



JOURNAL OF SCIENCE, TECHNOLOGY AND EDUCATION (JSTE)



**A PUBLICATION OF THE
DEPARTMENT OF SCIENCE,
TECHNOLOGY AND MATHEMATICS
EDUCATION (STME),
NASARAWA STATE UNIVERSITY, KEFFI, NIGERIA**



VOLUME 10

ISSN: 2651-5539

THERMALLY-ENHANCED EXTRACTION OF PHENOLIC-RICH ANTIOXIDANTS FROM *ACHYRANTHES ASPERA* LINN LEAVES: GREY WOLF MULTI-OBJECTIVE OPTIMIZATION AND GAUSSIAN PROCESS REGRESSION-BASED MODELLING APPROACHES

Ekot U. J.,¹ Akatobi K. N.,¹ and Adeyi O.^{1*}

¹Department of Chemical Engineering, Michael Okpara University of Agriculture, PMB 7267, Umudike, Abia State, Nigeria.

Corresponding author: adeyioladayo350@yahoo.com

Citation: Ekot U. J., Akatobi K. N., & Adeyi O. (2026). Thermally-enhanced extraction of phenolic-rich antioxidants from *Achyranthes Aspera* linn leaves: grey wolf multi-objective optimization and gaussian process regression-based modelling approaches. *Journal of Science, Technology, and Education (JSTE)*; www.nsukjste.com/. 10(33), 420 – 446.

Abstract

This research investigated the application of heat-assisted extraction (HAE) for the recovery of phenolic-rich antioxidants from *Achyranthes aspera* Linn leaves, employing Grey Wolf Optimizer (GWO) for multi-objective optimization and Response Surface Methodology (RSM) alongside Gaussian Process Regression (GPR) for predictive modelling. The experimental design was based on the central composite design (CCD) approach available in RSM. Key extraction parameters, including operating temperature (OT), solid-to-liquid ratio (S/L), and extraction time (ET), were optimized to maximize total polyphenol content (TPC), extract yield (EY), and antioxidant activity (AA). Both CCD-RSM and GPR with a Rational Quadratic kernel (GPR-RQ) demonstrated high predictive accuracy for the extraction outcomes, with CCD-RSM exhibiting slightly superior performance within the defined experimental domain and was therefore integrated with GWO for optimization purposes. Validation

experiments under optimal conditions achieved a TPC of 36.2023 mg GAE/g db, EY of 18.2291%, and AA of 6.2178 μ M AAE/g dw, with percentage relative standard deviations (% RSD) of 7.64%, 1.13%, and 7.46%, respectively, confirming excellent agreement between predicted and experimental results. The findings emphasize the effectiveness and simplicity of HAE as scalable extraction method while showcasing the robustness of GWO, CCD-RSM and GPR as advanced tools for optimization and modelling in bioactive compound extraction. This research provides key insights into the development of eco-friendly industrial methods for extracting phenolic antioxidants, with promising applications in the food, pharmaceutical, and cosmetic sectors.

Keywords: Heat-assisted extraction, phenolic antioxidants, *Achyranthes aspera* Linn, Gaussian Process Regression, Grey Wolf Optimizer

1. Introduction

Achyranthes aspera Linn, commonly known as Prickly Chaff Flower, is a plant of notable botanical and medicinal significance. This herbaceous

perennial belongs to the Amaranthaceae family and is distinguished by its prickly inflorescences and broad distribution across tropical and subtropical regions of Asia and Africa (Mandefro *et al.*, 2024). The plant is characterized by its erect, rigid stems

