

FRACTIONATION AND IDENTIFICATION OF BIOACTIVE CONSTITUENTS FROM *SAPIUM ELLIPTICUM* (HOCHST) LEAF EXTRACT

¹IGHODARO, Osasenaga Macdonald, ²AKINLOYE, Oluseyi Adeboye, ²UGBAJA, Regina Ngozi and ³OMOTAINSE, Samuel Olatunbosun

¹Department of Biochemistry, Lead City University, Ibadan, Nigeria.

²Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria.

³Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta, Nigeria.

Corresponding Author: Ighodaro, O. M. Department of Biochemistry, Lead City University, Ibadan, Nigeria. **Email:** macigho@gmail.com **Phone:** +234 8023658692

ABSTRACT

In view of antidiabetic and antioxidant properties observed in a previous study, crude ethanol leaf extract of Sapium ellipticum (SE) was fractionated using Silica gel F254 column chromatography to yield 164 fractions. Pooling together of fractions with similar thin layer chromatographic (TLC) mobility profile afforded five major fractions with masses ranging from 0.06 to 2.09 g, and retention factor (RF) ranging from 0.59 to 0.87. Bioactivity of the fractions obtained was evaluated against streptozotocin-induced oxidative stress and diabetes in adult Wistar rats. HPLC-MS analysis of active fractions in dynamic MRM mode using compound-specific parameters revealed the presence of lupeol, luteolin-7-O-glucosides, amentoflavone and α -tocopherol in S. ellipticum leaf extract. This array of phytochemicals present in the plant extract is arguably responsible for its pharmacological and medicinal properties.

Keywords: *Sapium ellipticum*, Bioactive constituents, Fractionation, Chromatography

INTRODUCTION

Over the years nature has been a source of important medicinal compounds and an impressive number of modern drugs have been isolated and produced from plant sources. The use of herbs and medicinal plants is becoming a universal phenomenon and has in recent times gain huge popularity and acceptance following meaningful validations from scientific investigations. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for therapeutic purposes (Otshudi *et al.*, 2000). A single plant or a combination of two or more may be used for the treatment of various disease conditions depending on the community. Several disease conditions including cardiovascular disorder, asthma, hypertension, rheumatism, sickle cell anaemia, fever, some forms of cancer, infectious pathologies in the respiratory and urinary tracts have been

effectively treated with traditional medicinal plants (Cousins and Huffman, 2002; Rios and Recio, 2005; Saganuwan, 2010).

The enormous significance of medicinal plants in respect to the health of individuals and communities cannot be over emphasized. About 3.4 billion people in the developing countries depend on plant-based medicines. This figure accounts for about 88 % of the global populace. According to the World Health Organization (WHO), more than 85 % of the world's population; especially millions of people in the vast rural areas of the developing world largely depend on traditional medicine for their primary health care (WHO, 2001). Ethnobotany is recognized as an effective way to discover future medicines. It has over time assumed a pivotal or central stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs. Medicinal plants contain substances that are bioactive, of therapeutic

potential, and are responsible for the medicinal functions elicited by plants. They have been found to be precursors for chemopharmaceutical semi-synthesis. Several secondary metabolites or natural products isolated from these medicinal plants are useful as clinically active drugs or serve as a drug leads (Agidew *et al.*, 2013).

The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, biochemists, microbiologists, botanists and natural-products chemists are combing the earth for phytochemicals and leads that could be developed for treatment of various diseases. In fact, according to WHO, approximately 25 % of modern drugs used in the United States have been derived from plants (WHO, 2009). Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 % showed positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Padmavathi, 2013). Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work (Offor and Uchenwoke, 2015).

Sapium ellepticum enjoy huge therapeutic application in the treatment of a number of disease conditions including diabetes (Adesegun *et al.*, 2008). It belongs to the family *Euphorbiaceae* and is commonly referred to as jumping seed tree. *S. ellipticum* is widely distributed in eastern and tropical Africa. In southwest part of Nigeria, particularly among the Ilorin indigenes, the plant is popularly known as *aloko-agbo*. A few scientific investigations have been carried out on it. Adesegun *et al.* (2008) in their *in vitro* study credited substantial antioxidant properties to the stem bark extract of the plant. Cytotoxicity screening of selected Nigerian plants used in traditional cancer treatment on HT29 (colon cancer) and MCF-7 (breast cancer) cell lines indicated that *Sapium ellipticum* leaf extract expressed the highest cytotoxic activity among

other plants with anticancer potential which was comparable to the reference drug, ciplastin (Sowemimo *et al.*, 2011). The phytochemical constituents, *in vitro* antioxidant capacities and antiplasmodial activities of *Sapium ellipticum* stem bark extracts were documented by Nana *et al.* (2013). Agidew and colleagues in their study demonstrated the presence of lupeol, lupeol acetate and stigmasterol in the stem bark extract of *Sapium ellipticum* (Agidew *et al.*, 2013).

