BIOCHEMICAL AND IN-SILICO STUDIES OF LYCOPENE EXTRACT AND Solanum lycopersicum-Daucus carota FOOD-MIX FOR CERVICAL CANCER PREVENTION

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A Ph.D. THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF Ph.D. DEGREE IN INDUSTRIAL CHEMISTRY OF COVENANT UNIVERSITY, OTA, OGUN STATE, NIGERIA

JULY, 2024

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Industrial Chemistry in the Department of Chemistry, College of Science and Technology, Covenant University, Ota, Nigeria.

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DECLARATION

I, ADEMOSUN, OLABISI THERESA (14PCC00650), declare that this research was carried out by me under the supervision of Prof. Kolawole O. Ajanaku and Prof. Adebayo H. Adebayo of the Department of Chemistry, Landmark University and Department of Biochemistry, College of Science and Technology, Covenant University, Ota respectively. I attest that this thesis has not been presented either wholly or partially for the award of any degree elsewhere. All the sources of materials and scholarly publications used in this thesis have been duly acknowledged.

ADEMOSUN, OLABISI THERESA

Signature and Date

CERTIFICATION

We certify that the thesis titled 'BIOCHEMICAL and In-silico STUDIES OF LYCOPENE EXTRACT and Solanum lycopersicum-Daucus carota FOOD-MIX FOR CERVICAL CANCER PREVENTION' is the original work carried out by ADEMOSUN, OLABISI THERESA (14PCC00650) in the Department of Chemistry, Covenant University, Ota, Ogun State, Nigeria under the supervision of Prof. Kolawole O. Ajanaku of Department of Chemistry and Prof. Abiodun H. Adebayo of Department of Biochemistry. We have examined and found this work acceptable as part of the requirements for the award of the degree of Doctor of Philosophy in Industrial Chemistry.

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DEDICATION

I dedicate this work to the Almighty God, my source of wisdom and strength, for His ever-abiding presence throughout this project. To Him alone be all the glory. Amen.

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LIST OF MAJOR ABBREVIATIONS

ARDS	Acute Respiratory Distress Syndromes
BHT	Butylated Hydroxytoluene
CC	Cervical Cancer
CVD	Cardiovascular Disease
CIN	Cervical Intraepithelial Neoplasia
DPPH	2,2-Diphenyl-1-picrylhydrazy
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic Acid
GC-MS	Gas Chromatography – Mass Spectrometry
FTIR	Fourier Transform infrared Spectroscopy
FRIN	Forestry Research Institute of Nigeria
FRAP	Ferric Reducing Ability of Plasma
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HSIL	High Grade Squamous Intraepithelial Lesion
IARC	International Agency for Research on Cancer
IC ₅₀	Half Maximal Inhibitory Concentration
IBD	Inflammatory Bowel Disease
LIC	Low- Income Countries
LSIL	Low Grade Squamous Intraepithelial
MS	Mass Spectrometry

MAE	Microwave Assisted Extraction
MT	Magness Taylor
MTT	(3-(4-5-dimethylthiazol-2-yl)-2,5- diphenyl-2H- tetrazolium bromide
NMR	Nuclear Magnetic Resonance
PAP	Papanicolaou Smear
ROS	Reactive Oxygen Specie
SCJ	Squamocolumnar Junction
SFE	Supercritical Fluid Extraction
SSC	Soluble Solid Content
TAC	Total Antioxidant Capacity
ТСА	Tomato – Carrots Aqueous Extract
TCE	Tomato – Carrots Ethanol Extract
UAE	Ultrasound- Assisted Extraction
WHO	World Health Organization

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ABSTRACT

Cervical cancer poses a significant global health burden, necessitating the development of effective chemopreventive strategies. This study investigates the nutritional, antioxidant, in-vitro cytotoxic assessment of a functionalised tomato (Solanum lycopersicum) -carrot (Daucus carota) food mix and the effect of extracted lycopene from tomato for apoptotic assessment on cervical cancer cell lines. Proximate analysis was conducted to determine the nutritional composition, while pH, titratable acidity, and bulk density measurements evaluated the physicochemical properties of the food samples. Trace metals analysis was used to assess the presence of potentially harmful metals, and yield quantification was used to determine the extraction efficiency using ethanol and aqueous solvents. Additionally, cytotoxic effects on cervical cancer cells and molecular docking analysis were performed to understand the biological activities and binding interactions of lycopene. The proximate analysis revealed the food samples' moisture content (0.44 to 0.54%), ash content (71.82 to 73.52 %), crude protein content (8.41 to 18.10 %), crude fiber content (3.787 to 6.547 %), crude fat content (0.10 to 8.72 %), carbohydrate content (0.628 to 6.193 %), and total energy content (87.012 to 136.892 kcal). The pH values ranged from 6.6 to 7.2, indicating slight acidity variation. Titratable acidity values ranged from 1.44 to 23.00 (lactic acid %), demonstrating a decrease in acidity. Bulk density analysis revealed values ranging from 0.44 g/cm³ to 0.59 g/cm³. Trace metals analysis showed a high iron (Fe) concentration, with no significant presence of lead (Pb) or cadmium (Cd). Yield quantification of the concentrate demonstrated higher extraction efficiency using 70% ethanol solvent compared to the aqueous solvent. Cytotoxicity testing on cervical cancer cells revealed that the ethanol extract of tomatoes exhibited the highest cytotoxic inhibition (40.28 %), followed by the aqueous extract (35.21%), while carrots displayed minimal cytotoxic effects. Furthermore, lycopene extract exhibited dose-dependent cytotoxicity, with the highest concentration (1000 µg/mL) showing remarkable inhibition (74.2%). Molecular docking analysis suggested favourable interactions between lycopene and the pro-apoptotic protein (BAX 1), indicating its potential to induce apoptosis in cervical cancer cells. However, the chemotherapeutic drug Camptothecin demonstrated stronger interactions. Molecular dynamics simulations confirmed the stability of lycopene-protein complexes throughout the 100-ns simulation, supporting their potential as anticancer agents. Overall, the study highlights the cytotoxic effects of tomato-carrot food extracts and lycopene on cervical cancer cells. The ethanol extract of tomatoes and the lycopene extract exhibited notable cytotoxicity, while the aqueous extract and carrots showed minimal inhibition. Molecular docking analysis revealed the potential of lycopene to promote apoptosis through interactions with the pro-apoptotic protein BAX 1. The stability analysis of lycopene-protein complexes further supported its anticancer properties. These findings contribute to our understanding of the molecular processes behind lycopene's anticancer activities and provide insights for future research on innovative chemo preventive treatments for cervical cancer. More so, the nutritional and physicochemical properties of the tomato-carrot food mix, along with its hydroethanolic extract as an efficient extraction medium for bioactive compounds, hold promise for its application in the food industry.

Keywords: Antioxidants, Cervical Cancer, Nutraceuticals, Lycopene, Tomato-carrot blend, Prophylactics