and spike-like clusters of greenish flowers. Its botanical relevance is evident in its diverse ecological roles and adaptations, making it a valuable subject for study in plant biology and ethnobotany (Sanguri *et al.*, 2011). Traditionally, *A. aspera* has been used across cultures for its therapeutic properties. It is used in traditional medicine systems for treating conditions such as fever, asthma, and gastrointestinal disturbances (Verma *et al.*, 2021; Ndhlala *et al.*, 2015). In Ayurvedic medicine, the plant is prized for its anti-inflammatory, analgesic, and diuretic effects, highlighting its role in managing inflammatory and pain-related ailments (Rehman *et al.*, 2018). The medicinal importance of *A. aspera* is underpinned by its rich phytochemical composition, which includes alkaloids, phenolic, flavonoids, and saponins (Sanguri *et al.*, 2011). These compounds contribute to its anti-inflammatory, antioxidant, and antimicrobial activities. Recent studies have validated its traditional uses, demonstrating significant efficacy in alleviating inflammation, reducing pain, and combating infections. Such findings underscore the plant's potential in modern therapeutic applications and its integration into contemporary pharmacological practices (Nargatti *et al.*, 2021).

Phenolic compounds are well known for their potent antioxidant effects, which are essential for counteracting cellular damage caused by free radicals and reducing the risk of long-term health disorders. These compounds, including flavonoids, phenolic acids, and tannins, possess remarkable free-radical-scavenging properties and effectively suppress oxidative processes. This protective action helps maintain cellular integrity and supports overall physiological health (Sun & Shahrajabian, 2023). The role of phenolic compounds as antioxidants is well documented in the scientific literature. Their mechanism of action involves neutralizing free radicals, binding to metal ions to prevent catalytic oxidative reactions, and modulating various oxidative stress pathways. Their ability to donate hydrogen atoms or electrons stabilizes free radicals, thus preventing cellular damage (Shahidi & Ambigaipalan, 2015). This antioxidant activity is instrumental in reducing the risk of health

conditions such as heart-related ailments, malignant growths, and nervous system disorders (Rahman *et al.*, 2021). The health benefits and applications of phenolic-rich extracts extend beyond their antioxidant capabilities. These extracts are utilized across the medical, beauty, and nutritional sectors owing to their properties that reduce inflammation, combat microbial infections, and inhibit tumor growth (Bhuyan & Basu, 2017). The therapeutic potential of phenolic-rich extracts is harnessed in developing functional foods, dietary supplements, and topical formulations, enhancing health and well-being through their bioactive components (Rasouli *et al.*, 2017). A wide range of phenolic compounds has been identified in *A. aspera* (Ndhlala *et al.*, 2015), and these need to be optimally recovered for industrial applications.

Extraction methods are essential in recovering bioactive compounds from plants, and various techniques have been developed to optimize this process. Traditional solvent extraction remains a widely adopted technique because of its straightforward approach and reliable efficiency. This technique involves using solvents with different polarities to selectively extract target compounds from plant materials. However, solvent extraction has some drawbacks, including the use of large volumes of potentially harmful organic solvents and longer extraction times, which can be inefficient and environmentally unfriendly (Adeyi *et al.*, 2022; Zakaria *et al.*, 2020). In response to these limitations, advanced methods such as ultrasound-assisted extraction (UAE) have been developed. UAE employs high-frequency sound waves to generate cavitation bubbles in the solvent, thereby rupturing cellular structures and promoting the release of bioactive constituents. This method is particularly advantageous because it reduces both solvent consumption and extraction time and is suitable for temperature-sensitive compounds (Zhang *et al.*, 2018). Other advanced techniques used for the recovery of bioactive compounds from plant matrices include microwave-assisted extraction (Oke *et al.*, 2023; Olalere *et al.*, 2021), supercritical fluid extraction (Lopez-Hortas *et al.*, 2022) and pressurised liquid extraction (Pereira *et al.*, 2019). All these methods have been reported to

enhance extract yield and decrease extraction time (Zhang *et al.*, 2018). Heat-assisted extraction (HAE) is a recent innovation in extraction technology that applies elevated temperatures to improve the solubility and diffusion rates of target compounds, thereby significantly increasing extraction efficiency. HAE is advantageous because it combines traditional solvent extraction with thermal energy, thereby reducing processing times and energy consumption. This method is particularly useful when dealing with thermally stable compounds, as heat can accelerate extraction of beneficial compounds from plant material without degrading the compounds of interest (Adeyi *et al.*, 2023). Frequently examined critical parameters in HAE include processing temperature, extraction duration, and the solid-to-solvent ratio. (Alanzi *et al.*, 2024). However, these factors needed to be optimized to obtain bioactive extracts of high quality and integrity (Olalere *et al.*, 2021; Oke *et al.*, 2001).

