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Hypoglycemic Effect Hydroacetone Extracts of *Treculia africana* Decne Root and Stem Bark in Alloxan-Induced Diabetic Rats

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ABSTRACT

The main objective of this study was to compare the hypoglycemic activity of ethylacetate soluble portion of aqueous acetone extract of the root bark (EtOAcfr TARB) and stem (EtOAcfr TASB) back of *Treculia africana* Decne in diabetic rats and also to characterize the ultraviolent and infrared spectra of an isolated constituent. Oral administration of the EtOAcfr TARB reduced fasting blood glucose level by 28.6% in 144hrs and 15.9% at 240 hrs in alloxan (100 mg/kg) induced rat of five groups (n=5), with the use of glucometer (one touch ultra-code 23) at predetermined intervals of time. Phytochemical screening showed little or no difference in the constituents of the stem and the root. This was also demonstrated in the TLC separation pattern of the extract. Indications from this study could suggest that *Treculia africana* could be a potential hypoglycemic herbal drug as both the stem and the root extracts showed a sustainable and better reduction of blood glucose level when compared with standard drug (glibenclamide, 0.5 mg/kg) at 144 hr. The activity appeared more in the root bark of the plant.

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Keywords*: Treculia africana,* Phytochemicals, Antidiabetic

INTRODUCTION

Diabetes mellitus (DM) is a global health problem and considered as a major factor of the immature morbidity and mortality worldwide (King *et al.*,1998). It is characterized by abnormal carbohydrate, lipid and protein metabolism resulting from insufficient action of insulin (WHO Expert Committee report, 1997). Type 2 DM is associated with the premature death, and disability such as nephropathy, neuropathy and microvascular or macrovascular disease (Alarcon *et al.*, 2003; Rameshkumar *et al*., 2004). The increase incidence of DM and the complicate effect on health, quality of life and life expectancy of its sufferers' worldwide has become a major health concern. Statistics showed that the global epidemic of DM is worse or greater in developing than the developed countries (Oputa, 2002). India, for example, is rated the leading country affected by DM epidemic with an estimate of 19 million diabetic subjects, while China and United States of America are rated second and third respectively (King *et al.,* 1998). About 1.9 million people ages 20 years or older were newly diagnosed with diabetes in 2010 in the United States (NIH, 2011) and 5.3 million in Africa, 6.6 million in former USSR, 12.6 million in Latin America,

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are also reported cases of DM (Greenfield and Chilshon, 2004). Different types of conventional diabetic drugs such as insulin, sulphonylurea, biguanides and thiazolidiones have been used in the management of type 1 and type 2 of this disease but their ability to manage the disease effectively is however, limited due to their pharmacokinetic characteristics. (Maggs *et al.,* 1998; Misbin *et al.,*1997). In addition, concerns of physician over the side effects observed with current medication in patients, has demanded for the need for novel approach to treatment (Oyelola *et al.,* 2007).

In response to this global health challenge, the WHO Expert Committee (1997) on diabetes mellitus recommended further evaluation of the folkloric method of managing the disease because of the high mortality and morbidity arising from its subjective complications and draw-backs associated with the use of the conventional antidiabetic drugs (Adeneye *et al.,*2006). In an attempt to pursue this goal, increasing number of medicinal plants is being investigated worldwide for their hypoglycemic efficacies (Anturtikar *etal.,*1995; Gao, 1989; Ogundipe *et al*., 2003). This can only be achieved by continuous screening of medicinal plants and adequate standardization of potential herbal remedies to ensure quality, safety, and efficacy.

Treculia africana Decne (Moraceae) commonly known as Africa breadfruit is among the several tropical African plants with ethnomedicinal and nutritive value, having the potential to provide important means or leads for a rational treatment and management of diabetes in tropical Africa countries and it is a plant mainly known for its food value. Several works have been done on the nutritional value of the plant. The antibacterial effect of the stem bark extract on the gastrointestinal (GIT) pathogens has been reported by Ogbonnia *et al.,* (2008). From a survey carried out among herbalists and number of patients in the University College Hospital Ibadan, the plant is reported to be an important component of one of the ancient anti-diabetic recipe used in the Western and Middle Belt of Nigeria (Oyelola *et al.,*2007). Evaluations of the nutritional values of the pod have been carried out (Edet *et al.,* 1985; Akubor and Badifu, 2004).

