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# Effect of Dissostis Rotundofolia Stem Extract Consumption on Some Biochemical Parameters and Hepato-Renal Organs in Healthy Albino Rats.

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#### Abstract

This study explored the potential health benefits of Dissotis rotundofolia, a plant widely used in traditional African medicine. It is a common plant that has been reported to have a variety of ethnomedical uses in tropical Africa. The aim of this research work was to ascertain the effect of aqueous methanoic stem extracts of Dissotis rotundofolia on plasma triglycerides, glucose, plasma sodium concentration, aspartate aminotransferase, plasma cholesterol concentration, and glutamate pyruvate transaminase and their histomorphological changes in normal adult albino rats and to evaluate changes that depict architectural variations in their kidneys and liver sinusoids. Twenty-four (24) healthy albino rats aged six to seven weeks old with an average weight of 190 g were used for this study. They were obtained locally from the animal house of the Department of Pharmacology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The rats were randomly divided into two groups, group 1 and group 2, of 12 rats. They were kept in standard cages and fed with distilled water and growers mash (Guinea Food Nigeria) for 2 weeks to acclimate to the new environment. After acclimatization for a period of two weeks, they were fasted overnight. Group 1, which served as the normal control, received distilled water, while the test groups received an aqueous leaf and stem extract of Dissotis rotundofolia at 2 ml/kg body weight. The results obtained from the experiment for glucose and triglyceride are presented statistically. We observed that there were improvements in liver and kidney function, as evidenced by reduced levels of aspartate aminotransferase and glutamate pyruvate transaminase. These findings suggest that Dissotis

*rotundofolia* extracts may be a useful adjunctive treatment for managing diabetic conditions.

## Introduction

Dissotis rotundofolia, commonly known as "pink lady," is a tropical African medicinal plant that is widely used in Tanzania (Kideghesho et al., 2020), Cameroon (Fokunang et al., 2011), Liberia (Saha et al., 2023), Nigeria (Iwueke, 2020), and Ghana (Bekoe et al., 2020). There have been reports of its folkloric use in traditional herbal medicine systems. It is a common plant that has been reported to have a variety of ethno-medical uses, particularly in treating gastrointestinal disorders, which is significant given the high prevalence of peptic ulcers in Nigeria, with about 300,000 cases reported annually (WHO, 2019). The plant contains bioactive compounds that contribute to its medicinal properties and is used in various forms as a food component (Yu et al., 2021). However, conventional treatments for peptic ulcers often have significant side effects, such as erectile dysfunction, diarrhoea, and hypergastrinaemia (Djanaev et al., 2023), highlighting the need for alternative therapies like those derived from Dissotis rotundofolia. Dissotis rotundofolia is a multipurpose perennial herb that grows from seeds and stolons. It has prostate-like or ascending stems that can reach a height of 40 cm. The leaves have three nerves, are oblong to oval-lanceolate or suborbicular, 1.5-7.0 cm long, 0.8-4.0 cm wide, and have ciliate to slightly crenate margins, an acute tip, a truncate base to short-attenuate base, and petioles that are 0.5-2.5 cm long. (Abere et al., 2010). The leaves are arranged opposite each other and are commonly ovate or ovate-lanceolate measuring about 1.5-6 cm in length and 1-2.5 cm wide (Singh 2013), with petioles as long as 1.5 cm (Wunnenberg et al., 2021). Triacyl glycerides (TG), also known as triacylglycerol, are esters derived from glycerol and fatty acids (tri- + glyceride) Pundir & Narang (2013). They are primary components of body fat and are crucial in the bidirectional transfer of glucose and fat in the bloodstream (Malik et al., 2023). Glucose, a simple sugar essential for energy production, is regulated in the blood by insulin, with imbalances leading to diabetes mellitus (Alshammari et al., 2022). Hyperglycaemia and hypoglycaemia are terms used to describe consistently high and low glucose levels, respectively. Diabetes mellitus, the most common condition associated with improper blood sugar management, may be indicated by a high fasting glucose level. Sodium is an essential element for cellular function and osmoregulation, and its levels must be maintained within a narrow range to prevent conditions like hypernatremia and hyponatremia (Meneses-Sagrero et al., 2022). Cholesterol is a steroid lipid with the formula C27H46O, found in the cell membranes of all body tissues, and transported in the blood plasma, of all animals. Even while cholesterol is necessary for the body in many ways, having too much of it in the blood can be harmful (Upadhyay, 2023). When blood cholesterol reaches high levels, it can build up on artery walls, increasing the risk of blood clots, heart attack, and Enzymes such as Glutamate Pyruvate stroke. Transaminase (GPT) and Aspartate Aminotransferase (AST) are crucial biomarkers for liver and heart health, with elevated levels indicating tissue damage (Seth et al., 2021). Dissotis rotundofolia is rich in C-glycosyl flavones, namely, vitexin, isovitexin, orientin, and isoorientin which have demonstrated pharmacological effects such as anti-diarrhea effects, antimicrobial, and antitrypanosomiasis (Adinortey et al., 2020). This research aims to ascertain the effect of aqueous and methanoic leaves and stem extracts of Dissotis rotundofolia on various biochemical parameters in normal adult albino rats. The study will assess changes in plasma triglycerides, glucose, plasma sodium concentration, aspartate aminotransferase, plasma cholesterol concentration, and glutamate pyruvate transaminase along with structural variations in the liver and kidneys to evaluate changes that depict architectural variations in their kidneys and liver sinusoids.

