NOVATEUR PUBLICATIONS
INTERNATIONAL JOURNAL OF INNOVATIONS IN ENGINEERING RESEARCH AND TECHNOLOGY
[IJIERT] ISSN: 2394-3696 Website: ijiert.org
VOLUME 8, ISSUE 9, Sep. -2021

FRACTIONATION AND BIOACTIVITY OF AQUEOUS EXTRACT OF CALOTROPS PROCERA LEAF

Ajiboso Semiu O.
(Ph.D), Department of Biochemistry & Molecular Biology,
Nasarawa State University, Keffi – Nigeria.
e-mail: semiuajiboso@gmail.com; +23407068391330

ABSTRACT
Calotropis procera, a multipurpose plant that is native to Asia and Africa has been reported to possess remedies for several temperate and tropical ailments. The use oits leaf for management of diabetes mellitus is popular among Nigerian populace, but investigation into its fraction which actually possesses the anti-diabetic property is unavailable. Thus, fractionation and bioactivity of aqueous extract of Calotropis procera leaf was carried out in the present study. The mean values of results showed that five (05) different fractions (F1 – F5) were obtained from the fractionation. Volatile oils constituted 60% of the crude extract fractions followed by alkaloids (10%), phenols (10%), saponins (10%) and glycosides (10%). The in-vivo toxicity study of the extract showed tha there were no extract-related moralities and pathological signs such as visual observations for behavioural pattern (restlessness, respiratory, distress, diarrhoea, convulsion, salivation, lethargy and sleep), changes in physical appearance, fur, injury, pain, and signs of illness in the normal rats administered different doses (25 mg/kg, 50 mg/kg and 100 mg/kg body weights) of the extract of throughout the period of the treatment. The experimental animals administered the fractions generally and significantly showed increase (at P>0.05) in body weight. Alloxan induction significantly increased (at P>0.05) the blood glucose level of the experimental animals while the fractions mostly the volatile oil fraction also significantly decreased (at P>0.05) the blood glucose level of the animals. The volatile oil fraction appeared to possess the most active principle(s) against hyperglycemia and eventual diabetes mellitus. Systemically, the animals administered the fractions were pharmaco-kinetic and pharmaco-dynamic stable. Therefore, an in-depth research work on the toxicity of the fractions is hereby recommended.

INTRODUCTION
Fractionation is the process of classification of an analyte or a group of analytes from a certain sample. The sample may be a mixture (gas, solid, liquid, enzymes, suspension or isotope) which is divided during a phase transition, into a number of smaller quantities (fractions) in which the composition varies according to a gradient (Houghton and Raman, 1998). Fractionation of plant results in isolation of individual constituent of the active chemical ingredients in the plant sample. It is a form of experimental procedure used to prepare medicinal plants for experimental purposes prior to biological testing (Abubakar and Haque, 2020).
Medicinal plants are plants that are used in herbalism for development of drug, drug synthesis and human cultures (Yudharaj et al., 2016). Medicinal plants can be classified according to the usage, active constituents and their herbs (Yudharaj et al., 2016); nature; physiologic activity (Wright, 2007); taxonomy (Shree, 2011) and biogenesis (Sangmai, 2010).
Medicinal plants play important roles in the development and growth of pharmaceutical industry. The medicinal properties of these plants can be harnessed through the extraction of its active chemical principles, ingredients and phytochemicals. A plant is said to be medicinal if any of its parts contains bioactive substances or compounds that are useful in the treatment of diseases or ailments; the plant parts that are commonly used include leaf, root, bark, seed, fruit, rhizome, flower and whole plant.
There are several hypoglycaemic drugs in the market today, these drugs are synthetic drugs and have associated side effects. Thus, sourcing alternative means to treat and manage diabetes mellitus from medicinal plants now becomes imperative. Many medicinal plants possess hypoglycaemic properties; these properties can be exerted through different mechanisms such as possession of minerals with insulinotropic property, phytochemicals, active principles etc. among these medicinal plants is Calotropis procera plant.
Calotropis procera plant with the common name Sodom Apple is an ancient tropical plant which is native to African and Asian countries. It is known as Tumfafiya in Hausa, Bomubomu in Yoruba and Epuko in Nupe languages, it is also regarded as ‘blessed plant’ in India due to its richness in medicinal properties.