Bioassay guided fractionation is one the key technique by which compounds with good biological activity has been isolated from medicinal plants (Agidew *et al.*, 2013). This present study sought to separate *Sapium ellipticum* leaf extract into fractions and identify the bioactive compounds present in the plant extract.

MATERIALS AND METHODS

Collection of *Sapium ellipticum* and Preparation of Leaf Extracts: Fresh *Sapium ellipticum* leaves were harvested in December 2012 from a forest in a suburb of Ibadan, southwest of Nigeria. The harvested leaves were taxonomically identified (TPL, 2010) and authenticated by a curator botanist (Mr. T. K. Odewo) at the Lagos University Herbarium (LUH), Nigeria, were a specimen was deposited to obtain a voucher specimen number LUH 5423. The plant material was freed of extraneous materials; air dried at room temperature to a constant weight and milled into a fine powder with a milling machine. Different extract fractions were prepared by macerating 50 grams of the dried powdery sample in 500 ml of each extracting solvent (distilled water and ethanol) at room temperature. The mixture was allowed to stand for 72 hours and stirred intermittently to facilitate extraction. The mixture was sieved using a muslin cloth of mesh size, 42 μ m. The resulting volume on sieving was reduced with a rotary evaporator. Final solvent elimination and drying was done using a water bath at 40 °C. The crude extracts were stored in sterile screwed (air-tight) bottles and aliquots were taken when required.

High Performance Liquid Chromatography Mass Spectrometric (HPLC-MS) Analysis of *S. ellipticum* Crude Extract: *S. ellipticum* crude extract was analysed by HPLC-MS according to the method described by Orcic *et al.* (2014). An Agilent 1100 HPLC Series System (Agilent, USA) equipped with a degasser, binary gradient pump, column thermostat, auto sampler and UV detector was used for HPLC-MS analysis of *S. ellipticum* crude extract. For the separation, a reverse-phase analytical column (Zorbax SB-C18, 100 x 3.0 mm i.d., 3.5 µm particles) was employed and the work temperature was set at 48 °C. The detection of the compounds was performed on both UV and MS mode. The UV detector was set at 330 nm until 17.5 minutes, then at 370 nm. The MS system operated using an electro spray ion source in negative mode. Chemical Station and Data Analysis software from Agilent (USA) were used in processing the chromatographic data. The sample was dissolved in mobile phase (ratio 1:1) to produce a final concentration of 2 mg/ml. The mobile phase was a binary gradient: methanol and acetic acid 0.1 % (v/v). The elution started with a linear gradient, beginning with 5 % methanol and ending at 42 % methanol, for 35 minutes; then 42 % methanol for the next 3 minutes. The flow rate was 1 ml/min and the injection volume was 5 µl (Anton *et al.*, 2013; Benedec *et al.*, 2013). The MS signal was used only for qualitative analysis based on specific mass spectra of each component compound. The MS traces/spectra of the analyzed sample were compared to spectra from library, which allows positive identification of compounds, based on spectral match. Component compound were distinguished and identified in MS detection (qualitative analysis) based on differences in their molecular mass and MS spectra. The detection limits were calculated as minimal concentration producing a reproductive peak with a signal-to-noise ratio greater than three.

Bioassay Guided Fractionation of *S. ellipticum* Crude Extract

Selection of the solvent-system with optimum performance for column fractionation: The crude leaf ethanol extract of *S. ellipticum* was reconstituted in absolute ethanol and spotted on pre-coated (Silica gel F254) thin layer chromatographic (TLC)

aluminium plate. Various solvent systems: Benzene/ethylacetate, Chloroform/ethanol, chloroform/ethylacetate, Hexane/ethylacetate, ethylacetate/ethanol, ethylacetate/methanol were used in different combination ratios as mobile phase; in order to determine the most suitable eluent for column fractionation of the extract. After each separation, the TLC plates were exposed to iodine fumes in a chamber. The eluent (solvent-system at a particular ratio) which gave the best separation or resolution was adopted.

Column chromatography fractionation of *S. ellipticum* crude extract: *S. ellipticum* crude extract (12 g) was subjected to column chromatography to separate the extract into its component fractions using a column size of 3.5 x 50 cm. Silica gel (60 G) was used as the stationary phase while varying solvent (hexane, ethyl acetate and methanol) combinations of increasing polarity were used as mobile phase. In setting up the experiment, the wet packing method was adopted in packing of the glass column. Firstly, the lower part of the column was stocked with glass wool with the aid of a glass rod and the column was held up in a vertically upright position on a retort stand. The slurry was prepared by mixing 170 g of silica gel with 350 ml of hexane, and this was carefully poured down into the column with the tap of the column left open to permit free flow of solvent into a conical flask placed on the base of the retort stand. The set-up was assessed to be appropriate when the solvent drained freely without eroding either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the packed column was allowed 24 hours to stabilize; after which the clear solvent on top of the silica gel was allowed to drain down to the silica gel meniscus.

