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Antidiabetic and Antioxidant Potential of *Detarium microcarpum* and *Zanthoxylum zanthoxyloides* and Combined Extract Efficacy in Rats

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ABSTRACT

Background and Objective: *Detarium microcarpum* and *Zanthoxylum zanthoxyloides* are both tropical medicinal plants used for the treatment and management of numerous ailments including diabetes. This study sought to evaluate the antidiabetic potential and efficacy, antioxidant and safety profile of the plants and combined extracts of the *Detarium microcarpum* and *Zanthoxylum zanthoxyloides*. **Materials and Methods:** The combined (*Detarium microcarpum* and *Zanthoxylum zanthoxyloides*) and separate plant parts were extracted with different solvents. Phytochemical analysis, radical scavenging activity and heavy metal content assessments were conducted. The antidiabetic potential of the extracts was further evaluated *in vivo* by employing the Oral Glucose Tolerance Test (OGTT) in 21 Wistar rats. The safety profile of the extracts was evaluated by assessing the effect of the extracts on the percentage change in body weights, hematological parameters and biochemical parameters as well. One-way and two-way analysis of variance followed by Dunnett's multiple comparison test was used at $p<0.05$. **Results:** Alkaloids, terpenoids, saponins and tannins were present in aqueous extracts. The combined aqueous extract showed better radical scavenging activity. In a glucose tolerance test on rats, the combined extract demonstrated the most effective glucose reduction (from 6.83-4.60 mmol L^{-1}). Combined extract also had better antioxidant and antihyperglycemic effects compared to individual extracts. **Conclusion:** All extracts were safe concerning hematological and biochemical parameters. Combined extracts showed better therapeutic potential compared to the individual extracts.

KEYWORDS

Zanthoxylum zanthoxyloides, *Detarium microcarpum*, antidiabetic, antioxidant, efficacy

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INTRODUCTION

Diabetes is a chronic metabolic condition characterized by the inadequate secretion of insulin or the development of insulin resistance. Hyperglycaemia, polyuria, polydipsia, polyphagia, altered metabolism of carbohydrates, proteins and lipids and increased risk of vascular complications are some of the conditions associated with diabetes. Diabetes can be classified into three types namely, type 1 diabetes, type 2 diabetes and gestational diabetes with most diagnosed cases of diabetes being cases of type 2 diabetes[1-4].

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In Sub-Saharan Africa, approximately 19.4 million individuals have diabetes. Projections indicate that by 2030, this number will rise to 28.6 million, reaching 34.2 million by 2040. In Ghana, around 6.46% of adults are with diabetes mellitus[5].

Although the control of diabetes is by synthetic oral hypoglycaemic drugs alongside insulin, these fail to reverse the course of the complication these drugs come along with and further exacerbate it by the expression of prominent side effects[6]. Aside from the prominent side effects of therapeutics, these drugs have high costs, with poor tolerability in patients and sometimes the development of therapeutic resistance[7].

Diabetes management varies by type, typically involving insulin injections for type 1 and antidiabetic drugs for type 2. Lifelong treatment and regular follow-ups are essential. This complex approach can lead to economic strain on individuals, particularly in low and middle income countries. In such contexts, individuals often turn to cost-effective traditional plant remedies[8]. These medicinal plants are valued due to their accessibility, affordability and availability, offering a dependable treatment option. They are also considered safe mainly due to their natural origin and traditional usage, though scientific systematic studies on their usage remain limited[9].

Zanthoxylum zanthoxyloides and *Detarium microcarpum* are both plants believed to have some medicinal properties. *Zanthoxylum zanthoxyloides* have been used in West African traditional medicine to manage and treat malaria, fever, sickle cell anaemia, tuberculosis, paralysis, elephantiasis, toothache, venereal diseases, dysmenorrhea, abdominal pain, cough, tuberculosis, urinary disorders, cancers and arthritis[10]. *Detarium microcarpum* has also been used traditionally in the treatment and management of numerous ailments such as stomach disorders, venereal diseases and gastrointestinal ailments. Investigations of the biological activities of *D. microcarpum* plants have shown the plant possesses some antimicrobial, antimalarial and cytotoxic effects[11].

To fully have the desired therapeutic effect and benefits of medicinal plants, their quality must be assured in terms of their safety which includes contamination by heavy metals as well as safety and toxicological profile and assessment[12].

Ensuring the therapeutic benefits of medicinal plants requires guaranteeing their quality in terms of safety, including heavy metal contamination, alongside overall safety and toxicity assessment[12]. Existing research on the anti-diabetic effects of medicinal plants primarily focuses on individual plants or specific plant parts. There's a lack of scientific data on the combined effects of multiple anti-diabetic plants. Consequently, the reliance on affordable, safe and effective natural remedies has gained importance. Thus, this study sought to evaluate the antidiabetic potential, efficacy, effect and safety profile of combined extracts of *Z. zanthoxyloides* and *D. microcarpum* on rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biochemistry and Biotechnology of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi between March 2021 and December, 2021.

Collection and identification of plant sample: The leaves, stems and roots of *Detarium microcarpum* and the leaves and stem bark of *Zanthoxylum zanthoxyloides* were collected in March 2021 at Ejura in the Ashanti Region with the help of a local herbalist. The plant was identified by Mr. Clifford Osafo Asare of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology.

Processing of plant samples: The leaves, stem and roots of *D. microcarpum* and the leaves and stem bark of *Z. zanthoxyloides* were washed and air-dried at room temperature under shade for 28 days. After drying, the samples were pulverized and stored in a zip lock bag and kept at room temperature until needed for use.

Extraction of plant samples: The extraction of the leaves, stems and roots of *D. microcarpum* and the leaves and stem bark of *Z. zanthoxyloides* was done by decoction for 48 hrs at room temperature. The aqueous, 50% ethanolic, methanolic, ethyl acetate and chloroform extraction of the various parts of *D. microcarpum* and *Z. zanthoxyloides* was carried out by suspending 60 g of the powder of the *D. microcarpum* leaves, stem and roots and *Z. zanthoxyloides* leaves and stem bark in 60 mL of the respective solvents namely distilled water, 50% ethanol (prepared in distilled water at 50:50 v/v), methanol, ethyl acetate and chloroform respectively. The extracts were then filtered through cotton wool and concentrated over a water bath at 60°C to obtain the various extracts.

The extraction of the best two performing extracts and their combination was done by decoction for 48 hrs at room temperature in the dark.

Phytochemical analysis: The extracts were screened for the presence of alkaloids, tannins, terpenoids, saponins, phenols, flavonoids and cardiac glycosides according to the methods described by Thilagavathi *et al.*[13] with slight modifications.

Glucose uptake assay: The assay was performed as employed by Jayatilake and Munasinghe[14] with some modifications. One gram of commercial baker's yeast was placed in a falcon tube and 10 mL of distilled water was added. Washing was through repeated centrifugation at 2800 g for 8 min, until the supernatant was clear. A 10% v/v solution of the extract was made with distilled water, discarding the pellets. Stock solutions of *D. microcarpum* and *Z. zanthoxyloides* extracts were formed by dissolving 20 mg in 2 mL of distilled water in Eppendorf tubes (2 mL). Concentrations of 1-5 mg mL^{-1} for each extract were then prepared. Metformin (500 mg), the standard antidiabetic drug, was dissolved in 50 mL of distilled water with concentrations of 1-5 mg mL^{-1}. Glucose solutions of 5, 10 and 25 mM were prepared by dissolving 4.5, 9 and 22.5 mg of glucose in 50 mL of distilled water.

