ursolic acid as a reference standard.

Phytochemical composition analysis of very active extracts

Zanthoxylum chalybeum, Xeroderris stuhlmannii, Syzygium cordatum and *Eureiandra fasciculate* showed significant inhibition >70% comparable to standards and these extracts were phytochemically profiled using Shinoda test for alkaloids, Meyer`s reagent test for alkaloids, Salkowski test for terpenoids and LC-MS/MS. An AB Sciex LC-MS/MS QTRAP 5500 equipped with four software's (i.e. Analyst 1.6.2, Peak View 2.2, Library View 1.0.1 and Master View) were used to analyse and determine the identities of the compounds in the extracts. It had a turbo v source with an electrospray ionisation probe operating in positive polarity. The HPLC was an Agilent 1260 LC system consisting of a vacuum degasser G4225A binary pump G1312B, auto sampler

Table 1: Participants occupation.

Occupation	Percentage (%)
Farmer	70
Registered herbalist	10
Unregistered herbalist	5
Retired teacher and farmer	4
Retired Nurse and farmer	6
Health worker	5

Table 2: General knowledge about the disease.

Knowledge sought	Number of people (%)
Definition	98
Illness Symptoms	96
Treated person behaviors	90
Causes	75

G1329B, an analytical column Phenomenex Synergi 4 µm Fusion-RP 100 Å, 50x2.0 mm and a guard column, Phenomenex Security Guard Cartridge Kit with Fusion-RP 4x2.0 mm cartridge. The mobile phase was A: Water/Methanol (90:10)+5 mM ammonium formate B: Methanol/Water (90:10)+5 mM ammonium formate operated through gradient elution. Source/Gas parameters were CUR: 30 psi, CAD: High, IS: 5500 V, TEM: 400°C GS1: 60 psi GS2: 60 psi, Q1 Scan rate=200Da/s, Enhance Product Ion (EPI) scan DP 75V and EP 10V.

Data management and analysis

The data were analyzed using SPSS version 20. The informant consensus factor corresponds to the percentage of informants that mention the use of the plant species for the management of DMT2, given by ICF=IFCx100/N; where IFC denotes the number of informants who mentioned the use of the plant species to treat DMT2 and N denotes the total number of informants in the ethnobotanical study.[18]

RESULTS AND DISCUSSION

Socio-demographic characteristics of the participants

A total of one hundred participants were identified and these agreed to participate in the study. The participants consist of 57 females and 43 males and most of them lived in a rural area (90%) and were small to medium enterprise farmers in their occupation. Out of the 100 participants 40% were of age >60, while 55% were between 40-59 years and 5% between 30-39 years. Those below 30 were not included in the study. The participants included, Table 1, exclusively farmers (70%), registered herbalist (10%, unregistered herbalist and farmers (5%), retired teachers and farmers (4%), retired nurses and farmers (6%) and health workers (5%). Also, from the population those who had directly interacted with the ethnomedicinal plants were, 68% of the population, 32% were people living with diabetes and using the ethnomedicines, 15% herbalist, 16% diabetes patients' relatives and 10% living with the patients and 5% herbalist relatives. Eight percent of the participants said they witnessed diabetes patients taking the ethnomedicines while 10% said they heard that the plants are used for DMT2 in social gatherings and 10% said they have used the plants for other ailments such as boosting immunity and other diseases other than DMT2.

General Knowledge about the disease

Table 2 shows that the participants' general knowledge of DMT2 was excellent with 98% of them managing to explain what DMT2 is. Most of the participants (90%) reported that the patient will only know that the ethnomedicine has worked by going back to the medical doctors and have their blood sugar tested while 10% said that the patient normally rise up from the sick bed and starts helping with house chores. This thorough knowledge of DMT2 can be attributed to the community health workers semi trained and appointed by Ministry of Health. The knowledge about the cause of DMT2 was also good with only 35% failing to respond to the interview questions correctly.

Diversities of ethnomedicinal plants used for the treatment DMT2

The current ethnomedical study results shows that species diversity in Cigaro resettlement area is very good. The study identified 30 ethnomedicinal plants which belonged to 18 different plant families. Fabaceae (7) and Anacardiaceae (4) were the most occurring plant families (Figure 1). *Zanthoxylum chalybeum* (82%), *Xeroderris stuhlmannii* (80%), *Elephantorrhiza elephantine* (68%), *Syzygium cordatum* (86%), *Eureiandra fasciculate* (63%), *Bauhinia petersiana* (62%), *Bridelia mollis* (60%), *Combretum fragrans* (62%), *Dalbergia melanoxylon* (63%) and *Dalbergia nyasae* (62%) were the most cited plant species (Table 3). Stem and root barks and leaves are the most frequently used plant parts. The plant material is powdered using a wooden mortar and pestle and sieved to homogenous powder. The ground powder is then given in half teaspoon dosage approximately 0.852g when it was weighed by the researchers however in majority of cases the herbalist just adds a random size in 2L or 1 L water and the patient asked to take 2 or 3 times a day. According to the participants' plants such as *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Elephantorrhiza elephantine, Syzygium cordatum* are very effective ethnomedicines for DMT2 with effectiveness cite frequency ranging from 80-100%. *Aloe greatheadii Schönland, Lipia Javanica, Eureiandra fasciculate, Rhus longipes* and *Pericopsis angolensis* efficacy reports were also high with cite frequency ranging from 55-63% see (Table 3).

Table 3: Diversities of ethnomedicinal plants used for the treatment DMT2.

Species name	Family name	ICF (%)	Plant part used	Dosage used	Efficacy cite quote (%)
Zanthoxylum chalybeum	Rutaceae	82	Root and stem bark powder.	½ teaspoon powdered added to water or porridge.	100
Xeroderris stuhlmannii	Fabaceae	80	Root bark	½ teaspoon and can be used in combination *Zanthoxylum chalybeum.*	100
Elephantorrhiza elephantine	Fabaceae	68	Stem bark	Teaspoon placed in porridge or 1L water.	82
Syzygium cordatum	Myrtaceae.	86	Root and stem bark powder.	½ teaspoon, can be used in combination with *Zanthoxylum chalybeum in equal amounts.*	70
Eureiandra fasciculate	Cucurbitaceae	63	Flowers and leaves.	Flowers or leaf portions infused in water and taken as cupful measures.	62
Bauhinia petersiana	Fabaceae	62	Fresh leaves	Flesh leaves are ground into paste infused in a cupful of water.	60
Bridelia mollis	Phyllanthaceae	60	Root and stem bark powder.	1 teaspoon powder added to water.	55
Combretum fragrans	Combretaceae	62	Dried and powered leaves.	Plant material added to 2L water.	61
Dalbergia melanoxylon	Fabaceae	63	Dried roots and leaves.	Infused in water.	54
Dalbergia nyasae	Papilionaceae	62	Dried leaves	Taken as powder or infused in water.	52
Aloe greatheadii Schönland	Aloaceae	46	Flesh leaves	Leaf potions placed in 1 cup water full.	58
Lipia Javanica	Verbenaceae	46	Fresh and dried leaves.	Leaf, portions placed in 1 cup warm water full.	52
Rhus longipes	Anacardiaceae	44	Fruits and leaves.	1 teaspoon powder added to water.	31
Pericopsis angolensis DC	Fabaceae	42	Dried stem and root bark.	Leaf, portions placed in 1 cup warm water full.	53
Carissa edulis	Apocynaceae	36	Roots bark, fruits and leaves.	Leaf, or powdered roots or fruits portions placed in 1 cup warm water full.	56
Hoodia currorii	Apocynaceae	32	Whole plant or extract.	Eaten fresh or infused in water	60
Portulaca oleracea	Portulacaceae	34	Leaves and stem.	Eaten raw or cooked.	55
Morus alba L.	Moraceae	26	Stem bark and leaves.	½ teaspoon of powdered plant material taken as powder or infused in water.	40
Vangueriopsis lanciflora	Rubiaceae.	18	Fruits and stem bark.	Extracted in water, fruits taken unripe or ripe.	52
Dioscorea steriscus	Dioscoraceae	10	Whole tuber cooked as fresh.	Added to diets.	55
Talinum tenuissimum	Portulacaceae	5	Stem and leaves	Eaten raw or cooked.	54