Previous report have revealed that the levels of blood glucose, triglycerides and LDL-cholesterol were lowered significantly after oral administration of aqueous root extract of *T. africana* in glucose load condition and in streptozotocin (STZ),-induced rabbits (Ojieh *et al.,*2009).

Evaluation of the effect of aqueous root extract of *T. africana* on the rate of plasma haemoglobin glycosylation in rabbits with STZ-induced diabetes and correlation with plasma lipid peroxidaion level has been carried out. (Ojieh *et al.,*2009). A member of the family Moraceae has been reported to yield glycosides with hypoglycemic activity e.g *Ficus bengalensis*. Dimethoxy derivatives of leucocyanidin 3-O-B-D, galactosyl callobioside and dimethoxy derivative of pelargonidin 3-O-A-L rhamnoside have demonstrated antidiabetic activity (Gosh *et al.,* 2004). Diethyl ether fraction and water soluble fraction of hydroacetone crude extract of the root bark of *T. africana* have been reported to have hypoglycemic activity (Oyelola *et al.,* 2007). There is no publication on the active compound that probably responsible for the hypoglycemic activity. However, phyllocoumarin , catechin and 6, 9-dihydroxy-megastigmane-3-one are the isolated compounds from *T. africana* reported to have been tested for their antimicrobial activity on gram positive and gram negative bacteria (Kuete *et al*, 2007). The focus of this study therefore; was to evaluate the comparative activity of the ethylacetate soluble extracts of *Treculia africana* stem bark and *Treculia africana* root bark (EtOAcTASB and EtacTARB) obtained from the partitioned hydroacetone crude extract in alloxan-induced diabetic rat

MATERIALS AND METHODS

Treculia africana root bark (TARB) and stem bark (TASB) were collected in July 2008 from Forestry Research Institute of Nigeria Ibadan (FRIN), and was authenticated at the Forestry Herbarium Ibadan. A voucher specimen (FHI No107359) is deposited at FRIN, Ibadan.

Plant Extraction

A portion of dried powder of *Treculia africana* stem bark (TASB) (1500 g) and *Treculia africana* root bark (TARB) (900 g) were macerated separately in 7.5 L and 4.5 L of extraction solvent 80% Acetone in a 8 L water tank and was allowed to stand for 96 hrs before it was decanted, filtered and yielded 3500 ml (3.79%) of TASB and 2000 ml (1.39%) of TARB respectively. Each of them was concentrated to a low volume of 500 ml to give the percentage yield. The concentrated aqueous acetone extracts were separately partitioned with ethyl acetate and the ethyl acetate soluble fraction as well as the aqueous fractions were evaporated to dryness to give a percentage yield of 2.65% of TASB 1.43% of TARB and then stored at 4^0 C.

Experimental animals

Twenty five wistar albino rats weighing between 130-200 g were obtained from the central animal house,

College of medicine, University of Ibadan and used for the study. They were grouped into 5 (five) consisting of 5 animals per cage, maintained at room temperature, 50% relative humidity, allowed free access to water and commercially produced diet (Ladokun Feed Ltd.) *ad libitum.* The animals were treated according to the international guidelines for the care of and use of laboratory animals.

Experimental design / Estimation of Blood glucose

The fasting blood glucose levels of all the animals were measured before inducement of hyperglycemia with alloxan. Group II- IV were made diabetic by administering a single injection of alloxan monohydrate at a dose of 100 mg/kg intravenously. The diabetic state of the animals was confirmed 5 days after administration of alloxan. The basal fasting blood glucose level of the diabetic groups was determined after which test extracts were administered to the diabetic treated group.