In the assay, 400 μL of extract and metformin were added to 400 μL of glucose solution in 2 mL Eppendorf tubes. Incubation followed at 37°C for 10 min. The reaction was initiated by adding 40 μL of yeast solution to each tube, which was vortexed and then incubated at 37°C for an hour. After incubation, tubes were centrifuged at 2500 g for 5 min. Then, 200 μL of each reaction mixture were transferred into 96-well plate wells in triplicate. Absorbance at 540 nm was measured using a microplate spectrophotometer (Synergy H1, USA). The percentage increase in glucose uptake by yeast cells was calculated using the equation:

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

EC_{50} values were determined by nonlinear regression analysis.

Determination of total phenolic content: The total phenolic content (TPC) was assessed using the Folin-Ciocalteu method as described by Singleton *et al.*[15] and procedures as employed by Torkornoo *et al.*[16]. This method relies on the reduction of the Folin-Ciocalteu reagent by phenolics, resulting in the production of molybdenum-tungsten blue[17]. The concentration of phenolic compounds in each sample

was determined using the gallic acid calibration curve and the gallic acid equivalence for each extract was calculated using the formula as mg of GAE/g of extract:

$$TPC = C \times \frac{V}{M}$$

where, 'C' is the concentration of gallic acid mg mL^{-1}, 'V' is the volume of plant extract in mL and 'M' is the weight of the extract in grams.

DPPH radical scavenging activity: The stock solutions of the aqueous extracts, hydro-ethanolic and methanolic extracts were used to determine the antioxidant activity as described by Brand-Williams *et al.*[18] with slight modifications. The percentage scavenging activity was determined by:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Absorbance of blank - Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

The mean percentage antioxidant activity for triplicates was graphically represented for both the standard and the extracts. The effective concentration at 50% (EC_{50}) values, indicating the amount of antioxidant required to reduce the initial DPPH concentration by 50%, were determined by nonlinear regression analysis.

Heavy metal analysis: Each powdered sample (raw powder and extract) of about 1g was weighed into a 50 mL digestion tube. Subsequently, 1 mL of H_2O, 2 mL HCl, 5 mL of 1:1 HNO_3:$HClO_4$ and 2 mL H_2SO_4 were added. Allowing the samples to stand at room temperature for about 20 minutes to facilitate the foam formed to settle. The samples underwent heating on a digestion block on a hot for about 2 hrs at a temperature of 150 until a cleared solution was attained. The resultant clear solution was then filtered into a 100 mL volumetric flask using a 0.45 pm pore size membrane filter paper (Whatman filter paper No. 41). The filtrate was adjusted to the mark by topping up with distilled water and digested samples were transferred into the plastic bottles and stored at 4°C prior to analysis with blanks prepared alongside. Heavy metals including chromium, copper, iron, nickel and lead was analysed using the atomic absorption spectrophotometer (Nov 400P, Analytik Jena GmbH, Jena, Germany).

Animals: Thirty Wistar rats were procured from School of Medicine, University of Ghana's animal holding facility. The rats were relocated to the Department of Biochemistry and Biotechnology at Kwame Nkrumah University of Science and Technology (KNUST), where they were housed in plastic cages with wood shavings. A 14-day acclimatization period to laboratory conditions was observed for the rats. The maintenance of optimal conditions of temperature between 24 and 26°C and 40-70% relative humidity was observed throughout the study. The Wistar rats were provided with unrestricted access to commercial feed and water. The identification of the rats involved in the study was by marking their tails with permanent makers. The conduct of the animal study followed the guidelines as set by the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA, New Delhi, India) and the Guide for Care and Use of Laboratory Animals[19].

Grouping of animals: Twenty-one Wistar rats (of average weight 117 g) were randomly divided into seven groups each containing three rats as shown in Table 1. The rats were allowed access to feed and water *ad libitum* for 28 days. Control group received distilled water only and the treatment groups received 100 and 250 mg kg^{-1} b.wt., using oral gavage. Treatment was done daily for 28 days.

Oral Glucose Tolerance Test (OGTT) in normoglycemic rats: Prior to the OGTT to determine the glucose lowering activity of the DMLAQ, ZZSAQ and their combined extracts, rats were fasted for 16 hrs

Table 1: Extract treatment groupings of rats

Group	Treatment
Group 1	Distilled water 1 mL
Group 2	DML 100 mg kg^{-1} b.wt., p.o for 28 days
Group 3	DML 250 mg kg^{-1} b.wt., p.o for 28 days
Group 4	ZZS 100 mg kg^{-1} b.wt., p.o for 28 days
Group 5	ZZS 250 mg kg^{-1} b.wt., p.o for 28 days
Group 6	COMBO 100 mg kg^{-1} b.wt., p.o for 28 days
Group 7	COMBO 250 mg kg^{-1} b.wt., p.o for 28 days

with access to water. Distilled water (normal control), a reference drug glibenclamide (5 mg kg^{-1} b.wt.) or each of the two varying doses of DMLAQ, ZZSAQ and their combined extract (100 and 250 mg kg^{-1} b.wt.) were administered to the rats by oral gavage to the various groups of rats each. After 30 min of the administration of the extracts and reference drugs to the various groups, glucose (10 g/kg) was administered to each rat. Blood samples from the twenty one rats were taken from the tail vein using a sterile lancet and blood glucose levels checked using Eco-Check Strips and Eco-Check Glucometer. The tail vein of all rats was cleaned and disinfected before and after use of the lancet. The blood glucose levels were taken before the administration of glucose, 1, 2 and 4 hrs after the administration of glucose as described by Larbie *et al.*[20].

Extract treatment effect on body weight: The body weight of each animal was measured before the start of extract treatment (day 0) and subsequently measured every other fourth day till the last day of extract treatment (day 28) using a mass balance (Zhejiang Haoyu, Jinhua, Zhejiang, China). The percentage change in body weights of the rats were computed using the following formula:

$$\text{Change in body weight (\%)} = \frac{\text{Weight}_n - \text{Weight}_{initial}}{\text{Weight}_{initial}} \times 100$$

where, Weight_n is the body weight on day 4, 8-28 and $\text{Weight}_{initial}$ is the body weight on D0.

Termination of treatment: The rats were sacrificed on the 28th day after an overnight fast. Animals were then exposed to cervical dislocation. With a sterile blade, incisions were made in the cervical region of the sacrificed rats and blood samples collected for haematological and biochemical analysis.

Evaluation of relative organ weight: The procured lungs, kidney, pancreas, liver and heart were washed with normal saline, blotted dried with tissue paper and weighed. The relative weight of the excised organs with respect to the body weight of the rat at sacrifice was calculated from the formula:

$$\text{Relative organ weight (\%)} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight on day of sacrifice (g)}} \times 100$$

Evaluation of haematological parameters: About 2 mL of blood samples were collected into EDTA K3 tubes for various haematological parameters using the Sysmex haematology system. The blood samples were evenly mixed to prevent blood clotting. The parameters assessed included white blood cell count (WBC), red blood cell (RBC) count, haemoglobin (Hgb), haematocrit, platelet count, lymphocytes, neutrophils, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), plateletcrit, Platelet Distribution Width (PDW) and Platelet Larger Cell Ratio (P-LCR).