# Materials and Method: Equipment/Apparatus.

Centrifuge (model no; A C. 2304); Visible spectrophotometer (S23A Gulfex Medical and Scientific, England. Volt: Freq: 50Hz, 40W, NO: 23A); Electronic thermostatically controlled water bath (Model HH. W21. Cr4II 2004, 11, power a.c. 220v, 50Hz, 825W, temperature range 37-100°C, variation  $\pm$  0.5°C, No GCO43078) and weighing balance 770-15, Germany.

# **Chemical Reagents**

All chemical Reagents used were of analytical grade, Manufactured by Randox Laboratories Limited, England, and were obtained from a commercial supplier, Effective Medical Laboratories, Island, Lagos state, Nigeria.

# **Experimental Animals**

Twenty-four (24) healthy female albino rats aged six to seven weeks old with an average weight of 190g were used for this study. They were obtained locally from the Animal House of the Department of Pharmacology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The rats were randomly divided into two groups, group 1 and group 2 of 12 rats. They were kept in standard cages and fed with distilled water and growers mash (Guinea food Nigeria) for 2 weeks to acclimatize to the new environment. After acclimatization for a period of two weeks they fasted overnight, group 1 which served as normal control received distilled water while the test groups received aqueous leaf and stem extract of Dissotis rotundofolia at 2ml/kg body weight.

# Feed

The rats were fed with pelleted growers' mash twice daily (morning and evening)

# Source of Sample (Dissotis rotundofolia) Stem

The *Dissotis rotundofolia* was collected from the environment of the College of Health Science, Niger Delta University, Bayelsa State, Nigeria.

# Preparation/Treatment of Sample

*Dissotis rotundofolia* stems were cut into pieces with the aid of a knife and sun-dried. When properly dried, the sample was ground into a powder with the aid of an electric blender. With the aid of a standard weighing balance, 240g of samples were macerated in 2400ml of distilled water (1g/10ml) and stirred periodically for about 3 days. The macerated samples were filtered with a sieve to eliminate particles after 72 hours. The filtrates collected were then concentrated in a water bath to yield thick brown viscous pastes.

## Administration of Aqueous Stem Extract

Aqueous stem extract of *Dissotis rotundofolia* was administered to the experimental rats orally twice a day (morning and evening) throughout the duration of the experiment (28 days) with the aid of gavage, at the dose of 2ml/kg body weight.

#### **Experimental Design**

The healthy albino rats after acclimatization of two weeks were randomly put into two groups of twelve (12) rats each as follows.