Species name	Family name	ICF (%)	Plant part used	Dosage used	Efficacy cite quote (%)
Nananthus aloides	Aizoaceae	10	Leaves and tubers.	Dried or fresh fused in water or taken as powder.	58
Ruschia rigens	Mesembryanthemaceae	12	Stem, leaves and flowers.	Dried or fresh fused in water or taken as powder.	42
Hagenia abyssinica	Rosaceae	5	Flowers and leaves.	Dried material fused in water.	36
Burkea Africana	Fabaceae	3	Stem bark	Dried bark fused in water.	33
Ozoroa insignis	Anacardiaceae	5	Stem and root bark.	Dried bark fused in water.	34
Searsia tenuinervis	Anacardiaceae	7	Dried and fresh leaves.	Infused in water.	41
Searsia dentate	Anacardiaceae	10	Fresh leaves and fruits.	Chewed or infused in water.	53
Annona stenophylla	Annonaceae	15	Leaves and fruits.	Infused in water.	56
Senna septemtrionalis	Fabaceae	6	Leaves	Crushed fresh leaves are mixed with water.	30

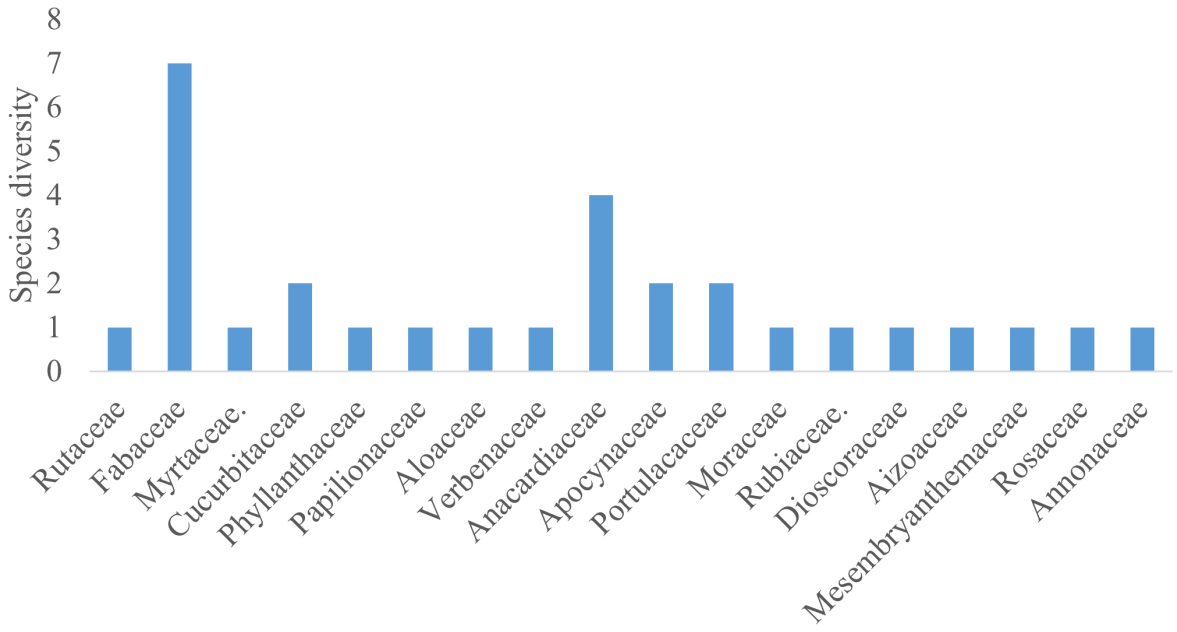

Figure 1: Antidiabetic plant species diversity in Cigaro resettlement area.

Traditional medicine knowledge and history of use

Key informants comprising of traditional medical practitioners and patients utilizing the plant species were also interviewed to ascertain history of use, safety and side effects encountered. Majority of the key informants reported that they had been using plant species such as, *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Elephantorrhiza elephantine, Syzygium cordatum, Lipia javanica, Eureiandra fasciculate, Rhus longipes* and *Pericopsis angolensis* for either prescribing to patients or using them as antidiabetic medicines for as long as 32 years with no side effects encountered (Table 4). *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Elephantorrhiza elephantine, Syzygium cordatum* can be used with conventional antidiabetic medicine metformin with no side effects encountered. Only *Lipia javanica* is not supposed to be used when on metformin. Using the two in combination results in dangerous low blood sugar levels resulting in collapsing. *Zanthoxylum chalybeum, Xeroderris stuhlmannii* and *Syzygium cordatum* may be used as single or in combination by mixing powder in equal proportions. When asked how they

would ascertain that their patients took the medicine herbalist and patients reported that usually serious ill patients stay at the herbalist's house until they feel better and are requested to visit a medical doctor to certify that the patient has been healed and there after a token of appreciation is paid to the herbalist. The researchers had an opportunity to interview some of the treated patients and one had this to say:

"I am grateful to this herbalist because without him I would have sold everything to pay medical doctors and buying medicines. I am still alive because of the herbs I got from him. I tried taking the medicines that I was given by the doctor it failed to work for me." Thus members of this communities tend to ran to ethnomedicines due to clinical failure of conventional medicines or due to higher costs.

Table 4: History of use and safety concerns.

