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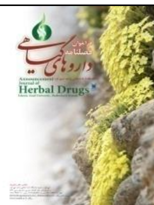

In-vitro antioxidant capacity and hepato-protective potential of *Blighia sapida* stem bark ether fractions in STZ induced diabetes rats

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ABSTRACT

Background & Aim: Diabetes mellitus is a metabolic disease that affects all systems in the body, including the liver. This study evaluated the *in-vitro* antioxidant capacity and liver function status of STZ-induced diabetic rats treated with petroleum and diethyl ether fractions of *Blighia sapida* stem bark for 14 days.

Experimental: The antioxidant ability of the petroleum and diethyl ether fraction of Blighia sapida stem bark was evaluated by total flavonoids and phenolic content (TFC and TPC) and DPPH scavenging activity using standard protocol. Thirty-five rats in seven groups were used. Plasma transaminases (ALT and AST) activities and bilirubin level was determined using standard procedure.

Results: The TFC and TPC of petroleum ether fraction of *B. sapida* (PEFBS) (47.16 mg QUE/100g and 39.87 mg GAE/100g) was observed to be higher compared to diethyl ether fraction of *B. sapida* (DEFBS) (37.44 mg QUE/100 g and 36.74 mg/GAE/100g). The DPPH scavenging activity of the fractions were significantly ($P<0.05$) reduced across the concentrations compared to the standard (gallic acid). STZ induced diabetes rats administered 2 ml/kg b. w. of normal saline significantly ($P<0.05$) increased plasma ALT, AST activities and bilirubin level compared to the normal control rats while treatment of diabetic rats with petroleum and diethyl ether fraction of *B. sapida* at both doses reduced the activities of these enzymes and level of bilirubin.

Recommended applications/industries: The results sustain the fact that, the fractions of *B. sapida* have an immense potential to be developed further into a therapeutic agent.

1. Introduction

Plants and their derivatives play key role in world health and have long been known to possess biological activity. Thirty percent of all modern drugs were derived from plants (Omoboyowa *et al.,* 2016). Several plant extracts are reported to have hypoglycaemic effect including *Blighia sapida* (Akee) an evergreen tree often cultivated for its edible fruit in many areas of the tropics and subtropics, especially in the Caribbean and West Africa. It has fragrant flowers and so is also grown as an ornamental and shade tree (Akintayo *et al.,* 2002).

The plant is used to treat anaemia and itching. In traditional medicine in Nigeria, *Blighia sapida* is

widely used for the treatment of diabetes, yellow fever, epilepsy and oedema, and as a laxative and diuretic.

Oxidative stress results from an imbalance between radical generating and radical scavenging systems that result in increased free radical production or reduced activity of antioxidant defenses or both phenomena (Robertson, 2004). It appears to be an important factor in the pathogenesis of a number of human metabolic disorders (Al-Omor *et al.,* 2004) including diabetes. Therefore, the potential of the antioxidant constituent of traditional plant for the management of life threatening diseases has raised interest among scientist.

Diabetes mellitus is one of the most common endocrine and metabolic disorders affecting over 170 million people worldwide (Njamen *et al.,* 2012). Diabetes is a chronic disease characterized by high blood glucose level and abnormal metabolism of carbohydrates, protein and fat associated with a relative or absolute insufficiency of insulin secretion and with various degrees of insulin resistance, accompanied by glycosuria, polydipsia, and polyuria (Omoboyowa *et al.*, 2016). Hepatic dysfunction is one of the underline complications of untreated diabetes which has increase the rate of mortality resulting from this disorder.

B. sapida fruit has been reported to inhibit certain enzymes (α-glucosidase and α-amylase) involve in carbohydrate metabolizing (Kazeem *et al*., 2014). The Root of the plant significantly lower blood glucose level in normoglycemic rats (Saidu *et al.,* 2012) and the bark extract possess ameliorating potential of pancreatic β-cell dysfunction (Ojo *et al.,* 2017). However, possible *in-vitro* antioxidant capacity and possible effect of petroleum and diethyl ether fractions of *B. sapida* stem bark on hepatic functions of STZ-induced diabetes rats has not been reported. Hence, this study was carried out to ascertain the ability of the plant to scavenge free radical and evaluate its hepato-protective potential in STZ induced diabetes animal model.