Optimizing extraction parameters in natural product processing is fraught with challenges due to the multifaceted nature of the processes involved. The primary difficulty lies in the need to simultaneously optimize multiple parameters such as temperature, solvent type, pH, and extraction time, all of which can interact in complex ways to influence the yield, purity, and bioactivity of the extracted compounds (Zhang *et al.*, 2018). Traditional optimization methods, which often rely on a one-variable-at-a-time approach, fail to account for these interactions, leading to suboptimal results (Bezerra *et al.*, 2008). Additionally, the variability in raw materials, such as differences in plant species, maturity, and environmental conditions, further complicates the optimization process, making it difficult to achieve consistent and reproducible outcomes. In this context, multi-objective optimization (MOO) has emerged as a critical tool for enhancing the efficiency of extraction processes (Rangaiah *et al.*, 2020). MOO enables simultaneous optimization of several competing objectives, for example, maximizing yield while maintaining high purity and antioxidant activity (Oke *et al.*, 2002; Adeyi *et al.*, 2022; Tiwari *et al.*, 2009). This approach not only significantly enhances extraction performance but

also ensures that bioactive compounds are preserved in their most potent form, thereby improving the therapeutic efficacy of the extracts. However, implementing MOO presents its own set of challenges, including the need for extensive experimental data and computational resources to build accurate models, as well as the potential for overfitting, which can reduce the generalizability of the optimization results across different batches of raw material or processing conditions (Cerdeira-Flores *et al.*, 2022).

Optimization techniques are fundamental in refining extraction processes to optimize both the efficiency and quality of the compounds extracted. Common optimization approaches include Response Surface Methodology (RSM) (Weremfo *et al.*, 2023; Chakraborty *et al.*, 2020), Genetic Algorithms (GA) (Chen *et al.*, 2023; Zhang *et al.*, 2022), and Particle Swarm Optimization (PSO) (Poyraz *et al.* 2021; Sarma *et al.*, 2018). RSM is frequently used for its ability to model and understand relationships between variables and responses, enabling fine-tuning of extraction parameters for optimal results. However, RSM can be limited by its dependency on polynomial equations, which may not adequately capture complex interactions (Mirjalili *et al.*, 2014). GA and PSO, both heuristic techniques, offer flexibility and adaptability in searching large solution spaces but can be computationally intensive and prone to premature convergence (Mirjalili *et al.*, 2014). Inspired by the hierarchical structure and predatory tactics of grey wolves, the Grey Wolf Optimizer (GWO) is an innovative meta-heuristic algorithm. It stands out for its simplicity, ease of implementation, and robust performance in handling nonlinear, multi-modal optimization problems (Faris *et al.*, 2018). GWO is particularly advantageous in multi-objective optimization, where it effectively balances exploration and exploitation, enabling simultaneous optimization of conflicting objectives, such as maximizing yield while minimizing impurity and energy consumption (Mirjalili *et al.*, 2016). Its flexibility and efficiency make GWO a valuable tool for optimizing complex extraction processes, where traditional methods might struggle to achieve balanced solutions. Multiple decision-making approaches exist for

multi-objective optimization, aiding in the prioritization and ranking of solutions by balancing the trade-offs among conflicting objectives. These approaches include methods such as the Analytical Hierarchy Process (AHP), Multi-Objective Optimization by Ratio Analysis (MOORA), Weighted Sum Model (WSM), Weighted Product Model (WPM), Pareto Optimality, and the Technique for Order Preference by Similarity to Ideal Solution (TOPSIS).