Plant species	Time (years)	Safety
Zanthoxylum chalybeum	5-30	None known unless over dose
Xeroderris stuhlmannii	>20	None known
Elephantorrhiza elephantine	5-10	None known
Syzygium cordatum	2-20	Do not over dose
Lipia Javanica	2-30	Do not over dose and use when taking metformin
Eureiandra fasciculate	5-32	Vomiting and Diarrhea in case of over dose
Rhus longipes	10-25	None known
Pericopsis angolensis	10-20	None known

The diversity of antidiabetic plants confirmed by literature review

Thirty-five scientific studies confirming utilization of the 30 species for diabetes identified in Cigaro resettlements were identified in literature using search engines Google scholar, PubMed, Science finder and Scopus. The most referenced species was *Morus alba L* appearing in 12 scientific studies. The details of the literature search for the 30 plant species showing part used, bioactivity assays, clinical trials, compounds identified and number of citations are presented in Table 5. The major bioactive assay that has been conducted to investigate the antidiabetic activity of the plants include alpha amylase and glucosidase assays and *in vivo* assays involving the use of alloxan or streptozotocin diabetes induced rats. Since DMT2 is an insulin depended ailment resulting in the body failing to utilize insulin efficiently, assays such as anti PTP 1B and DPP IV activity must be investigated as these are models to collect information about insulin secretion and utilization. In most studies compound identification was not done. In the few studies compounds such as alkaloids, triterpenes, steroidal glycosides and flavonoids were identified as the active compounds showing either inhibitory activity against alpha amylase and glucosidase or anti-hyperglycemic in alloxan or streptozotocin diabetes induced rats. From the 30 plant species identified in the Cigaro

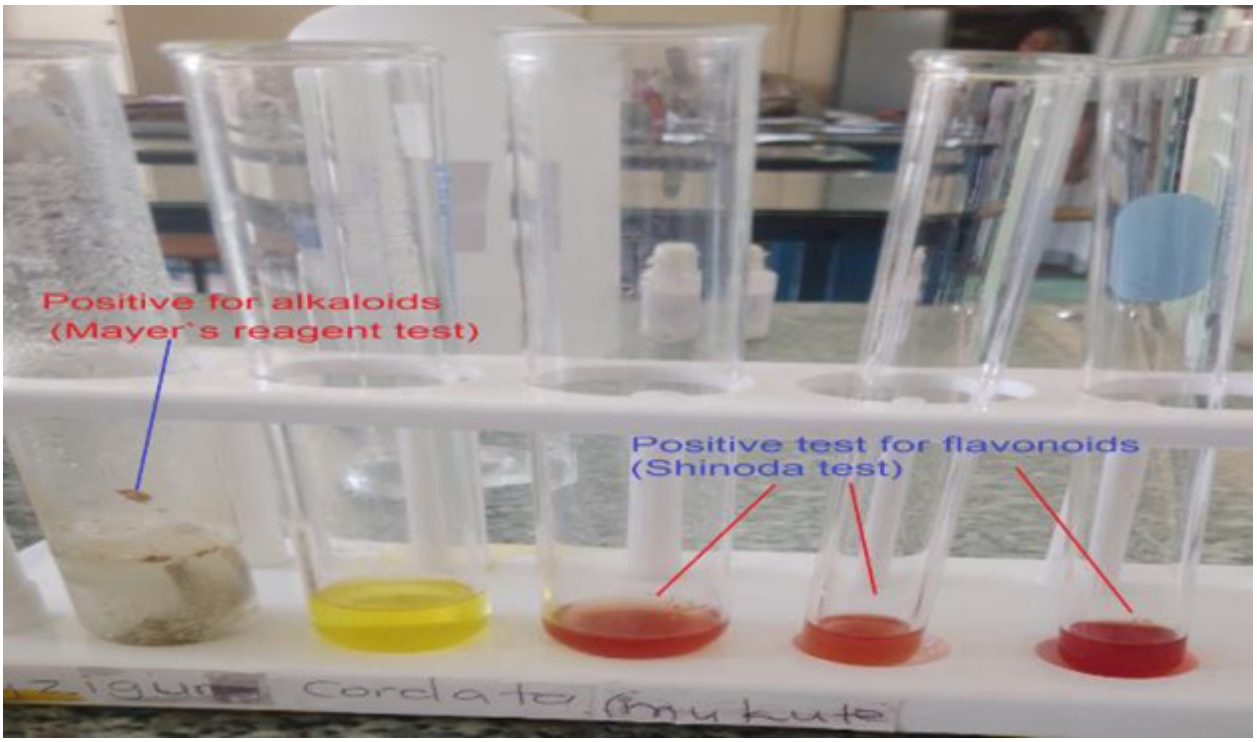

Figure 2: Mayer's reagent and Shinoda test positive results.

Table 5: Diversity of plants with antidiabetic activity as confirmed by scientific literature study (PubMed, Science finder, Scopus).

Species name	Family name	Part used	Bioactivity assays	Clinical study	Compounds identified	Number of scientific citations	Reference
Zanthoxylum chalybeum	Rutaceae	L and SB, RB	Alpha amylase and glucosidase inhibition *In vivo* analysis using alloxan and Streptozotocin rats.	NE	Alkaloids, Triterpenes, Lignans.	3	Agwaya *et al.*[20] Kimani *et al.*[21] Ochieng *et al.*[22]
Xeroderris stuhlmannii	Fabaceae	ST and RB	Alpha amylase and glucosidase inhibition.	NE	NE	1	Nyathi *et al.*[23]
Elephantorrhiza elephantine	Fabaceae	L, RT	Alpha amylase Glucose utilization assay	NE	NE	1	Olaokuna *et al.*[24]
Syzygium cordatum	Myrtaceae.	L	Use of streptozotocin diabetes induced rats.	NE	Oleanolic Acid	2	Musabayane *et al.*[25] Mapanga *et al.*[26]
Eureiandra fasciculate	Cucurbitaceae	NE	NE	NE	NE	0	0
Bauhinia petersiana	Fabaceae	NE	NE	NE	NE	0	0
Bridelia mollis	Phyllanthaceae	NE	NE	NE	NE	0	0
Combretum fragrans	Combretaceae	L	Alpha glucosidase inhibition.	NE	Combretin A and B	1	Dawe *et al.*[27]
Dalbergia melanoxylon	Fabaceae	L	NE	NE	NE	1	Rahideh *et al.*,[28]
Dalbergia nyasae	Papilionaceae	NE	NE	NE	NE	NE	NE
Aloe greatheadii Schönland	Aloaceae	L	Use of streptozotocin-induced diabetes rat model.	NE	NE	1	Loots *et al.*[29]
Lipia Javanica	Verbenaceae	L	Use of diabetic mice.	NE	Flavonoids and saponins	1	Usai *et al.*[30]
Rhus longipes	Anacardiaceae	Fr P	Alpha glucosidase inhibition assay and mRNA levels of Insulin (INS) and Glucose Transporter type-4 (GLUT-4) genes assays. Randomized clinical trials using insulin determination by immunoassay method, measurement of glucose by enzymatic method-MDA and PON1 activity calorimetrically and hs-CRP turbidimetrically.	1	NE	2	Mohammadi *et al.*[31] Rahideh *et al.*[32]
Pericopsis angolensis DC	Fabaceae	NE	NE	NE	NE	NE	NE

