

***IN SILICO* PREDICTION AND BIOCHEMICAL VALIDATION OF
PROTEIN TARGETS IN *Anopheles gambiae***

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DECEMBER, 2022

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PROTEIN TARGETS IN *Anopheles gambiae***

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**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE
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OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND
TECHNOLOGY, COVENANT UNIVERSITY, OTA, OGUN STATE,
NIGERIA**

DECEMBER, 2022

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 Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria.

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DECLARATION

I, **ADEDEJI, EUNICE OLUWATOBILOBA (17PCP01674)** declare that this research was carried out by me under the supervision of Prof. Olubanke O. Ogunlana of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria and Dr. Segun A. Fatumo of the Department of Non-communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom. I attest that the thesis has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this thesis are duly acknowledged.

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Signature and Date

CERTIFICATION

We certify that this thesis titled “*IN SILICO* PREDICTION AND BIOCHEMICAL VALIDATION OF PROTEIN TARGETS IN *Anopheles gambiae*” is an original research work carried out by **ADEDEJI, EUNICE OLUWATOBILOBA (17PCP01674)** 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 Dr. Segun A. Fatumo. 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

This work is dedicated to God Almighty, the source of my life, strength, and wisdom. In Him alone I have my being.

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LIST OF ABBREVIATIONS

1Elf	Elongation factor 1-alpha
2Elf	Elongation factor 2
3HKT	3-hydroxykynurenine transaminase
ABC transporters	ATP-binding cassette transporters
AChE	Acetylcholinesterase
AgAQP3	<i>An. gambiae</i> 's Aquaporin 3
AgaCA	<i>An. gambiae</i> 's β -class carbonic anhydrase
AgTreT	<i>An. gambiae</i> 's Trehalose transporter
AMP	Antimicrobial peptides
<i>An. gambiae</i>	<i>Anopheles gambiae</i>
Arg	Arginase
APL1	<i>Anopheles Plasmodium</i> responsive leucine-rich repeat protein 1
ATP	Adenosine triphosphate
BiGG reconstruction	Biochemical, genetic and genomic reconstruction
Cas	Clustered regularly interspaced palindromic repeats associated protein
CEs	Carboxylesterases
CLIPs	Clip domain serine proteases
CRISPR	Clustered regularly interspaced palindromic repeats
crRNA	CRISPR RNA
CTLs	C-type lectin-like proteins
CYP 450s	Cytochrome P450s
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
dsRNA	Double-stranded RNA
FBA	Flux Balance Analysis
FREP 1	Fibrinogen related proteins 1
GABA	Gamma-aminobutyric acid

GILT	gamma-interferon–inducible lysosomal thiol reductase
GLUT	Glucose transporter
GPCRs	G protein-coupled receptors
GPR	Gene-protein-reaction
gRNA	Guide RNA
GSMM or GEM	Genome-scale metabolic network model
GSTs	Glutathione S-transferases
HNH	Histidine asparagine histidine
HR	Homology directed repair
HSP	Heat shock 70kDa protein 1/8
IAPs	Inhibitor of apoptotic proteins
ILPs	Insulin-like peptides
IRS	Indoor residual spraying
ITNs	Insecticide-treated nets
KMO	Kynurenine 3-monooxygenase
LLINs	Long-lasting insecticidal nets
LRIM1	Leucine-rich repeat immune protein 1
LRRs	Leucine-rich repeat proteins
mRNA	messenger RNA
MRTC	Malaria Research and Training Center
NHEJ	Non homologous end joining
NO	Nitric oxide
NOS	Nitric oxide synthase
NOX5	NADPH oxidase 5
<i>P. berghei</i>	<i>Plasmodium berghei</i>
<i>P. falciparum</i>	<i>Plasmodium falciparum</i>
PAH	Phenylalanine-4-hydroxylase
PAM	Protospacer adjacent motif
PAMP	Pathogen-associated molecular patterns
Pbf	Post-blood-feeding
Pbm	Post blood meal
PCR	Polymerase chain reaction
PDB	Protein data bank

