

Pharmacognostic Evaluation and Anti-Diabetic Activity of Ethanol Extract of *Triumfetta cordifolia* A. Rich (Tiliaceae) Leaves

ABSTRACT

Background: The therapeutic effect of bioactive compounds from plant are limitless hence, the use of medicinal plants in treating diseases such as diabetes cannot be over emphasized. The study was aimed at evaluating some pharmacognostic parameters and investigates the antidiabetic activity of ethanol extract of *Triumfetta cordifolia*

Methods: The pharmacognostical profiling of *Triumfetta cordifolia* leaves was carried out using some standard pharmacognostic tools for crude drug standardization such as qualitative and quantitative microscopy, analytical evaluation and phytochemical screening. The plant material was extracted using cold maceration method in ethanol and fractionation was carried out using n-hexane, ethylacetate and butanol. The acute toxicity study was done following standard method. 120 mg/kg body weight of alloxan monohydrate was used to induce diabetes in albino wistar rats intraperitoneally. The diabetic rats were treated with 200 and 400 mg/kg of the ethanol crude extract and 400mg/kg of each of the fractions (N-hexane, ethylacetate and butanol). Glibenclamide was used as the standard drug (5mg/kg) and diabetic rats without treatment as negative control. The procedure was also similarly performed using the non-diabetic rats. The administration of all treatments was done orally, once daily for 21 days and at the end of the treatment period, the blood sera of the blood samples from rats across the groups were collected and biochemical assays were carried out on them to evaluate the AST, ALT and ALP concentrations.

Results: The results of the qualitative microscopical evaluation of *Triumfetta cordifolia* leaf revealed anisocytic stomata, unicellular trichomes, wavy wall epidermal cells and prismatic calcium oxalate. Quantitative microscopical study gave 23.67 ± 0.58 stomata number, 0.023 ± 0.00058 stomata index, 18.33 ± 1.53 Palisade ratio and 10.67 ± 0.58 vein-islet number while the analytical standard revealed 9.3 total ash, 3.5 water soluble ash and 1.34 acid insoluble ash. The phytochemical analysis revealed the presence of alkaloids, glycosides, Tannins, flavanoids steroids and terpenoids in the *Triumfetta cordifolia* leaves ethanol extract (TCEE). A significant reduction ($P \leq 0.05$) in fasted blood sugar level of diabetic rats was observed during treatment with *Triumfetta cordifolia* leaves extract and the blood sugar level lowering potential was comparable to the standard drug (glibenclamide). There was improvement of body weight in TCEE treated groups and ethylacetate fraction group. The Leaf extract of *Triumfetta cordifolia* showed a high significant ($P \leq 0.05$) ameliorating potential on liver's degenerating hepatocytes evidenced by the reduced AST, ALT and ALP levels when compared with the diabetic group.

Conclusion: The overall results showed that *Triumfetta cordifolia* leaf possesses blood sugar lowering and liver hepatocytes regenerating potentials while the pharmacognostical profiling of the plant can serve as a reference and guide for future researchers.

Key words: *Triumfetta cordifolia*, Diabetes, Glibenclamide, Ash value, Stomata, Trichome, Microscopy

INTRODUCTION

Diabetes is a disease that is estimated to affect about 2-3% of the world population and is commonly referred to as diabetes mellitus. It is a clinical syndrome with global prevalence and increasing incidence all over the world. It is one of the major health challenges that occur in all age groups and it has severe effect on most organs of the body resulting to serious complications. Diabetes is characterized by abnormal hyperglycemia (WHO, 1980; WHO, 1985) and is caused by inherited and/or acquired deficiency in production of insulin by the pancreatic islet, or by the ineffectiveness of the insulin produced (Neelesh, 2010). The endocrine hormone, insulin operates

at various sites throughout the body regulating majorly carbohydrate metabolism by controlling entry of glucose into the blood stream. Chronic hyperglycemia damages some vital organs such as the eye, heart and kidney (Mayfield, 1998).