Group I Normal control rat (Normal saline)
Group II EtOAcTARB extracts (10 mg/kg
Group III Glibenclamide (Standard drug 5 mg/kg)
Group IV EtOAcTASB extracts (10 mg/kg)
Group V Diabetic untreated rat

Fasting blood glucose was monitored for 4 hrs (interval of 2 hrs) after the initial administration of the extract and standard drug using a glucometer (One Touch Ultra) code "23". Subsequent administration of the extracts was carried out daily for the next 6 days after the fasting blood sugar level have been recorded. Administration of the extracts was stopped on the 7th day after which the animals were observed for additional 3 days. The last fasting blood sugar level was recorded on the 10th day of the experiment. The response of the animals to the various treatments was then compared.

Determination of phytochemical constituent

The phytochemical screening of the secondary metabolites including the total alkaloids, tannin, saponin and flavonoids was evaluated using standard procedures (Trease and Evans, 1996).

Statistical analysis

Results were expressed as mean +/- SEM of five determinations. The significance of the differences between the tested group and the control animal groups were established by student's t- test and value lower than 0.05 were considered to be significant.

RESULTS

Single administration (single dose) of aqueous extracts of EtacTARB (10 mg/kg p.o) in diabetic rat showed reduction in FBG level after 4 hrs and 24 hrs from 123.2± 16 - 92.6± 4.2 (Table 1). Glibenclamide (0.5 mg/kg p.o) showed maximum reduction 35.9% and 29.9% after 4 hrs and 24 hrs respectively. On the subsequent administration (subacute treatment), either of the gibenclamide or the tested extracts showed activity after 144 hrs when compared with the diabetic untreated group. Only the EtacTARB extract showed a significant sustainable reduction in FBG level after the drug administration is stopped on the seventh (7th) day, at both 144 hrs and 240 hrs ($P<0.05$), 28.6% and 15.9% at 144 hrs and 240 hrs respectively (Fig 1). In this study, there is similarity in the Thin Layer Chromatography (TLC) pattern of both the TASB and TARB.

Table1:
Effect of extracts on fasting blood glucose level of diabetic rat.

	Basal level	2hrs	4hrs	24hrs	72hrs	144hrs	240hrs
Group I Normal control rat	107.6 ±4.9	120.4 ±4.8	108.2 ±6.3	93.6 ±6.4	112.6 ±9.5	102.6 ±3.8	115 ±6.0
Group II EtacRB Frc (10 mg/kg)	134.6 ± 17	*134.2 ±11.2	123.2 ± 16	92.6 ±4.2	124 ± 13	*94.2 ± 5.1	*101.6 ± 3.7
Group III Standard drug (5mg/kg) (Glibenclamide)	165.2 ± 29.2	*103.4 ± 9.3	*97.4 ± 19	*69.6 ± 8	113.2 ± 13	105 ± 13	189 ±43
Group IV EtacSB Frc (10 mg/kg)	146.2 ± 9.6	221.4 ±36.7	230.6 ± 47.1	99 ±14	122 ±5.3	*102 ±10.1	124 ±11.9
Group V Diabetic untreated rat	164.2 ±35.3	160.75 ±34.2	152 ±39.9	99.25 ±14.3	110.5 ±14.3	132 ±13.2	120.75 ±7.6

*Values are Mean± S.E.M, * Shows significant difference in sugar level of treated rats (p<0.05).*

