

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

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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 $\geq 60\%$. The ethanolic/water (60/40) mixture extract of *Zanthoxylum chalybeum*, *Xeroderris stuhlmannii*, *Syzygium cordatum* and *Eureiandra fasciculata* 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 fasciculata* 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: Ethnomedicines, Antidiabetic activity, *In vitro* enzyme inhibition studies.

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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



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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 phytochemicals. 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 phytochemicals. 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-maltotrioxide, 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-maltotrioxide. 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 μ L of phosphate buffer (100 mM, 6.9 pH). Nearly, 20 μ L of extract/acarbose at 1,000 μ g/mL was added to 10 μ L of enzyme solution and incubated for 15 min at 37°C. Then, 20 μ L 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 μ L 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 μ L. Crude extracts or sitagliptin (standard inhibitor of DPPIV) were diluted to various concentration in the range 0-100 μ g/mL using 50 mM Tris HCl buffer at pH 7.5 to a final volume of 35 μ L. Absorbance of the solutions were recorded at 405 nm followed by adding 15 μ L 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 μ L 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 μ L 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 μ L) total volume, composed of extracts in the concentration range 0-100 μ g/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 μ L 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 fasciculata* 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 μ m 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 $ICF = IFC \times 100 / N$; 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 (%) |
|------------------------------------|----------------|---------|--------------------------------|--|-------------------------|
| <i>Zanthoxylum chalybeum</i> | Rutaceae | 82 | Root and stem bark powder. | ½ teaspoon powdered added to water or porridge. | 100 |
| <i>Xeroderris stuhlmannii</i> | Fabaceae | 80 | Root bark | ½ teaspoon and can be used in combination <i>Zanthoxylum chalybeum</i> . | 100 |
| <i>Elephantorrhiza elephantine</i> | Fabaceae | 68 | Stem bark | Teaspoon placed in porridge or 1L water. | 82 |
| <i>Syzygium cordatum</i> | Myrtaceae. | 86 | Root and stem bark powder. | ½ teaspoon, can be used in combination with <i>Zanthoxylum chalybeum</i> in equal amounts. | 70 |
| <i>Eureiandra fasciculata</i> | Cucurbitaceae | 63 | Flowers and leaves. | Flowers or leaf portions infused in water and taken as cupful measures. | 62 |
| <i>Bauhinia petersiana</i> | Fabaceae | 62 | Fresh leaves | Flesh leaves are ground into paste infused in a cupful of water. | 60 |
| <i>Bridelia mollis</i> | Phyllanthaceae | 60 | Root and stem bark powder. | 1 teaspoon powder added to water. | 55 |
| <i>Combretum fragrans</i> | Combretaceae | 62 | Dried and powered leaves. | Plant material added to 2L water. | 61 |
| <i>Dalbergia melanoxydon</i> | Fabaceae | 63 | Dried roots and leaves. | Infused in water. | 54 |
| <i>Dalbergia nyasae</i> | Papilionaceae | 62 | Dried leaves | Taken as powder or infused in water. | 52 |
| <i>Aloe greatheadii Schönland</i> | Aloaceae | 46 | Flesh leaves | Leaf portions placed in 1 cup water full. | 58 |
| <i>Lipia Javanica</i> | Verbenaceae | 46 | Fresh and dried leaves. | Leaf, portions placed in 1 cup warm water full. | 52 |
| <i>Rhus longipes</i> | Anacardiaceae | 44 | Fruits and leaves. | 1 teaspoon powder added to water. | 31 |
| <i>Pericopsis angolensis</i> DC | Fabaceae | 42 | Dried stem and root bark. | Leaf, portions placed in 1 cup warm water full. | 53 |
| <i>Carissa edulis</i> | Apocynaceae | 36 | Roots bark, fruits and leaves. | Leaf, or powdered roots or fruits portions placed in 1 cup warm water full. | 56 |
| <i>Hoodia currorii</i> | Apocynaceae | 32 | Whole plant or extract. | Eaten fresh or infused in water | 60 |
| <i>Portulaca oleracea</i> | Portulacaceae | 34 | Leaves and stem. | Eaten raw or cooked. | 55 |
| <i>Morus alba</i> L. | Moraceae | 26 | Stem bark and leaves. | ½ teaspoon of powdered plant material taken as powder or infused in water. | 40 |
| <i>Vangueriopsis lanciflora</i> | Rubiaceae. | 18 | Fruits and stem bark. | Extracted in water, fruits taken unripe or ripe. | 52 |
| <i>Dioscorea steriscus</i> | Dioscoraceae | 10 | Whole tuber cooked as fresh. | Added to diets. | 55 |
| <i>Talinum tenuissimum</i> | Portulacaceae | 5 | Stem and leaves | Eaten raw or cooked. | 54 |