Species name	Family name	Part used	Bioactivity assays	Clinical study	Compounds identified	Number of scientific citations	Reference
Carissa edulis	Apocynaceae	L	Use of normal and Streptozotocin (STZ) diabetic rats.	NE	NE	1	El-Fiky *et al.*[33]
Hoodia currorii	Apocynaceae	S and L	Sorbitol dehydrogenase and glucosidase inhibition assays, *in vivo* assays using rats.	NE	Steroidal glycosides pregnane glycoside.	2	Rubin *et al.*[34] Deutschländer *et al.*[35]
Portulaca oleracea	Portulacaceae	S and L	Alloxan induced diabetic rats.			3	Ramadan *et al.*[36] Bai *et al.*[37] Lee *et al.*[38]
Morus alba L.	Moraceae	L, R and FrP	Glucosidase inhibition assays, invivo assays using alloxan diabetes induced mice.	8	Farnesylated 2-arylbenzofurans alkaloids flavonoids.	12	Ha *et al.*[39] Kang *et al.*[40] Andallu and Suryakantham[41] Zhong and Furne[42] Mudra and Ercan-Fang[43] Asai and Nakagawa[44] Kimura and Nakagawa[45] Liu and Liu[46] Banu and Jabir[47] Phiri and Chagonda[48] Zhao *et al.*[49] Thondre *et al.*[50]
Vangueriopsis lanciflora	Rubiaceae.	NE	NE	NE	NE	NE	NE
Dioscorea steriscus	Dioscoraceae	NE	NE	NE	NE	NE	NE
Talinum tenuissimum	Portulacaceae	NE	NE	NE	NE	NE	NE
Nananthus aloides	Aizoaceae	NE	NE	NE	NE	NE	NE
Ruschia rigens	Mesembryanthemaceae	NE	NE	NE	NE	NE	NE
Hagenia abyssinica	Rosaceae	L	Use of streptozotocin-induced diabetic mice.	NE	NE	1	Kifle and Belayneh[51]
Burkea africana	Fabaceae	R	Alpha amylase inhibition.		Abietane-type diterpenoid, rubesanolidic acid.	1	Tamfu *et al.*[52]
Ozoroa insignis	Anacardiaceae	NE	NE	NE	NE	NE	NE
Searsia tenuinervis	Anacardiaceae	NE	NE	NE	NE	NE	NE
Searsia dentate	Anacardiaceae	NE	NE	NE	NE	NE	NE

Species name	Family name	Part used	Bioactivity assays	Clinical study	Compounds identified	Number of scientific citations	Reference
Annona stenophylla	Annonaceae	R	Translocation of GLUT 4 assay using FITC fluorescence measured Use of alloxan induced diabetes rats.	NE	NE	NE	Morales Ramos *et al.*[53] Taderera *et al.*[54] Taderera *et al.*[55]
Senna septemtrionalis	Fabaceae	NE	NE	NE	NE	NE	NE

NE: No evidence, SB: Stem bark, RB: Root bark, ST: Stem tuber, RT: root tuber, L: Leaves, S: Stem, FrP: Fruit powder.

Table 6: Preliminary antidiabetic *in vitro* studies of (60/40) ethanol/water extract (*n*=3).

Plant	Protein tyrosine phosphatase 1B (%)	Dipeptidyl Peptidase-4 (%)	Alpha glucosidase (%)	Alpha amylase (%)
Zanthoxylum chalybeum	92.1±0.5	87.2±0.7	90.3±0.3	85.4±0.6
Xeroderris stuhlmannii	88.1±0.3	80.0±0.5	87.2±0.3	83±0.6
Elephantorrhiza elephantine	76.2±0.4	78.3±0.7	76.0±0.3	77.2±0.5
Syzygium cordatum	84.1±0.5	93.3±0.6	84.1±0.8	92.3±0.5
Eureiandra fasciculate	72.3±0.2	76.2± 0.5	77.0±0.3	74.1± 0.6
Bauhinia petersiana	76.4±0.3	70.5±0.7	68.1±0.3	62.3±0.4
Bridelia mollis	68.3±0.4	66.4±0.6	76.3±0.5	52.4±0.6
Combretum fragrans	70.2±0.5	55.3±0.5	46.3±0.2	38.3±0.5
Dalbergia melanoxylon	55.2±0.6	60.0±0.3	56.1±0.3	70.3±0.5
Dalbergia nyasae	53.2±0.3	43.6±0.3	44.4±0.5	56.5±0.2
Aloe greatheadii Schönland	67.3±0.5	70.6±0.3	68.1±0.3	78.2±0.5
Lipia Javanica	76.0±0.3	67.6±0.5	62.2±0.5	70.1±0.2
Rhus longipes	45.2±0.5	56.3±0.5	72.1±0.4	38.1±0.5
Pericopsis angolensis DC	66.4±0.6	63.2±0.3	47.2±0.3	44.4±0.3
Carissa edulis	76.7±0.3	67.3±0.6	66.6±0.7	74.2±0.3
Hoodia currorii	56.1±0.5	55.2±0.5	40.4±0.3	60.2±0.2
Portulaca oleracea	78.1±0.6	72.2±0.3	66.4±0.3	52.3±0.3
Morus alba L.	77.3±0.3	76.3±0.6	62.5±0.5	58.0±0.6
Vangueriopsis lanciflora	63.2±0.3	65.1±0.5	70.6±0.5	73.4±0.2
Dioscorea steriscus	66.3±0.5	47.0±0.3	44.0±0.2	67.1±0.5
Talinum tenuissimum	53.0±0.3	44.3±0.6	38.2±0.3	66.0±0.6
Nananthus aloides	56.2±0.5	58.0±0.5	46.3±0.3	43.0±0.5
Ruschia rigens	43.0±0.6	23.2±0.3	34.2±0.2	38.2±0.3
Hagenia abyssinica	35.1±0.3	55.3±0.4	49.0±0.5	50.2±0.4
Burkea Africana	66.1±0.3	64.4±0.5	63.6±0.5	68.7±0.3
Ozoroa insignis	32.0±0.3	39.3±0.5	60.4±0.5	42.2±0.1
Searsia tenuinervis	44.3±0.4	69.6±0.3	66.0±0.5	73.3±0.3
Searsia dentate	56.2±0.3	45.1±0.3	50.2±0.3	61.2±0.2
Annona stenophylla	60.2±0.2	68.5±0.5	72.3±0.5	68.0±0.2
Senna septemtrionalis	65.0±0.5	67.2±0.3	48.2±0.4	51.3±0.2
Standards	72.2±0.5	83.6±0.3	79.1±0.6	72.2±0.3

Table 7: LC-MS/MS characterisation of *Zanthoxylum chalybeum* crude extracts.