The liver is an important organ that is involve in glucose and lipid homeostasis and its tissues are insulin dependent (Neelesh *et al.*, 2010). Diabetic patients suffers from severely affected liver which in turn affect the uptake and metabolism of free fatty acids as well as the biosynthesis of cholesterol, triglycerides and phospholipids (Neelesh *et al.*, 2010). The abnormal functionality of a diabetic patient's liver results in increased lipid peroxidation due to hyperlipidemia (Neelesh *et al.*, 2010).

The primary health care needs of about 80% of African population are dependent on the use of herbal remedies (Ladele & Bisi-Amosun, 2014) and the variety of climate and vegetations made African herbal medicine form an important part of her communal culture (Erinoso & Aworinde, 2018). In most developing countries of the World most especially Sahel region of West Africa, traditional herbal medicine is often used side by side orthodox medicine with herbal medicine having more preference most especially when the cost of western medicine is beyond reach of poor people (Busia, 2005; Faye *et al.*, 2010). The reason for this recent trend is not farfetched from the enormous bioactive compounds that are embedded in different plant species. Herbal medicine is the last resort for common man due to its availability, accessibility and potency in the treatment of diseases such as diabetes.

Anti-diabetic agents help to correct the anomaly in blood sugar level in diabetic patient. Conventional antidiabetic medicines such as injectable insulins, sulfonylureas, biguanides, glucosidase inhibitors and glinides etc. have been used in managing the major symptom of diabetes but the undesirable side effects of these antidiabetic agents which may be as a result of their degradation products has led to the search for alternative therapy (Satyanarayana, 2006)

Herbal medicine has always proven to be a reliable alternative to orthodox medicine generally in disease control and therapeutics. Despite the relentless efforts by researchers from different part of the world toward drug development as well as discovery of novel compounds that are bioactive in regulating the sugar level in the blood stream of diabetic patient, no patient of diabetes disease had been reportedly recovered totally from it (Li *et al.*, 2004). Hence, successful treatment of diabetes still remains a major challenge to scientific researchers and the world population at large. Huge numbers of plants have been recorded in different official monographs to possess marked blood sugar lowering potentials in diabetic patients. It is therefore pertinent to say that the total remedy to this clinical syndrome called diabetes lies in herbal medicine.

Triumfetta cordifolia is a fast growing shrub that is commonly found in Central and Southern America and in moist area of Tropical Africa where most local communities know it for its versatile medicinal properties or use it as food (Brink and Achigan-Dako, 2012). In Nigeria, It is commonly found in the Southern region such as Akwa Ibom, Bayelsa (Brink and Achigan-Dako,

2012). It belongs to the Tiliaceae family and is commonly called cord-leaf-burbark and Burweed.

The leaves of *Triumfetta cordifolia* are used as psychotropic (Borokini *et al.*, 2012) and sap of the leafy twigs is used to treat digestive disorders, dysentery, diarrhoeal, diabetes, rhinitis, mental disorders, fever etc. (Orodeh *et al.*, 2019). The leaves are also used in the treatment of amenorrhoea and extract from macerated fruit in water or alcohol is used to treat delayed labour (Odewo *et al.*, 2018; Orodeh *et al.*, 2019). Nwafor *et al.* (2011) reported that *Triumfetta cordifolia* fruit is locally used to treat gastrointestinal disorders and ulcers. The stem of *Triumfetta cordifolia* is reported to have anti-hyperlipidemic property and induces weight loss (Brink and Achigan-Dako, 2012).

METHODOLOGY

Collection and preparation of plant sample

The sample of *Triumfetta cordifolia* leaves was collected from Nsukka, Enugu State, Nigeria in March, 2021 and authenticated by a taxonomist Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD). A voucher specimen (esut/cog/202203) was preserved in the herbarium of the Department of Pharmacognosy, Enugu State University of Science and Technology, Enugu, Nigeria. The leaves were dried at room temperature for 10 days and ground into coarse powder form. The powdered leaves sample was stored in an airtight container ready for extraction.