Statistical and machine learning models play a crucial role in predicting and optimizing extraction outcomes by enabling the analysis of complex datasets and the identification of patterns that influence the efficiency and quality of the extraction process. Techniques such as regression analysis (e.g. RSM) (Chelladurai *et al.*, 2021; Lee *et al.*, 2006), neural networks (Dahmoune *et al.*, 2015; Golpour *et al.*, 2021), multi-gene genetic algorithm (Alanzi, *et al.*, 2024; Adeyi *et al.*, 2022), and support vector machines (Liao *et al.*, 2016; Wang *et al.*, 2028) have been widely used to model the relationships between extraction parameters and outcomes, allowing for the fine-tuning of processes to achieve desired results. These models help in minimizing experimental trials, reducing costs, and enhancing the scalability of extraction processes (Jankovič *et al.*, 2024). Gaussian Process Regression (GPR) is a robust statistical approach frequently employed in process modelling, especially for systems where data is limited or expensive to acquire. GPR provides a probabilistic approach to predicting outcomes by defining a distribution over functions, which allows it to model complex, non-linear relationships effectively. This method is particularly advantageous in optimizing extraction processes, as it can account for uncertainty in predictions and provide confidence intervals for the estimates (Wang, 2023). GPR has been effectively deployed in a wide range of disciplines, including energy prediction and bioprocess optimization, where it has demonstrated its capability to deliver accurate and reliable predictions (Shi *et al.*, 2022). Its flexibility and robustness make GPR an invaluable tool in the optimization of extraction processes.

Despite all the pharmacological tendencies demonstrated by *A. aspera* leaves, the extraction

protocol which takes into cognizance/consideration the optimization of the key extraction variables for the optimal recovery of antioxidants from *A. aspera* leaves has not been investigated. Also the core engineering studies of the modelling and prediction of the process of antioxidants recovery from *A. aspera* leaves are seldom reported. These important aspects of study are core centre of reference for further encompassing engineering investigations such as techno-economic analysis of phenolic-rich antioxidants from *A. aspera* leaves, control, prediction and optimization of the process of antioxidant recovery from *A. aspera* leaves and which are conspicuously absent from the literature. Therefore, this present study is aimed at filling these gaps. The key objectives of this investigation were to (i) determine the effect of major extraction process parameters on the extractability of phenolic-rich antioxidants from *A. aspera* leaves using CCD in RSM (ii) optimize the process of phenolic-rich antioxidant extraction from *A. aspera* leaves using Grey wolf optimizer (iii) model and compare the process of antioxidant extraction from *A. aspera* leaves using RSM and GPR (iv) characterize the recovered optimum extract using HPLC.

2. Materials and Methods

2.1 Plant Materials and reagent

Fresh matured *A. aspera* leaves were sourced from the area around Michael Okpara University of Agriculture, Umudike, Nigeria (Latitude: 5.4773° N and Longitude: 7.5428° E). The leaves were detached from their stems, thoroughly rinsed under running tap water to eliminate any debris, and left to air dry at room temperature until they reached a stable weight. Once dried, the leaves were ground into a fine powder using an electric blender. The resulting powder was sieved through a 0.105 mm mesh and kept in a sealed dark container prior to extraction. The sample's moisture content was recorded at 10% before storage.

2.2. Chemicals and reagents

All laboratory reagents used were of high-purity quality and sourced from Sigma Aldrich, Poole, United Kingdom. These included sodium carbonate (Na_2CO_3), ascorbic acid, Folin-Ciocalteu phenol

reagent, glacial acetic acid, 2,4,6-tripyridyl-s-triazine, sodium acetate trihydrate, hydrochloric acid (HCl), and iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$). In this study, the HPLC analysis was calibrated using standards such as betulinic acid, gallic acid, caffeic acid, ellagic acid, chlorogenic acid, ferulic acid, rutin, and quercetin, while distilled water was consistently used throughout the experiments.