Retention Time	Compound Name	Match Score	CAS#	Compound class	Formula
5.898	4 - vinyl-guaiacol	99.6	2000071-77-8	Phenolic acid	$C_9H_{10}O$
7.331	phenol 2 4-bis(1 1-dimethylethyl)-	98.6	96-76-4	Phenolic acid	$C_{14}H_{22}O$
10.014	Quercetin	98.5	117-38-5	Flavonoid	$C_{15}H_{10}O_7$
11.625	Luteolin-3',4',6,8-tetramethyl ether.	98.9	855-97-0	O-alkylated flavonoid.	$C_{19}H_{18}O_6$
12.881	Quercetin-3-O-α-rhamnoside.	99.2	55696-57-6	O-glycosylated flavonoid.	$C_{21}H_{20}O_{11}$
17.719	Luteolin-4'-O-β-neohesperidoside.	98.3	25694-72-8	O-glycosylated flavonoid.	$C_{27}H_{30}O_{15}$
20.539	Rutin	98.8	153-18-4	O-glycosylated flavonoid.	$C_{27}H_{30}O_{16}$
22.591	Luteolin-7-O-β-glucoside	99.5	5373-11-5	O-glycosylated flavonoid.	$C_{21}H_{20}O_{11}$
25.221	N-(2-Hydroxy-2-methylpropyl)-3-phenyl-acrylamide.	98.3		Alkaloid	$C_{13}H_{17}NO_2$
25.863	Fagaramide	99.2	495-86-3	alkaloid	$C_{14}H_{17}NO_3$
27.012	Skimmianine	98.2	83-95-4	Alkaloid	$C_{14}H_{13}NO_4$
28.247	4-Cyano-6-(Benzylamino)-2,7-diphenyl-pyrimido[5,4-c]pyridazine-3,8-dione.	98.6	2000819-63-4	Alkaloid	$C_{26}H_{18}N_6O_2$

Table 8: LC-MS/MS characterisation of *Xeroderris stuhlmannii* crude extracts.

Retention Time	Compound Name	Match Score	CAS#	Compound class	Formula
11.717	5-Methyl-2,2,4-triethyl-2H-imidazole	96.7	2000108-88-5	Alkaloid	$C_{10}H_{18}N_2$
12.524	Quercetin	97.9	117-39-5	Flavonoid	$C_{15}H_{10}O_7$
16.549	Isoquercetin	96.5	482-35-9	O-glycosylated flavonoid	$C_{23}H_{32}O_7$
17.563	Vitexin	92.5	3681-93-4	C-glycosylated flavonoid	$C_{21}H_{20}O_{10}$
18.649	Isovitexin	94.2	3895-3-85-4	C-glycosylated flavonoid	$C_{21}H_{20}O_{10}$
20.262	Orientin	90.2	28608-75-5	C-glycosylated flavonoid	$C_{21}H_{20}O_{11}$
24.168	Isoorientin	91.9	4261-42-1	C-glycosylated flavonoid	$C_{21}H_{20}O_{11}$

resettlement area 14 (47%) were newly reported for their use as anti DMT2 medicine with zero scientific studies reported. The species are, *Eureiandra fasciculate, Bauhinia petersiana, Bridelia mollis, Dalbergia nyasae, Pericopsis angolensis DC, Vangueriopsis lanciflora, Dioscorea steriscus, Talinum tenuissimum, Nananthus aloides, Ruschia rigens, Ozoroa insignis, Searsia tenuinervis, Searsia dentate* and *Senna septemtrionalis*. Leaves, root and stem bark and fruits are the utilized parts. While some effort has be made in trying to scientifically show the effectiveness of the plant species as anti DMT2 medicine under laboratory studies, very few clinical trials have been performed. Among the 30 species only 3 species have been tested clinically using volunteer human subjects under controlled scientific studies. These are *Portulaca oleracea, Rhus longipes* and *Morus alba. Morus alba* has the highest clinical studies. Lack of clinical studies data hinders utilization of the plants in conventional medicine set ups.[19] This is because of inadequate knowledge of their mode of action, potential adverse reactions, contraindications and possible interactions with existing conventional medicines.

Table 9: LC-MS/MS characterisation of *Syzygium cordatum* crude extracts.

Retention Time	Compound Name	Match Score	CAS#	Compound class	Formula
9.123	Isovitexin-7-O-glucosyl-2"O-rhamnoside	98.7	72036-50-1	O-glycosylated flavonoid	$C_{21}H_{19}O_{10}$
10.412	N-(3-methyl-2-buten-1-yl)-adenosine	96.3	7724-76-7	alkaloid	$C_{15}H_{21}N_5O_4$
12.001	Methylsalicylate	97.5	119-36-8	Phenolic acid	$C_8H_8O_3$
15.231	Vitexin	92.5	3681-93-4	C-glycosylated flavonoid	$C_{21}H_{20}O_{10}$
17.512	Isovitexin	94.2	3895-3-85-4	C-glycosylated flavonoid	$C_{21}H_{20}O_{10}$

Table 10: LC-MS/MS characterisation of *Eureiandra fasciculate* crude extracts.

Retention Time	Compound Name	Match Score	CAS#	Compound class	Formula
2.681	Rutin	90.2	153-18-4	O-glycosylated flavonoid	$C_{27}H_{30}O_{16}$
3.231	Vitexin	92.5	3681-93-4	C-glycosylated flavonoid	$C_{21}H_{20}O_{10}$
4.367	Isovitexin	94.2	3895-3-85-4	C-glycosylated flavonoid	$C_{21}H_{20}O_{10}$
9.633	5-caffeoylquinic acid	94.6	327-97-9	Phenolic acid ester	$C_{16}H_{18}O_9$
10.182	Isoorientin	91.9	4261-42-1	C-glycosylated flavonoid	$C_{21}H_{20}O_{11}$

Preliminary antidiabetic *in vitro* studies of (60/40) ethanol/water extract

Table 6 summarizes percentage inhibitory activity of the 30 plant species against 4 enzymes, protein tyrosine phosphatase 1B, dipeptidyl peptidase-4, alpha glucosidase and alpha amylase that are usually used as models for testing antidiabetic activity. All the 30 species showed inhibitory activity against the 4 enzymes supporting their potency claim in Cigaro resettlement area in Chegutu district. *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Syzygium cordatum* and *Eureiandra fasciculate* showed remarkable inhibition > 70% comparable to standards. *Zanthoxylum chalybeum, Xeroderris stuhlmannii* and *Syzygium cordatum* showed superior inhibitory activity against the 4 enzymes > 80. These also received the highest percentage ICF and efficacy cite quote, Table 3, in ethnomedicinal studies proving their usefulness as DMT2 medicines.

Phytochemical composition analysis of very active extracts

Figure 2 shows the positive colors that were obtained when Shinoda, Meyer and Salkowski tests were carried out to investigate the presence of flavonoids, alkaloids and terpenoids. Using LC-MS/MS analysis of crude extracts of *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Syzygium cordatum* and *Eureiandra fasciculate* showing remarkable inhibition many bioactive compounds were identified Tables 7-10.