Pharmacognostic Evaluation of plant sample

Pharmacognostic features of the *Triumfetta cordifolia* leaf were examined using standard methods described by Obi *et al.* (2021), Evans (2009) and Inya-Agha (2006).

Extraction and Fractionation

A 2 kg of the powdered sample was extracted using ethanol of analytical standard and 100g of the extract was subjected to column chromatography using n-hexane, ethylacetate and butanol respectively. The individual fractions were concentrated at a maximum temperature of about 40°C using a rotary evaporator.

Chemicals

All chemicals and drugs used in this study were obtained commercially and are of analytical grade. Alloxan monohydrate (Sigma Chemical Company, St. Louis, USA), glibenclamide (Aventis Pharma Ltd, Goa, India).

Phytochemical screening

Phytochemical screening of the plant extracts was done using standard procedures as outlined by Evans (2009) and Harborne (1998) for the presence of secondary metabolites such as; alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, etc.

Preparation of experimental animal

Adult albino Wistar rats weighing between 78 to 129g and mice weighing between 25 to 38g of both sexes were procured and housed from/in the Department of Pharmacology standard animal house, Enugu State University of Science and Technology. The animals were fed and allowed to acclimatize for 4 days before the experimental procedures were carried out. The animals were fed with standard rat pellet diet and the experimental protocols were in accordance with the Enugu State University of Science and Technology's Ethics committee guideline (approved ref. no.: ESUT/021/009). The care and handling of animals was in line with the internationally accepted principles for laboratory animal use and care.

Acute toxicity study (LD₅₀)

Acute toxicity test of the plant extract in rat was determined using standard method as described by Lorke (1983) with slight modification by Patrick *et al.* (2022).

Oral glucose tolerance test in normal mice

The effect of ethanol extract on blood glucose level was also evaluated using oral glucose challenge. Total of twenty (20) mice was used for this study. They were grouped into four groups of five mice per group. Prior to the test, the mice were fasted overnight and basal fasting blood glucose determined. The mice were treated with different concentrations of the ethanol extract 30 minutes before the administration of 2 g/kg glucose solution in sterile water orally. Group 1 received 10ml/kg distilled water and served as negative control, group 2 and 3 received 200 and 400mg/kg crude extract respectively while group 4 received 5mg/kg glibenclamide and served as positive control. At zero minute, 30, 60, 120 and 180 minutes after the administration of glucose, blood samples were collected by tail vein and the glucose concentrations were determined using glucometer (Ibrahim *et al.*, 2014).

Induction of diabetes in rat and mice

Diabetes was induced using standard methods as described by Odoh *et al.* (2014) and Patrick *et al.* (2022).

Antidiabetic study in normal and diabetic rats

The diabetic rats were divided into eight groups of five rats each with group 1 and 2 receiving 200 and 400mg/kg of the plant extracts respectively. Group 3, 4 and 5 received 400mg/kg each of n-hexane, ethylacetate and butanol fractions respectively while group 6 was diabetic rats group that served as negative control. Group 7 received 5mg/kg glibenclamide (standard anti diabetic) and group 8 was normal non-diabetic rats group. All the treatments were done once daily for 21 days and blood samples were drawn from rat's tail vein and the fasted blood glucose levels were determined at three days interval after overnight fasting of rats.

Collection of blood samples and evaluation of biochemical parameters

Blood samples were drawn from overnight fasted albino wistar rats on day 21 of the study period through orbital puncture of retro-orbital plexus vein using standard method and parameters such

alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT) were determined from the blood serum using standard methods as described by Patrick *et al.*(2022)

Statistical analysis

Results gathered from the study was presented as mean \pm deviation, while the raw data were analyzed statistically using one way analyses of variance (ANOVA).