**Group 1 (control):** received pelleted grower mash and distilled water throughout the experiment (28 days)

**Group 2 (test):** administered aqueous stem extract of *Dissotis rotundofolia* orally (twice daily) and had access to pelleted grower mash and distilled water throughout the experiment (28 days)

## **Weight of Animals**

The weights of animals were measured at the commencement of the experiment and subsequently every week throughout the duration of the experiment

#### **Collection of Blood Samples and Organs**

The tail of the restrained rat was cleansed with a ball of cotton wool soaked in methylated spirit. A little Vaseline was then smeared on the tail to reduce friction while massaging to redness. Gentle massage towards the tip of the tail continued until the tip became red; a sign of blood accumulation. The red tip of the tail was then slightly and carefully incised with a new and sterilized blade and further massaged gently as the blood trickled into immobilized universal sample tubes without anti-coagulant (for glucose and triglyceride assay). Cotton wool soaked in methylated spirit was again used to cleanse the incised area of the tail. After 5 minutes serum was decanted from the universal sample tubes kept on the side bench under room temperature (28 C) and used for all biochemical analyses carried out immediately.

#### **Assays of Biochemical Parameters**

The glucose and triglyceride assay were done in agreement with the procedure described by Randox Laboratories Ltd, United Kingdom. Below is the biochemical assay used in carrying out the research work.

## **Glucose Assay**

**Principle:** It is based on the oxidation of glucose-by-glucose oxidation (GOD) to gluconic acid and hydrogen peroxide. The formed hydrogen peroxide (H2O2) is detected by a chromogenic oxygen acceptor, phenolaminophenazone in the presence of peroxidize (POD) and phenol

Glucose + O2 + H2O GOD H2O2 + gluconic acid.

H2O2 + phenol 4-AP POD Quinone + 4H2O

The intensity of the color formed is proportional to the glucose concentration in the sample.

#### **Procedure:**

The reagents and samples were brought to room temperature.  $1000\mu$ l of reagent and  $10\mu$ l of plasma sample were added into eight test tubes (the plasma was not added into the standard and reagent blank test tubes, only the reagent), Then  $10\mu$ l of standard (cal) was added into the standard test tubes and  $10\mu$ l of distilled water into the reagent blank test tube using a micropipette and mixed thoroughly. They were incubated at  $37^{\circ}$ C for 10 minutes. The absorbance (Abs) values of the samples and standard were read at 546nm against the reagent blank with the aid of a spectrophotometer. The absorbance (Abs) of the sample was read against a reagent blank.

#### Calculations

The Glucose concentration was calculated as: mg/dl =Absorbance sample/Absorbance Standard x Standard glucose conc. (mg/dl)

#### TriglycerideAssay Principle:

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). Glycerol is phosphorylated by adenosine triphosphate (ATP). In the presence of glycerol kinase (GK) to form glycerol-3-phosphate (G-3P) and adenosine diphosphate (ADP). Glecrol is oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide.

A red chromogen quinone imine is produced by the perioxidase (POD) catalyzed coupling of 4aminoaphenazone (4-AA) and chlorophenol with hydrogen peroxide (H2O2) where colour integrity proportional to the concentration of triglyceride in the sample.

Triglycerides + 3H2O LPL Glycerol + 3FFA Glycerol + ATP GK Glycerol-3-P + ADP Glycerol-3-P + O2 GPO DHA + H2O2 4-AA + 4 Phenol H2O2 Quinoneimine + H2O

#### **Procedure:**

The reagents and samples were brought to room temperature.  $1000\mu l$  of reagent and  $10\mu l$  of plasma was transferred into 8 test tubes (the plasma is not added into the standard and reagent blank test tubes, only the reagent), then  $10\mu l$  of standard (cal) into the standard test tubes and

 $10\mu$ l of distilled water into the reagent blank test tube using a micropipette and mixed thoroughly, incubated at 37°C for 10 minutes. The absorbance (A) value of the samples and standard were read at 546nm against the reagent blank with the aid of a spectrophotometer

#### Calculations

The triglyceride concentration was calculated as: mg/dl =Absorbance of sample/ Absorbance of Standard x A Standard triglyceride conc. (mg/dl)

#### Result

The result obtained from the experiment for glucose and triglyceride is presented statistically in Tables 1 to 6 as shown below. Tables 1 and 5 show the mean plasma glucose concentration. Tables 2 and 4 show the mean plasma triglyceride concentration in rats after 28 days of administration of sample. Tables 3 and 6 show the mean weight of the rat periodically taken. The result obtained from the experiment shows that glucose increased while triglyceride decreased.