In Nigerian folklore traditional medicine, Calotropis procera plant parts are used in the treatment and management of various illnesses, diseases and ailments. Calotropis procera leaf is a source of income and livelihood to decoction vendors and herbalists. Also, the popularity of Calotropis procera leaf in herbal practice in the management of diabetes mellitus in Nigeria is enormous and thus a dire need for scientific validation.

Therefore, the objective of the study was to determine the fractional constituents of aqueous extract of Calotropis procera leaf and anti-diabetic property of the fractions obtained.

MATERIALS AND METHODS

80 clinically healthy adult white rats (Rattus norvegicus) of both sexes with mean weight 105.00±0.10g were used for the experiment. The animals were collected from the animal house of Bingham University Karu, Nasarawa State – Nigeria. The animals were kept (same sex) in steel cages and allowed to acclimatise for two weeks, during this period they were fed ad libitum (allowed to free food – rat basal diet (Vital, GCOML and water).

Collection and authentication of plant sample

Matured leaves of Calotropis procera plant were harvested from the plant around the Institute of Molecular and Infectious Diseases, Nasarawa State University, Keffi - Nasarawa State in May, 2021. The plant sample was authenticated by a plant scientist in the Department of Plant Science, Nasarawa State University, Keffi - Nasarawa State.

Chemicals and Reagents

All the chemicals and reagents used were of analytical grade, Poole – England, the reagents were prepared with distilled water except otherwise stated. The reagents were kept in cleaned reagent bottles.

Induction of diabetes

Diabetes mellitus was induced using the procedure described by Yakubu et al., (2010). Blood glucose levels of the rats were determined before and after the administration of alloxan.

Determination of blood glucose and body weight

Blood glucose and body weight were determined on days 0, 5 and 10 using Bayer ContourTM blood glucose kit, a product of Bayer Consumer Care AG, Postfash, Basel, Switzerland and digital weighing balance (model: YP-B10002, Nederland) respectively.

Extract and doses preparation

1kg of fresh leaves of Calotropis procera were chopped into pieces, air dried to constant weight at room temperature for 72 hours. The dried sample was grinded to fine particle size using an electric blender, Phillips HR1727 Holland model. The grinded sample was allowed to pass through a sieve range of 75µm mesh size. The filtered sample of a known weight (560g) was dissolved in 1000 ml distilled water for 12 hours with intermittent shaking. Extract obtained was filtered through Whatman No. 1 filter paper, followed by evaporation using Lyophilizer (AVAIN LABS, India) at 55^{o}C for 30 minutes to give a yield of 14.96 g.

Calculated amount of the residue was weighed and constituted in distilled water to give the required doses of 25, 50 and 100 mg/kg body weight. The doses used in this study were as obtained from the ethno-botanical survey carried out on the plant within our locality.

Fractionation of crude extract of Calotropis procera leaf

The procedure described by Deblangey et al., (2013) was used to determine the fractions in both aqueous and hexane extracts of Calotropis procera leaves.

Procedure

Dried sample (150g) of Calotropis procera leaves was macerated with 1000ml of distilled water for 72 hours. After 72 hours, the extract was filtered and the residue was labelled crude A, crude A was further macerated with hexane (1000ml), left undisturbed for another 72 hours. After 72 hours, the extract was

filtered and the residue was labelled as crude B. each of the crude extracts was concentrated using rotary evaporator. The concentrated crude extracts A and B were then poured into a beaker containing 30 g silica gel; 100ml of distilled water was also added to make slurry of the silica. The silica gel with absorbed concentrated crude extracts A and B were transferred into one packet column, using a solvent comprising of water and hexane 2:1 (v/v) ratio under increased pressure (flash gradient column chromatography). The extracting solvents were collected at the outlet flask with the concentrated crude extract remaining in the feeder flask. The TLC solvent system was prepared from 15ml of hexane and 10ml of distilled water. The solvent system was poured into a TLC chamber to a depth of less than 0.5cm; the chamber was covered and swirled. The prepared TLC plate in the TLC chamber was left undisturbed for 2 hours. After 2 hours, the plate was removed and the solvent front was immediately mark with a pencil and was then allowed to dry. The TLC plate was treated with spray reagent of 10% concentrated H_2SO_4 in ethanol to enhance visualization. The development of each TLC plate was then compressed with each other for extract with similar development and retention factor (R_f).

