

Optimization of Ultrasonic-Assisted Extraction (UAE) of Phytochemicals from *Securinega virosa* through Response Surface Methodology, Characterization and *In-vitro*, *In-silico* Modelling Antioxidant Evaluation

Muhammed, Taofeeq Olayemi¹, Opeyemi N. Avoseh¹

¹ Lagos State University, Ojo, Lagos State, Nigeria.

1 INTRODUCTION & JUSTIFICATION

Background

- Natural products have been pivotal in drug discovery due to their diverse chemical structures and broad pharmacological activities.
- Bioactive constituents such as alkaloids, flavonoids, terpenoids, saponins and phenolic acids contribute to their therapeutic potential.
- Ultrasonic-Assisted Extraction (UAE) is a green and efficient technique with interacting parameters that affect extraction efficiency.
- Response Surface Methodology (RSM) is a statistical tool used to model variables, evaluate interactions and predict optimal conditions.



Securinega virosa leaves

Problem Statement / Justification

- Securinega virosa* is widely used in traditional medicine, yet lacks sufficient scientific validation.
- Conventional methods (maceration, Soxhlet) are inefficient, time-consuming, solvent-intensive and may degrade thermolabile compounds.
- The combined effects of key process variables on yield, TPC, TFC and TAC remain inadequately optimized and not scientifically established.
- Limited report on *in-vitro* antioxidant and *in-silico* molecular docking to validate the pharmacological potential of the plant's compounds.



Air-dried leaf powder

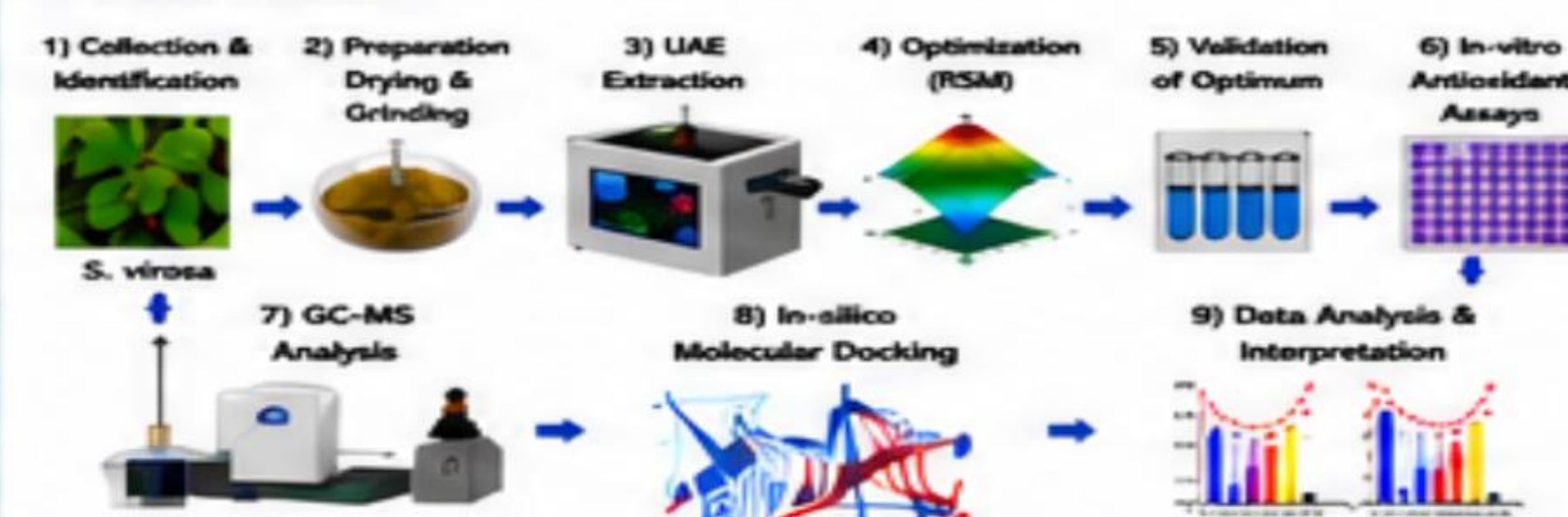
Aim

- To optimize the extraction of phytochemicals from *Securinega virosa* using RSM, characterize its constituents via GC/MS and assess the antioxidant properties *in-vitro* and *in-silico* molecular modelling approach.