Evaluation of biochemical parameters: About 3 mL each of blood samples were put in the gel-activated tubes, allowed to clot, centrifuged at 1500 g and serum used for biochemical analyse. The biochemical

parameters that were assessed were total cholesterol, High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), triacylglycerols, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and bilirubin (total bilirubin (TBil) and direct bilirubin (DBil)). The levels of indirect bilirubin (I-Bil) was estimated as the difference between total bilirubin (TBil) and direct bilirubin (DBil).

Statistical analysis: Data were analysed by GraphPad Prism 8 for Windows and the results were expressed as Mean±Standard Error (SEM). Data was then assessed by one-way and two-way analysis of variance followed by Dunnett's multiple comparison test. Values for which $p<0.05$ was considered significant. The EC_{50} values were determined by nonlinear regression analysis.

RESULTS

Phytochemical constituent: The aqueous extract of DML and ZSS had all phytochemicals assayed present except flavonoids. However, the combined extract of these two extracts had all phytochemicals present apart from phenols just as the aqueous extract of ZZS. The phytochemical constituents present in the various extracts were presented in Table 2.

Glucose uptake assay: The aqueous extract of *D. microcarpum* leaves exhibited a higher glucose uptake activity at various concentrations, except for the 3-5 mg mL^{-1} range where the aqueous extract of *Z. zanthoxyloides* stem bark showed greater uptake at 5 mM glucose concentration. The glucose uptake activity of *D. microcarpum* leaves was not statistically different ($p<0.05$-0.001) from the

Table 2: Phytochemical constituents of various extracts of *D. microcarpum* and *Z. anthoxyloides* and the combined extract

Plant	Extract	Alkaloids	Flavonoids	Phenols	Saponins	Tannins	Terpenoids	Cardiac glycosides
DM	LAQ	+++	-	++	+	+++	++	+
	LHE	+	+	++	++	+++	+	-
	LME	-	+	+++	+	+++	+	-
	LEA	-	-	-	-	+	-	-
	LCF	++	-	+	-	+	-	+
	SAQ	+	++	+	++	-	+	-
	SHE	+	-	+	++	-	+	++
	SME	+	-	++	+	+	++	-
	SEA	+	-	-	-	-	-	-
	SCF	+	-	-	-	+	-	-
	RAQ	+	++	-	++	-	-	-
	RHE	+	+	-	++	+	+	-
	RME	+	-	++	+	+	-	++
	REA	+	-	+	-	-	-	-
	RCF	+	-	-	-	+	-	+
ZZ	LAQ	+	++	+	++	+++	++	-
	LHE	+	-	++	+++	+++	+	-
	LME	+	-	++	++	+++	-	+++
	LEA	-	-	+	-	+++	-	-
	LCF	++	-	+	-	+	-	+
	SAQ	+++	++	-	++	+	+	-
	SHE	+	-	+	+	++	+	-
	SME	-	-	++	-	++	-	-
	SEA	+	-	-	-	+	-	-
	SCF	+	-	-	-	+	-	-
COMBO	-	+	++	-	++	++	+	+

-: Not present, +: Present in small concentration, ++: Present in moderate concentration, +++: Present in high concentration, DM: *Detarium microcarpum*, ZZ: *Zanthoxylum zanthoxyloides*, COMBO: Combined aqueous extract of DML and ZZS, LAQ: Leaf aqueous extract, LHE: Leaf hydroethanolic extract, LME: Leaf methanolic extract, LEA: Leaf ethylacetate extract, LCF: Leaf chloroform extract, SAQ: Stem aqueous extract, SHE: Stem hydroethanolic extract, SME: Stem methanolic extract, SEA: Stem ethylacetate extract, SCF: Stem chloroform extract, RAQ: Root aqueous extract, RHE: Root hydroethanolic extract, RME: Root methanolic extract, REA: Root ethylacetate extract and RCF: Root chloroform extract

Table 3: Effect of various plant extracts on the percent increase in glucose uptake by yeast cell at 5 mM glucose concentration

		Concentration of extracts (mg mL^{-1}) Percent glucose uptake by yeast cells at different extract concentrations				
Plant	Extract	1	2	3	4	5
DM	LAQ	88.74±0.34	88.46±0.19	83.61±0.19	77.75±0.92	73.09±0.52
	LHE	80.08±0.09[a]	71.79±0.86[a]	61.27±0.73[a]	55.40±0.41[a]	42.08±0.89[a]
	LME	84.64±0.16[a]	80.82±0.24[a]	77.84±0.37[a]	75.51±0.41[a]	70.77±1.29[a]
	LEA	49.53±1.56[a]	7.26±3.65[a]	-	-	-
	LCF	78.21±1.54	64.06±1.22[a]	69.28±0.70[a]	58.94±0.74[a]	54.00±0.19[a]
	SAQ	67.32±0.28[a]	56.52±4.48[a]	46.56±1.56[a]	21.51±2.06[a]	3.82±4.72[a]
	SHE	71.23±0.56[a]	50.56±1.90[a]	28.40±1.22[a]	6.99±1.68[a]	12.57±0.58[a]
	SME	81.19±0.18[a]	72.72±0.33	62.94±1.89[a]	56.33±0.65[a]	49.53±0.34[a]
	SEA	72.35±3.76[a]	66.76±1.54[a]	57.73±2.34[a]	49.16±2.22[a]	39.20±2.35[a]
	SCF	71.14±1.63[a]	75.14±1.01	56.89±2.91[a]	53.28±4.02[a]	46.00±0.61[a]
	RAQ	71.69±1.07[a]	53.26±5.30[a]	67.32±0.98[a]	59.87±0.49[a]	23.09±1.45[a]
	RHE	77.19±0.52[a]	65.17±0.37[a]	54.47±0.16[a]	41.71±1.21[a]	32.22±1.31[a]
	RME	-	-	-	-	-
	REA	67.78±0.95[a]	36.87±0.81[a]	-	-	-
	RCF	82.03±0.52	78.49±0.28	69.83±0.85[a]	58.38±0.98[a]	53.17±1.80[a]
ZZ	LAQ	19.09±0.83[a]	16.01±0.52[a]	66.11±0.25[a]	47.11±2.87[a]	33.14±1.34[a]
	LHE	79.42±0.52[a]	71.70±0.25[a]	62.94±1.10[a]	51.58±2.23[a]	43.58±1.13[a]
	LME	78.40±0.47[a]	67.69±0.76	60.62±0.74[a]	48.32±0.42	43.11±0.89[a]
	LEA	41.71±0.61[a]	-	-	-	-
	LCF	67.50±3.27[a]	45.16±1.93[a]	60.99±3.23[a]	56.98±0.43[a]	57.64±1.25[a]
	SAQ	67.60±0.28[a]	60.15±1.46[a]	86.87±0.28	86.03±0.70	89.02±0.09[a]
	SHE	81.19±0.18[a]	72.72±0.33[a]	62.94±1.89[a]	56.33±0.65[a]	49.53±0.34[a]
	SME	82.96±0.56[a]	81.01±0.00[a]	75.89±0.37[a]	68.90±0.79[a]	59.31±0.57[a]
	SEA	77.19±0.74[a]	63.81±1.39[a]	45.06±0.65[a]	13.69±1.06[a]	3.54±1.71[a]
	SCF	45.99±13.52[a]	28.77±2.50[a]	1.12±4.86[a]	0.93±1.30[a]	-
SD	MET	87.24±0.88	84.21±0.40	82.73±0.40	79.62±0.84	72.36±3.12