Figure 1. Diagram of *Blighia sapida*

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used in this study were of the analytical grade (BDH, England). Reagents used for all the assays were commercial kits and products of Randox, USA.

2.2. Plant materials

Fresh stem barks of *B. sapida* were collected randomly from trees within the Medicinal Plant Garden, Olusegun Agagu University of Science and Technology (OAUSTECH), Okitipupa, Ondo State and authenticated by the plant taxonomist, Department of Biological Sciences, OAUSTECH, Nigeria and a voucher specimen number (OAUSTECH/0625) was assigned and submitted to the OAUSTECH Herbarium. The identity and authenticity of the plant was also confirmed with the one deposited in http://www.theplantlist.org databases (Figure 1). The stem barks were air-dried and pulverized into powdered form.

2.3. Animals

In bred male albino wistar rats weighing between 120 ± 5 g were obtained from the Animal House of Department of Biochemistry, University of Ilorin, Kwara State, Nigeria and used for the protocols. The animals were kept in well ventilated rodent cubicles under 12 hours light/ dark cycles and fed with rats mash (Top feeds, Nigeria) and water *ad libitum*. The caring and experimental uses of the animals were according to the guidelines of National institute of health guidelines for cares of laboratory animals.

2.4. Preparation of extract and fractions

The dried plant material (100 g) was heated in 500 ml HCl (2 M) for 30 min in boiling water bath. The extract (300 ml) was cooled, filtered and divided into two equal portions. One portion (150 ml) was extracted with petroleum ether. The other portion (150 ml) was extracted with diethyl ether to obtain the phenolic fractions in both samples (Eseyin *et al.,* 2018). The fractions were filtered with whatman filter paper and concentrate in vacuum with the aid of a rotary evaporator.

2.5. *In-vitro* antioxidant analysis of fractions. Determination of total phenolic content

The total phenolic contents of the fractions were measured using the Folin-Ciocalteau colorimetric method as described by Ainsworth and Gillespie (2007) with gallic acid as standard. Basically, 0.2 ml of fractions was mixed with 1 ml of Folin-Ciocalteau phenol reagent (10- fold diluted). After 4 min, 0.8 ml of saturated sodium carbonate Na_2CO_3 (7.5%) solution was added and the mixture was allowed to stand for 2 h. Absorbance was measured at 765 nm. The same procedure was repeated with standard garlic acid solutions (10-200 µg/ml). The amounts of total polyphenols in different fractions were expressed as µg of garlic acid equivalent (GAE)/ mg fraction.

2.6. Determination of total flavonoids content

Total flavonoid content was determined following a method by Park *et al*. (2008). In a 10 ml test tube, 1 ml of sample (1 mg/ml), 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$ (0.5M) and 0.15 ml of $AlCl_3.6H_2O$ (0.3 M) were mixed. After 5 minutes, 1 ml of NaOH was added. The solution was mixed thoroughly and the absorbance was measured against the reagent blank at 506 nm. The total flavonoid content was determined from Quercetin standard calibration curve. The total flavonoids were expressed as mg of Quercetin equivalent per g of the fractions.

2.7. Determination of antioxidant activity by DPPH radical scavenging assay

The free radical scavenging activity of fractions was evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging assay according to the method reported by Karadag *et al.* (2009). Briefly, 0.25, 0.5, 0.75, 1.0 ml of the fractions was diluted with distilled water to make up to 1 ml. The diluted fractions were added with DPPH reagent (2.4 mg in 200 ml methanol). Methanol and gallic acid were used as control and antioxidant-standards, respectively. Absorbance was measured at 517 nm after holding for 30 min at room temperature. The absorbance of the control and samples were measured, and the DPPH scavenging activity in percentage was calculated according to the following formula:

$$Scavenging\ effect\ (\%) = \frac{AC - AS}{AC} \times 100$$

Where Ac: control absorbance and As: absorbance in presence of sample (fraction).