2 EXPERIMENTAL DESIGN & METHODOLOGY

A. Overall Workflow



B. UAE Process Schematic



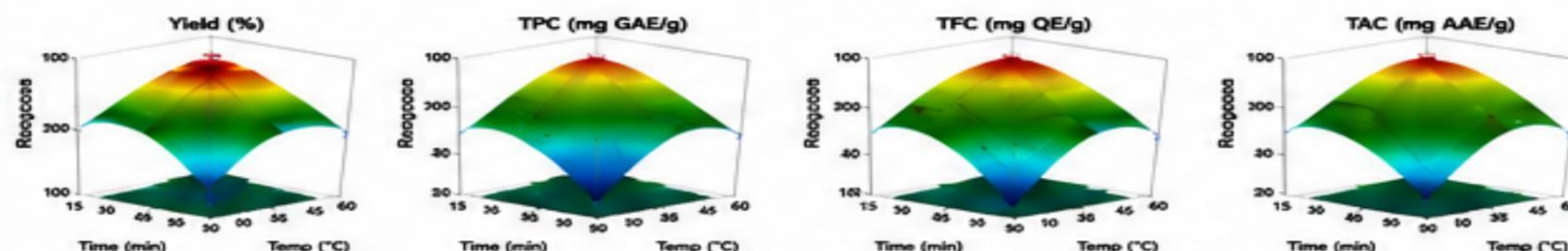
C. Experimental Design (Box-Behnken D-H)

Independent Variables & Levels				
Variables	Code	Low (-1)	Center (0)	High (+1)
Temperature (°C)	A	30	45	60
Time (min)	B	15	30	45
Solvent/Solid Ratio (mL/g)	C	25	35	45

Response Optimized

- Extraction Yield (%)
- Total Phenolic Content (TPC)
- Total Antioxidant Capacity (TAC)

3.1 Response Surface Optimization (3D Surface Plots)



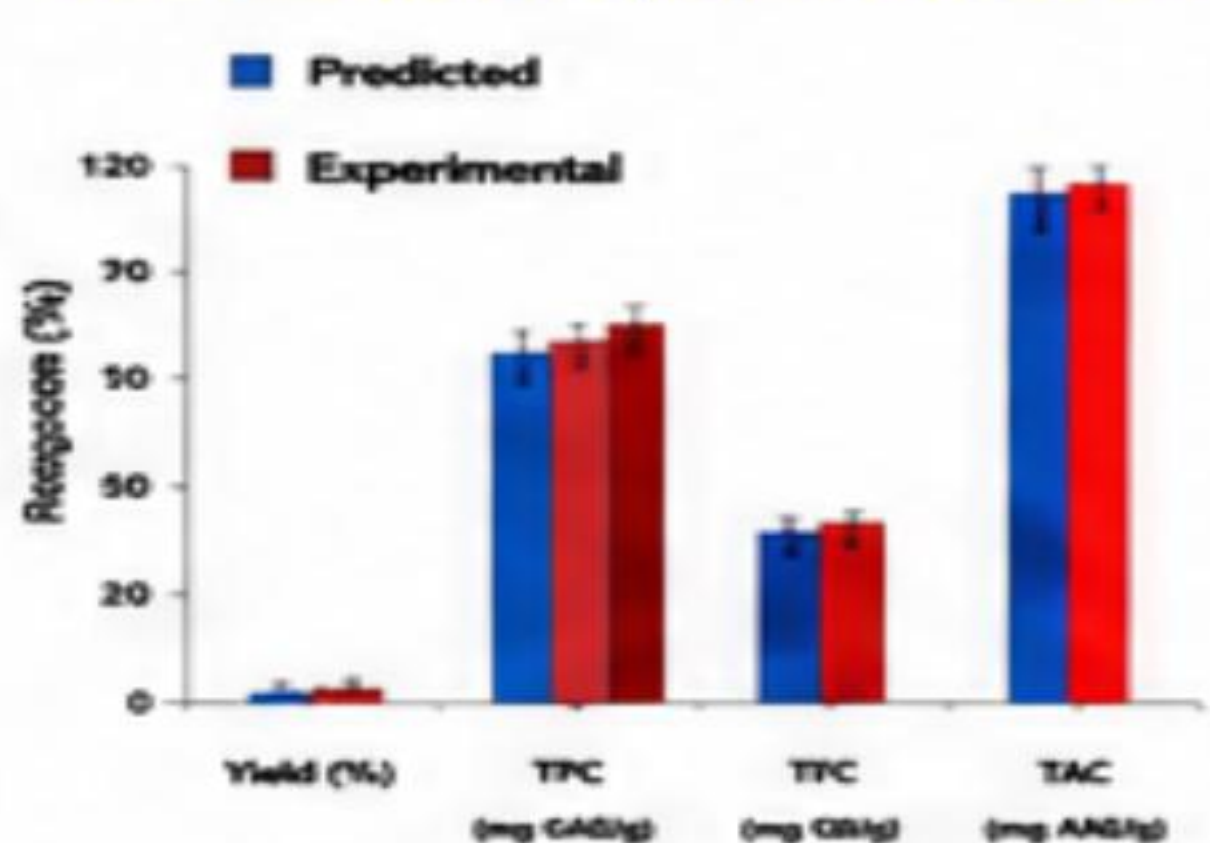
3D plots showing significant effects and interactions of independent variables on responses.