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

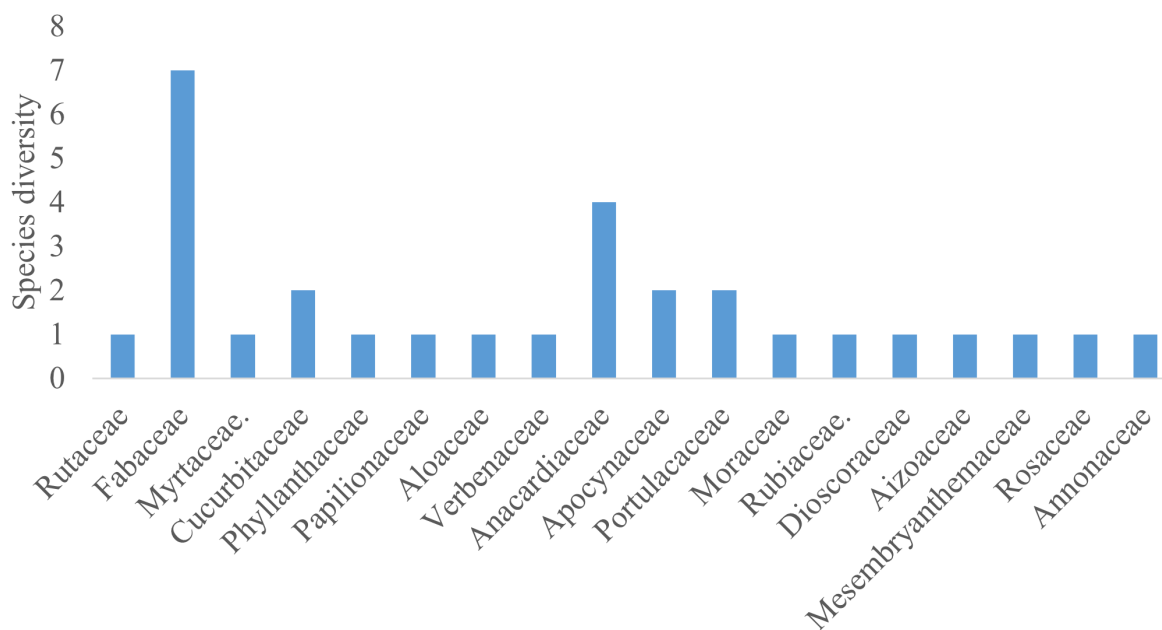


Figure 1: Antidiabetic plant species diversity in Cigaro resettlement area.

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 fasciculata*, *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.

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

Table 4: History of use and safety concerns.