DM-*Detarium microcarpum*, ZZ: *Zanthoxylum zanthoxyloides*, SD: Standard drug, LAQ: Leaf aqueous extract, LHE: Leaf hydroethanolic extract, LME: Leaf methanolic extract, LEA: Leaf ethylacetate extract, LCF: Leaf chloroform extract, SAQ: Stem aqueous extract, SHE: Stem hydroethanolic extract, SME: Stem methanolic extract, SEA: Stem ethylacetate extract, SCF: Stem chloroform extract, RAQ: Root aqueous extract, RHE: Root hydroethanolic extract, RME: Root methanolic extract, REA: Root ethylacetate extract, RCF: Root chloroform extract, MET: Metformin and [a]Significant difference: a ($p<0.05$-0.001) from metformin

standard drug metformin across all concentrations as seen in Table 3. In contrast, the aqueous extract of *Z. zanthoxyloides* stem bark exhibited statistically different activity at all concentrations. Notably, the glucose uptake enhancement by DML aqueous extract at 5 mM concentration resembled that of the standard metformin. However, while glucose uptake increased with concentration in the aqueous extract of *Z. zanthoxyloides* stem bark, there was a noticeable decrease in uptake. Generally, glucose uptake declined as concentration increased for all extracts at both 5 and 10 mM glucose concentrations. Among the five different solvent extracts, *D. microcarpum* leaves demonstrated the highest glucose uptake activity, except for the ethyl acetate extract where *Z. zanthoxyloides* stem bark showed better activity.

The glucose uptake activity of *D. microcarpum* extracts remained relatively consistent at 5 and 10 mM glucose concentrations, with minor variations across concentrations, unlike the extracts of *Z. zanthoxyloides* as seen in Table 3 and 4. The maximum increase in glucose uptake by yeast cells was observed at 1 mg mL^{-1} for most of the analyzed extracts.

Furthermore, based on the IC_{50} values from the glucose uptake studies, the aqueous extracts of DML and ZZS (0.33±0.090 and 0.67±0.056), respectively demonstrated the highest effectiveness for a glucose concentration of 5 mM, compared to the standard antidiabetic drug metformin (0.38±0.093). Similarly, the aqueous extract of DML exhibited the lowest concentration for stimulating glucose uptake at the 10 mM glucose concentration, with an IC_{50} value of 0.32±0.019, compared to the standard metformin's value of 1.88±0.382 as seen in Table 5 and 6.

Table 4: Effect of various plant extracts the percent increase in glucose uptake by yeast cell due to the at 10 mM glucose concentration

		Concentration of extracts (mg mL^{-1}) Percent glucose uptake by yeast cells at different extract concentrations				
Plant	Extract	1	2	3	4	5
DM	LAQ	88.89±0.33[a]	88.61±0.18[a]	83.64±0.18[a]	78.05±0.90[a]	73.46±0.51[a]
	LHE	80.53±0.51[a]	73.92±0.24[a]	64.28±0.56[a]	57.76±0.40[a]	49.40±1.20[a]
	LME	84.76±0.72[a]	80.26±2.11[a]	79.43±0.51[a]	74.65±2.62[a]	72.73±0.16[a]
	LEA	59.04±0.90	24.97±1.44[a]	-	-	-
	LCF	73.64±4.05	71.72±0.33	62.90±1.75[a]	55.65±2.07	47.66±2.45[a]
	SAQ	67.77±0.27	57.12±4.42	47.47±1.37[a]	22.59±2.03[a]	0.46±2.80[a]
	SHE	75.48±0.48[a]	54.09±0.91[a]	31.04±1.74[a]	4.50±0.78[a]	3.31±4.86[a]
	SME	81.91±0.66[a]	71.53±2.85	53.44±15.18[a]	65.38±2.67[a]	60.06±1.57[a]
	SEA	82.28±1.02[a]	79.25±1.57[a]	75.39±0.80[a]	72.82±0.91[a]	67.31±1.94
	SCF	72.54±1.26	67.22±1.96	61.34±2.47[a]	45.00±5.34	32.51±2.24
	RAQ	67.95±7.37	37.46±13.92[a]	67.77±0.97[a]	60.42±0.49	24.15±1.43
	RHE	75.48±0.27[a]	67.95±0.18[a]	55.46±0.51[a]	44.72±0.82[a]	39.48±0.93[a]
	RME	-	-	-	-	-
	REA	72.27±1.57[a]	48.58±2.93[a]	16.99±0.49	-	-
	RCF	76.76±0.37[a]	72.54±0.46	53.90±5.07[a]	53.72±0.32	38.02±1.83[a]
ZZ	LAQ	20.47±0.64[a]	17.63±0.55[a]	66.39±0.27[a]	49.96±3.21	34.43±1.10
	LHE	78.05±0.40[a]	69.79±0.09[a]	62.26±0.16[a]	54.73±0.64[a]	47.47±0.60[a]
	LME	78.24±0.84[a]	68.32±0.48	60.00±0.49[a]	51.06±0.79	39.30±0.66[a]
	LEA	51.33±1.04	4.96±1.83[a]	-	-	-
	LCF	76.76±0.36[a]	72.54±0.46	53.90±5.07[a]	53.72±0.32	38.02±1.83
	SAQ	23.97±0.57[a]	8.08±1.99[a]	58.31±1.83[a]	57.30±0.69	52.53±0.88[a]
	SHE	80.08±0.09[a]	71.79±0.86[a]	61.27±0.73[a]	55.40±0.41[a]	42.08±0.89[a]
	SME	84.48±0.37[a]	82.46±0.18[a]	78.42±0.18[a]	68.78±0.09[a]	56.47±0.55[a]
	SEA	78.42±2.44[a]	72.36±2.23[a]	59.14±7.80[a]	58.31±1.37[a]	44.08±0.84[a]
	SCF	68.87±0.28	54.73±1.46	9.18±0.28	-	-
SD	MET	58.13±0.57	60.97±1.01	24.52±1.39	49.95±0.90	25.99±0.72

DM: *Detarium microcarpum*, ZZ: *Zanthoxylum zanthoxyloides,* SD: Standard drug, LAQ: Leaf aqueous extract, LHE: Leaf hydroethanolic extract, LME: Leaf methanolic extract, LEA: Leaf ethylacetate extract, LCF: Leaf chloroform extract, SAQ: Stem aqueous extract, SHE: Stem hydroethanolic extract, SME: Stem methanolic extract, SEA: Stem ethylacetate extract, SCF: Stem chloroform extract, RAQ: Root aqueous extract, RHE: Root hydroethanolic extract, RME: Root methanolic extract, REA: Root ethylacetate extract, RCF: Root chloroform extract, MET: Metformin and [a]Significant difference: a ($p<0.05$-0.001) from metformin

Table 5: IC_{50} values of extracts of glucose uptake studies at 5 mM glucose concentration