3.2 Optimization Summary

Response	Model R ²	Adjusted R ²	Predicted R ²	Adeq. Precision	Lack of Fit (P-value)	Optimum Condition			Predicted Value	Experimental Value	% Error
						Temp (°C)	Time (min)	Solvent/Solid (mL/g)			
Yield (%)	0.9863	0.9721	0.9304	24.56	0.142	62.3	45.5	34.2	18.76	18.42	1.81
TPC (mg GAE/g)	0.9912	0.9817	0.9516	28.74	0.098	63.1	46.2	33.8	92.47	90.81	1.80
TFC (mg QE/g)	0.9895	0.9768	0.9391	26.11	0.118	64.0	47.0	34.9	48.63	47.69	1.93
TAC (mg AAE/g)	0.9904	0.9792	0.9463	27.68	0.106	62.7	45.8	34.5	115.32	112.98	2.03

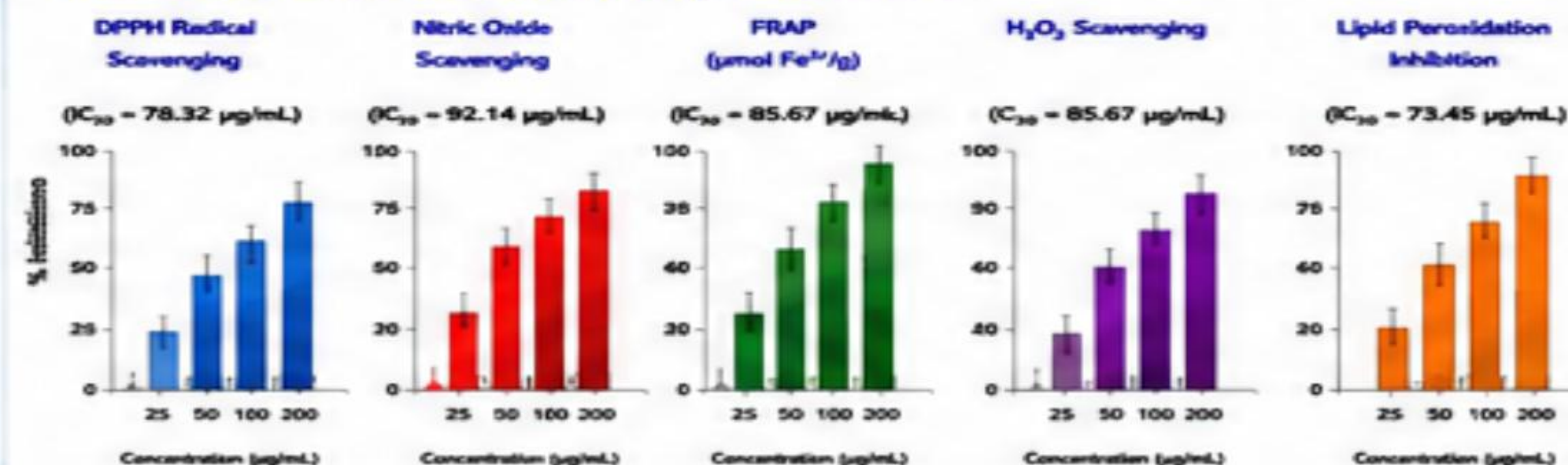
R² > 0.98 indicates excellent model fit and reliability.