The data were presented as mean of triplicate and the concentration required for a 50% (IC_{50}) reduction of DPPH radical was determined graphically.

2.8. Ethical approval

The procedure for this work was ratified by the Ethics Committee, Animal Care and Usage Research, Olusegun Agagu University of Science and Technology (OAUSTECH /ACUR/2019/062). All research practices in this experiment were accomplished according to the National Institute of Health Guide for Care and Use of Research animals (NIH, 1985).

2.9. Diabetes mellitus induction

Diabetes mellitus was induced by intra-peritoneal injection of 50 mg/kg b.w. of streptozotocin (Sigma Chemicals, USA) in citrate buffer (pH 4.5) to fasted Wistar albino rats. After 4 days, blood glucose concentration of induced rats above 250 mg/dL were considered diabetic, and used in the experiment. Diabetes induced and control rats were treated with the petroleum and diethyl ether fractions for 14 days.

2.10. Experimental design: a curative study

Following successful diabetes induction, a total of thirty-five (35) wistar albino rats (130 ± 5 g) were randomly selected and distributed into 7 groups of 5 animals each, and they received treatment as follows:

Group 1: Normal control (received normal saline orally)
Group 2: Negative control (STZ induced + 2ml/kg b.w. of normal saline)
Group 3: Standard control (STZ-induced + 5 mg/kg b. w. of Metformin)
Group 4: STZ induced + 100 mg/kg b. w. of PEFBS
Group 5: STZ induced + 200 mg/kg b. w. of PEFBS
Group 6: STZ induced + 100 mg/kg b. w. of DEFBS
Group 7: STZ induced + 200 mg/kg b. w. of DEFBS

2.11. Collection of samples

After the fourteen day of treatment, the blood samples were collected through the ocular veins into sample bottles. The animals were sacrifice by cervical dislocation and the livers excised for histopathological examination.

2.12. Biochemical analysis

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using the method of Reitman and Frankel (1957). Total bilirubin concentration was determined by the method of Jendrassik and Grof (1938).

2.13. Liver histology

The liver tissues were completely fixed in 10% formalin; blocks were embedded in paraffin and sections cut at 5 µm and stained with haematoxylin and eosin, mounted in Canada balsami microscope at ×100 and × 400 magnifications (Avwioro, 2010).

2.14. Statistical analysis

Data were reported as means ± SD, where appropriate. One-way analysis of variance (ANOVA) was used to analyze the experimental data and Turkey multiple test range was used to compare the group means obtained after each treatment with control measurements using GraphPad Prism 6. Differences were considered significant when p≤0.05.

3. Results and discussion

The reported evidences on the efficacy of diverse medicinal plants used for management of both human and experimentally induced diabetes have improved the acceptance of herbal remedies for the treatment of life threatening diseases. Therefore, it is necessary to evaluate the safety of these herbs. In this study, the hepatic function of STZ-induced diabetes rats treated with petroleum and diethyl ether fractions of *B. sapida* stem bark and the anti-oxidant capacity of the fractions were assessed to ascertain the protective role of the plant on liver dysfunction resulting from STZ induction of diabetes and radical scavenging capacity of the plant.

3.1. Antioxidant activities of B. sapida stem bark

Oxidative stress is caused by a relative overload of oxidants, this impairs cellular functions and contributes to the pathophysiology of many diseases (Bonnefort-Rousselot *et al.,* 2000; Omoboyowa *et al.,* 2017), the complication of diabetes seem to be partially mediated by generation of reactive oxygen species (ROS) (Omoboyowa et al., 2017). The ability of petroleum and diethyl ether fractions of *B sapida* stem bark to generate antioxidants was studied. The total flavonoids content of petroleum ether fraction of the plant (47.16 mg QUE/100g) was observed to be higher compared to that of diethyl ether fraction (37.44 mg QUE/100 g). Also, the total phenolic content of petroleum ether fraction of *B. sapida* stem bark (39.87 mg GAE/100g) was higher compared to the diethyl ether fraction of the stem bark (36.74 mg/GAE/100g) (Table 1). The anti-oxidative effect of *B. sapida* stem bark might be due to phenolic compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Shadidi *et al.,* 1992) and may have contributed to the observed hepato-protective activity of the plant observed in STZ-induced diabetes rats. The DPPH scavenging activity of the fractions were significantly (P<0.05) reduced across the concentrations compared to the standard (gallic acid) as shown in Figure 2.