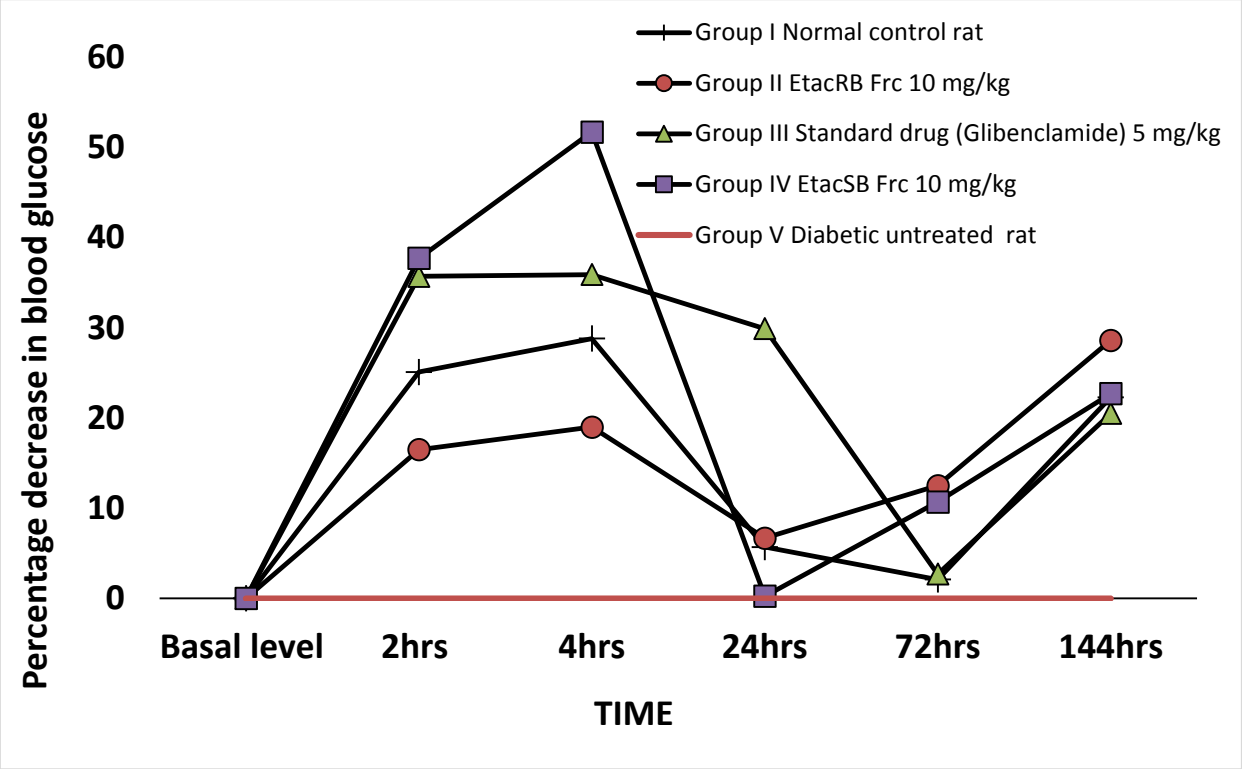

Fig. 1:
Percentage (%) decrease in fasting blood sugar level of rats treated with extracts.

Table 2
Effect of fractions obtained from column chromatography of EtOAcfrTARB on fasting blood glucose (FBG) level of diabetic rats.

	0hr	2 hr	4 hr	6 hr	24 hr	72 hr	144hr	240hr
Normal control	101.2 ±7.96	102.6 ±7.4	108.6 ±5.9	110.2 ±6.3	115.2 ±4.7	109 ±6.9	116.2 ±7.4	110.6 ±1.6
Diabetic control	234.6 ±33.1	327.6 ±34.4	384.8 ±36.4	348.8 ±47.1	415.8 ±45	493 ±43.9	486 ±39.6	360 ±33
F1	236.2 ±36.3	305.6 ±42.5	297.8 ± 41	313.6 ±42.4	252.8 ±58.7	*234.8 ±51.5	*235.3 ±55	*239 ±57
F2	148.3 ±4.8	*176.3 ±12.9	*152.3 ±17.5	160.8 ±4.8	169 ±21.3	156.3 ±11.9	*144.8 ±9.8	*140.8 ±17.2
F5	231.4 ±37.4	295.2 ±53.2	393.2 ±58.5	366.2 ±43.4	330.2 ±45.4	357.2 ±42.2	*227.8 ±35.2	*196.6 ±29.3
F6	390.3 ±70.6	464.3 ±40.7	552.5 ±23.5	557 ±19	498.8 ±27.8	412.5 ±55.8	*150.3 ±18	*130 ±14.8
Glibenclamide	435.2 ±56.8	428.4 ±42.7	538.8 ±33.3	558.8 ±15.4	531.8 ±22.9	408 ±57.3	*399 ±45.7	*280.5 ±81.7

± *Shows S.E.M. Values, * Shows significant difference in sugar level of treated rats ($p<0.05$).*
F(n) Fractions obtained from column chromatography.