RESULTS

Figure 1: Effect of ethanol extract of *Triumfetta cordifolia* leaves and Standard (Glibenclamide) on blood glucose level in Oral Glucose Tolerance Test (OGTT) in normal mice. Bars represent Mean \pm SEM

Figure 2: Effect of ethanol extract and fractions of *Triumfetta cordifolia* leaves and Standard (Glibenclamide) on fasted blood sugar levels of diabetic rats for 3 weeks. EETC = Ethanol extract of *Triumfetta cordifolia*; HFTC = N-hexane fraction of *Triumfetta cordifolia*; BFTC = Butanol Fraction of *Triumfetta cordifolia*

Figure 3: Effect of ethanol extract and fractions of *Triumfetta cordifolia* leaves and Standard (Glibenclamide) on body weight of rats for 3 weeks. Bars represent Mean \pm SEM

Figure 4: Effect of daily oral doses of *Triumfetta cordifolia* leaves extract on serum AST level (mg/dl) of alloxan-induced diabetic wistar rats. and normal control. Bars represent mean \pm SEM

Figure 5: Effect of daily oral doses of *Triumfetta cordifolia* leaves extract on serum ALT level (mg/dl) of alloxan-induced diabetic wistar rats. and normal control. Bars represent mean \pm SEM

Figure 6: Effect of daily oral doses of *Triumfetta cordifolia* leaves extract on serum ALP level (mg/dl) of alloxan-induced diabetic wistar rats and normal control. Bars represent mean \pm SEM

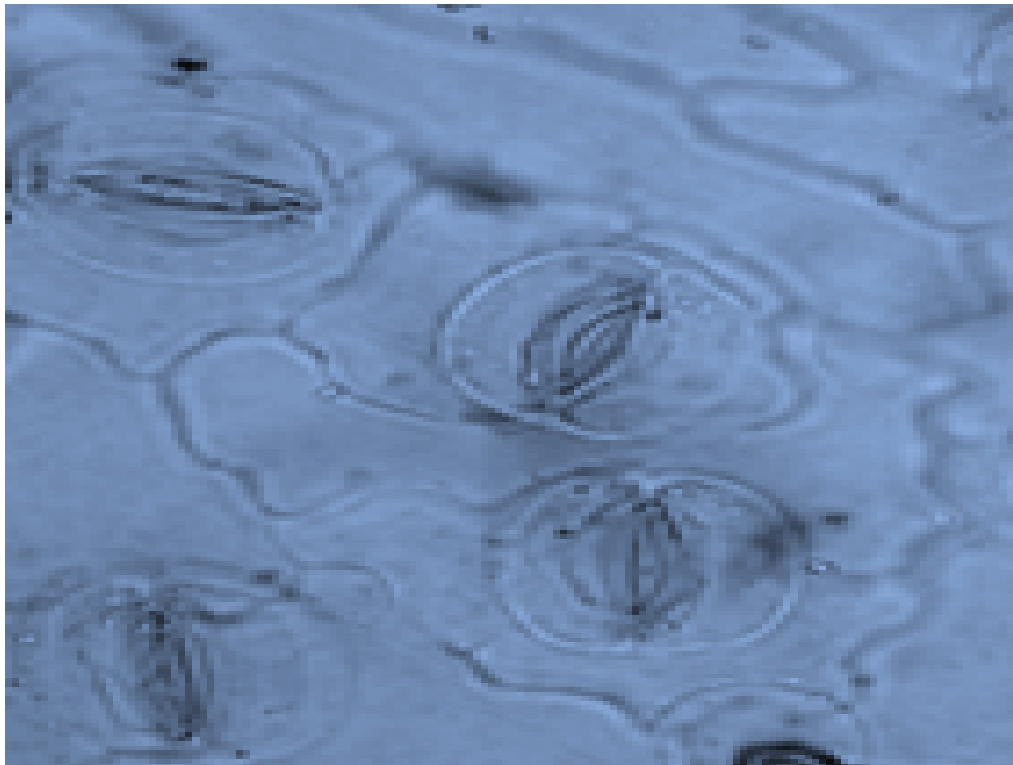


Plate 1: Photomicrograph of the lower epidermis of *Triumfetta cordifolia* leaf showing Anisocytic stomata and wavy wall epidermal cells (x 400)