2.3 Heat-assisted Extraction of Bioactive Compounds

The process of recovering bioactive antioxidants from *A. aspera* leaves using a thermally-enhanced technique (heat-assisted extraction (HAE) method) was conducted following the procedure described by Adeyi *et al.*, (2022). Approximately one gram of *A. aspera* leaves, ground to a particle size of 0.105 mm, was placed in a 200 mL beaker. Distilled water was then added to the beaker, and the mixture was placed in a water bath and maintained at specific temperature and time conditions for extraction, as determined by the Central Composite Design-Response Surface Methodology (CCD-RSM) experimental design. After the extraction time elapsed, the leaf fibers were separated from the mixture at room temperature using a laboratory centrifuge set at 500 rpm for 5 minutes. The resulting extract was decanted, measured, and stored for subsequent evaluation.

2.4 Central Composite Experimental Design

Design Expert software (Version 7.1.6, Stat-Ease Inc., and Minneapolis, USA) was utilized for the experimental design, data analysis, and development of predictive models. The extraction of bioactive compounds from *A. aspera* leaves via HAE was planned and analyzed employing the CCD-RSM. The HAE process was governed by three primary factors: operating temperature (OT), solid-to-liquid solvent ratio (S:L), and time of extraction (ET). These parameters were investigated across three graded levels, designated as -1, 0, and +1. Specifically, OT was set at 35, 45, and 55°C; S/L was 20, 35, and 50 g/mL; and ET was 100, 150, and 200 min. These parameter levels were chosen based on prior research and initial experimental findings.

This research focused on three key response variables. These included the total phenolic content (TPC), measured in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g d.w); the antioxidant activity (AA), expressed in micromoles of ascorbic acid equivalents per gram of dry weight ($\mu\text{M AAE/g d.w}$); and the extract yield (EY), represented as a percentage (%). A second-degree polynomial equation was formulated for each individual response, as shown in Eq. (1).

$$Y = b_0 + \sum_{i=1}^3 b_i X_i + \sum_{i < j=1}^3 b_{ij} X_i X_j + \sum_{i=1}^3 b_{ii} X_i^2 \quad (1)$$

Within this framework, the symbols X_i correspond to independent factors that affect the outcome variables, denoted as Y . The coefficients b_0 , b_i , b_{ii} , and b_{ij} represent the constants associated with the model, where b_0 is the intercept, b_i accounts for linear influences, b_{ii} reflects quadratic contributions, and b_{ij} captures the interaction effects between variables. The number “3” indicates the total extraction parameters evaluated in the study. Model coefficients were analyzed and the assessment of statistical significance was conducted with a confidence interval of 95%. This analysis was performed utilizing the Design-Expert software to evaluate the reliability of the results. The model's validity was evaluated through the coefficient of determination (R^2), adjusted R-squared (R^2_{adj}), and predicted R-squared (Pred R^2).

2.5 GPR-based Predictive Modelling Theory and Model Development

2.5.1 GPR-based Theory

Gaussian Process Regression (GPR) is a powerful and adaptable non-parametric approach used in computational learning for regression tasks. It leverages the properties of Gaussian processes (GPs) to define a distribution over functions, making it suitable for modelling complex and non-linear relationships (Adeyi *et al.*, 2022b). The fundamental concept of Gaussian Process Regression (GPR) involves utilizing a Gaussian process to establish a prior assumption about the function space. This prior is subsequently refined and updated through

the incorporation of observed data, resulting in a posterior distribution (Kotlar *et al.*, 2019).

A Gaussian Process (GP) is a set of random variables where any finite subset of them follows a joint Gaussian distribution. The GP is entirely defined by its mean function, denoted as $m(x)$, and its covariance function, $k(x, x')$, which together describe the relationship between the variables. These are expressed in Eq. (2) and (3).

$$m(x) = \mathbb{E} [f(x)] \quad (2)$$

$$k(x, x') = \mathbb{E} [(f(x) - m(x))(f(x') - m(x')))] \quad (3)$$

Here, $\mathbb{E}$ denotes the mathematical expectation of the expression within the parentheses.