Table 1. Antioxidant potential of *Blighia sapida* stem bark

	PEFBS	DEFBS
TFC (mg QUE/100g)	47.16 ± 2.32	37.44 ± 8.45
TPC (mg GAE/100g)	39.87 ± 1.08	36.74 ± 9.68

TFC: Total flavonoids content; TPC: Total phenolic content; PEFBS: petroleum ether fraction of *Blighia sapida*; DEFBS: Diethyl ether fraction of *Blighia sapida*.

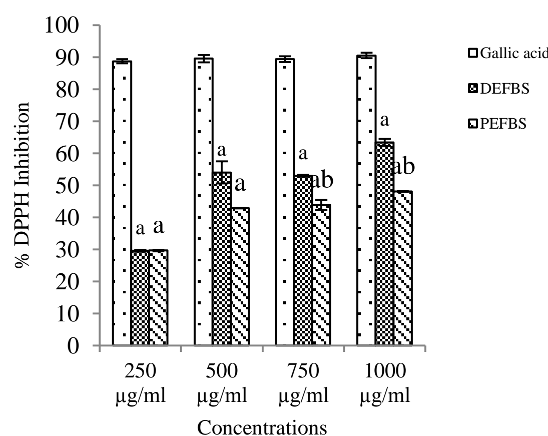

Figure 2: DPPH scavenging activity of petroleum and diethyl ether fractions of *B. sapida* stem bark

3.2. Biochemical assays

The results of this study revealed that, the STZ-induced diabetes rats showed significantly (P<0.05) higher plasma AST and ALT activities and bilirubin level compared to the normal control rats (Figure 3a-c).

The elevation in the enzyme activities in diabetes induced untreated rats could be as a result of leakage from the hepatocytes that were damaged by biotransformation of streptozotocin in the liver which might result from production and accumulation of free radicals since CYP2EI-dependent oxidative stress following induction of CYP2EI by STZ has been reported to be one of the causes of hepatotoxicity (Jaeschke *et al.,* 2002; Saeed *et al*., 2008). This result is consistent with several findings which showed STZ induced diabetes results in elevated AST activity and bilirubin level (Zafar *et al.,* 2009; Omonkhua *et al.,* 2014).

Treatment of STZ induced rats with petroleum and diethyl ether fractions of *B. sapida* reduced the plasma AST and ALT activities and bilirubin level compared to the STZ induced diabetes rats administered 2 ml/kg b.w of normal saline. This result revealed that treatment of diabetes rats with fractions of *B. sapida* stem bark at both 100 and 200 mg/kg b.w ameliorates the hepatic damage induced by STZ. The ether fractions have been reported to be rich in phenolic compounds (Eseyin *et al.,* 2018). Phenolic compounds such as caffeic acid, ferulic acid, quercetin, catechin, myricetin, P-coumaric acid etc might be present in the fractions and these compounds have been observed to poses anti-oxidative activity (Eseyin *et al.,* 2018). The ability of the ether fractions of *B. sapida* stem bark to ameliorate the liver dysfunction resulting from STZ metabolism might be due to the synergistic activity of the phenolic compounds to scavenge free radicals generated and prevent their accumulation in the liver thereby stabilizing the cell membrane of the hepatocytes and prevent the leakage of the enzymes. The results obtained in this study agreed with the findings of Saeed *et al*., (2008) who reported the attenuation of biochemical parameters in STZ-induced diabetes rats by oral administration of extracts and fractions of *Cephalotaxus sinensisi*.