Active *Zanthoxylum chalybeum* extract consisted of alkaloids, flavonoids and phenolic acids. Alkaloids, N-(2-Hydrox y-2-methylpropyl)-3-phenyl-acrylamide, fagaramide and skimmianine have been reported previously to have antidiabetic activity by inhibiting alpha amylase and glucosidase.[55] Flavonoids such as quercetin, luteolin-3', 4', 6, 8-tetramethyl ether and rutin exhibited *in vivo* anti-diabetic activity.[56] C-glycosylated flavonoids have shown good stability in biological systems therefore making them useful active compounds.[57] The active, *Xeroderris stuhlmannii, Syzygium cordatum* and *Eureiandra fasciculate* extract was found to be rich in O and C-glycosylated flavonoids vitexin, orientin and rutin. Phenolic acids, 4-vinyl-guaiacol and 5-caffeoylquinic acid were found in *Zanthoxylum chalybeum* and *Eureiandra fasciculate* extracts. 5-caffeoylquinic acid showed antidiabetic activity in streptozotocin induced diabetic rats.[58]

CONCLUSION

The study found out that 30 plant species are used as ethnomedicines against DMT2 in Cigaro resettlement area of Chegutu district. The most popular plant families are Fabaceae and Anacardiaceae. *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Elephantorrhiza elephantine, Syzygium cordatum, Eureiandra fasciculate, Bauhinia petersiana, Bridelia mollis, Combretum fragrans, Dalbergia melanoxylon* and *Dalbergia nyasae* were the most popular plants. *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Elephantorrhiza elephantine, Syzygium cordatum* were reported to be very effective plants against DMT2 with *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Elephantorrhiza elephantine, Syzygium cordatum* receiving superior commendation. In history of use and safety concerns it was found out that people in the area have been using the plants for longer periods with few side effects unless overdose i.e. half teaspoon dosage approximately 0.852g of plant material when it was weighed by the researchers. The 30 plant species also showed good enzyme inhibitory activity against protein tyrosine phosphatase 1B, dipeptidyl peptidase-4, alpha glucosidase and alpha amylase in preliminary *in vitro* antidiabetic studies. *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Syzygium cordatum and Eureiandra fasciculate* showed remarkable inhibition > 70% that was comparable to standard drugs. *Zanthoxylum chalybeum, Xeroderris stuhlmannii* and *Syzygium cordatum* showed superior inhibitory activity than standards > 80%. The active compounds identified in the extracts are alkaloids, C and O glycosylated flavonoids and phenolic acids. Thus, the present findings has laid the necessary foundational knowledge for future studies to support the medicinal use of the 30 identified plant species.

ACKNOWLEDGEMENT

Authors would like to thank all people including, the herbalists who participated in this study, Bindura University of Science Education and Chinhoyi University of Technology for providing laboratory space and reagents and Hilbright Science College for financial support.

FUNDING SOURCES

Financial aid provided by Hilbright Science College.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DMT2: Diabetes Mellitus type 2; **LC-MS/MS:** Liquid Chromatography Tandem Mass Spectrometry; **PTP 1B:** Protein tyrosine phosphatase 1B; **DPP IV:** Dipeptidyl peptidase-4.

SUMMARY

In the present study, the ethnobotanical survey identified 30 medicinal plant species belonging to 18 plant families of which 14 (46.7)% are newly reported here for their claim as anti DMT2 medicine. From the 30 plant species, 10 were very popular as antidiabetic remedies with informant consensus factor of ≥ 60%. The ethanolic/water (60/40) extract of *Zanthoxylum chalybeum, Xeroderris stuhlmannii, Syzygium cordatum and Eureiandra fasciculate* showed the greatest *in vitro* antidiabetic activity, > 70% for almost all enzymes' models used. Standard chemical tests and LC-MS/MS analysis showed that the extracts consist of mostly C-glycosylated flavonoids and to lesser extent O-glycosylated flavonoids, alkaloids and phenolic acids.

ETHICAL CLEARANCE

The ethical clearance was obtained from the Chinhoyi University of Technology ethical clearance office and the Medicinal Council Association of Zimbabwe (MCAZ). Oral consent was first obtained from the community head man, health worker and participants after an explanation of the aims and purposes of the study was provided and their confidentiality was assured. The participants were then made to sign consent forms. Participation was voluntary after an explanation of the purpose of the study.

AUTHOR CONTRIBUTIONS

PM and PD designed the study and carried out the field studies. PM carried out the *in vitro* assays. PM wrote the first draft. PD and SN managed the literature and wrote the second draft. PM wrote the final draft. All authors approved the final draft.

REFERENCES

1. Ramesh S, Kosalram K. The burden of non-communicable diseases: A scoping review focus on the context of India. J Educ Health Promot. 2023;12:41. doi: 10.4103/jehp.jehp_1113_22, PMID 37113407.
2. O'Hearn M, Lara-Castor L, Cudhea F, Miller V, Reedy J, Shi P, *et al.* Incident type 2 diabetes attributable to suboptimal diet in 184 countries. Nat Med. 2023;29(4):982-95. doi: 10.1038/s41591-023-02278-8, PMID 37069363.
3. Shrestha N, Lohani SP, Angdembe MR, Bhattarai K, Bhattarai J. Cost of diabetes mellitus care among patients attending selected outpatient clinics. JNMA J Nepal Med Assoc. 2013;52(190):343-8. doi: 10.31729/jnma.2114, PMID 24362657.
4. Padhi S, Nayak AK, Behera A. Type II diabetes mellitus: a review on recent drug-based therapeutics. Biomed Pharmacother. 2020;131:110708. doi: 10.1016/j.biopha.2020.110708, PMID 32927252.
5. Vieira R, Souto SB, Sánchez-López E, Machado AL, Severino P, Jose S, *et al.* Sugar-lowering drugs for type 2 diabetes mellitus and metabolic syndrome-review of classical and new compounds: Part-I. Pharmaceuticals (Basel). 2019;12(4):152. doi: 10.3390/ph12040152, PMID 31658729.
6. Kahn SE, Cooper ME, Prato SD. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present and future. Lancet. 2014;383:1068-83. doi: 10.1016/S0140-6736.
7. National Center for Complementary and Integrative Health. Complementary, alternative, or integrative health: what's in a name? [cited Jan 10, 2021] Available from: https://nccih.nih.gov/sites/nccam.nih.gov/files/Whats_In_A_Name_06-16-2016.pdf.
8. Cahyana Y, Adiyanti T. Flavonoids as antidiabetic agents. Indones J Chem. 2021;21(2):512-26. doi: 10.22146/ijc.58439.
9. Adhikari B. Roles of alkaloids from medicinal plants in the management of diabetes mellitus. J Chem. 2021; 2021:2021zArticle, 10 pages. doi: 10.1155/2021/2691525.

