

BIOCHEMICAL AND MOLECULAR STUDIES of *Tapinanthus cordifolius* AND *Irvingia wombolu* LEAVES IN HIGH-FAT DIET AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

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JUNE, 2023

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR AWARD OF DOCTOR OF PHILOSOPHY (Ph.D) DEGREE IN BIOCHEMISTRY, DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA, OGUN STATE, NIGERIA.

JUNE, 2023

ACCEPTANCE

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DECLARATION

I, CHIKE-EKWUGHE, AMARACHI (08CO07510), hereby state that I conducted this research under the guidance and supervision of Prof. Olubanke O. Ogunlana and Prof. Abiodun H. Adebayo from the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria. I confirm that this thesis has not been submitted, in its entirety or in part, for the purpose of obtaining any other degree. Proper recognition has been given to all the sources of materials and scholarly publications utilized in this thesis.

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Signature and Date

CERTIFICATION

We certify that this thesis titled "**BIOCHEMICAL AND MOLECULAR STUDIES OF *Tapinanthus cordifolius* AND *Irvingia wombolu* LEAVES IN HIGH-FAT DIET AND STREPTOZOTOCIN-INDUCED DIABETIC RATS**" is an original work conducted by **CHIKE-EKWUGHE, AMARACHI (08CO07510)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Prof. Olubanke O. Ogunlana and Prof. Abiodun H. Adebayo. We have examined and found this work acceptable as part of the requirements for the award of Doctor of Philosophy (Ph.D) degree in Biochemistry.

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DEDICATION

I dedicate this thesis to my Creator, Saviour and Helper – God Almighty, for making me who I am today. He alone made this work a reality. To God be the glory.

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LIST OF ABBREVIATION

Bgl	Blood Glucose Levels
Bmi	Body Mass Index
Chrec	Covenant Health Research Ethics Committee
Cftr	Cystic Fibrosis Mutated Chloride Channel
Di	Diabetes Insipidus
Dm	Diabetes Mellitus
Gdg	Guideline Development Group
Hnf4a	Hepatocyte Nuclear Factor 4 Alpha
Hfd	High-Fat Diet
Idf	International Diabetes Federation
Iw	<i>Irvingia Wombolu</i>
Lpl	Lipoprotein Lipase
Mody	Maturity-Onset Diabetes in the Young
Nice	National Institute for Health and Care Quality
Niprd	National Institute for Pharmaceutical and Research Development
Ros	Reactive Oxygen Species
Snps	Single Nucleotide Polymorphisms
Stz	Streptozotocin
Sur	Sulfonylurea Receptor
Tc	<i>Tapinanthus Cordifolius</i>
T2d	Type 2 Diabetes
Tzds	Thiazolidinediones
Ins	The Insulin Hormone
Whr	Waist-To-Hip Circumference
Who	World Health Organization

ABSTRACT

Over 80% of the population from developing countries depend on medicinal plants as the primary health care source for managing diabetes. Globally, the prevalence of diabetes is rapidly on the increase and the high cost of treatment is a significant cause for concern. This study investigated the *in vitro*, *in silico* and *in vivo* phytochemical, antioxidant, toxicological and antidiabetic activities of *Irvingia wombolu* (IW) and *Tapinanthus cordifolius* (TC) leaf extracts. The leaves of TC and IW were extracted singly using 85% ethanol and fractionated sequentially using n-hexane, ethyl-acetate, butanol, and water. Phytochemical screening, *in vitro* antioxidant and antidiabetic studies of the extract and solvent fractions, were carried out using standard methods. The extract with the highest *in vitro* antioxidant and antidiabetic activities was further used for the *in silico* and *in vivo* studies. Bioactive compounds were identified using gas chromatography/mass spectrometry (GC/MS). *In silico* assessment of the identified compounds was carried out by molecular docking. Furthermore, acute and sub-chronic toxicity studies were assessed in male and female mice and rats respectively. The animal diabetes model was induced using a high-fat diet and a single low dose of streptozotocin (40 mg/kg). Blood glucose levels of diabetic rats were monitored at various time intervals for 6 h after TC extract administration (200, 400 and 800 mg/kg/day) and then every 7 days till the 21st day. The oral glucose tolerance test (OGTT) was carried out on normal rats by administering 2000 mg/kg glucose solution and measuring the blood glucose value every hour for 2 h. At the end of the toxicological and diabetes studies, the animals were euthanized and sacrificed. Organs were harvested for histological and molecular studies, while serum was collected for haematological and biochemical analyses. The preliminary phytochemical screening revealed the presence of phenols, tannins, flavonoids, steroids, and anthraquinones in both plants; saponin, terpenes in IW with cardiac glycosides and alkaloids in TC. Quantitative analysis of the plant showed that the crude extract of TC is richer in phenols, flavonoids and tannins than IW. The TC showed the highest antioxidant activity and exhibited significant inhibition on α -amylase, α -glucosidase and lipase with IC₅₀ values of 22.72, 542 and 494 μ g /mL, respectively, when compared to IW with values of 37.89, 966.75 and 910.49 μ g /mL respectively. From the *insilico* studies, the alpha-tocopherol-beta-D-mannoside, 5-ergosterol, acetosyringone, benzaldehyde, 4-(ethylthio)-2,5-dimethoxy, 5-trimethoxybenzoic acid, and campesterol of the 44 identified GC-MS phytoconstituents from TC extract were established as potent inhibitors of α -amylase, α -glucosidase and lipase. The acute and sub-chronic toxicity studies showed no deaths at the highest extract dose. No significant ($p < 0.05$) change was observed in haematological and biochemical parameters. The histological architecture of all organs remains unchanged. The 21-day antidiabetic study showed gross alteration in blood glucose level and biophysical, liver and kidney function parameters. Organ oxidative stress, inflammatory cytokines, and hormonal and molecular parameters in diabetic rats were reversed with TC, most notably at 800 mg/kg. This study validates the ethnobotanical use of TC as an antidiabetic agent.

Keywords: *Antidiabetic, in silico, Blood glucose, Irvingia wombolu, Tapinanthus cordifolius, Toxicological studies, Molecular studies*