	IC_{50} (mg mL^{-1}±SEM)					
	DML	DMS	DMR	ZZL	ZZS	Metformin
Aqueous	0.33±0.090	1.78±0.089	3.40±0.646	4.81±0.194	0.67±0.056	0.38±0.093
Hydroethanol	1.30±0.09	1.78±0.087	3.09±0.091	4.32±0.190	5.13±0.170	-
Methanol	0.49±0.024	5.13±0.170	-	3.99±0.123	0.63±0.155	-
Ethylacetate	0.99±0.278	3.71±0.269	1.26±0.362	1.397±0.121	2.34±0.140	-
Chloroform	7.15±1.145	4.66±0.587	5.98±0.422	1.85±0.171	1.00±0.210	-

DML: *Detarium microcarpum* leaves, DMS: *Detarium microcarpum* stem, DMR: *Detarium microcarpum* roots, ZZL: *Zanthoxylum zanthoxyloides* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark and COMBO: Combined aqueous extract of DML and ZZS

Table 6: IC_{50} values of extracts of glucose uptake studies at 10 mM glucose concentration

	IC_{50} (mg mL^{-1}±SEM)					
	DML	DMS	DMR	ZZL	ZZS	Metformin
Aqueous	0.32±0.019	2.06±0.205	2.52±1.449	4.49±1.565	3.74±0.502	1.88±0.382
Hydroethanol	5.34±0.228	1.93±0.111	3.48±0.113	4.88±0.164	4.36±0.189	-
Methanol	0.48±0.085	7.91±0.399	-	3.88±0.143	7.64±0.950	-
Ethylacetate	1.12±0.314	0.59±0.080	1.74±0.324	1.01±0.277	4.67±0.228	-
Chloroform	5.30±0.622	3.37±0.279	6.63±1.064	3.82±0.274	2.04±0.186	-

DML: *Detarium microcarpum* leaves, DMS: *Detarium microcarpum* stem, DMR: *Detarium microcarpum* roots, ZZL: *Zanthoxylum zanthoxyloides* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark and COMBO: Combined aqueous extract of DML and ZZS

Table 7: Total phenolic content *Detarium microcarpum* and *Zanthoxylum zanthoxyloides* and the combined extract

	Total phenolic content (mg mL^{-1})					
	DML	DMS	DMR	ZZL	ZZS	COMBO
Aqueous	27.55±0.33	22.03±0.01	37.82±0.85	74.99±0.99	52.34±5.17	33.13±1.44
Hydroethanol	43.04±1.78	58.82±0.88	42.44±3.60	81.47±1.65	60.16±0.59	-
Methanol	46.51±0.73	34.98±2.71	39.63±0.51	63.90±1.71	42.46±1.82	-
Chloroform	00.63±1.99	8.42±2.72	06.95±2.25	35.30±1.41	26.49±8.07	-

DML: *Detarium microcarpum* leaves, DMS: *Detarium microcarpum* stem, DMR: *Detarium microcarpum* roots, ZZL: *Zanthoxylum zanthoxyloides* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark and COMBO: Combined aqueous extract of DML and ZZS

Table 8: Antioxidant activities of ascorbic acid and extracts of *Detarium microcarpum* and *Zanthoxylum zanthoxyloides* and the combined extract

	EC_{50} (mg mL^{-1})						
	DML	DMS	DMR	ZZL	ZZS	COMBO	Ascorbic acid
Aqueous	4.28±0.456[a]	-	5.32±0.457[a]	2.14±0.357[a]	3.24±0.313[a]	3.04±0.182[a]	0.26±0.128
Hydroethanol	0.74±0.049	0.54±0.056	0.57±0.056	0.28±0.052	0.29±0.042	-	-
Methanol	1.19±0.086[a]	0.39±0.059	0.12±0.041	0.27±0.04	1.04±0.074	-	-

DML: *Detarium microcarpum* leaves, DMS: *Detarium microcarpum* stem, DMR: *Detarium microcarpum* roots, ZZL: *Zanthoxylum zanthoxyloides* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark, COMBO: Combined aqueous extract of DML and ZZS and [a]Significant difference: a ($p<0.05$-0.001) from ascorbic acid

Table 9: Heavy metal content of *D. microcarpum* and *Z. zanthoxyloides* raw powder, aqueous extracts and combined extracts

	Concentration (mg mL^{-1} Mean±SD)							
Heavy metal	DML raw	DMS raw	DMR raw	ZZL raw	ZZS raw	DMLAQ	ZZSAQ	COMBO
Chromium (Cr)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Copper (Cu)	BDL	0.08±0.017	0.18±0.033	0.21±0.016	BDL	BDL	0.04±0.034	0.03±0.011
Iron (Fe)	6.39±0.110	4.20±0.056	7.71±0.131	8.39±0.139	11.25±0.165	3.78±0.059	4.53±0.097	4.56±0.065
Nickel (Ni)	2.75±0.223	2.22±0.301	2.44±0.159	2.89±0.079	3.77±0.404	4.51±0.468	4.69±0.058	4.53±0.373
Lead (Pb)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL connotes below detection limit, Detection limit- 0.00001 mg L^{-1}, DML: *Detarium microcarpum* leaves, DMS: *Detarium microcarpum* stem, DMR: *Detarium microcarpum* roots, ZZL: *Zanthoxylum zanthoxyloides* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark and COMBO: Combined aqueous extract of DML and ZZS

Total phenolic content: The ZZL plant part exhibited the highest total phenolic content among all samples. The hydroethanolic extract of ZZL had the greatest content (81.47±1.65 mg mL^{-1}), followed by ZZS with its highest content in the hydroethanolic extract (60.16±0.59 mg mL^{-1}). While the combined aqueous extract showed higher phenolic content than DML aqueous extract, it was lower than ZZS aqueous extract. Hydroethanolic extracts had the highest phenolic content, followed by methanolic extracts, except for ZZL and ZZS extracts which had the highest content. This suggests higher phenolic content in *Z. zanthoxyloides* compared to *D. microcarpum* as seen in Table 7.

DPPH radical scavenging activity: All the extracts reduced DPPH (2,2-diphenyl-1-picrylhydrazyl) to diphenylpicrylhydrazine at 517 nm. From the Table 8, the methanol extract of ZZL had the best DPPH radical scavenging activity followed closely by the hydroethanolic extract of ZZS and ZZL. However, the effective concentration (EC_{50}) at which half (50%) of free radicals were scavenged was low in all the aqueous extracts compared to the hydroethanolic and methanolic extracts. Of the aqueous extract, ZZL and the combined extract performed better with all the aqueous extract being statistically different from the standard ascorbic acid. All aqueous extracts of both plants and the combined extracts were statistically different from the standard ascorbic acid.

Heavy metal analysis: From the heavy metal analysis by the Atomic Absorption Spectrophotometry (AAS), two out of the five metals analysed, that is chromium (Cr) and lead (Pb) were both below detection limit in all raw samples and extracts. However, copper was below the detection limit in both the DML and ZZS raw samples but present in very low concentration in the ZZS aqueous extract and the combined extract as well from Table 9.