Calculation:

R_f = Distance travelled by the compound / Distance travelled by the solvent front

Anti-diabetic activity of extracted fractions

30 rats (5 normal, 25 alloxan induced-diabetic rats) were distributed into six groups – A, B, C, D, E & F, each group contained five rats each (same sex) after diabetes had been confirmed.

Calculated amount of each fraction was weighed and constituted in distilled water to give the required doses of 100 mg/kg body weight; fractions were administered orally to respective groups.

A= non-diabetic rats given 0.5ml of distilled water
B= diabetic administered 100mg/kg b.w of alkaloids
C= diabetic administered 100mg/kg b.w of saponins
D= diabetic administered 100mg/kg b.w of volatile oil
E= diabetic administered 100mg/kg b.w of phenols
F= diabetic administered 100mg/kg b.w of glycosides

In-vivo toxicity study

The procedure described by Builders et al., (2012) was used to determine the in-vivo toxicity study of the extract.

20 normal (non-induced) rats were randomly groupe d as follows:

A= rats administered 0.5ml of distilled water
B= rats administered 25 mg/kg b.w. of extract
C= rats administered 50 mg/kg b.w. of extract
D= rats administered 100 mg/kg b.w. of extract

As a follow – up to this, administration of the aqueous extracts of Calotropis procera leaf to the rats was continued daily for 10 days at the doses stated. The body weight was recorded at the beginning and end of the experiment.

RESULTS

Fractionation of aqueous extract of Calotropis procera leaf

The result of fractionation of aqueous extract of Calotropis procera leaf is presented in Figure 1. Five (5) fractions were obtained from the aqueous extract of Calotropis procera leaf. Volatile oils constituted 60% of the crude extract fractions followed by alkaloids (10%), phenols (10%), saponins (10%) and glycosides (10%).

Effect of alloxan on blood glucose level of administered rats

The results of effect of alloxan on blood glucose level of administered rats were presented in Figure 2. There was a marked increase in the blood glucose levels of alloxan administered rats with range of mean values of 14.50 – 15.10 mg/dl. The increase was about 86% and significantly different at $p>0.05$ when compared to the mean blood glucose value (2.13mg/dl) of non – alloxanised rats.

Effects of administration of fractions of aqueous extract of Calotropis procera leaf on blood glucose level of diabetic rats

The effects of administration of fractions of aqueous extract of Calotropis procera leaf on blood glucose level of diabetic rats is shown in Figure 3. The result revealed that each fraction at the administered dose of 100 mg/kg body weight caused significant reduction in blood glucose levels of the experimental rats. The reduction in blood glucose levels of diabetic rats administered the fractions as progressive throughout the 10 days treatment. At the end of the treatment, the results revealed 62.20%, 53.94%, 85.59%, 39.29%, and 18.29% reductions in blood glucose by alkaloids, saponins, volatile oil, phenols and glycosides respectively. Volatile oil significantly reduced ($p>0.05$) blood glucose levels in the administered groups to value similar to non-diabetic group. There was no significant difference ($p>0.05$) in blood glucose values of diabetic rats administered volatile oil and non-diabetic rats.

Result for in-vivo toxicity study of different doses of aqueous extract of Calotropis procera leaf in normal rats

There were no extract-related moralities and pathological signs such as visual observations for behavioural pattern (restlessness, respiratory, distress, diarrhoea, convulsion, salivation, lethargy and sleep), changes in physical appearance, fur, injury, pain, and signs of illness in the normal rats administered different doses (25 mg/kg, 50 mg/kg and 100 mg/kg body weights) of aqueous extract of Calotropis procera leaf throughout the period of the treatment.

Effects of different doses of the extract on body weight of normal rats

The effects of administration of aqueous extract of Calotropis procera leaf on body weight of normal rats are presented in Figure 4. The result revealed a significant increase ($P>0.05$) in body weight of all the experimental rats (distilled water and extract administered). The body weight gain was sustained and progressive throughout the treatment period. By the end of the treatment period, the extract at the doses of 25, 50 and 100 mg/kg body weight had increased the body weight of the treated rats by 10.23 %, 13.43 %, and 14.80 % respectively. The performance of the extract at different doses tested and distilled water from the computed percentages was in the order of 100 mg/kg body weight > 50 mg/kg body weight > 25 mg/kg body weight of the extract > distilled water (10.06 %).