PO	Phenoloxidase
PRR	Pattern recognition receptors
PULPZ	Percentage of unreached products larger than zero
QPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RNAi	RNA interference
RT-PCR	Real-time polymerase chain reaction
RuvC	Resistance to ultraviolet C
SBML	Systems Biology Markup Language
sgRNA	Single guide RNA
siRNA	small interfering RNA
TALEN	Transcription activator-like effector nucleases
<i>T. cinnabarinus</i>	<i>Tetranychus cinnabarinus</i>
TEPs	Thioester-containing proteins
trancRNA	Trans-activating CRISPR RNA
Tre	Trehalase
tRNA	Transfer RNA
XA	Xanthurenic acid
YFP	Yellow florescent dye
ZFN	Zinc finger nucleases

ABSTRACT

Malaria, an endemic disease in sub-Saharan Africa, is transmitted by female *Anopheles* mosquitoes. The major malaria vector control strategy has remained the use of insecticides. However, resistance in *Anopheles* to all classes of existing insecticides motivates the identification of novel targets for malaria vector control. *Anopheles* metabolic proteins represent a repertoire of possible targets, however, finding potential targets using experimental methods alone is a tall order. This study aimed to identify and modulate genes central for *An. gambiae*'s survival or *Plasmodium berghei* clearance in the mosquito using *in silico* and biochemical techniques. A multi-compartment central metabolic model of *An. gambiae* was constructed using Reconstruction, Analysis and Visualization of Metabolic Networks (RAVEN) toolbox, manual curation was performed, and essential genes were predicted using chokepoint and percentage of unreached products larger than zero criteria. Experimental validation of selected genes was carried out using RNA interference (RNAi) and chemical inhibition methods. Double-stranded RNA (138 nL of 5 $\mu\text{g}/\mu\text{L}$) for selected genes and LacZ was injected into respective groups of between 2 to 4 days old female *An. gambiae* mosquitoes. Seventy (70) female mosquitoes per treatment were injected for survival experiments, and survival was monitored from day 2 post-injection until the death of all mosquitoes. Two hundred (200) female mosquitoes per treatment group (LacZ and Arginase) were injected for experiments involving infection with *P. berghei*. Parasite infection was performed 48 h after injection, and oocyte count was determined. Validamycin was administered at varying concentrations to third-stage (L3) larvae and first-stage (L1) larvae, then biochemical parameters and effect on gene-expression were investigated. Appropriate statistical analyses were carried out on the results. The central metabolic model consisted of 570 reactions, 471 metabolites, and 833 genes distributed across cytoplasm, mitochondria, and extracellular compartments. Three genes out of the 106 genes predicted from the network were selected, and three other non-metabolic genes for experimental validation in *An. gambiae* G3 mosquitoes based on established literature alluding to their essentiality. The six genes validated were Trehalase, Arginase, 3-hydroxykynurenine transaminase (3HKT), Heat shock 70kDa protein (HSP), elongation factor 2 (2elf), and elongation factor 1-alpha. Knockdown of HSP and 2elf resulted in a significant reduction ($p < 0.05$) in the percentage survival of mosquitoes compared to control groups. Similarly, knockdown of arginase led to a marked reduction ($p < 0.05$) in the number of oocytes count per midgut compared to the control group. In addition, larval treatment with validamycin A, an inhibitor of trehalase resulted in marked larval death at 48 h in a dose-dependent manner and retarded development of mosquitoes. Lethal concentration 50 (LC_{50}) was 167.1 ppm in L3 larvae and 30.71 ppm in L1 larvae. Treatment with validamycin increased trehalose concentration at all concentrations considered and expression of insulin-like peptide2 at 500 ppm, 24 h after validamycin A exposure. This study suggests trehalase as a possible larvicide target, revealed the importance of HSP and 2elf for mosquito survival, and arginase for parasite development. These may serve as potential targets for vector control. Further studies to identify suitable inhibitors for these targets is recommended.

Keywords: *Anopheles*, *Elongation factor*, *Heat shock*, *Plasmodium*, *Trehalase*, *Validamycin*, *Vector control*.