The sample was prepared in a ceramic mortar by adsorbing 12.0 g of the crude extract to 22.0 g of powder silica gel (60 G) in methanol and dried on a regulated hot plate. Continuous stirring of the adsorbed sample to dryness was done with spatula while guarding against thermal degradation. The resultant dry sample was allowed to cool and then gently layered on

top of the column. A layer of purified sand was used to cover the sample to avoid direct contact with eluent. The tap was then opened to allow the eluent to flow at a controlled rate of 40 drops per minute. Elution of the extract was done with solvent systems of gradual increasing polarity. The following ratios of solvent combinations were sequentially used in the elution process; Hexane: ethylacetate 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. The same ratios were used for Ethyl acetate: methanol combination. A measured volume (4 ml) of each solvent mixture was collected with 10 ml syringe and sprayed uniformly by the sides of the glass into the column each time. This was done to prevent the solvent droplets from falling directly and disturbing the topmost layer of the column. Distortion of the layer would result in non-uniform drain of the fractions. The eluted fractions were collected in aliquots of 20 ml in small sterile bottles.

Analytical thin layer chromatography (TLC) and pooling of fractions: Precoated silica gel (F254) aluminium plate was used as a stationary phase and hexane: ethyl acetate (7:3) as mobile phase for the thin layer chromatography analysis of extract fractions. With the aid of a sharp lancet, the plate was cut into uniform strips. Using a 1.0 μ l micro pipette, a spot of the sample was applied on the TLC plate about 1.0 cm from its origin. Care had to be taken not to overload the plate so as to prevent streaking. The plate was dried under a steady current of air at room temperature. This allowed the sample application to be made as thin as possible and thus improved separation. The plates (strips) were developed in a glass chromatographic tank; containing the solvent system and lined with thick filter paper. This facilitated saturation of the chromatographic tank with solvent vapour and thus improved separation and consequently reduced the time taken for the plates to develop. The tank was covered with a glass lid and the solvent was allowed to ascend until the solvent front was about 3/4 of the length of the plate. The plate was removed and dried by a hot air dryer and viewed under UV lamp at 365 and 254 nm to

identify the fluorescing spot. The fluorescent spot was marked and then sprayed with spray reagent (0.16 g vanillin in 30mL concentrated tetraoxosulphate (IV) acid, H₂SO₄). The plate was placed in a hot oven at 110 °C for 5 seconds for visibility of fluorescent spots or bands. The colour reaction was recorded and the relative Retention Factor (RF) value was calculated based on the formula described by Sherma and Fried (2003) thus: $R_f = \frac{\text{front}}{\text{Distance travelled by the sample from the starting point} \div \text{Distance travelled by the solvent from the starting point to the solvent}}$

Based on their thin layer chromatography profile vis-à-vis their relative retention factor (RF) value, fractions that showed similar TLC mobility and band formation pattern were pooled together into a pre-weighed beaker and were subjected to another round of TLC analysis to determine the number of spots or bands. The pooled fractions were then concentrated in rotavaporator at 40 °C and 210 milibar and the mass of the sample was determined using a sensitive electronic weigh balance (sensitivity range of 0.01-200g) model.

Antidiabetic and Antioxidant Assays of *S. ellipticum* Fractions: Four physiologically normal rats and 16 streptozotocin-induced diabetic rats were used to assess the anti-diabetic effects of four of the fractions (SEF1, SEF2, SEF3 and SEF5) obtained from SE crude extract. Diabetes Mellitus was induced in normoglycaemic rats starved for 16 h by injecting with a single intra peritoneal (i.p) dose of freshly prepared streptozotocin (STZ), 55 mgKg⁻¹BW of rats in 0.1 M citrate buffer, pH 4.5. Control rats were injected with citrate buffer alone (1 mL). The 'diabetic' animals were maintained on 5% glucose solution overnight to overcome the drug induced hypoglycaemia. 72 h after STZ injection, acutecheck active glucometer with disposable test strips was used to determine the fasting blood glucose level and to confirm the diabetic level of rats. Animals with 200 mg/dL and above were considered to be diabetic and used for the study.

Treatment of experimental diabetic rats with extract fractions (SEF1, SEF2, SEF3 and SEF5) was done once daily at a dose of 50

mg/kg BW through oral route (p.o). Metformin (12 mg/kg BW) was used as a reference anti-diabetic drug. Blood samples were collected from the tails of animals for glucose estimation on the first, third and seventh day (final) after treatments commenced. After the last blood glucose level determination, the animals were fasted overnight and weighed and then sacrificed by cervical dislocation.

Collection of Blood and Organ Samples:

Blood samples were collected and serum was prepared from each sample by centrifugation at 3000 rpm. The liver and kidney were immediately suspended in the homogenizing buffer (ice cold Tris-HCL buffer, 0.1M, pH 7.4) and thereafter homogenized using a Potter Elvehjem type homogenizer. The homogenization was done using 3 ml buffer to 1 g of tissue. The homogenate was centrifuged at 10,500 x g for 30 minutes at 4 °C to obtain the post mitochondrial fraction (PMF).The post mitochondrial fraction (PMF) obtained in each case was used to determine protein and MDA concentrations as well as SOD and CAT activities in the tissues by methods earlier described.