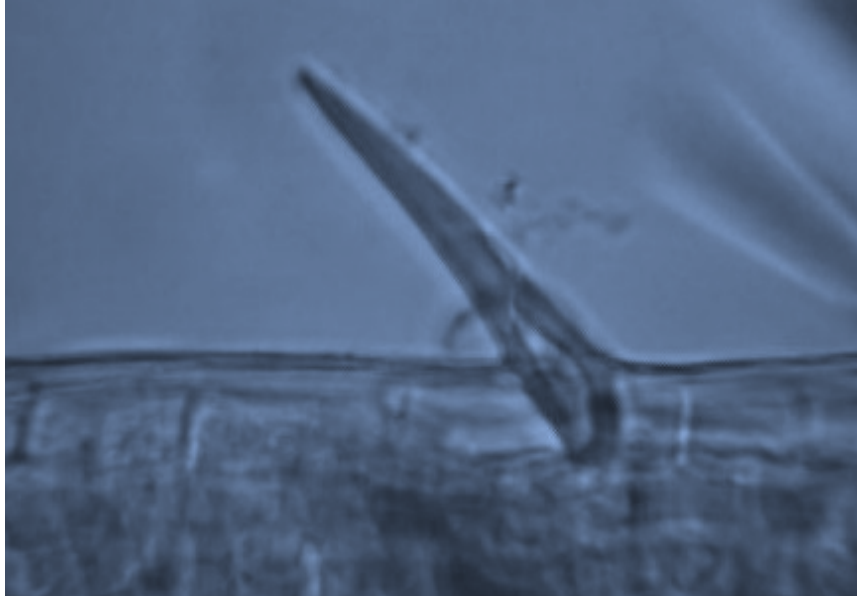


Plate 2: Photomicrograph of *Triumfetta cordifolia* leaf showing non-glandular Unicellular trichome (x 400)

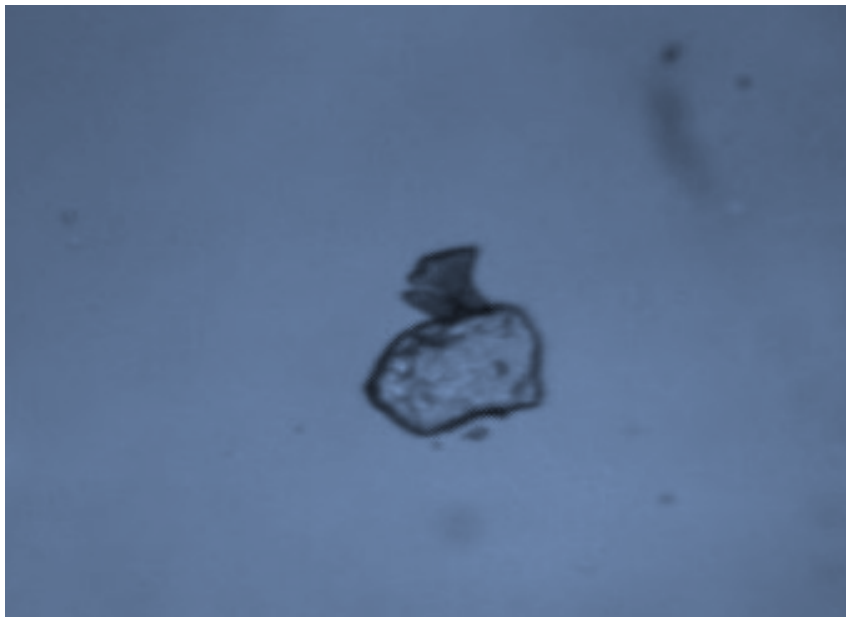


Plate 3: Photomicrograph showing calcium oxalate crystal from *Triumfetta cordifolia* leaf (x 400)