DISCUSSION

In seeking the functionality of extracts applicable to medicine, aqueous extract of Calotropis procera leaf was found to possess anti- hyperglycaemic property. The isolation of alkaloids, phenols, saponins, glycosides from the extract suggests the isolated phytochemicals as the chemical constituents of the extract. Alkaloids, tannins, glycosides, saponins, steroids, phenols, phlobatannins, cardenolides, saponins, triterpenes, anthocyanins, flavonoids and polyacetylenes are some of the chemical constituents of plants. In the fractionation of leaves, solvents that are commonly used include hexane, chloroform, ethyl acetate, methanol, ethanol, ether, ethanoic acid and water (Lii et al., 2004). Volatile oil, being the first three fractions in the seven fractions isolated is considered the most abundant fraction in the extract. The quantity of oil isolated also correlates positively with the total fat content in the proximate composition of Calotropis procera leaf. The amount oil in the leaf can also be linked to its volatility, waxy nature and strong scent of the leaves (Wang et al., 2010). Volatile or essential oils are the odorous principles found in various plants and plant parts. Essential oils comprise the volatile, distillable or expressible fraction responsible for the characteristics odour found in many plants (Li et al., 2011).

Alloxan is a hyperglycaemic chemical substance used to induce diabetes in experimental animals through intraperitoneal route of administration. According to Cheekati et al., (2017), alloxan causes a massive reduction in insulin release by the destruction of the β-cells of the islets of Langerhans, inducing hyperglycemia. Thus, leading to increased blood glucose from carbohydrates metabolic derangement due to insulin deficiency.

Earlier reports reveal that alloxan-induced diabetic animals may exhibit most of the diabetic complications mediated through oxidative stress (Punitha and Manoharan, 2006). The lowering effects of the fractions on blood glucose suggest that the fractions possess anti-hyperglycemic property. Alkaloids have been reported to exhibit anti-diabetic effect against alloxan-induced diabetes through reduction of oxidative damage, modulation of antioxidant enzymes and potentiation of pancreatic secretion of insulin (Shukla et al., 2012).

Phenols are potent inhibitor of a-glucosidase and radical-scavenging activities (Wang et al., 2013). The anti-diabetic activity of saponins has been linked to its combined antioxidant and antiglycation properties (Xi, 2008). Glycosides exhibit their hypoglycaemic activity by suppressing the transfer of glucose from the stomach to the small intestine and inhibiting glucose transport at the brush border of the small intestine. The eventual mechanism responsible for the highest hypoglycemic effects of volatile oil in the present study may result from the ability to induced insulin release or increase peripheral uptake of glucose (Hamden et al., 2008). Thus, apart from treating anti-inflammatory disease such as rheumatoid arthritis and asthma, ingestion of the leaves may also suppress lipid accumulation (Okie et al., 2009). The hypoglycaemic activity of the fractions suggests potential use of Calotropis procera leaf in the treatment of diabetes mellitus and other associated complications.

This study evaluates the toxicity of the aqueous extract of Calotropis procera leaf in an animal model regardless of the pharmacological beneficial effects of the extract. According to Duke (1997), in screening natural products for the pharmacological activity, assessment and evaluation of the toxic characteristics of a natural product extract, fraction or compound are usually an initial step.

The non-mortality record and no visual observations for behavioural pattern (salivation, fur, lethargy, and sleep), changes in physical appearance, pain, and signs of illness that are considered to be of toxicological significance in the extract administered groups suggests that the extract at the tested doses may be safe for consumption and non toxic to the administered rats.

For centuries, natural products, such as medicinal plants have been the basis for the treatment of various ailments (Kader et al., 2013). Many medicinal plants have been reported to be toxic to both humans and animals (Chokshi, 2007). Furthermore, toxicity studies on medicinal plants or preparations derived from should be obtained in order to increase the confidence in ts safety for humans use particularly for the development of pharmaceuticals (Lafontan and Langin, 2009).

The body weight changes serve as a sensitive indication of the general health status of animals (Michael et al., 2007). The observed body weight gain in the extract administered groups may be attributed to the nutritional composition of the extract (El Hilaly et al., 2004) and the significant increments in body weight gain (Jo et al., 2009). The loss of appetite is often synonymous with weight loss due to disturbances in the metabolism of carbohydrate, protein, or fat (El Hilaly et al., 2004). Therefore, the normal food and water intake without loss of appetite are suggested as been responsible for the increment in body weight in this present study. Adipose tissue is a dynamic organ that plays an important role in organism's fatness (Lafontan and Langin, 2009). Additional supply of nutritional and metabolic requirements or increase in appetite of the rats by higher doses of the extract may contribute to more body weight gain in rats treated in higher doses (50 mg/kg and 100 mg/kg body weight) of the extract.