The STZ-induced diabetes rats administered 100 mg/kg b.w of petroleum ether fraction of *B. sapida* stem bark was observed to be more potent as it shows reduction in AST and ALT activities and bilirubin level compared to other treated groups. Therefore, the active compound(s) responsible for the plant efficacy might be present in high concentration in the petroleum fraction of the stem bark.

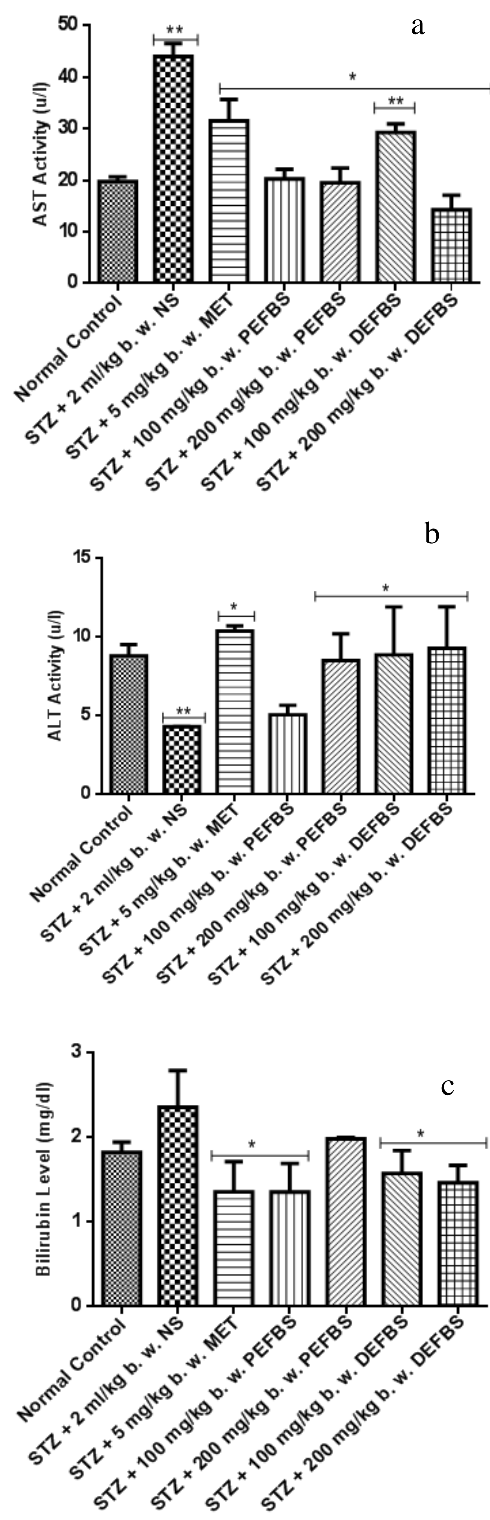

Fig 3a-c: Liver function parameters of streptozotocin induced diabetes rats treated with Fractions of B. sapida stem bark. ** (P<0.05) Significant compared with Normal control rats; * (P<0.05) significant

compared with streptozotocin (STZ) induced rats administered 2 ml/kg b. w. of normal saline. NS: Normal saline; MET: Metformin; PEFBS: Petroleum ether fraction of *B. sapida*; DEFBS: diethyl ether fraction of *B. sapida*.