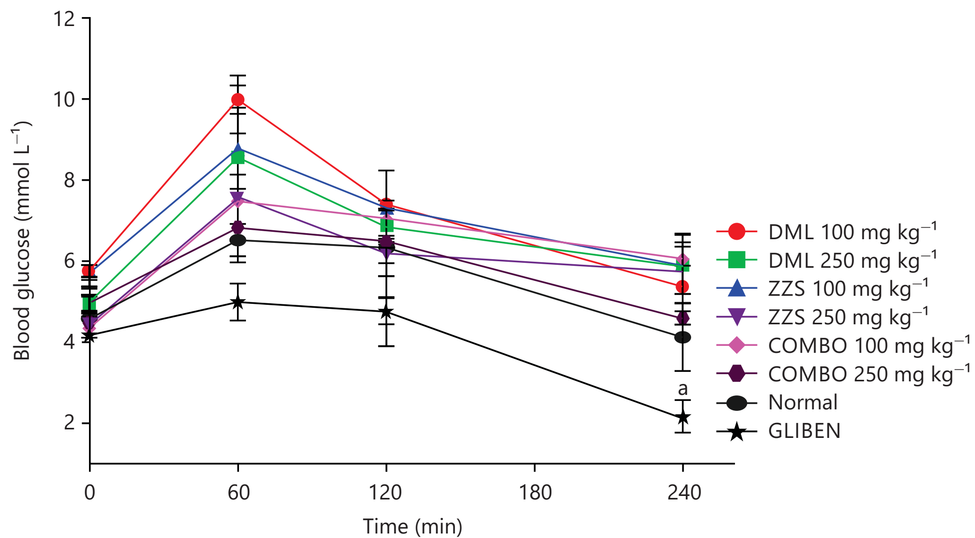

Fig. 1: Effects of extract treatments on oral glucose tolerance test

DML: *Detarium microcarpum* leaves, DMS: *Detarium microcarpum* stem, DMR: *Detarium microcarpum* roots, ZZL: *Zanthoxylum zanthoxyloides* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark and COMBO: Combined aqueous extract of DML and ZZS

Table 10: Effect of extract treatment on blood glucose levels on OGTT

	Oral glucose tolerance (OGTT) (mM)							
Time (min)	DML 100	DML 250	ZZS 100	ZZS 250	COMBO 100	COMBO 250	Normal	GLIBEN
0	5.77±0.15	5.00±0.35	5.73±0.186	4.43±0.32	4.37±0.12	4.97±0.19	4.57±0.15	4.17±0.67
60	10.00±0.35	8.57±2.02	8.80±1.002	7.57±1.59	7.50±0.64	6.83±0.09	6.53±0.39	5.00±0.46
120	7.40±0.12	6.87±0.39	7.33±0.913	6.20±1.11	7.07±0.44	6.50±0.58	6.33±0.69	4.77±0.87
240	5.37±0.03	5.87±0.83	5.93 ±0.73	5.73±0.75[a]	6.07±0.29	4.60±0.15	4.13±0.84	2.17±0.41

DML: *Detarium microcarpum* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark, COMBO: Combined aqueous extract of DML and ZZS, GLIBEN: Glibenclamide and [a]Significant difference: a ($p<0.05$-0.001) from normal

Oral Glucose Tolerance Test (OGTT): From Fig. 1 and Table 10, after oral glucose administration, blood glucose levels in rat groups peaked at 60 min and returned to baseline at 240 min for the normal group. The glibenclamide group showed a further reduction, while most extract groups maintained elevated levels. The natural decline in blood glucose occurred in the normal group, resulting in no significant differences between normal and positive control (glibenclamide) groups, except for a significant decrease at 240 min ($p<0.05$-0.001).

At the 240 min mark, the 250 mg kg^{-1} combined extract effectively suppressed postprandial hyperglycemia, with final blood glucose levels nearly matching the normal group. Notably, at 2 hrs, the 250 mg kg^{-1} ZZS extract achieved better glucose reduction (6.20±1.11 mmol L^{-1}) than the normal control (6.33±0.69 mmol L^{-1}) and other extracts (excluding glibenclamide).

Percentage change in body weight: At end of the study (day 28), the normal group had the highest increase in body weight (64.51±4.94%) compared to all the six extracts. The DML extract (100 and 250 mg) had the least change in body weight compared to the normal group and other extract groups. The normal and all extracts showed a maximal increase in body weight on day 8 as seen in Fig. 2. Further, on day 16, there was a significant decrease ($p<0.05$-0.001) only between the DML 100 mg and the normal. From day 20-28, there was a significant decrease ($p<0.05$-0.001) between all extracts except ZZS 250 mg and COMBO 100 mg.

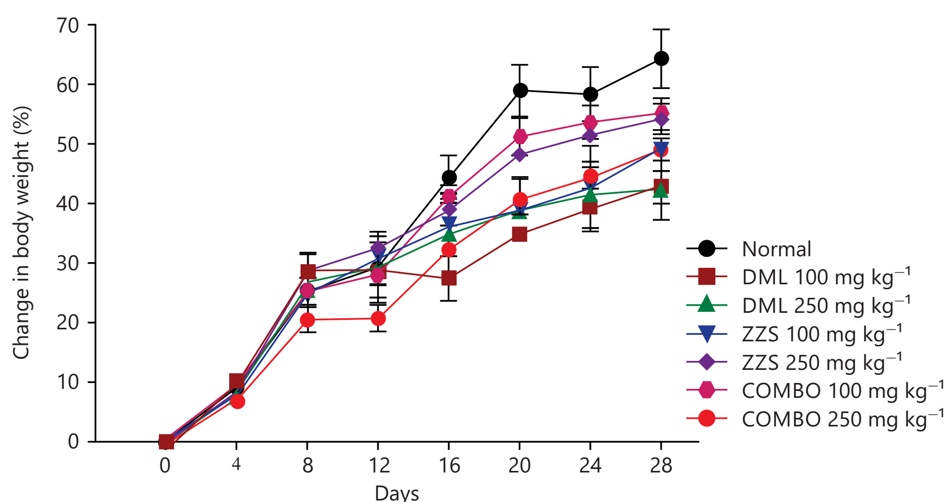

Fig. 2: Effect of extract treatment on percentage change in body weight

DML: *Detarium microcarpum* leaves, DMS: *Detarium microcarpum* stem, DMR: *Detarium microcarpum* roots, ZZL: *Zanthoxylum zanthoxyloides* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark and COMBO: Combined aqueous extract of DML and ZZS

Table 11: Effect of extract treatment on relative organ weight

	Relative organ weight (%)						
Organ	DML 100	DML 250	ZZS 100	ZZS 250	COMBO 100	COMBO 250	Normal
Lungs	0.78±0.11	0.65±0.10	1.07±0.28[a]	0.60±0.04	0.61±0.01	0.67±0.14	0.52±0.07
Kidney	0.65±0.00	0.67±0.02	0.63±0.04	0.57±0.02	0.59±0.03	0.63±0.03	0.54±0.01
Pancreas	0.63±0.17	0.74±0.05	1.00±0.11	0.87±0.22	0.86±0.12	1.09±0.08	0.74±0.05
Liver	2.65±0.13	2.78±0.11	2.84±0.27	2.68±0.13	2.63±0.07	2.74±0.18	2.52±0.13
Heart	0.35±0.02	0.36±0.01	0.34±0.03	0.35±0.01	0.35±0.01	0.32±0.02	0.32±0.01
Spleen	0.32±0.05	0.28±0.01	0.32±0.05	0.27±0.01	0.23±0.02	0.24±0.01	0.28±0.06

DML: *Detarium microcarpum* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark, COMBO: Combined aqueous extract of DML and ZZS and Significant difference: a ($p<0.05$-0.001) from normal

Table 12: Effect of DML, ZZS and COMBO (100 and 250 mg kg^{-1}, p.o) on blood biochemistry parameters in Wistar rats after 28 days of administration compared to control