HPLC–MS Analysis of Bioactive *Sapium ellipticum* Fractions:

The method of Orcic *et al.* (2014) was employed in the HPLC–MS analysis of selected fractions of *S. ellipticum* (SEF2, SEF3 and SEF5). The choice of fractions was on the basis of observed biological activity. Agilent Technologies 1200 series HPLC coupled with Agilent 6410B series electrospray ionization triple-quad MS/MS was used to perform the analyses. The samples were in each case first subjected to separation using a Zorbax Eclipse XDB-C18 (Agilent) rapid resolution column (50 mm x 4.6 mm i.d., 1.8 μm particle size) held at 50 °C. 0.05 % aqueous formic acid (A) and methanol (B) constituted the binary mobile phase. Samples were dissolved in mobile phase (ratio 1:1) to obtain a final concentration of 2 mg/ml. Working standards were prepared by serial dilutions (1:1) of standard mixture with solvents A and B.

The injection volume was 5L, and elution was 1 ml/min with gradient program as follows: 0 min 30 % B, 6 min 70 % B, 9 min 100 % B, 12 min 100 % B, re-equilibration time 3 min. Eluted components were detected by MS, using the ion source parameters as follows: nebulization gas (N₂) pressure was 40 psi, drying gas (N₂) flow was 9 l/min and temperature 350 °C, capillary voltage 4 kV, negative polarity. Data were acquired in dynamic MRM mode, using the optimized compound-specific parameters (retention time, precursor ion, product ion, fragmentor voltage, collision voltage). Agilent Mass Hunter Workstation software-Qualitative Analysis (ver.B.03.01) was used to determine the peak areas for samples.

RESULTS

HPLC-MS Chromatogram: Figures 1 and 2 represent the chromatograms of high performance liquid chromatography and mass spectrophotometry (HPLC-MS) analysis of *S. ellipticum* extract. The retention time (minutes and seconds) of the individual components are shown in Figure 1. The presence of three chromophoric compounds represented by major peaks with retention time ranging from 6:40 to 8:20 min: sec is shown in Figure 2.

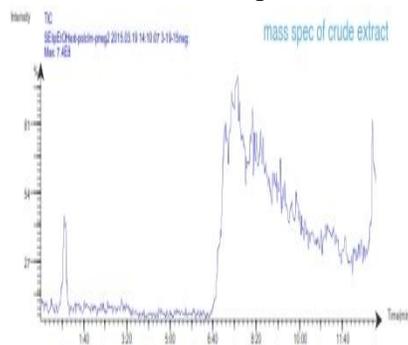


Figure 1: Chromatogram of HPLC-MS analysis of *Sapium ellipticum* extract

The MS-chromatogram represented by Figures 3, 4 and 5 depicts the presence of high molecular weight compounds (ranging from 300 to 950 g) in the extract.

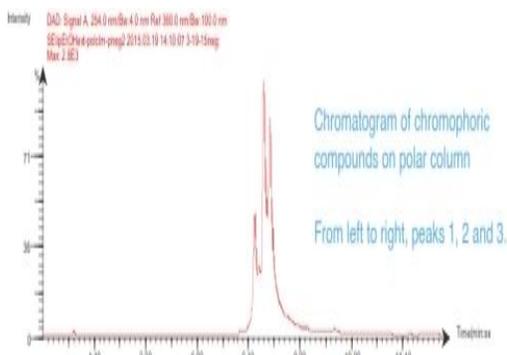


Figure 2: Chromatogram of HPLC-MS analysis of *Sapium ellipticum* extract showing the major peaks with retention time ranging from 6:40 to 8:20 min: sec

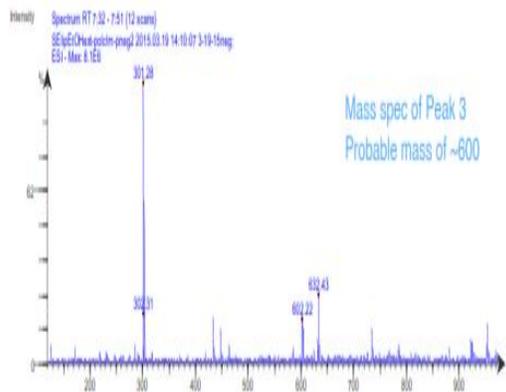


Figure 5: Chromatogram of LC-MS analysis of *Sapium ellipticum* extract showing peak 3