Table 1: Results of qualitative microscopy of *Triumfetta cordifolia* leaf

S/n	PARAMETERS	OBSERVATION	TYPES / SHAPES
1.	Stomata	Present	Anisocytic
2.	Calcium oxalate	Present	Prismatic
3.	Trichome	Present	Unicellular /non glandular
4.	Epidermal cells	Present	Wavy wall

Table 2: Results of quantitative microscopy of *Triumfetta cordifolia* leaf

S/N	PARAMETERS	Composition (mm ²)
1.	Stomata number (Lower epidermis)	23.67 ± 0.58
2.	Stomata index	0.023 ± 0.00058
3.	Palisade ratio	18.33 ± 1.53
4.	Vein islet number	10.67 ± 0.58

*values are mean ± Standard deviation of three replicate analyses

Table 3: Results of Pharmacognotic analytical standard of *Triumfetta cordifolia* leaf

S/N	PARAMETERS	VALUES (%)
1.	Total Ash (inorganic)	9.3 ± 0.014
2.	Water soluble Ash	3.5 ± 0.0082
3.	Acid insoluble Ash	1.34 ± 0.0082
4.	Moisture content	9.50 ± 0.015
5.	Water extractive	22.03 ± 0.096
6.	Alcohol extractive (Ethanol)	17.59 ± 0.0096
7.	N-hexane extractive	2.8 ± 0.013
8.	Percentage yield	15.97

*values are mean ± Standard deviation of four replicate analyses

Table 4: Results of Phytochemical screening of *Triumfetta cordifolia* leaf

S/N	CONSTITUENTS	Ethaol crude extract of <i>Triumfetta cordifolia</i> leaf extract	N-hexane Fraction of <i>Triumfetta cordifolia</i> leaf extract	Ethylacetate Fraction of <i>Triumfetta cordifolia</i> leaf extract	Butanol Fraction of <i>Triumfetta cordifolia</i> leaf extract
1.	Alkaloids	+	-	-	+
2.	Glycosides	+	-	+	+
3.	Tannins	+	-	+	+
4.	Flavonoids	+	-	+	+
5.	Steroids	+	+	-	-
6.	Terpenoids	+	+	-	-

+ = Present - = Absent

Table 5: Effects of Ethanol extract of *Triumfetta cordifolia* leaves on some biochemical parameters of albino wistar rats (blood sera) from the various groups after 21 days of treatment

GROUP	AST (iu/L)	ALT (iu/L)	ALP (iu/L)
Group 1: Alloxan + 200mg/kg ethanol extract	41.88 ± 1.28	24.01 ± 1.45	43.75 ± 3.18
Group 2: Alloxan + 400mg/kg ethanol extract	43.16 ± 3.85	18.66 ± 1.10	45.25 ± 1.38
Group 3: N-Hexane fraction (400mg/ml)	69.67 ± 2.11	50.99 ± 1.97	51.54 ± 2.79
Group 4: Ethylacetate Fraction (400mg/ml)	45.70 ± 2.16	19.41 ± 1.54	39.01 ± 1.93
Group 5: Butanol Fraction (400mg/ml)	45.45 ± 1.57	44.98 ± 1.57	50.42 ± 0.57
Group 6: Glibenclamide	40.99 ± 2.74	31.98 ± 1.54	46.67 ± 2.17
Group 7: Diabetic control	79.61 ± 0.78	59.90 ± 1.5	82.5 ± 0.68
Group 7: Normal control	30.76 ± 1.41	16.97 ± 1.46	27.70 ± 1.64

Values represented in Mean±Standard deviation of five observations. P≤0.05 (ANOVA) AST; ALT; ALP.