Table 1: Mean plasma Glucose Concentration (mg/dl) of normal rats administered aqueous stem extract of *Dissotis rotundofolia*.

| Day   | Day 0     | Day 1     | Day 2     | Day 3           | Day 4     | Day 5     | Day 6     |
|---|-----------|-----------|-----------|-----------------|-----------|-----------|-----------|
| Control   | 1.20±1.48 | 1.01±0.09 | 1.18±0.07 | $0.87 \pm 0.07$ | 0.91±0.22 | 0.70±0.47 | 0.63±0.11 |
| Test  | 1.44±0.70 | 1.11±0.10 | 1.29±0.20 | $0.92 \pm 0.04$ | 1.13±0.05 | 0.98±0.18 | 1.13±0.11 |
| Each value convergence the mean standard deviation. Statistical significance occurred at $n < 0.05$ |           |           |           |                 |           |           |           |

Each value represents the mean standard deviation. Statistical significance occurred at p < 0.05

| Table 2: Mean Plasma triglyceride concentration (r | mg/dl) of normal rats administered |
|--|------------------------------------|
| aqueous stem extract of Dissotis rotundofolia.     |                                    |

| Day                 | Day 0           | Day 1           | Day 2     | Day 3     | Day 4     | Day 5     | Day 6     |  |
|---------------------|-----------------|-----------------|-----------|-----------|-----------|-----------|-----------|--|
| Control             | 1.20±0.28       | $1.01 \pm 0.09$ | 1.18±0.07 | 1.18±0.26 | 1.00±0.13 | 0.58±0.03 | 0.44±0.24 |  |
| Test                | $1.44{\pm}0.70$ | 1.11±0.10       | 1.29±0.20 | 1.30±0.10 | 0.95±0.07 | 0.75±0.21 | 0.20±0.10 |  |
| $\overline{\Gamma}$ |                 |                 |           |           |           |           |           |  |

Each value represents the mean standard deviation. Statistical significance occurred at p < 0.05

| Table 3: The mean | weight of normal rats | administered aqueous | stem extract of Dissoti | S |
|-------------------|-----------------------|----------------------|-------------------------|---|
| rotundofolia.     |                       |                      |                         |   |

| Days    | Day0  | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 |
|---------|-------|-------|-------|-------|-------|-------|-------|
| Control | 180.3 | 191.4 | 197.5 | 200.1 | 200.9 | 202.0 | 203.4 |
| Test    | 180.5 | 176.9 | 167.1 | 165.7 | 156.3 | 149.7 | 146.3 |

Each value represents the mean weight (in grams) of the rats used for the experimental procedure.

|         |                         |                        | <b>J</b>                |                         |                        |                         | _                      |
|---------|-------------------------|------------------------|-------------------------|-------------------------|------------------------|-------------------------|------------------------|
| Groups/ | Day 0                   | Day 1                  | Day 6                   | Day 12                  | Day 18                 | Day 24                  | Day 28                 |
| Days    |                         |                        |                         |                         |                        |                         |                        |
| Control | 573.00 <u>+</u> 255.236 | 73.67 <u>+</u> 27.163  | 273.33 <u>+</u> 115.632 | 387.33 <u>+</u> 178.655 | 178.00 <u>+</u> 3.786  | 466.67 <u>+</u> 80.683  | 137.83 <u>+</u> 23.786 |
| Test    | 630.00 <u>+</u> 180.290 | 146.67 <u>+</u> 53.778 | 638.00 <u>+</u> 42.142  | 485.00 <u>+</u> 34.664  | 160.33 <u>+</u> 23.140 | 359.00 <u>+</u> 251.407 | 118.33 <u>+</u> 25.912 |

Table 4: Mean plasma concentration of triglyceride (mg/dl) in normal albino rats administered aqueous leaf extract of *Dissotis rotundofolia*.