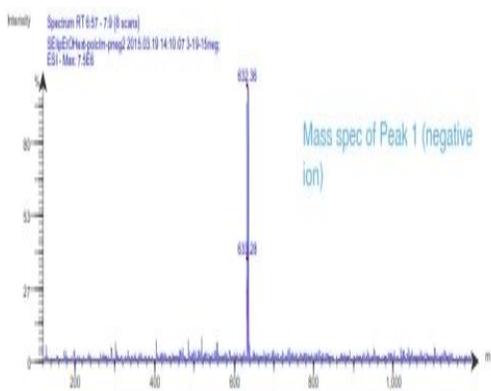


Figure 3: Chromatogram of LC-MS analysis of *Sapium ellipticum* extract showing peak 1

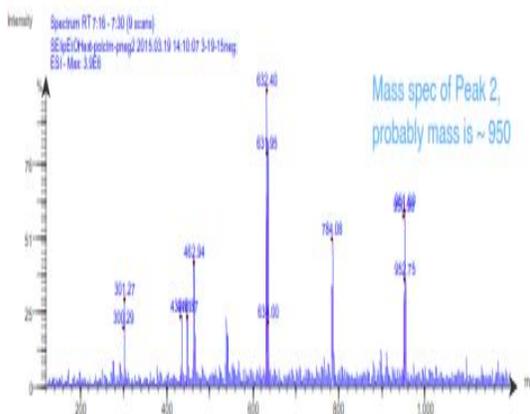


Figure 4: Chromatogram of LC-MS analysis of *Sapium ellipticum* extract showing peak 2

Column and Thin Layer Chromatographic Fractions of *Sapium ellipticum* Extract: Fractionation of *S. ellipticum* crude extract using column chromatography yielded 164 fractions. Pooling together of the fractions with similar TLC mobility profile; afforded five major fractions with masses ranging from 0.06 to 2.09 g (Table 1) and R_f values ranging from 0.59 to 0.87 (Figure 6). The colour reactions of the fractions following exposure to a solution of vanillin and concentrated tetraoxosulphate (vi) acid varied from gray, bluish brown, reddish brown, to light brown (Figure 7).

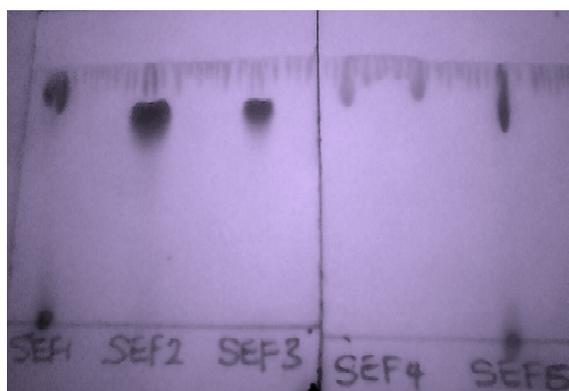


Figure 6: TLC mobility pattern of fractions of *Sapium ellipticum* extract as viewed under ultraviolet lamp at 365 nm

Table 1: Characteristics of column fractions of *Sapium ellipticum*

Fractions	Quantity (g)	Rate of flow (RF)	TLC Coloration UV 254nm	Track/Spot (Number)	356nm
SEF1	1.16	0.84	Gray	One	Absorbed
SEF2	1.78	0.62	Bluish brown	One	Absorbed
SEF3	2.09	0.59	Reddish brown	One	Absorbed
SEF4	0.06	-	-	-	-
SEF5	1.59	0.87	Light brown	One	Absorbed

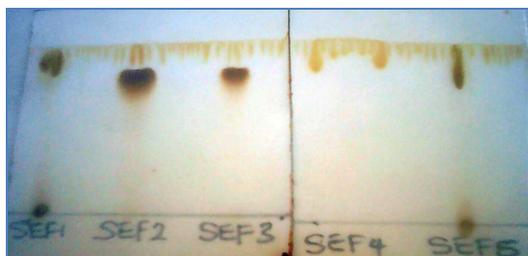


Figure 7: Colour reactions of fractions of *Sapium ellipticum* extract as viewed under Ultraviolet lamp at 254nm

Antidiabetic and Antioxidant Activities of *Sapium ellipticum* Extract Fractions: Figure 8 represents the effects of SE fractions on fasting blood glucose (FGB) level in STZ diabetic rats. Of all the fractions (SEF1, SEF2, SEF3 and SEF5) only SEF2 and SEF3 showed some degree of hypoglycaemic activity, affording 12.9 and 14.8 % FGB reduction respectively in comparison with the level of reduction (4.73 %) noted in the diabetic control rats. These effects were markedly lower than that of METF which produced 59.3 % reduction in FBG during the same duration of treatment.

Effects of *Sapium ellipticum* extract fractions on tissue protein: The effects of SE fractions on protein concentration in the liver and pancreas of STZ diabetic rats are summarized in Figure 9. STZ diabetic rats demonstrated significant loss of protein in both tissues with more severity noted in the pancreas. Although treatment with *S. ellipticum* extract fractions (particularly SEF3 and SEF5) significantly enhanced pancreatic protein content in diabetic rats; it however failed to replicate the same effect in the liver.