DISCUSSION

The results of Pharmacognostic analytical standards as shown in Table 3 revealed that the powdered sample of *Triumfetta cordifolia* leaf was exhaustively extracted with percentage yield of 13.16 % which suggest that the solvent can extract reasonable percentage of the total secondary metabolites in the plant sample. The total ash value of 9.3% revealed the total plant derived inorganic salts and mineral matters majorly phosphate, carbonates and silicates or contaminants such as silicious earth materials that likely adhere to the surface of the plant (Inya-Agha, 2006). The low value of acid insoluble ash (1.34%) clearly showed that the sample material was properly handled because acid insoluble ash revealed the exact amount of silicious contaminants majorly as a result of improper handling of crude drugs while the water soluble ash value (3.5%) reveal the presence of adulterants from other plants that bear close resemblance to the authentic plant sample (Inya-Agha, 2006). The value of water, n-hexane and alcohol extractives of 22.03%, 2.8% and 17.59% respectively revealed the best solvent of extraction for secondary metabolites in plant samples of which alcohol extractive gave second to the best yield after water. Water extracts are difficult to manage due to too much moisture that encourages microbial degradation unlike alcohol extracts hence, the choice of alcohol in this research work. Solvent extractive values are also important to detect drugs which may be used fraudulently as substitutes or adulterant in the genuine drug samples (Elujoba, 2006).

The results of quantitative microscopy shown in Table 2 showed that *Triumfetta cordifolia* leaf sample has stomata number of 23.67, stomata index (0.023) palisade ratio (18.33) and vein-islet number of 10.67. The parameters of quantitative microscopy help to evaluate accurate cellular

micrometry of all the essential tissues in crude drugs (Wallis, 1920) while the results of qualitative microscopy as presented in Table 1 and Plate 1-3 showed the presence of anisocytic stomata, prismatic calcium oxalates, non glandular unicellular trichomes and wavy wall epidermal cells. These features are important in pharmacognostic evaluation because knowledge of all the diagnostic characters of any plant is the main essence of qualitative microscopy and it helps to detect contaminants and substitutes in plant crude drugs (Mukherjee, 2002).

The presence of alkaloids, glycosides, tannins, flavonoids, steroids and terpenoids were observed during phytochemical screening of the plant crude extract as displayed in Table 4 while the various solvent's fractions contain some of the aforementioned secondary metabolites based on individual elution power as shown in Table 4. Some glycosides have been reported by Sudhanshu *et al.* (2018) to be involved in pancreatic β cells restoration and secretion of insulin as well as control of insulin secretion and glycogen synthesis processes while Some triterpenoids are known natural antioxidants and have been reported to ameliorate lipoprotein lipase expression, dyslipidemia and insulin sensitivity (Eu *et al.*, 2010; Gao *et al.*, 2009; Sheng and Sun, 2011; Takagi *et al.*, 2010). Flavonoids are polyphenolic compounds that are reportedly implicated to be actively involved in the restoration of pancreatic β -cell and insulin secretion (Sudhanshu *et al.*, 2018). Some also have the ability to lower the risks of chronic and degenerative diseases such diabetes mellitus (Duthie and Brown, 1994; Milner, 1994). Aniszewski (2015) reported several alkaloids with antidiabetic activity.

The results of acute toxicity studies of ethanol extract of *Triumfetta cordifolia* recorded no death and visible signs of toxicity such as decrease feed intake, locomotion and sensitivity to touch and pain even at the highest dose level of 5000 mg / kg, all animal displayed normal behavioral profile.