10. Oboh M, Govender L, Siwela M, Mkhwanazi BN. Anti-diabetic potential of plant-based pentacyclic triterpene derivatives: progress made to improve efficacy and bioavailability. Molecules. 2021;26(23):7243. doi: 10.3390/molecules26237243, PMID 34885816.
11. WHO. World Health Organization global report on traditional and complementary medicine. Geneva: World Health Organization; 2019; License: CC BY-NC-SA 3.0 IGO.
12. Foley JA. Increasing production of artemisinin by demethylation of A. annua Hairy Root cultures. Worcester Polytechnic Institute; 2007. Available from: https://digitalcommons.wpi.edu/mqp-all/386.
13. Maroyi A. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. J Ethnobiol Ethnomed. 2013;9:31. doi: 10.1186/1746-4269-9-31, PMID 23642285.
14. Abdel-Hady H, El-Wakil EA, Morsi E. Characterization of ethyl acetate and methanol extracts of *Commiphora myrrha* and evaluating *in vitro* anti-diabetic and anti-obesity activities. J App Pharm Sci. 2019;9(9):38-44. doi: 10.7324/JAPS.2019.90906.
15. Shai LJ, Magano SR, Lebelo SL, Mogale AM. Inhibitory effects of five medicinal plants on rat alpha-glucosidase: comparison with their effects on yeast alpha-glucosidase. J Med Plants Res. 2011;5(13):2863-7.
16. Bharti SK, Krishnan S, Kumar A, Rajak KK, Murari K, Bharti BK, *et al.* Antihyperglycemic activity with DPP-IV inhibition of alkaloids from seed extract of Castanospermum australe: investigation by experimental validation and molecular docking. Phytomedicine. 2012;20(1):24-31. doi: 10.1016/j.phymed.2012.09.009, PMID 23063145.
17. Mohammadi S, Kouhsari SM, Feshani AM. Antidiabetic properties of the ethanolic extract of *Rhus coriaria* fruits in rats. DARU J Pharm Sci. 2010;18(4):270-5. PMID 22615627, PMCID PMC3304353.
18. Suleman S, Beyene Tufa T, Kebebe D, Belew S, Mekonnen Y, Gashe F, *et al.* Treatment of malaria and related symptoms using traditional herbal medicine in Ethiopia. J Ethnopharmacol. 2018;213:262-79. doi: 10.1016/j.jep.2017.10.034, PMID 29102764.
19. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. doi: 10.3389/fphar.2013.00177, PMID 24454289.
20. Agwaya MS, Vuzi PC. Hypoglycemic activity of aqueous root bark extract *Zanthoxylum chalybeum* in alloxan-induced diabetic rats. J Diabetes Res. 2016; 2016:Article ID 8727590, 5 pages. doi: 10.1155/2016/8727590, PMID 27069932.
21. Kimani NM, Ochieng CO, Ogutu MD, Yamo KO, Onyango JO, Santos CB. Inhibition kinetics and theoretical studies on *Zanthoxylum chalybeum* Engl. Dual inhibitors of α-glucosidase and α-amylase. J Xenobiot. 2023;13(1):102-20. doi: 10.3390/jox13010009, PMID 36976158.
22. Ochieng CO, Nyongesa DW, Yamo KO, Onyango JO, Langat MK, Manguro LA. α-amylase and α-glucosidase inhibitors from *Zanthoxylum chalybeum* Engl. root bark. Fitoterapia. 2020;146:104719. doi: 10.1016/j.fitote.2020.104719, PMID 32889048.
23. Nyathi B, Bvunzawabaya JT, Venissa P Mudawarima CV, Manzombe E, Tsotsoro K, Selemani MA, *et al.* Inhibitory and *in silico* molecular docking of *Xeroderris stuhlmannii* (Taub.) Mendonca and E.P. Sousa phytochemical compounds on human α-glucosidases. J Ethnopharmacol. 2023;312(312):116501. doi: 10.1016/j.jep.2023.116501.
24. Olaokun OO, Alaba AE, Ligege K, Mkolo NM. Phytochemical content, anti-diabetes, anti-inflammatory antioxidant and cytotoxic activity of leaf extracts of *Elephantorrhiza elephantina* (Burch.) Skeels. S Afr J Bot. 2020;128:319-25. doi: 10.1016/j.sajb.2019.11.030.
25. Musabayane CT, Mahlalela N, Shode FO, Ojewole JA. Effects of *Syzygium cordatum* (Hochst.) [Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2005;97(3):485-90. doi: 10.1016/j.jep.2004.12.005, PMID 15740884.
26. Mapanga RF, Tufts MA, Shode FO, Musabayane CT. Renal effects of plant-derived oleanolic acid in streptozotocin-induced diabetic rats. Ren Fail. 2009;31(6):481-91. doi: 10.1080/08860220902963558, PMID 19839826.
27. Dawé A, Mbiantcha M, Yakai F, Jabeen A, Ali MS, Lateef M, *et al.* Flavonoids and triterpenes from *Combretum fragrans* with anti-inflammatory, antioxidant and antidiabetic potential, Aus der. Z Naturforsch C J Biosci. 2018;. https:;73(5-6): 211-9. doi: 10.1515/znc-2017-0166, PMID 29451865.
28. Rahideh ST, Shidfar F, Khandozi N, Rajab A, Hosseini SP, Mirtaher SM. The effect of sumac (*Rhus coriaria* L.) powder on insulin resistance, malondialdehyde, high sensitive C-reactive protein and paraoxonase 1 activity in type 2 diabetic patients. J Res Med Sci. 2014;19(10):933-8. PMID 25538775.
29. Loots DT, Pieters M, Islam MS, Botes L. Antidiabetic effects of *Aloe ferox* and *Aloe greatheadii* var. davyana leaf gel extracts in a low-dose streptozotocin diabetes rat model. S Afr J Sci. 2011; 107(7/8):7-8. doi: 10.4102/sajs.v107i7/8.532.
30. Usai R, Majoni S, Rwere F. Natural products for the treatment and management of diabetes mellitus in Zimbabwe-a review. Front Pharmacol. 2022;13:980819. doi: 10.3389/fphar.2022.980819, PMID 36091798.
31. Muhammad A, Tel-Cayan G., Öztürk M., Nadeem S., Duru M. E., Anis I., *et al.* Biologically active flavonoids from *Dodonaea viscosa* and their structure-activity relationships. Ind. Crop. Prod. 2015;78:66-72. https://doi.org/10.1016/j.indcrop.2015.10.011.
32. Rahideh ST, Shidfar F, Khandozi N, Rajab A, Hosseini SP, Mirtaher SM. The effect of sumac (*Rhus coriaria* L.) powder on insulin resistance, malondialdehyde, high sensitive C-reactive protein and paraoxonase 1 activity in type 2 diabetic patients. J Res Med Sci. 2014;19(10):933-8. PMID 25538775.
33. El-Fiky FK, Abou-Karam MA, Afify EA. Effect of *Luffa aegyptiaca* (seeds) and *Carissa edulis* (leaves) extracts on blood glucose level of normal and streptozotocin diabetic rats. J Ethnopharmacol. 1996;50(1):43-7. doi: 10.1016/0378-8741(95)01324-5, PMID 8778506.
34. Rubin ID, Bindra JS, Cawthorne MA. Extracts, compounds and pharmaceutical compositions having ant-diabetic activity their use, United States Patent 2002. Us 2002O146468A1.
35. Deutschländer MS, Lall N, van de Venter M. Plant species used in the treatment of diabetes by South African traditional healers: an inventory. Pharm Biol. 2009;47(4):348-65. doi: 10.1016/j.jep.2009.04.052.
36. Ramadan BK, Schaalan MF, Tolba AM. Hypoglycemic and pancreatic protective effects of *Portulaca oleracea* extract in alloxan induced diabetic rats. BMC Complement Altern Med. 2017;17(1):37. doi: 10.1186/s12906-016-1530-1, PMID 28077129.
37. Bai Y, Zang X, Ma J, Xu G. Antidiabetic effect of *Portulaca oleracea* L. polysaccharide and its mechanism in diabetic rats. Int J Mol Sci. 2016;17(8):1201. doi: 10.3390/ijms17081201, PMID 27463713.
38. Lee JH, Park JE, Han JS. *Portulaca oleracea* L. extract reduces hyperglycemia via PI3k/Akt and AMPK pathways in the skeletal muscles of C57BL/Ksj-db/db mice. J Ethnopharmacol. 2020;260(5):112973. doi: 10.1016/j.jep.2020.112973, PMID 32416244.
39. Ha MT, Shrestha S, Tran TH, Kim JA, Woo MH, Choi JS, *et al.* Inhibition of PTP1B by farnesylated 2-arylbenzofurans isolated from *Morus alba* root bark: unraveling the mechanism of inhibition based on *in vitro* and *in silico* studies. Arch Pharm Res. 2020;43(9):961-75. doi: 10.1007/s12272-020-01269-4, PMID 32978714.
40. Kang CW, Park M, Mulberry LHJ. Mulberry (Morus alba L.) Leaf Extract and 1-Deoxynojirimycin Improve Skeletal Muscle Insulin Resistance via the Activation of IRS-1/PI3K/Akt Pathway in db/db Mice. Life (Basel). 2022;12(10):1630. https:. doi: 10.3390/life12101630, PMID 36295064.
41. Andallu B, Suryakantham V. Effect of mulberry (*Morus indica* L.) therapy on plasma and erythrocyte membrane lipids in patients with type 2 diabetes. Clin Chim Acta. 2001;314:47.e53. doi: 10.1016/S0009-8981(01)00632-5.
42. Zhong L, Furne JK. An extract of black, green and mulberry teas causes malabsorption of carbohydrate but not of triacylglycerol in healthy volunteers. Am J Clin Nutr. 2006;84:551.e555.
43. Mudra M, Ercan-Fang N, Zhong L, Furne J, Levitt M. Influence of mulberry leaf extract on the blood glucose and breath hydrogen response to ingestion of 75 g sucrose by type 2 diabetic and control subjects. Diabetes Care. 2007;30(5):1272-4. doi: 10.2337/dc06-2120, PMID 17303787.
44. Asai A, Nakagawa K. Effect of mulberry leaf extract with enriched 1-deoxynojirimycin content on postprandial glycemic control in subjects with impaired glucose metabolism. J Diabetes Investig. 2011;2:318.e323. doi: 10.1111/j.2040-1124.2011.00101.x.
45. Kimura T, Nakagawa K, Kubota H, Kojima Y, Goto Y, Yamagishi K, *et al.* Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. J Agric Food Chem. 2007;55(14):5869-74. doi: 10.1021/jf062680g, PMID 17555327.
46. Liu Q, Liu J. The treatments of total alkali from *Morus folium* jiangtang capsule combined with diet and exercise program for type 2 diabetes mellitus equivalence randomized controlled study. J Pract Tradit Chin Intern Med. 2013;27:24.e27.
47. Banu S, Jabir NR. Reduction of post-prandial hyperglycemia by mulberry tea in type-2 diabetes patients. Saudi J Biol Sci. 2015;22:32.e36. doi: 10.1016/j.sjbs.2014.04.005.
48. Phiri J, Chagonda LS. Hypoglycemic effects of *Annona stenophylla* and *Morus alba* Plant extracts in alloxan-induced diabetic mice. J Biol Act Prod Nat. 2012;2(6):377-81. doi: 10.1080/22311866.2012.10719146.
49. Zhao X, Fu Z, Yao M, Cao Y, Zhu T, Mao R, *et al.* Mulberry (*Morus alba* L.) leaf polysaccharide ameliorates insulin resistance- and adipose deposition-associated gut microbiota and lipid metabolites in high-fat diet-induced obese mice. Food Sci Nutr. 2022;10(2):617-30. doi: 10.1002/fsn3.2689, PMID 35154697.
50. Thondre PS, Lightowler H, Ahlstrom L, Gallagher A. Mulberry leaf extract improves glycaemic response and insulaemic response to sucrose in healthy subjects: results of a randomized, double blind, placebo-controlled study. Nutr Metab (Lond). 2021;18(1):41. doi: 10.1186/s12986-021-00571-2, PMID 33858439.
51. Kifle ZD, Belayneh YM. Antidiabetic and anti-hyperlipidemic effects of the crude hydromethanol extract of *Hagenia abyssinica* (Rosaceae) Leaves in streptozotocin-induced diabetic mice. Diabetes Metab Syndr Obes. 2020;13(13):4085-94. doi: 10.2147/DMSO.S279475, PMID 33149648.
52. Tamfu AN, Ntchapda F, Gade IS, Mbane MN, Tagatsing MF, *et al.* A new abietane-type diterpenoid from roots of *Burkea africana* Hook (Fabaceae) with α-amylase inhibitory potential. Nat Prod Res. 2021. doi: 10.1080/14786419.2021.1976176.
53. Morales Ramos JG, Esteves Pairazamán AT, Mocarro Willis ME, Collantes Santisteban S, Caldas Herrera E. Medicinal properties of *Morus alba* for the control of type 2 diabetes mellitus: a systematic review. F1000Res. 2021;10:1022. doi: 10.12688/f1000research.55573.1, PMID 34912543.