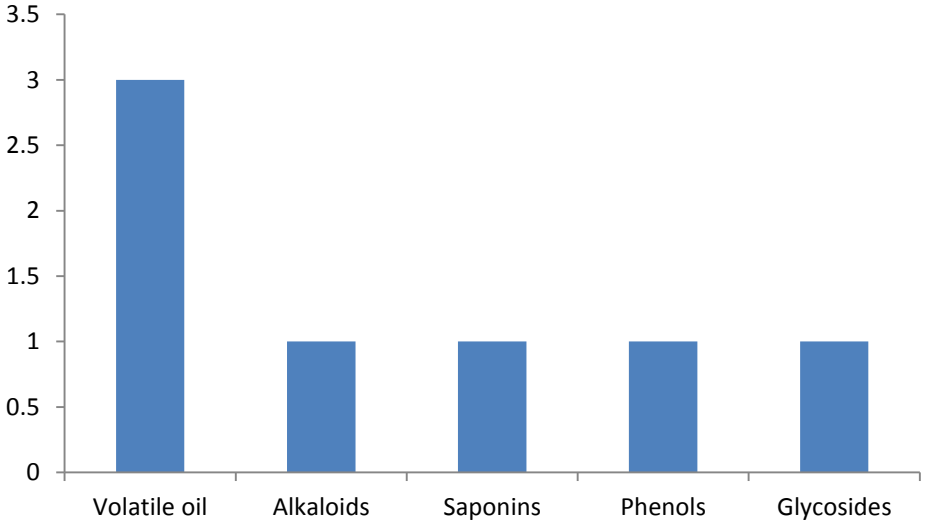

Figure 1: Showing the fractions obtained through fractionation of crude extract of Calotropis procera leaf

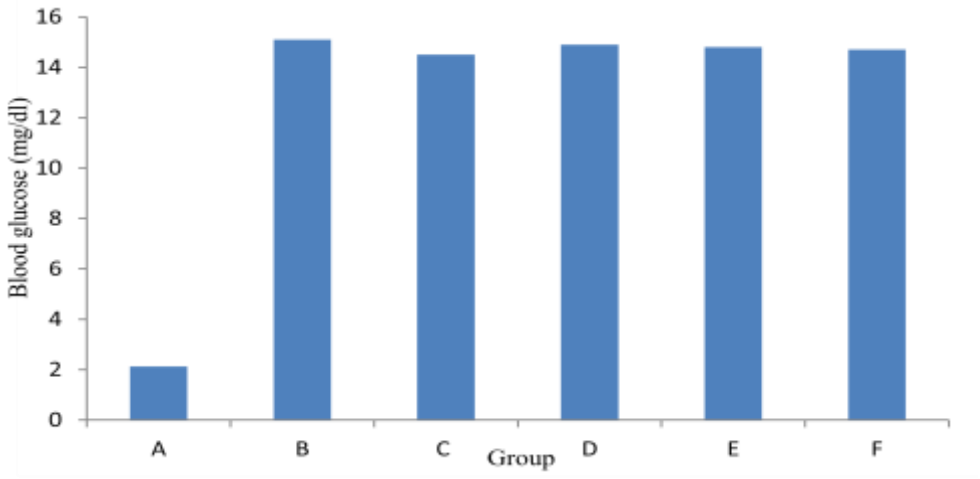

Figure 2: Showing the effect of alloxan on blood glucose level of administered rats