The result in table 2 shows the hypoglycemic effect of the various fractions obtained from column chromatography of EtOAcTARB (5 mg/kg p.o) compared with glibenclamide (0.5 mg/kg p.o) and control group given distilled water (dw) (5 ml/kg p.o) following the same method of administration described above.

Nearly all the groups of alloxan induced rats showed severe hyperglycemia at single dose (IP) of 100 mg/kg except the group treated with fraction 2 (F_2) for which the reason cannot yet be explained.

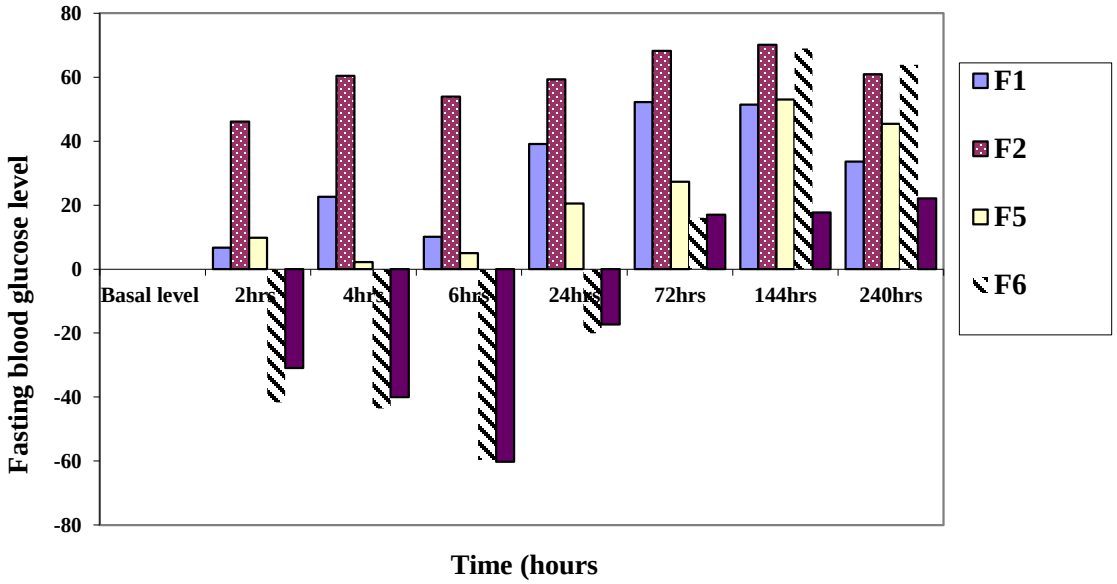

Fig 2
Percentage decrease in fasting blood sugar of rats treated with column chromatographic fractions