54. Taderera T, Chagonda LS, Gomo E, Katerere D, Shai LJ. *Annona stenophylla* aqueous extract stimulate glucose uptake in established C2CI2 muscle cell lines. Afr Health Sci. 2019;19(2):2219-29. doi: 10.4314/ahs.v19i2.47, PMID 31656507.
55. Taderera T, Gomo E, Chagonda LS. The antidiabetic Activity of an Aqueous Root Extract of *Annona stenophylla* Engl. and Diels in Nondiabetic Control and Alloxan-induced diabetic rats. J Biol Act Prod Nat. 2016;6:4.
56. Cahyana Y, Adiyanti T. Flavonoids as antidiabetic agents. Indones J Chem. 2021;21(2):512-26. doi: 10.22146/ijc.58439.
57. Brazier-Hicks MB, Evans KM, Gershater MC, Puschmann H, Steel PG, Edwards R. The C-glycosylation of flavonoids in cereals. J Biol Chem. 2009;284(27):17926-34. doi: 10.1074/jbc.M109.009258, PMID 19411659.
58. Singh AK, Rana HK, Singh V, Chand Yadav TC, Varadwaj P, Pandey AK. Evaluation of antidiabetic activity of dietary phenolic compound chlorogenic acid in streptozotocin induced diabetic rats: molecular dynamics, *in silico* toxicity, *in vitro* and *in vivo* studies. Comput Biol Med. 2021;134:104462. doi: 10.1016/j.compbiomed.2021.104462, PMID 34148008.

Cite this article: Mugari P, Nyoni S, Dzomba P. Ethnomedicinal Plants, Associated Indigenous Knowledge and Phytochemical Composition of Extracts with Significant *in vitro* Antidiabetic Activity. Pharmacog Res. 2024;16(4):769-83.