Conversely, diabetic animals treated with METF showed significant protein recovery in both tissues.

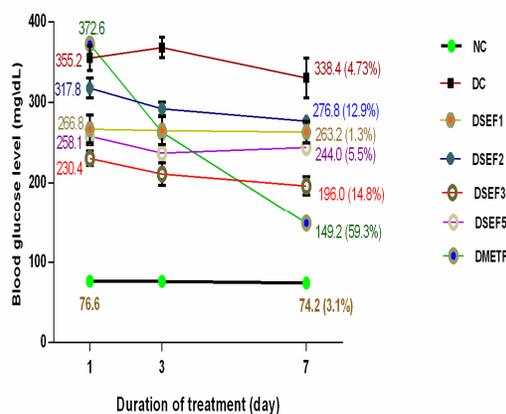


Figure 8: Curative effects of *Sapium ellipticum* fractions and METF on fasting blood glucose of STZ-Treated rats

Values are expressed as mean \pm SEM of 4 rats. Figures in parentheses represent mean percentage reduction in fasting blood glucose level (FGBL). NC = Normal control, DC= Diabetic control, SEF = *Sapium ellipticum* fraction, DSEF1 = Diabetic animals treated with SEF1 (50mg/kg BW), DSEF2 = Diabetic animals treated with SEF2 (50 mg/kg BW), DSEF3 = Diabetic animals treated with SEF3 (50mg/kg BW), DSEF5= Diabetic animals treated with SEF5 (50 mg/kg BW), DMETF = Diabetic animals treated with metformin (12 mg/kg BW).

Effects of *Sapium ellipticum* fractions on SOD and CAT activities: The effects of SE fractions on SOD and CAT activities in STZ diabetic rats are represented by Figures 10 and 11. Compared to normal control rats (CN), SOD activity was substantially lowered in the liver and pancreas of diabetic control animals (DC), with more suppression observed in the pancreas.

Treatment of diabetic rats with SE fractions with the exception of SEF1 significantly improved SOD activities in both tissues when compared to diabetic control animals. These effects were greater than that offered by METF. Of all the fractions SEF5 was outstanding in enhancing SOD activities in both tissues. Meanwhile, SEF2 and SEF3 along with METF promoted pancreatic SOD activity but their effects were relatively lower than that provided by SEF5 in the same tissue. In like manner, both hepatic and pancreatic CAT activities were markedly decreased in STZ-diabetic rats. This alteration was only corrected (to near normal) in pancreas of rats separately treated with SEF5 and METF, with the former eliciting greater effect. Treatment with METF and SE fractions did not significantly improve hepatic CAT activity. Though SEF2 and SEF3 also provided some improvement on pancreatic CAT activity, the changes were mild and non-significant when compared to diabetic control animals.

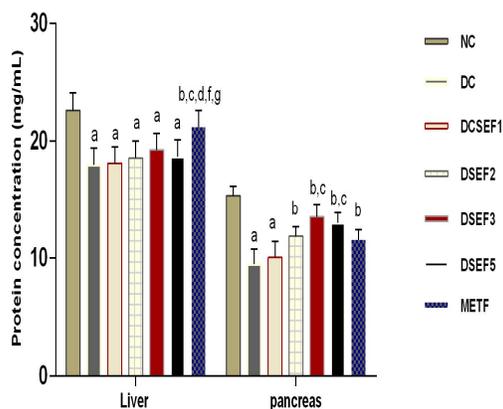


Figure 9: Effects of *Sapium ellipticum* column fractions on liver and pancreas protein concentration in STZ-diabetic rats

Values are expressed as mean \pm SEM of 4 rats. Figures in parentheses represent mean percentage reduction in fasting blood glucose level (FGBL). NC = Normal control, DC= Diabetic control, SEF = *Sapium ellipticum* fraction, DSEF1 = Diabetic animals treated with SEF1 (50mg/kg BW), DSEF2 = Diabetic animals treated with SEF2 (50mg/kg BW), DSEF3 = Diabetic animals treated with SEF3 (50mg/kg BW), DSEF5 = Diabetic animals treated with SEF5 (50mg/kg BW), DMETF = Diabetic animals treated with metformin (12 mg/kg BW). a =significant when compared to NC b=significant when compared to DC c = significant when compared to DSEF1, d= significant when compared to DSEF2, f = significant when compared to DSEF3, g = significant when compared to DSEF5, k= significant when compared to DMETF.