The induced alloxan damages pancreas beta cells which results in reduced insulin secretion leading to increase in blood sugar level, total cholesterol and triglyceraldehyde (Szkudelski, 2001; Vivek *et al.*, 2010). The oral glucose tolerance test (OGTT) is one of the experimental designs used to evaluate the antidiabetic potentials of *Triumfetta cordifolia* leaves sample and the results as shown in Figure 1 revealed that the 400mg/kg and 200mg/kg of the ethanol extract of *Triumfetta cordifolia* leaves produced significant reduction of blood sugar level that are comparable to the standard drug (glibenclamide). The initial fasted blood sugar levels were 91.00, 90.25, 98.00 and 94.00 mg/dl for the Normal control, 400mg/kg ethanol crude extract of *Triumfetta cordifolia* leaves, 200mg/kg ethanol crude extract of *Triumfetta cordifolia* leaves and the Standard control (glibenclamide) respectively. Blood glucose levels of 122.00, 75.00, 113.25, 120.50, and 121.25mg/dl respectively were recorded immediately after administration of 2 g/kg glucose solution to the rats in all groups. Steady reduction in blood glucose levels were later recorded after 30 minutes, 1¹/₂ hours and 2¹/₂ hours of administration of 2 g/kg glucose solution leading to final blood sugar levels of 110.25, 94.25, 98.75 and 95.75mg/dl respectively. Sulphonylureas like glibenclamide are reportedly implicated for hypoglycemia even in lower than normal level of glucose concentration threshold for glucose stimulated insulin release

provided the beta-cells of the pancreas are fully functional (Krentz and Bailey, 2005). It is therefore pertinent to suggest that *Triumfetta cordifolia* leaves extract may possess similar mode of action in lowering sugar level in diabetic patient as glibenclamide.

The results of blood sugar lowering effect of *Triumfetta cordifolia* leaves on alloxan induced hyperglycemia in albino rats as shown in figure 2 gave a significant reduction in the ethanol crude extract groups (200mg/kg and 400mg/kg), fractions (N-hexane, ethylacetate and Butanol) and standard drug (glibenclamide). The sugar level reduction in crude extract groups (200mg/kg and 400mg/kg), n-hexane fraction and ethylacetate fraction are highly comparable to the standard drug, glibenclamide (initial blood sugar level after diabetes induction of 330.75mg/dl and final sugar level of 83mg/dl) with initial blood sugar levels after induction of diabetes of 424.25mg/dl, 364.75mg/dl, 489.25mg/dl and 382.25mg/dl and subsequent reductions to 98.5mg/dl, 89.75mg/dl, 100.5mg/dl and 104.30mg/dl respectively after treatment for 21 days. Sustained reduction of blood sugar level in hyperglycemia can lead to eventual decrease in risk of developing microvascular and macrovascular complications (Muniapan *et al.*, 2004).

It is an established fact that the activity of free radicals generated from injected alloxan are majorly responsible for degeneration of liver hepatocytes (Eze *et al.*, 2012). Lipid and protein oxidation often lead to oxidative stress in the vascular wall triggering atherosclerosis which can eventually result in cardiovascular disease (Goldstein *et al.*, 1973). The effects of the crude plant extract, individual fractions and standard drug (glibenclamide) on some hepatic panels were evaluated and the results as presented in Table 5 and Figure 4-6 revealed various significant level of ameliorating activities on parameters such as AST, ALT and ALP. The abnormal increase in the volume of AST, ALT and ALP enzymes in blood serum as observed in the diabetic group are strong indicators of degenerating states of the livers and subsequent significant lesser values of these liver enzymes in group 1, 2, 3 and 6 when compared to the diabetic control group showed a clear justification of various significant ameliorating abilities of the *Triumfetta*'s ethanol crude extract and individual fractions. The butanol fraction has the least ameliorating potential whereas the crude extract and ethylacetate fraction showed better ameliorating power. Though, these observations are quite lower than the standard control drug as presented in Table 5 and Figure 4-6.

CONCLUSION

This study has established the blood sugar lowering potential and the ameliorating activities of *Triumfetta cordifolia* leaves extract justifying its use in the treatment of diabetes and liver diseases. The Pharmacognostic parameters can be used as tool for précised identification and purity authentication of *Triumfetta cordifolia* leaves sample. The over results of this study can be a reference for future researchers that wish to do further studies on this plant and the plant extract can be used as alternative natural anti hyperglycemia source due to its high safety level.

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