The GP according to Lin *et al.*, (2019) is written as:

$$f(x) \sim GP(m(x), k(x, x')) \quad (4)$$

In this context, $m(x)$ denotes the mean value of the process at point x , which is often assumed to be zero for convenience. Meanwhile, $k(x, x')$ is the covariance function, also referred to as the kernel, which captures the underlying assumptions about the function's characteristics, including properties like smoothness and periodicity.

The selection of the kernel function $k(x, x')$ is crucial because it determines the characteristics of the functions that the GP is capable of representing (Shabani *et al.*, 2020). The kernel essentially governs how the model interprets and captures relationships within the data. Some commonly used kernels include Squared-Exponential kernel (Eqn. 5), Rational Quadratic kernel (Eq. 6), Periodic kernel (Eq. 7) and Matern kernel (Eq. 8) (Tolba *et al.*, 2019):

$$k_{SE}(x, x') = \sigma^2 \exp(-(x - x')^2 / 2l^2) \quad (5)$$

$$k_{RQ}(x, x') = \sigma^2 (1 + (x - x')^2 / 2al^2)^{-\alpha} \quad (6)$$

$$k_{Per}(x, x') = \sigma^2 \exp\left(\frac{2\sin^2(\pi(x - x')/P)}{l^2}\right) \quad (7)$$

$$k_{Mv}(x, x') = \sigma^2 \frac{1}{2^{v-1}\Gamma(v)} \left(\sqrt{2v} \frac{|x - x'|}{l}\right)^v \cdot B_v\left(\sqrt{2v} \frac{|x - x'|}{l}\right) \quad (8)$$

In this case, σ denotes the amplitude, while l represents the characteristic length of correlation. In

Eq. (7), P denotes the period within the function under study. In Eq. (8), the parameters Γ and B_v correspond to the standard Gamma function and the modified Bessel function of the second kind of order v , respectively. The parameter v governs the level of smoothness of the resulting GP.

By assigning values of $v = 1/2$ and $v = 5/2$ in Eq. (8), we obtain the exponential kernel in Eq. (9) and the Matern 5/2 kernel in Eq. (10).

$$K_E(x, x') = \sigma^2 \exp(-|x - x'|/l) \quad (9)$$

$$k_{M_{5/2}}(x, x') = \sigma^2 \left(1 + \sqrt{5} \frac{|x - x'|}{l}\right) \exp\left(-\sqrt{5} \frac{|x - x'|}{l}\right) \quad (10)$$

Given a dataset in Eq. (11),

$$D = \{x_i, y_i\}_{i=1}^n \quad (11)$$

where $x_i \in R^d$ are input points and $y_i \in R$ are the corresponding outputs.

GPR assumes:

$$y = f(x) + \varepsilon \quad (12)$$

In this equation, x represents the input variable, f is the regression function, and y is the observed outcome. The term ε follows a Gaussian distribution, denoted as $\varepsilon \sim N(0, \sigma_\varepsilon^2)$, where ε is independent and has a variance of σ_ε^2 .

The prior distribution over the function values at the training points X is:

$$\mathbf{f} = [f(x_1), \dots, f(x_n)]^T \sim N(\mathbf{m}, \mathbf{K}) \quad (13)$$

Here, $\mathbf{m}$ represents a vector of the mean function values at each input point, denoted as $[m(x_1), \dots, m(x_n)]^T$. Additionally, $\mathbf{K}$ is the covariance matrix, where each element K_{ij} corresponds to the covariance between input points x_i and x_j , given by $k(x_i, x_j)$.