3.3 Validation of Optimized Conditions



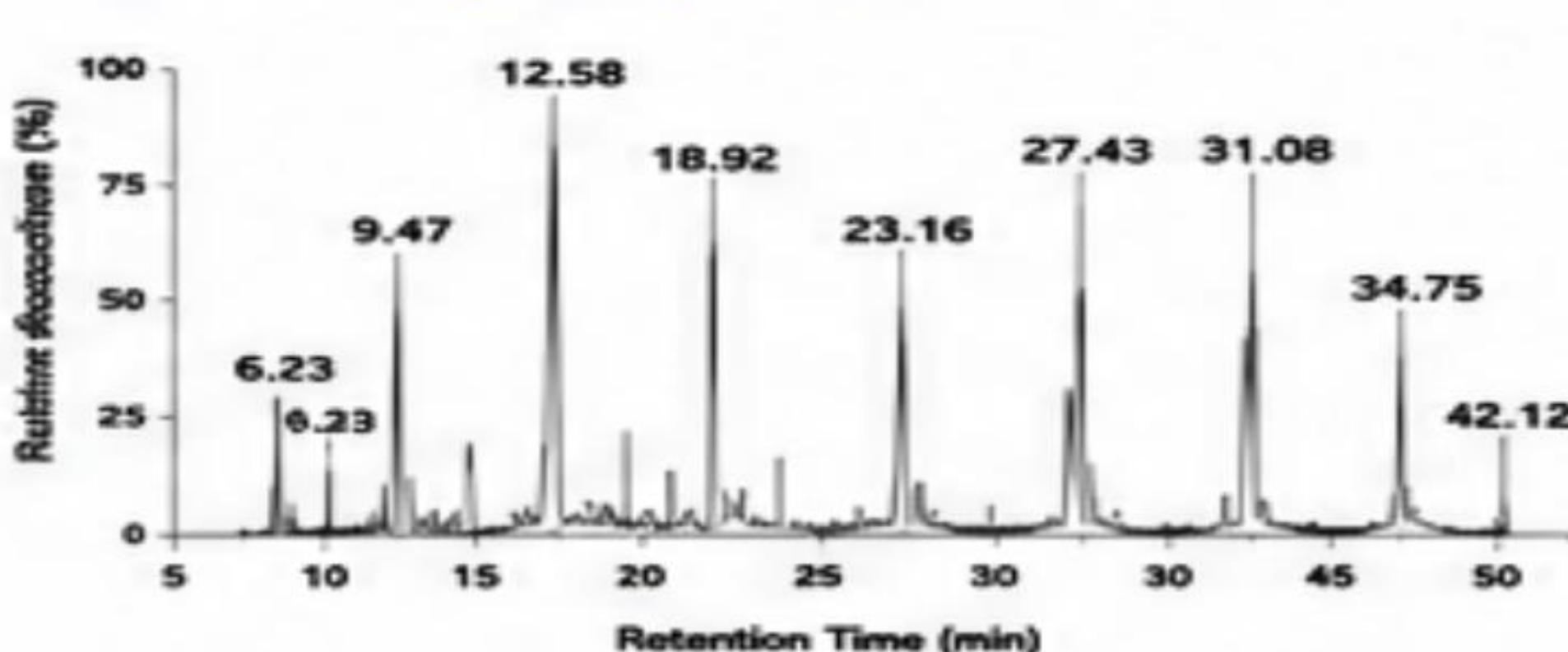
Close agreement between predicted and experimental values confirms adequacy of the model.

3.4 *In-vitro* Antioxidant Activity of Optimized Extract



Dose-dependent antioxidant activity of *S. virosa* extract in multiple *in-vitro* assays.

3.5 GC-MS Analysis of Optimized Extract



GC-MS chromatogram and major bioactive constituents identified in the optimized extract.