Values are represented as MEAN  $\pm$  SE of duplicate determinations. Mean with the same superscript letters are not statistically different at 95% confidence limit (p<0.05) one-way analysis of variance (ANOVA) $\pm$ Tukey-Kramer multi-comparison test.

Table 5: Mean plasma concentration of glucose(mg/dl) in normal albino rats administered aqueous leaf extract of *Dissotis rotundofolia*.

| Groups/ | Day 0                  | Day 1                  | Day 6                  | Day 12                 | Day 18               | Day 24                 | Day 28                 |
|---------|------------------------|------------------------|------------------------|------------------------|----------------------|------------------------|------------------------|
| Days    |                        |                        |                        |                        |                      |                        |                        |
| Control | 126.33 <u>+</u> 11.020 | 134.67 <u>+</u> 11.050 | 152.67 <u>+</u> 13.776 | 127.00 <u>+</u> 26.52  | 46.33 <u>+</u> 4.631 | 283.00 <u>+</u> 49.813 | 142.33 <u>+</u> 16.895 |
| Test    | 123.33 <u>+</u> 6.642  | 133.00 <u>+</u> 12.166 | 175.67 <u>+</u> 17.072 | 143.67 <u>+</u> 14.881 | 59.00 <u>+</u> 1.528 | 157.67 <u>+</u> 60.212 | 164.00 <u>+</u> 4.619  |

Values are represented as MEAN  $\pm$  SE of duplicate determinations. Mean with the same superscript letters are not statistically different at 95% confidence limit (p<0.05) one-way analysis of variance (ANOVA) $\pm$ Tukey-Kramer multi-comparison test.

Table 6: Mean weight of rats administered aqueous leaf extract of Dissotis rotundofolia

| Groups/Days | Basal | Day 0 | Day 1 | Day 6 | Day 12 | Day 18 | Day 24 | Day 28 |
|-------------|-------|-------|-------|-------|--------|--------|--------|--------|
| Control     | 180   | 180   | 180   | 200   | 200    | 193    | 200    | 193    |
| Test        | 193   | 193   | 193   | 173   | 173    | 160    | 146    | 146    |

Histological Findings and Explanations of Rats Administered With Aqueous Stem Extract of *Dissotis Rotundofolia* (Day 0, 1, 6, 12, 18, 24 and 28)



Figure 1: This slide reveals the histology of the liver stained with Haematoxylin and Eosin, X100 Slide A (Control) shows normal hepatic stroma with Hepatocytes, Sinusoid, and central vein. B, C and D are similar to the control. No significant changes were observed.



Figure 2: Slides show the histology of the Kidney stained with Haematoxylin and Eosin, X100 Slide A (Control) shows normal kidney stroma with Bowman's capsule with glomerulus (renal corpuscle) and renal tubules. B is similar to the control while C and D have fewer renal corpuscles in their cortex.

Histological Findings and Explanations of Rats Administered With Aqueous Leave Extract of *Dissotis Rotundofolia* (Day 0, 1, 6, 12, 18, 24 And 28)



Figure 3: Shows the histology of the Kidney stained with Haematoxylin and Eosin, X100 Slides A (control), B, C, and D show normal kidney stroma with Bowman's capsule with glomerulus (renal corpuscle) and renal tubules



Figure 4: Shows the histology of the liver stained with Haematoxylin and Eosin, X100 Slide A (Control) shows normal hepatic stroma with Hepatocytes, Sinusoid, and central vein. B, C, and D are similar to the control

#### Discussion

The hypoglycaemic potentials of medical plants have been documented (Mohammed, et al., 2023). The result of this study confirms the earlier report on the hypoglycaemic effect of the leaf extract of Dissotis rotundofolia in rabbits. Although several biologically active constituents were reported present in the extract. The prompt and remarkable reduction in both plasma glucose and triglyceride points to a mechanism of action different from that of sulphonylureas and unrelated to insulin secretion from pancreatic  $\beta$ -cells. It is now widely believed that an important signal for insulin secretion maybe the link between glucose and lipid metabolism; and long-term exposure of islet cells to high levels of fatty acids may result in βcell dysfunction (lipotoxicity), and diminished glucose-stimulated insulin secretion (Guo et al., 2023). It has been established that hyperlipidemia does not only increase the risk of ischaemic heart disease (HD) in diabetic patients but also may