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

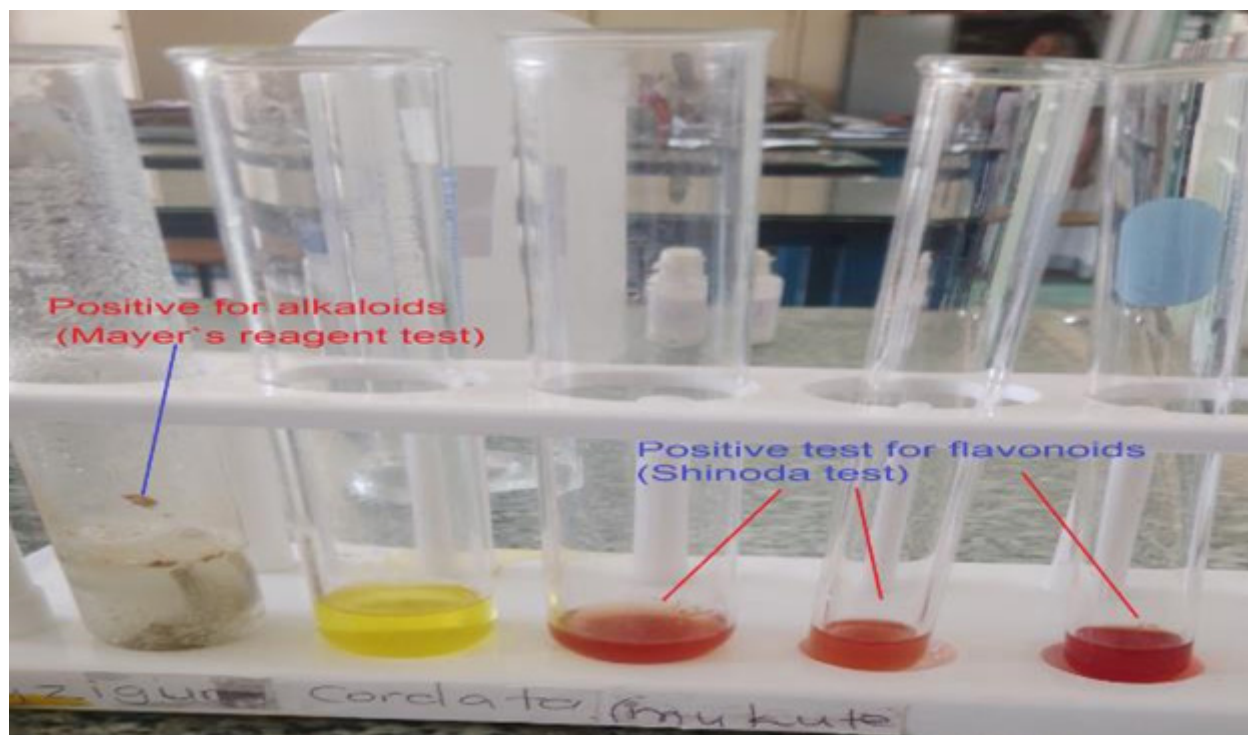


Figure 2: Mayer's reagent and Shinoda test positive results.

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 |
|------------------------------------|----------------|--------------|---|----------------|----------------------------------|--------------------------------|---|
| <i>Zanthoxylum chalybeum</i> | Rutaceae | L and SB, RB | Alpha amylase and glucosidase inhibition <i>In vivo</i> analysis using alloxan and Streptozotocin rats. | NE | Alkaloids, Triterpenes, Lignans. | 3 | Agwaya <i>et al.</i> ^[20] Kimani <i>et al.</i> ^[21] Ochieng <i>et al.</i> ^[22] |
| <i>Xeroderris stuhlmannii</i> | Fabaceae | ST and RB | Alpha amylase and glucosidase inhibition. | NE | NE | 1 | Nyathi <i>et al.</i> ^[23] |
| <i>Elephantorrhiza elephantina</i> | Fabaceae | L, RT | Alpha amylase Glucose utilization assay | NE | NE | 1 | Olaokuna <i>et al.</i> ^[24] |
| <i>Syzygium cordatum</i> | Myrtaceae. | L | Use of streptozotocin diabetes induced rats. | NE | Oleanolic Acid | 2 | Musabayane <i>et al.</i> ^[25] Mapanga <i>et al.</i> ^[26] |
| <i>Eureiandra fasciculata</i> | Cucurbitaceae | NE | NE | NE | NE | 0 | 0 |
| <i>Bauhinia petersiana</i> | Fabaceae | NE | NE | NE | NE | 0 | 0 |
| <i>Bridelia mollis</i> | Phyllanthaceae | NE | NE | NE | NE | 0 | 0 |
| <i>Combretum fragrans</i> | Combretaceae | L | Alpha glucosidase inhibition. | NE | Combretin A and B | 1 | Dawe <i>et al.</i> ^[27] |
| <i>Dalbergia melanoxydon</i> | Fabaceae | L | NE | NE | NE | 1 | Rahideh <i>et al.</i> ^[28] |
| <i>Dalbergia nyasae</i> | Papilionaceae | NE | NE | NE | NE | NE | NE |
| <i>Aloe greatheadii Schönland</i> | Aloaceae | L | Use of streptozotocin-induced diabetes rat model. | NE | NE | 1 | Loots <i>et al.</i> ^[29] |
| <i>Lipia Javanica</i> | Verbenaceae | L | Use of diabetic mice. | NE | Flavonoids and saponins | 1 | Usai <i>et al.</i> ^[30] |
| <i>Rhus longipes</i> | 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 <i>et al.</i> ^[31] Rahideh <i>et al.</i> ^[32] |