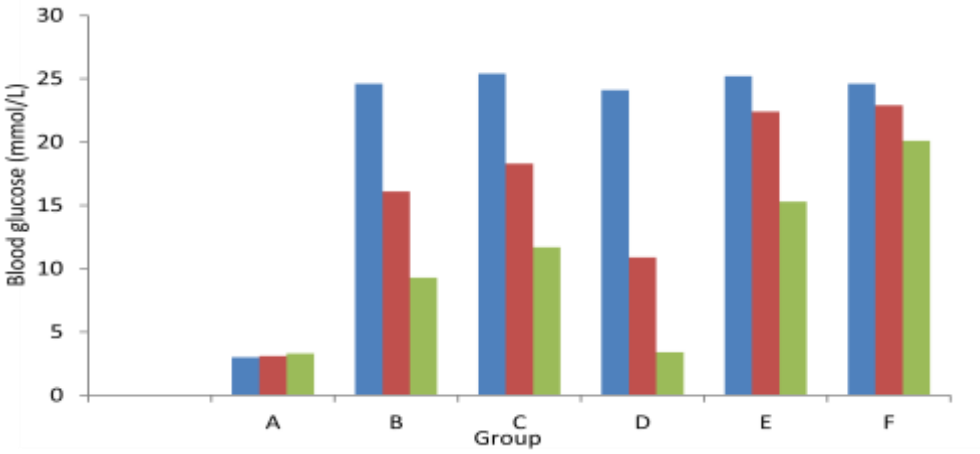

Figure 3: Showing the effect of administration of fractions of aqueous extract of Calotropis procera leaf on blood glucose level of diabetic rats

Key: A= Non-diabetic; B= Diabetic + 100mg/kg b.w. of Alkaloids; C= Diabetic + 100mg/kg b.w. of Saponins;
D= Diabetic + 100mg/kg b.w. of Volatile oil; E= Diabetic + 100mg/kg b.w. of Phenols and F= Diabetic + 100mg/kg b.w. of Glycosides. Blue, brown and lemon colours represent Days 0, 5 and 10 respectively.

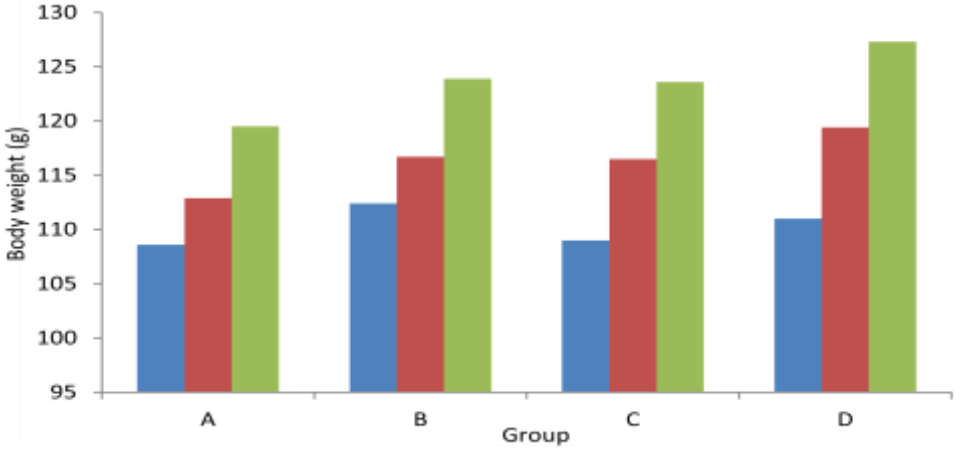

Figure 4: Showing the effect of different doses of aqueous extract of Calotropis procera leaf on body weight of normal rats

Key: A= Normal rats + Distilled water; B= Normal rats + 25mg/kg b.w. of the extract; C= Normal rats + 50mg/kg b.w. of the extract; D= Normal rats + 100mg/kg b.w. of the extract. Blue, brown and lemon colours represent Days 0, 5 and 10 respectively.

CONCLUSION

From the work carried out, virtually almost all the fractions obtained from the fractionation of calotropis procera leaf possessed anti-diabetic activity. The volatile oil fraction appeared to possess the most active principle(s) against hyperglycemia and eventual diabetes mellitus. Systemically, the animals administered the fractions were pharmacokinetic and pharmacodynamic stable.

RECOMMENDATION

An in-depth research work on the toxicity of the fractions is hereby recommended.