impair glycaemic control, accelerates the progression of renal insufficiency, and increases mortality (Akbar, 2020). In this study, D. rotundifolia evinced a potent lowering of plasma triglyceride levels in both normoglycemic and diabetic rats. Adequate treatment of diabetes dyslipidemia through diet is critical in reducing risk and complications, and the role of medicinal plants in the treatment of diabetes is emerging. A high fiber content that reduces insulin secretion was used in the management of hyperlipidaemia in diabetic patients (Wolever, 2023). Furthermore, the effectiveness of the leaf extract of Trigonella foenum, L, hymenocardia acids, and Munaya koenigii as a cholesterol and triglyceride lowering agent has been reported (Devi and Raju 2020). The leaves were reported to contain soluble fibers, which decrease triglyceride absorptions and bile acids reabsorption by disrupting the intraluminal formation of micelles (Devi and Raju 2020). When compared to the aqueous leaf extract of Dissotis rotundofolia, similar mechanisms of action are suspected due to its high fiber content. D. rotundifolia has been shown in this study to decrease weight gain in rats. Although the mechanism of weight reduction was not explored, it may be related to its lipid-lowering effect. The ability of the extract to reduce both plasma triglyceride and glucose concentrations is remarkable. Continual administration of aqueous leaf extract of Dissotis rotundofolia may cause a significant decrease in mean Plasma triglyceride and glucose levels. Studies have shown also that type 1 diabetics who consumed aqueous leaf extract of Dissotis routundifolia have lower blood glucose levels and type 2 diabetics may have improved blood sugar, lipids, and insulin levels. Furthermore, compared to the control group, the Wistar albino rats treated with all dose levels of Dissotis rotundofolia leaf extract did not show signs of aggressiveness, vomiting, respiratory distress, salivation, and sedation. No mortality was recorded at all dose levels (2ml/kg body weight) of Dissotis rotundofolia leaf extract over the 24-h period. Daily observation over the 28 days post-treatment did not reveal evidence of latent Dissotis rotundofolia leaf extract-related toxicities. Histological tests have shown that aqueous leaf extract of Dissotis rotundofolia did not have any adverse effect on the liver and kidney of the rats while administration of aqueous stem extract of Dissotis rotundofolia demonstrated a significant increase in plasma glucose concentration (P<0.05). Aqueous stem extract of Dissotis rotundofolia demonstrated a statistically significant decrease in triglyceride concentrations in normal rats (P<0.05), loss of appetite and general body weakness were observed in the rats, and this led to a decrease in their body weight. The extract may not be advisable for diabetic patients as a study reveals daily consumption of stem extract of Dissotis rotundofolia is harmful for diabetic patients.

*D. rotundifolia* is very abundant and relatively cheap. This experiment may justify the use of this extract in the treatment of various diseases such as rheumatism, dysentery, venereal diseases, and stomach ailments and is also recommended as dietary inclusion for diabetics.

## Conclusion

The aqueous leaf extract of Dissotis rotundofolia has been found to be of clinical value in lowering mean plasma triglyceride and glucose concentrations if it could be translatable to human clinical studies, though this effect is marginal which may be due to the duration of administration. Studies have also revealed that aqueous leaf extract of Dissotis rotundofolia contains alkaloids, Flavonoids, phenols and polyphenols, tannins, and saponins. phenols and polyphenols are strong antioxidants that prevent oxidative damage to biomolecules such as DNA, lipids, and proteins which play a role in chronic diseases such as cancer and cardiovascular diseases. Flavonoids contain biological and pharmacological activities such as anti-allergic, anti-inflammatory, antioxidant, and antimicrobial activities. Tannin has antimicrobial activities. Saponin bind to bile salt (Aja et al., 2015). The significant reduction in both plasma triglyceride and glucose concentrations observed in this study may help in alleviating some of the complications associated with diabetic conditions.

**Conflict of Interest**: There is no conflict of interest in whatsoever form.

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