| <i>Pericopsis angolensis</i> 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 |
|---------------------------------|---------------------|--------------|---|----------------|--|--------------------------------|---|
| <i>Carissa edulis</i> | Apocynaceae | L | Use of normal and Streptozotocin (STZ) diabetic rats. | NE | NE | 1 | El-Fiky <i>et al.</i> ^[33] |
| <i>Hoodia currorii</i> | Apocynaceae | S and L | Sorbitol dehydrogenase and glucosidase inhibition assays, <i>in vivo</i> assays using rats. | NE | Steroidal glycosides pregnane glycoside. | 2 | Rubin <i>et al.</i> ^[34] Deutschländer <i>et al.</i> ^[35] |
| <i>Portulaca oleracea</i> | Portulacaceae | S and L | Alloxan induced diabetic rats. | | | 3 | Ramadan <i>et al.</i> ^[36] Bai <i>et al.</i> ^[37] Lee <i>et al.</i> ^[38] |
| <i>Morus alba</i> L. | Moraceae | L, R and FrP | Glucosidase inhibition assays, <i>in vivo</i> assays using alloxan diabetes induced mice. | 8 | Farnesylated 2-arylbenzofurans alkaloids flavonoids. | 12 | Ha <i>et al.</i> ^[39] Kang <i>et al.</i> ^[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 <i>et al.</i> ^[49] Thondre <i>et al.</i> ^[50] |
| <i>Vangueriopsis lanciflora</i> | Rubiaceae. | NE | NE | NE | NE | NE | NE |
| <i>Dioscorea steriscus</i> | Dioscoraceae | NE | NE | NE | NE | NE | NE |
| <i>Talinum tenuissimum</i> | Portulacaceae | NE | NE | NE | NE | NE | NE |
| <i>Nananthus aloides</i> | Aizoaceae | NE | NE | NE | NE | NE | NE |
| <i>Ruschia rigens</i> | Mesembryanthemaceae | NE | NE | NE | NE | NE | NE |
| <i>Hagenia abyssinica</i> | Rosaceae | L | Use of streptozotocin-induced diabetic mice. | NE | NE | 1 | Kifle and Belayneh ^[51] |
| <i>Burkea africana</i> | Fabaceae | R | Alpha amylase inhibition. | | Abietane-type diterpenoid, rubesanolidic acid. | 1 | Tamfu <i>et al.</i> ^[52] |
| <i>Ozoroa insignis</i> | Anacardiaceae | NE | NE | NE | NE | NE | NE |
| <i>Searsia tenuinervis</i> | Anacardiaceae | NE | NE | NE | NE | NE | NE |
| <i>Searsia dentate</i> | Anacardiaceae | NE | NE | NE | NE | NE | NE |