REFERENCES

1) Abubakar, A.R. and Haque, M. (2020). Preparation of medicinal extraction and fractionation experimental purposes. Journal of Pharmacy & BioAllied Sciences, 12(1): 1-10.
2) Cheekati, R. R., Rao, A. S. & Vijayaraghavan, R. (2017). A histological study of alloxan-induced diabetes on experimental male Wistar rats. National Journal of Physiology, Pharmacy and Pharmacology, 7(12): 1329 – 1334.
3) Chokshi, D. (2007). Subchronic oral toxicity of a standardized white kidney bean (Phaseolus vulgaris) extract in rats. Food and Chemical Toxicology, 45(1): 32-40.
4) Deblangey, A., Roger, J.M., Palagos, B., Grenier, G. And Bendoula, B. (2013). Comparative study of two methods (hexane extracts and NMR) for the determination of oil content in an individual olive fruit. European Journal of Lipid Science and Technology, 115(9): 1070-1077.
5) Duke, J.A. (1997). The Green Pharmacy: new discoveries in herbal remedies for common diseases and conditions from the world's foremost authorities in healing herbs. St. Martins Press.
6) El Hilaly, J., Israili, Z. H. and Lyoussi, B. (2004). Acute and chronic toxicological studies of Ajuga seed in experimental animals. Jornal of Ethnopharmacology, 91(1): 43-50.
7) Hamden, K., Carreau, S., Lajmi, S., Aloulou, D., Kchaou, D. and Elfeki, A. (2008). Protective effect of 17 β-estradiol on hyperglycemia, stress oxidant, liver dysfunction and histological changes induced by alloxan in ale rat pancreas and liver. Steroids, 94: 495-501.
8) Houghton, P.J. and Raman, A. (1998). Laboratory handbook for fractionation of natural extracts. Chapman and Hall, London, P.199.
9) Jo, J., Gavrilova, M.O and Pack. (2009). Hypertrophy and hyperplasia: dynamics of adipose tissue growth. Plos Computational Biology, 5(3): 324-329.
10) Lafontan, M. And Langin, D. (2009). Lipolysis and lipid mobilization in human adipose tissue. Progress in Lipid Research, 48(5): 275-297.
11) Michael, B., Yano, B. and Sellers, R.S. (2007). Evaluation of organ weights for rodent and non- rodent toxicity studies: a review of regulatory guidelines and survey of current practices. Toxicology Pathology, 35(5): 742-750.
12) Okiei, W., Ogunlesi, M., Ofor, E. And Osibote, E. A. S. (2009). Analysis of essential oil constituents in hydro-distillates of Calotropis procera (Ait.) R.Br. Research Journal of Phytochemistry, 3: 44-53.
13) Punitha, R. and Manoharan, S. (2006). antihyperglycemic and antilipidperoxidative effects of Pongamia pinnata (Linn.) Pierre flowers in alloxan induced diabetic rats. J. Ethnopharmacol., 105: 39-46.
14) Sangmai TK, Et Al. Diuretic property of aqueous extract of leaves of Mimosa pudica Linn on experimental albino rats. Journal of Natural Product, 3, 2010, 173-178.
15) Shree Devi MS. Acute toxicity and diuretic activity of Mangifera indica linn bark extracts. International Journal of Pharma and Bio Sciences, 2(3), 2011, 141-146.
16) Shukla, A., Bigoniya, P., And Srivastava, B. (2012). hypoglycaemic activity of Lepidium sativum linn seed total alkaloid on alloxan induced diabetic rats. Research Journal of Medicinal Plant, 6(8): 587-596.

17) Wang, Z,. Chinoy, Z.S., Anbre, S.G., Peng. W., Mcbride, R., Deviries, R.P., Glushka, J., Paulson, J. and Greert-Jan, B. (2013). A general strategy for the chemo enzymatic synthesis of asymmetrically branched n-glycans. Science, 341(6144): 379 – 383.
18) Wright, C.J. Herbal Medicines As Diuretics: a review of the scientific evidence, Journal of Ethnopharmacology, 114(1), 2007, 1-31.
19) Xi, M.(2008). Antioxidant and antiglycation properties of total saponins extracted from traditional chinese medicine used to treat diabetes mellitus. Phytother. Res., 22(2): 228 – 237.
20) Yakubu, T.M., Akanji, M.A. and Nafiu, M.O. (2010). Antidiabetic activity of aqueous extract of Cochlospermum planchonii root in alloxan-induced diabetic rats. Cameroon Journal of Experimental Biology, 6(2): 91-100.
21) Yudharaj, P., Shankar, M., Sowjana, R., Sireesha, B., Ashok Naik, E. & Priyadarshini, R.J. (2016). Importance and uses of medicinal plants – an overview. International Journal of Preclinical & Pharmaceutical Research, 7(2): 67-73.