Parameter	Normal	DML 100 mg kg^{-1}	DML 250 mg kg^{-1}	ZZS 100 mg kg^{-1}	ZZS 250 mg kg^{-1}	COMBO 100 mg kg^{-1}	COMBO 250 mg kg^{-1}
GLU (mmol L^{-1})	2.84±0.43	2.81±0.03	3.14±0.22	2.38±0.15	3.09±0.39	2.93±0.09	2.74±0.20
T-bil (μmol L^{-1})	4.72±1.33	2.93±0.10	3.37±0.25	2.72±0.32	2.81±0.17	5.52±0.85	2.84±0.07
D-bil (μmol L^{-1})	2.05±0.76	1.41±0.02	1.71±0.43	1.52±0.10	1.20±0.05	2.80±0.39	1.15±0.08
IBIL (mmol L^{-1})	2.67±0.83	1.53±0.08	1.66±0.24	1.21±0.37	1.61±0.20	2.72±0.47	1.69±0.15
ALT (U L^{-1})	73.37±11.84	34.03±2.66	56.67±10.90	32.73±1.44[a]	36.10±2.03[a]	46.47±5.15	40.33±4.95[a]
AST (U L^{-1})	332.00±3.40	349.40±14.16	106.77±73.62[a]	231.63±8.15[a]	249.20±5.12[a]	329.27±9.38	311.80±28.37
ALP (U L^{-1})	242.20±10.43	227.50±10.61	339.87±69.78[a]	263.20±43.71	348.53±11.84[a]	334.23±42.34[a]	314.07±50.82[a]
HDL-C (mmol L^{-1})	1.56±0.33	1.49±0.16	0.92±0.06	0.86±0.20	0.84±0.19	1.11±0.46	1.19±0.30
TC (mmol L^{-1})	3.20±0.19	2.60±0.36	2.67±0.48	2.01±0.61	2.26±0.64	2.70±0.43	3.11±0.23
LDL (mmol L^{-1})	1.20±0.11	0.60±0.21	1.35±0.48	0.66±0.44	1.01±0.37	1.01±0.66	1.57±0.47
VLDL (mmol L^{-1})	0.45±1.33	0.44±0.01	0.40±0.00	0.49±0.05	0.41±0.10	0.57±0.02	0.35±0.08
CR	3.05±0.50	2.38±0.09	4.02±0.77	3.14±0.26	3.65±0.26	5.05±2.60	4.17±1.20
TRIG (mmol L^{-1})	0.98±0.13	0.99±0.00	0.89±0.00	1.08±0.11	0.90±0.22	1.27±0.05	0.77±0.17

DML: *Detarium microcarpum* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark, COMBO: Combined aqueous extract of DML and ZZS, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, T-bil: Total bilirubin, D-bil: Direct bilirubin, I-bil: Indirect bilirubin, TRIG: Triglyceride, TC: Total cholesterol, LDL: Low density lipoprotein, HDL-C: High density lipoprotein, VLDL: Very low-density lipoproteins , GLU: Globulin, CR: Creatinine and Significant difference: a ($p<0.05$-0.001) from normal

Relative organ weight: There was no significant difference in the weight of the organs in all groups between the normal group and the various extract groups except for a significant increase ($p<0.05$-0.001) in the weight of lungs for the ZZS 100 mg kg^{-1} extract compared to the normal as seen in Table 11.

Table 13: Effect of DML, ZZS and COMBO (100 and 250 mg kg^{-1}, p.o) on haematological indices in Wistar rats after 28 days of administration compared to control

Parameter	Normal	DML 100 mg kg^{-1}	DML 250 mg kg^{-1}	ZZS 100 mg kg^{-1}	ZZS 250 mg kg^{-1}	COMBO 100 mg kg^{-1}	COMBO 250 mg kg^{-1}
WBC ($\times 10^3$ µL^{-1})	11.27±1.16	9.15±0.43	12.40±2.40	9.97±1.24	12.57±1.52	10.30±1.31	9.77±0.43
RBC ($\times 10^6$ µL^{-1})	8.02±0.48	8.09±0.28	8.10±0.12	8.14±0.08	8.31±0.30	8.60±0.28	8.60±0.10
HGB (g dL^{-1})	14.57±0.45	14.80±0.52	14.47±0.29	14.20±0.38	14.80±0.46	15.40±0.35	14.60±0.35
HCT (%)	61.27±2.79	58.50±0.87	58.67±1.48	57.07±1.02	60.37±1.96	63.90±1.91	61.23±0.97
MCV (fL)	76.60±1.72	72.25±1.36	72.37±0.82	70.10±1.39	72.67±1.44	74.33±0.43	71.13±1.83
MCH (pg)	18.23±0.60	18.25±0.03	17.87±0.12	17.47±0.38	17.80±0.17	17.93±0.26	16.97±0.48
MCHC (g dL^{-1})	24.13±0.69	25.30±0.52	24.67±0.13	24.87±0.41	24.53±0.27	24.13±0.44	23.83±0.34
PLT ($\times 10^3$ µL^{-1})	846.67±51.19	989.50±135.97[a]	920.67±168.68	1129.67±173.25[a]	1239.33±9.40[a]	797.33±106.16	879.00±173.72
LYM (%)	50.03±6.39	64.30±8.78	55.57±10.10	62.57±10.79	61.43±6.96	61.10±2.42	63.80±4.38
MXD (%)	7.77±0.80	7.15±2.51	7.57±2.09	4.53±1.38	7.00±2.80	7.70±0.61	6.33±1.34
NEUT (%)	42.20±5.66	28.80±6.12	36.87±0.50	32.90±9.40	31.57±4.52	31.20±2.02	29.87±3.98
LYM# ($\times 10^3$ µL^{-1})	5.53±0.43	6.00±1.10	7.13±0.91	6.47±1.70	7.53±0.35	6.30±0.85	6.20±0.21
MXD# ($\times 10^3$ µL^{-1})	0.90±0.17	2.10±1.10	1.00±1.45	0.43±0.09	0.97±0.52	0.80±0.06	0.63±0.15
NEUT# ($\times 10^3$ µL^{-1})	4.83±0.97	2.55±0.43	4.27±0.82	3.07±0.48	4.07±1.01	3.20±0.46	2.93±0.48
RDW-SD (fL)	48.50±1.00	50.70±1.56	48.90±0.35	49.43±2.09	50.80±0.76	44.87±0.89	44.50±0.46
RDW-CD (%)	16.93±0.38	20.15±0.03	18.90±0.32	20.03±1.33	20.27±0.96	16.23±0.47	17.17±0.88
PDW (fL)	10.17±0.32	10.20±0.29	10.63±2.14	10.87±0.84	11.37±0.52	10.10±0.25	10.53±0.09
MPV (fL)	8.57±0.12	8.55±0.14	8.80±0.18	8.80±0.52	9.27±0.26	8.46±0.15	8.60±0.00
P-LCR (%)	15.40±0.82	15.90±0.92	17.57±0.22	17.80±3.71	20.90±2.08	14.77±1.00	16.33±0.22
PCT (%)	0.73±0.05	0.84±0.10	0.82±0.15	1.01±0.21	1.15±0.03	0.68±0.08	0.75±0.15

DML: *Detarium microcarpum* leaves, ZZS: *Zanthoxylum zanthoxyloides* stem bark, COMBO: Combined aqueous extract of DML and ZZS, WBC: White blood cell, RBC: Red blood cell, HGB: Haemoglobin, HCT: Haematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, PLT: Platelet, LYM: Lymphocyte, NEUT: Neutrophil, RDW: Red cell distribution width, PDW: Plate volume distribution width, MPV: Mean platelet volume, P-LCR: Platelet large cell volume, PCT: Plateletcrit and [a]Significant difference: a (p<0.05-0.001) from normal

Blood biochemistry: Generally, there was no significant difference between the control group and the six extract in all blood biochemistry parameters except for the liver enzymes parameters namely ALT, AST and ALP for the various extracts as seen in Table 12.