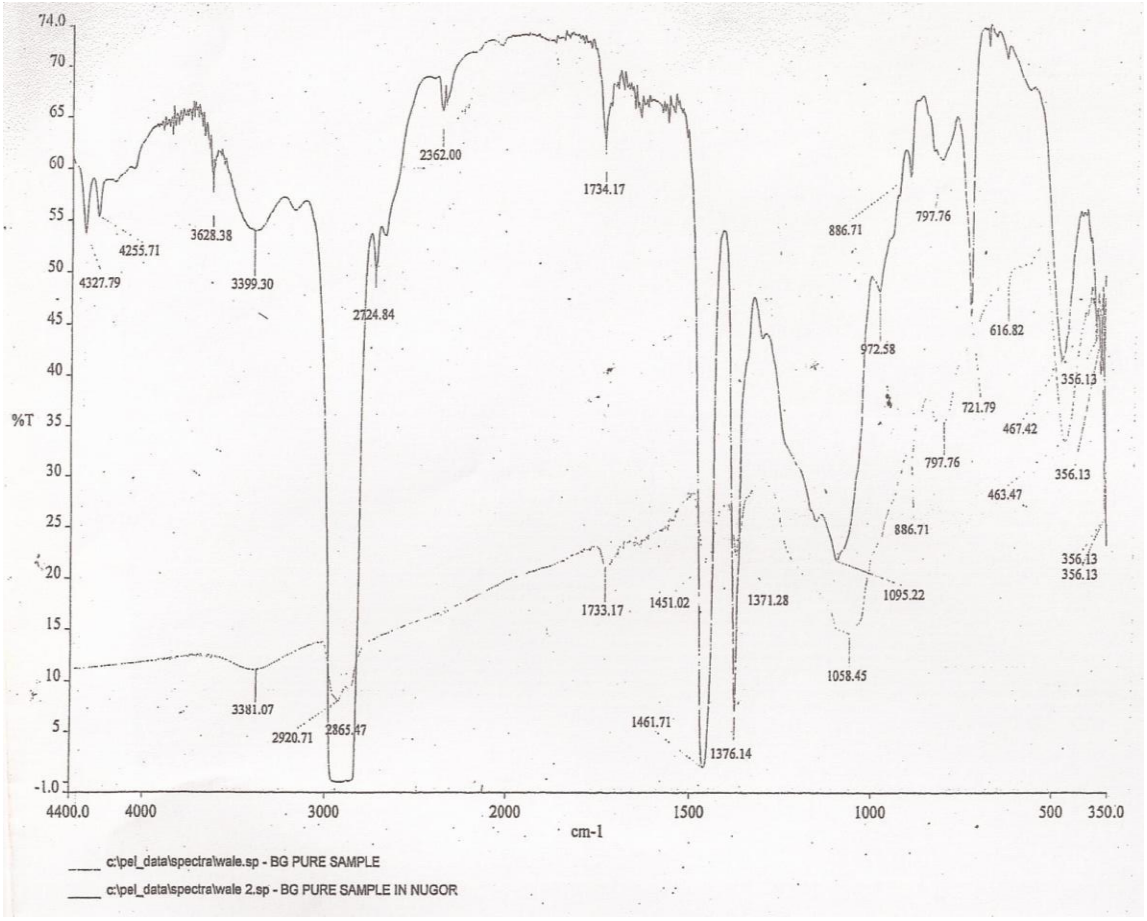

Plate 1: Infrared spectrum of the pure sample and the pure sample Nujol

Single administration of the glibenclamide exerted hypoglycemic effect 2 hrs after the administration. At 6 hrs, animals in group V and VI shows a notable severe hyperglycemia in FBG diabetic rat having the peak values 559 ± 19.0 and 558 ± 15.4 respectively. So, there is no definite percentage decrease in the fasting blood glucose from 4 hrs to 24 hrs.

Subacute treatments of (0.5 mg/kg p.o) glibenclamide, 5 mg/kg p.o of F_5 and F_6 for seven consecutive days significantly ($P<0.05$) lowered the FBG level. The effect of this could be due to different types of bioactive principles of which possess effective hypoglycemic activity in a diabetic animal

Infrared spectrum of the sample showed a kind of overlap in its bands with that of Nujol as shown in Plate 1. Absorption Infrared (IR) spectra showed C-H stretching and C-H bending at 2865.47 cm^{-1} 1371.28 cm^{-1} respectively. There are two notable peaks of C-H bending which probably indicates the spectrum of methyl ($-CH_3$) groups at different peaks of 1451.02cm^{-1} and 1371.28cm^{-1}. Also, the band around 3381cm^{-1} possibly shows a characteristic -OH stretchings. Carbonyl (C=O) stretching of either ketone or aldehyde group at the peak value of 1733.17cm^{-1} was also found present in the IR spectra.