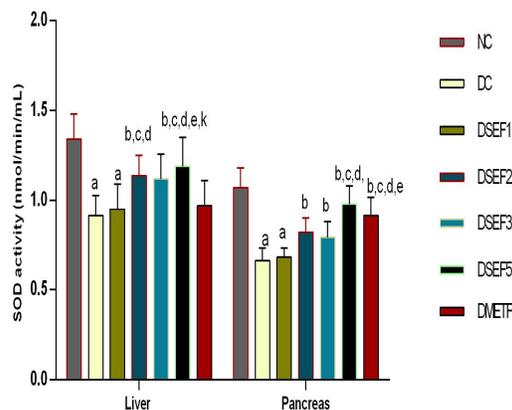


Figure 10: Effects of *Sapium ellipticum* column fractions on SOD activity in liver and pancreas of STZ – treated rats

Values are expressed as mean \pm SEM of 4 rats. NC = Normal control, DC= Diabetic control, SEF = *Sapium ellipticum* fraction, DSEF1 = Diabetic animals treated with SEF1 (50mg/kg BW), DSEF2 = Diabetic animals treated with SEF2 (50mg/kg BW), DSEF3 = Diabetic animals treated with SEF3 (50mg/kg BW), DSEF5= Diabetic animals treated with SEF5 (50mg/kg BW), DMETF = Diabetic animals treated with metformin (12 mg/kg BW). a =significant when compared to NC b=significant when compared to DC c=significant when compared to DSEF1, d= significant when compared to DSEF2, f = significant when compared to DSEF3, g = significant when compared to DSEF5, k= significant when compared to DMETF.

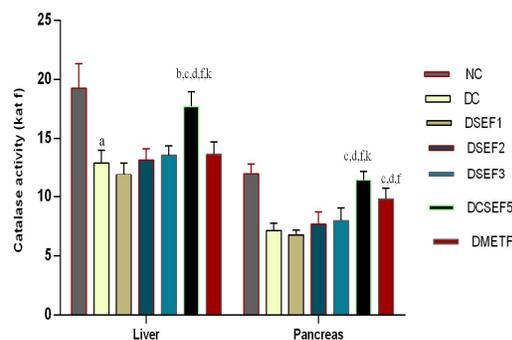


Figure 11: Effects of *Sapium ellipticum* column fractions on CAT activity in liver and pancreas of STZ – treated rats

Values are expressed as mean \pm SEM of 4 rats. NC = Normal control, DC= Diabetic control, SEF = *Sapium ellipticum* fraction, DSEF1 = Diabetic animals treated with SEF1 (50mg/kg BW), DSEF2 = Diabetic animals treated with SEF2 (50mg/kg BW), DSEF3 = Diabetic animals treated with SEF3 (50mg/kg BW), DSEF5= Diabetic animals treated with SEF5 (50mg/kg BW), DMETF = Diabetic animals treated with metformin (12 mg/kg BW). a =significant when compared to NC b=significant when compared to DC c=significant when compared to DSEF1, d= significant when compared to DSEF2, f = significant when compared to DSEF3, g = significant when compared to DSEF5, k= significant when compared to DMETF.

Effects of *Sapium ellipticum* Fractions on

MDA Concentration: Figure 12 depicts the effects of SE fractions on hepatic and pancreatic MDA level in STZ diabetic rats. The extract fractions with the exception of SEF1 significantly reduced MDA concentration in both tissues following STZ-induced elevation. This effect was significantly greater than that of METF in both tissues over the same period of treatment.

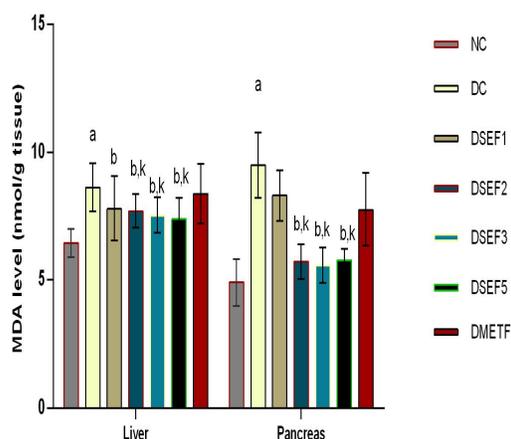


Figure 12: Effects of *Sapium ellipticum* column fractions on hepatic and pancreatic MDA levels in STZ – treated rats

Values are expressed as mean ± SEM of 4 rats. NC = Normal control, DC= Diabetic control, SEF = *Sapium ellipticum* fraction, DSEF1 = Diabetic animals treated with SEF1 (50mg/kg BW), DSEF2 = Diabetic animals treated with SEF2 (50mg/kg BW), DSEF3 = Diabetic animals treated with SEF3 (50mg/kg BW), DSEF5= Diabetic animals treated with SEF5 (50mg/kg BW), DMETF = Diabetic animals treated with metformin (12 mg/kg BW). a =significant when compared to NC b=significant when compared to DC c=significant when compared to DSEF1, d= significant when compared to DSEF2, f = significant when compared to DSEF3, g = significant when compared to DSEF5, k= significant when compared to DMETF.

Bioactive Compounds in *Sapium ellipticum*

Fractions: Table 2 shows the compounds that were detected in different bioactive fractions of *S. ellipticum* extract in dynamic MRM mode, using the optimized compound-specific parameters (precursor ion, product ion, fragmentor voltage, and collision voltage and retention time).