Haematology: There was no significance difference between the control group and both COMBO extracts in all haematological parameters. Furthermore, there were no significance difference between DML and ZZS extract for all haematological parameters except significant difference in platelet count for both extracts. There were significant increases (p<0.05-0.001) in platelet count for the DML 100 extracts and both ZZS extracts (100 and 250 mg) as seen in Table 13.

DISCUSSION

The mechanism of glucose transport across yeast cell membranes has garnered attention as an *in vitro* method for assessing plant extracts' hypoglycaemic potential. Interestingly, this study noted a general decline in glucose uptake by yeast cells as sample and glucose concentrations increased. Glucose uptake by cells is indicated by residual glucose in the medium after a specific time. Most extracts enhanced yeast cell glucose uptake, though some ethyl acetate extracts did not at 5 and 10 mM glucose concentrations[21]. This glucose uptake enhancement holds promise for better blood glucose level management and hyperglycaemia control[22].

Yeast cell glucose uptake may differ from that of human cells. Yeast cell glucose transport might involve facilitated diffusion, unlike phosphotransferase enzyme systems seen in human cells. Muscle cell glucose uptake is driven by insulin-triggered glucose transport molecules accumulation in cell membranes. In yeast cells, glucose uptake is influenced by variables such as intracellular glucose concentration and metabolism. Increased glucose metabolism favours higher glucose uptake. Yeast cell glucose uptake with the aqueous extract of *D. microcarpum* leaves and *Z. Zanthoxyloides* stem bark extract likely involves facilitated

diffusion and high levels of glucose metabolism. Investigating *in vivo* activity of these extracts could reveal enhanced glucose uptake in muscle and adipose tissues[23].

The FBS, OGTT and HbA1c are clinically used to diagnose and manage prediabetes and diabetes. The OGTT is a clinically sensitive criterion that helps in the detection of early irregularities in the disposal of glucose[24]. In a diabetic state, the ability of insulin to stimulate the uptake of glucose from the bloodstream into cells is compromised. As a result, after the intake of carbohydrate-rich foods, maintaining the optimum blood glucose levels becomes challenging which reduces the body's ability to response to insulin which is released. This leads to the rise in blood glucose levels resulting in postprandial hyperglycaemia (PPHG)[25].

Studies in relation to the effects of drugs on the reduction of PPHG have been one of the numerous key aspects in management of diabetes mellitus that has been a well-focused therapeutic approach up to date[23]. Most extracts known to have antidiabetic properties exert its abilities by suppressing postprandial hyperglycaemia. To manage PPHG, the digestion of carbohydrates into monosaccharides by the carbohydrate digestive enzymes and the absorption of these monosaccharides by the intestinal glucose transporters can be targeted[25]. From the results of OGTT, all extracts helped in the clearance of glucose after four hours to levels compared to normal and the standard drug glibenclamide, without any negative effect such as hypoglycaemia as seen with the standard drug glibenclamide. It could mean that the extracts especially the combined extract (COMBO) work in a similar mechanism like the standard drug and as well increase the permeability of glucose into the cells by increasing the activity of glucose transporters to take up more glucose into the cells[25,26].

Drug toxicity can lead to body weight abnormalities, with decreased body weight and altered organ weight as indicators of toxicity with subtle changes in weight significant. During the study, all extracts increased rat body weight compared to control. Substantial body weight loss can be fatal[9]. Herbal products might impact organs like the liver, kidneys, spleen, brain and heart. Varied body weights and mostly unchanged relative organ weight, except lungs for ZZL 100 mg kg^{-1}, suggest non-toxicity[27].

All extracts were safe from heavy metals such as lead (Pb) and chromium (Cr), toxic metals absent in detectable amounts. The Pb poses risks like reproductive and renal system issues[28]. The absence of Pb suggests no harm to the renal system from extracts. With respect to all heavy metals analysed, the various plant extract and raw powder will not cause any negative effect resulting from excess concentration, when taken in as medicine.

Serum transaminase enzymes, ALT and AST, indicate hepatocyte toxicity. Elevated ALT and AST levels signal liver damage. The ALT is mainly found in cytoplasm of hepatocyte. The AST is found in heart, kidney, liver, muscle, pancreas and red blood cells[26]. The observed significant decline in levels of ALT in ZZS and DML extracts, as well as the non-significant decline of the combined extracts suggest possible hepatoprotective effects. All extracts at 100 mg kg^{-1} showed non-significant triglyceride reduction and 250 mg kg^{-1} extracts showed non-significant total cholesterol reduction, indicating potential lipid-lowering effects, dose-dependent for cholesterol[29].

The methanolic extracts of both plants exhibited better radical scavenging activity in the DPPH antioxidant assay, having the most effective EC_{50} value. This was higher than the aqueous extract and combined aqueous extract, with the methanolic DMR extract having a better activity compared to ascorbic acid. The combined aqueous extract (COMBO) performed better than the individual components (aqueous DML and ZZS extracts). The extracts having the most potent value of EC_{50} values of extracts can be attributed to extracts rich in bioactive phytochemicals like phenols, alkaloids, terpenoids and flavonoids. The lower

EC_{50} of the COMBO extract compared to individual components underscores the synergistic and/or antagonistic interactions of antioxidant-capable phytochemicals[30,31]. Future recommendations studies can be carried out to separate the active components exclusive to the combined extract of *Zanthoxylum zanthoxyloides* leaf extract and *Detarium microcarpum* stem bark and isolate the components to study the molecular interactions that contribute to the synergy and efficacy of the combine extract. Researches on the antidiabetic potential can be carried *in vitro* by glucose uptake activity with HEPG2 from rat diaphragm and L6 myoblast and *in vivo* in the STZ/NA rat model as the antidiabetic potential of the combined extract has been evaluated.

CONCLUSION

The combined aqueous extract (COMBO) had the best effect of suppressing postprandial hyperglycaemia than the individual aqueous extract of DML and ZZS and the best *in vitro* antioxidant activity due to the presence of phenols, alkaloids, terpenoids and flavonoids. The combined extracts also had lipid lowering and hepatoprotective potential. This indicates that the combined extract (COMBO) has a better therapeutic potential as an antidiabetic agent.

SIGNIFICANCE STATEMENT

Prior researches predominantly have delved into the antidiabetic attributes of individual plants or their specific plant parts. However, critical gaps exist in understanding the combined effects and efficacy of multiple antidiabetic plants. Recognizing the growing reliance of the individuals on a cost effective and safe natural remedies, this study sought to assess the collective antidiabetic potential, efficacy and safety profile of combined extracts from *Zanthoxylum zanthoxyloides* and *Detarium microcarpum* which seeks to provide basis for future development in the use of plant combinations for enhanced therapeutic benefits.

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