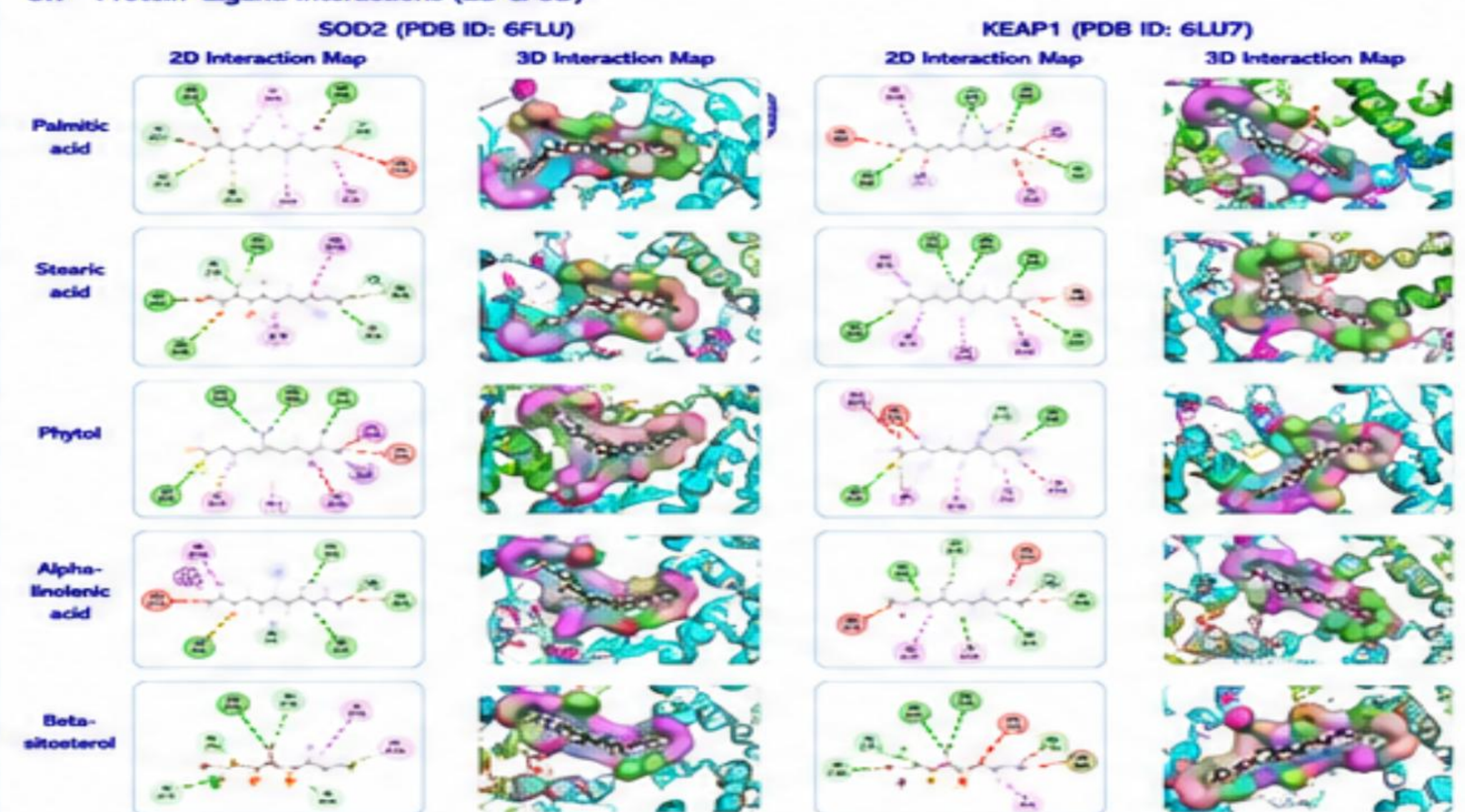
Major Compounds Identified

No.	Compound Name	RT (min)	Area (%)	Molecular Formula
1	Palmitic acid	15.74	18.32	C ₁₆ H ₃₂ O ₂
2	Stearic acid	18.92	15.76	C ₁₈ H ₃₆ O ₂
3	Phytol	23.16	12.43	C ₂₉ H ₆₀ O
4	Alpha-linolenic acid	27.43	9.85	C ₁₈ H ₃₀ O ₂
5	Beta-sitosterol	31.08	8.67	C ₂₇ H ₅₀ O

3.6 *In-silico* Molecular Docking (Docking Scores: kcal/mol)

Ligand	SOD2 (PDB ID: 6FLU)		KEAP1 (PDB ID: 6LU7)	
	Binding Energy (kcal/mol)	RMSD (Å)	Binding Energy (kcal/mol)	RMSD (Å)
Palmitic acid	-5.79	1.42	-6.01	1.36
Stearic acid	-6.12	1.35	-6.38	1.31
Phytol	-6.48	1.28	-6.72	1.22
Alpha-linolenic acid	-6.93	1.19	-7.21	1.15
Beta-sitosterol	-7.80	1.05	-8.43	1.02

3.7 Protein-Ligand Interactions (2D & 3D)



Interactions

- Conventional H-bond
- Carbon H-bond
- PI-Alkyl
- PI-PI Stacked
- PI-Sigma
- Hydrophobic (Alkyl)
- PI-PI T-shaped

4 CONCLUSION, IMPLICATION & SDG IMPACT

Key Findings

- UAE conditions were successfully optimized using RSM to maximize yield, TPC, TFC and TAC.
- The optimized extract exhibited strong antioxidant activities across multiple *in-vitro* assays.
- GC-MS revealed the presence of bioactive phytochemicals with known antioxidant potential.
- In-silico* docking showed strong binding affinity of key compounds with SOD2 and KEAP1 targets, suggesting cytoprotective and anti-oxidative mechanisms.

Implication

- S. virosa* is a source of natural antioxidants and supports its traditional use.
- Provides scientific evidence for development of nutraceuticals and phytopharmaceuticals.
- Promotes green extraction technology and sustainability in alignment with SDG 3 (Good Health & Well-being).