DISCUSSION

The result of the study showed that both the root and stem bark of *T. africana* have potential for anti hyperglyceamia but that of root bark shows a better sustainable effect.

In-vivo models (alloxan or sreptozotocin- induced diabetic rats) are used in evaluating medicinal plants with suspected hypoglycemic potentials. In this study, DM was induced using intraperitoneal injection (IP) of alloxan at a single dose of 80 mg/kg/bw. Significant hyperglycemic effect of this dose was not actually seen after observing the rats for over ten (10) days post-induction. Another inducement was done at the same dosage and hyperglycemia was actually established five (5) days post-induction. However, an unexpected reduction in the fasting blood glucose (FBG) of the diabetic untreated group at 24 hrs from 152 ± 39.9 – 99.25 ± 14.3 (mg/dil) was observed before it started rising again at the 72 hrs (3rd day). The reason for this unusual reduction cannot yet be explained. Literature shows that alloxan induces DM by selectively destroying the pancreatic β cell, which are involved in the synthesis, storage and release of insulin, the peptide hormone regulating carbohydrate, protein and lipid metabolism (Malaise, 1982; Sharfiir, 1997).

Animals in group IV showed increase in FBG within the first 4hrs from the basal values. This could probably be that there is variation in the hormonal susceptibility and resistance against the inducement of alloxan among rats of different sex. Considering the long-term significant reduction ($P<0.05$) of the TARB in FBG at both the 144 hrs and 240 hrs, it could be inferred that the root bark extract may possess composite active ingredients that can exert a long-term pharmacokinetic effect even if the drug administration is stopped after short period of acute or sub-acute crisis treatment. The anti- hyperglycemic mechanism might be due to increase in peripheral glucose uptake in an alloxan- induced diabetic rats, or that the metabolism of the drug is slow in the body therefore, give it a room to have a prolonged activity in the blood stream.

The similarity in the TLC pattern of both TASB and TARB could probably suggest the relativity in the hypoglycemic effect of both the TASB and TARB. A pure compound has been isolated from fraction 1 (F1), usingpreparative TLC. Partial structural analysis was d one to identification and characterization. The characteristic progression in the electronic spectra ranging between the wavelength of 215nm and 237nm as shown in the Plate 1, indicates the possibility of the presence of conjugated dienes and trienes associated with some functional groups which could be alkane alkene and alkyl group (C=C-C=C, C=C-C=O).

Considering the effects of TARB on the rate of plasma haemoglobin glycosylation, triglycerides and LDL-cholesterol by Ojieh *et al.*,(2009), and being in consonance with that reported for the root bark by Oyelola *et al*., (2007), the observed hypoglycemic effect of the TARB in this study is an indication that TARB really contains active principle with potent hypoglyceamic property which could justify the ancestral use in the management of suspected type ii diabetic patients. This result has lead to further work of column chromatography of the EtacTARB extract and isolation of active principle(s), which would constitute areas of future research.

Diabetes mellitus is a well known clinical disease with various late complications like retinopathy, neuropathy and nephropathy. The results generated in this study show that hydroacetone root extract of *Treculia africana* possesses hypoglyceamic principles (thus inhibiting the attendant haemoglobin glycosylation resulting from the formation of lipid peroxides in diabetes that was proved by Ojieh *et al.,* 2009), and in consonance with the hypoglycemic activity of the diethyl ether fraction and water soluble fraction of hydroacetone crude extract of the root bark of *T. africana* (Oyelola *et al.,* 2007). This could serve as an effective adjunct in the management of diabetes mellitus. Although there is not enough evidence to identify specifically the type of compound which is absorbed in the various wavelength – absorbance graph

from the IR and absorption spectra from the UV, one could only suspect the presence of functional groups in the compound. Further work on the Nuclear Magnetic Resonance (NMR) of the isolated compound would be able to reveal more information about the nature of the compound.

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