Histopathology study

As shown in Figure 4 and 5, the control rats showed normal central venules without congestion (white arrow) , the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (black arrow), no pathological lesion was seen. Relative to control across groups; STZ induced rats treated with 5 mg/kg of metformin shows normal central venules without congestion, the morphology of the hepatocytes appear normal, the sinusoids appear normal and not infiltrated, no pathological lesion seen. STZ induced rats treated with 2ml/kg of normal saline showing portal vein with congestion and some periportal infiltration is of evidence, the morphology of the hepatocytes appear distorted with chromatolytic appearance, the sinusoids appear infiltrated with hemorrhagic red cells (red arrow). STZ induced rats treated with 100 mg/kg of petroleum ether fraction of *B. sapida*: showing portal vein with mild congestion and moderate periportal infiltration is observable, the morphology of the hepatocytes appear normal except for observable appreciable clustered regenerating cells, the sinusoids appear normal and not infiltrated. STZ induced rats treated with 200 mg/kg of petroleum ether fraction of *B. sapida*: showing central venules with localized congestion within the walls of portal vessels, the morphology of the hepatocytes appear normal, the sinusoids appear clear and not infiltrated. STZ induced rats treated with 100 mg/kg of diethyl ether fraction of *B. sapida*: showing mild area of haemorrhage and necrosis, the morphology of the hepatocytes appear normal, the sinusoids appear slightly infiltrated by inflammatory cells. STZ induced rats treated with 200 mg/kg b.w of diethyl ether fraction of *B. sapida*: showing normal central venules without congestion, the sinusoids appear clear and and infiltrated by inflammatory cells, the morphology of the hepatocytes appear normal with distinct layering. Areas with observable cytomorpholog ical alteration is indcated with red arrow.

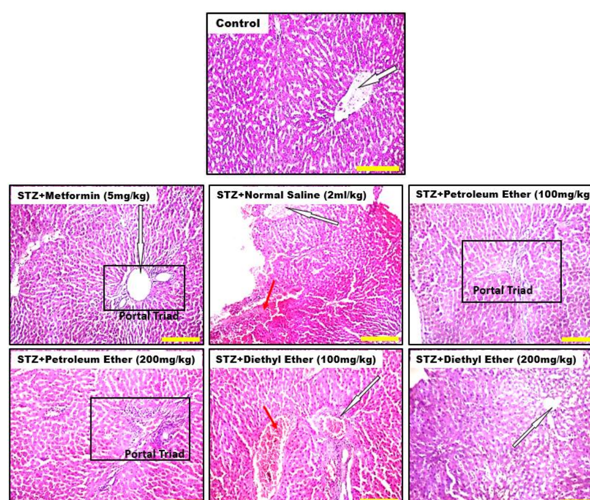

Figure 4: Photomicrographs of liver micromorphological sections stained by Hematoxylin and Eosin (scale bar: 100µm)

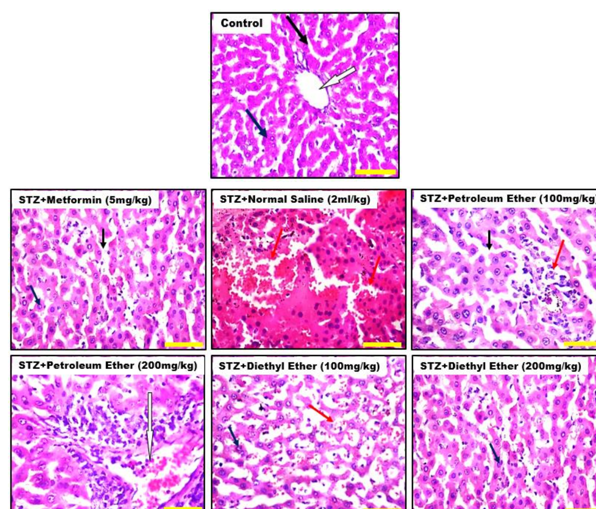

Figure 5: Photomicrographs of liver micromorphological sections stained by Hematoxylin and Eosin (scale bar: 200µm).

4. Conclusion

This study showed that STZ induced diabetes caused hepatocellular damage in rats, which was ameliorated by treatment with petroleum and diethyl ether fractions of *B. sapida* stem bark at 100 and 200 mg/kg body weight. However, treatment of diabetic rats with petroleum ether fraction of *B. sapida* at 100 and 200 mg/kg body weight elicited the highest level of liver protection. Therefore, further study should be carried out to isolate the phenolics compounds present in the

petroleum ether fraction for the purpose of development of anti-diabetes drug with less cytotoxicity.

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