| Species name | Family name | Part used | Bioactivity assays | Clinical study | Compounds identified | Number of scientific citations | Reference |
|------------------------------|-------------|-----------|---|----------------|----------------------|--------------------------------|---|
| <i>Annona stenophylla</i> | Annonaceae | R | Translocation of GLUT 4 assay using FITC fluorescence measured Use of alloxan induced diabetes rats. | NE | NE | NE | Morales Ramos <i>et al.</i> ^[53] Taderera <i>et al.</i> ^[54] Taderera <i>et al.</i> ^[55] |
| <i>Senna septemtrionalis</i> | 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 (%) |
|------------------------------------|-------------------------------------|----------------------------|-----------------------|-------------------|
| <i>Zanthoxylum chalybeum</i> | 92.1±0.5 | 87.2±0.7 | 90.3±0.3 | 85.4±0.6 |
| <i>Xeroderris stuhlmannii</i> | 88.1±0.3 | 80.0±0.5 | 87.2±0.3 | 83±0.6 |
| <i>Elephantorrhiza elephantina</i> | 76.2±0.4 | 78.3±0.7 | 76.0±0.3 | 77.2±0.5 |
| <i>Syzygium cordatum</i> | 84.1±0.5 | 93.3±0.6 | 84.1±0.8 | 92.3±0.5 |
| <i>Eureiandra fasciculata</i> | 72.3±0.2 | 76.2±0.5 | 77.0±0.3 | 74.1±0.6 |
| <i>Bauhinia petersiana</i> | 76.4±0.3 | 70.5±0.7 | 68.1±0.3 | 62.3±0.4 |
| <i>Bridelia mollis</i> | 68.3±0.4 | 66.4±0.6 | 76.3±0.5 | 52.4±0.6 |
| <i>Combretum fragrans</i> | 70.2±0.5 | 55.3±0.5 | 46.3±0.2 | 38.3±0.5 |
| <i>Dalbergia melanoxyloides</i> | 55.2±0.6 | 60.0±0.3 | 56.1±0.3 | 70.3±0.5 |
| <i>Dalbergia nyasae</i> | 53.2±0.3 | 43.6±0.3 | 44.4±0.5 | 56.5±0.2 |
| <i>Aloe greatheadii</i> Schönland | 67.3±0.5 | 70.6±0.3 | 68.1±0.3 | 78.2±0.5 |
| <i>Lipia Javanica</i> | 76.0±0.3 | 67.6±0.5 | 62.2±0.5 | 70.1±0.2 |
| <i>Rhus longipes</i> | 45.2±0.5 | 56.3±0.5 | 72.1±0.4 | 38.1±0.5 |
| <i>Pericopsis angolensis</i> DC | 66.4±0.6 | 63.2±0.3 | 47.2±0.3 | 44.4±0.3 |
| <i>Carissa edulis</i> | 76.7±0.3 | 67.3±0.6 | 66.6±0.7 | 74.2±0.3 |
| <i>Hoodia currorii</i> | 56.1±0.5 | 55.2±0.5 | 40.4±0.3 | 60.2±0.2 |
| <i>Portulaca oleracea</i> | 78.1±0.6 | 72.2±0.3 | 66.4±0.3 | 52.3±0.3 |
| <i>Morus alba</i> L. | 77.3±0.3 | 76.3±0.6 | 62.5±0.5 | 58.0±0.6 |
| <i>Vangueriopsis lanciflora</i> | 63.2±0.3 | 65.1±0.5 | 70.6±0.5 | 73.4±0.2 |
| <i>Dioscorea steriscus</i> | 66.3±0.5 | 47.0±0.3 | 44.0±0.2 | 67.1±0.5 |
| <i>Talinum tenuissimum</i> | 53.0±0.3 | 44.3±0.6 | 38.2±0.3 | 66.0±0.6 |
| <i>Nananthus aloides</i> | 56.2±0.5 | 58.0±0.5 | 46.3±0.3 | 43.0±0.5 |
| <i>Ruschia rigens</i> | 43.0±0.6 | 23.2±0.3 | 34.2±0.2 | 38.2±0.3 |
| <i>Hagenia abyssinica</i> | 35.1±0.3 | 55.3±0.4 | 49.0±0.5 | 50.2±0.4 |
| <i>Burkea Africana</i> | 66.1±0.3 | 64.4±0.5 | 63.6±0.5 | 68.7±0.3 |
| <i>Ozoroa insignis</i> | 32.0±0.3 | 39.3±0.5 | 60.4±0.5 | 42.2±0.1 |
| <i>Searsia tenuinervis</i> | 44.3±0.4 | 69.6±0.3 | 66.0±0.5 | 73.3±0.3 |
| <i>Searsia dentate</i> | 56.2±0.3 | 45.1±0.3 | 50.2±0.3 | 61.2±0.2 |
| <i>Annona stenophylla</i> | 60.2±0.2 | 68.5±0.5 | 72.3±0.5 | 68.0±0.2 |
| <i>Senna septemtrionalis</i> | 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 ₉ H 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 ₆ O 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 fasciculata*, *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 been 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 ₅ O ₄ |
| 12.001 | Methylsalicylate | 97.5 | 119-36-8 | Phenolic acid | C ₈ H ₈ O 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 fasciculata* 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 fasciculata* 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 fasciculata* 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-Hydroxy-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 fasciculata* 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 fasciculata* 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 elephantina*, *Syzygium cordatum*, *Eureiandra fasciculata*, *Bauhinia petersiana*, *Bridelia mollis*, *Combretum fragrans*, *Dalbergia melanoxylon* and *Dalbergia nyasae* were the most popular plants. *Zanthoxylum chalybeum*, *Xeroderris stuhlmannii*, *Elephantorrhiza elephantina*, *Syzygium cordatum* were reported to be very effective plants against DMT2 with *Zanthoxylum chalybeum*, *Xeroderris stuhlmannii*, *Elephantorrhiza elephantina*, *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 fasciculata* 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.

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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 $\geq 60\%$. The ethanolic/water (60/40) extract of *Zanthoxylum chalybeum*, *Xeroderris stuhlmannii*, *Syzygium cordatum* and *Eureiandra fasciculata* 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.

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