DISCUSSION

The complexities of crude extracts due to the wide range of compounds contained in them sometimes interfere with their efficacy in treating disease conditions. There is also the possibility of undesired side effects or herbal toxicity as a result of the presence of certain components in plants whole extracts. This explains why bioassay guided fractionation of crude extracts is a significant step in optimising the therapeutic effectiveness of medicinal plants and in overcoming herbal toxicity which s a major concern in the medicinal application of herbs or plant extracts. In the process of fractionation, phytoconstituents which are not relevant to the medicinal potential of given crude extract in treating a particular ailment are precluded.

For instance, in the present study, of all the SE fractions obtained , only two fractions (SEF2 and SEF3) showed some degree of hypoglycemic activity, affording 12.9 and 14.8 % FBG reduction respectively which appear significant when compared to diabetic control rats (4.73 %) but significantly low relatively to METF performance (59.3 %). This result also suggests that the hypoglycemic capability of SE leaf extract diminished with separation of the extract into its component fractions. Though the bioactive components identified in the SEF2 (Amentoflavone) and SEF3 (lupeol and luteolin-7-O-glucoside) in the current study are compounds that have been associated with antidiabetic functions, findings of this study suggest that the antidiabetic potential of SE depends on the collective or synergistic effort of some of the component bioactive compounds present in it.

The active antidiabetic constituents detected in SE fractions are not novel compounds. A number of investigations have previously informed of their presence in different plants. Patil *et al.* (2011) identified amentoflavone as the antidiabetic principle in *Biophytum sensitivum*. Lupeol has been reported to be one of the antidiabetic compounds in *Coccinia indica* (Kumar *et al.*, 2013) and two other *Coccinia* species (Ocvirk, 2013).

Table 2: Compounds detected in LC-MS analysed *Sapium ellipticum* fractions

SE fraction	Precursor m/z	Product m/z	V Collision (V)	V Fragmentor (V)	Compound	Time (min:sec)
SEF3	426	298	27	212	Lupeol	5.67
	447	285	29	230	Luteolin-7-O-glucoside	2.11
SEF2	536	373	33	220	Amentoflavone	5.30
SEF5	431	309	21	199	α -tocopherol	5.78

Interestingly, Agidew *et al.* (2013) had reported the presence of lupeol along with its acetate derivative and stigmasterol in the stem extract of SE. Hence, identification of lupeol in the leaf extract in this study corroborates their report. The presence of Luteolin-7-O -glucoside in a number of medicinal plants is popular (Hashmi *et al.*, 2015).

It has been established that oxidative stress forms the foundation for the induction of multiple cellular pathways that can ultimately lead to both the onset and subsequent complications of diabetes. This is due to persistent and chronic hyperglycaemia which depletes the antioxidant defense systems and thus promotes *de novo* free radical generation (Giacco and Brownlee, 2010). This explains why diabetic persons tend to have more oxidative cell and organism environments than healthy individuals (Guzik *et al.*, 2002; Rains and Jain, 2011). In chronic hyperglycaemic condition, production of reactive species (RS) is perpetuated and hence, the antioxidant enzymes and non enzymatic antioxidants are severely suppressed in various tissues, which further exacerbate oxidative stress (Maritim *et al.*, 2003; Rains and Jain, 2011). Streptozotocin (STZ), a well known diabetic agent is also known for its free radical generating potential *in vivo*.

SOD, CAT and GPx, along with some minerals (Se, Mn, Cu and Zn) constitute the first line antioxidant defense system against free radical attack. SOD catalyzes the dismutation of superoxide anion radicals ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) (Fukai and Ushio-Fukai, 2011), while catalase and Glutathione peroxidase (using glutathione as substrate) breakdown hydrogen peroxide to water and molecular oxygen (Wu and Cederbaum, 2003).

The collective role of these enzymes is therefore critical in the entire defense strategy and mechanism against free radicals that are continually generated in biological systems, either by accident of chemistry or on purpose

In the present study, evaluation of the antioxidant potential of SE fractions (SEF1, SEF2, SEF3 and SEF5) against free radical-generating STZ in diabetic rats showed that the active principle (s) underlying the antioxidant activities of the leaf extract is located mainly in fraction 5 (SEF5). In all, SEF5 appeared to possess greater antioxidant effects than other fractions. This observation is substantiated and accommodated by the results of HPLC-MS spectrometric analysis of SE fractions that revealed the presence of α -tocopherol in SEF5. Alpha tocopherol is a well known endogenous antioxidant renowned for its high proficiency against free-radical induced oxidative stress both *in vitro* and *in vivo*. This arguably provides the scientific basis for the significant antioxidant effects demonstrated by SEF5 in this study. Overall, findings from this study buttressed the fact that the arrays of phytochemicals present in plant extracts are arguably responsible for their pharmacological and medicinal properties.

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