Given a new test point x_* , the joint distribution of the observed outputs $\mathbf{y}$ and the function value at x_* is:

$$\begin{bmatrix} \mathbf{y} \\ f(x_*) \end{bmatrix} \sim N\left(\begin{bmatrix} \mathbf{m} \\ m(x_*) \end{bmatrix}, \begin{bmatrix} \mathbf{K} + \sigma_\varepsilon^2 \mathbf{I} & \mathbf{k}_* \\ \mathbf{k}_*^T & k(x_*, x_*) \end{bmatrix}\right) \quad (14)$$

where $\mathbf{k}_* = [k(x_1, x_*), \dots, k(x_n, x_*)]^T$ and $\mathbf{I}$ represents the identity matrix.

The posterior distribution for the function value at x_* is then given by:

$$p(f(x_*)|\mathbf{X}, \mathbf{y}, x_*) = N(\mu_*, \sigma_*^2) \quad (15)$$

with mean and covariance:

$$\mu_* = \mathbf{m}(x_*) + \mathbf{K}_*^T(\mathbf{K} + \sigma_n^2 \mathbf{I})^{-1}(\mathbf{y} - \mathbf{m}) \quad (16)$$

$$\sigma_*^2 = k(x_*, x_*) - \mathbf{k}_*^T(\mathbf{K} + \sigma_n^2 \mathbf{I})^{-1} \mathbf{k}_* \quad (17)$$

The covariance function includes parameters known as hyper parameters. These hyper parameters are estimated and refined throughout model training by optimizing the marginal log-likelihood or through the cross-validation approach. Equation (18) provides the marginal log-likelihood formula.

$$\log - \text{likelihood} = \sum_{i=1}^n \log \left(\frac{1}{\sigma \sqrt{2}} e^{-\frac{1}{2} \left(\frac{x_i - \mu}{\sigma} \right)^2} \right) \quad (18)$$

Here, $\log$ represents the natural logarithm, σ stands for the standard deviation, x is the observed data's function value, and μ signifies the model's mean.

2.5.2 GPR-based Model Development

GPR-based predictive models were developed using 120 experimental extraction datasets for the responses of TPC, EY, and AA, alongside HAE process parameters of OT, S/L, and ET. The training process was conducted using the Gaussian Processes for Machine Learning Library (GPML) within the MATLAB 2017 environment. These trained models were then used to predict the extraction data of *A. aspera* leaves, with input variables including OT, S/L, and ET, and output variables comprising TPC, EY, and AA of the extract. Four different kernels were considered to define the Gaussian Process: the Squared-Exponential kernel, the Rational Quadratic kernel, the Marten kernel, and the Exponential kernel. To optimize the hyper parameters of these kernels and prevent over fitting, the cross-validation method in MATLAB 2017a was applied. Consequently, four GPR models were defined based on their respective kernels: GPR-SE for the Squared-Exponential kernel, GPR-RQ for the Rational Quadratic kernel, GPR-M 5/2 for the Marten 5/2 kernel, and GPR-Ex for the Exponential

kernel. To evaluate the accuracy of the models, several statistical parameters were used, including the Root Mean Square Error (RMSE), Coefficient of Determination (R^2), and Mean Absolute Error (MAE), with definitions provided in Eqs. (19), (20), and (21).

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^n (x_i - y_i)^2} \quad (19)$$

$$R^2 = 1 - \frac{\sum_{i=1}^n (x_i - y_i)^2}{\sum_{i=1}^n (x_i - \bar{x})^2} \quad (20)$$

$$\text{MAE} = \frac{1}{n} \sum_{i=1}^n |x_i - y_i| \quad (21)$$

Here, x_i and y_i represent the observed and predicted outputs, while $\bar{x}$ denotes the mean of the observed data, and n indicates the number of observations.

2.6.1 Multi-Objective Grey Wolf Optimizer Theory

The Multi-Objective Grey Wolf Optimizer (MOGWO) is a cutting-edge algorithm that takes its inspiration from the social structure and hunting tactics of grey wolves. This method is specifically engineered to tackle complex optimization challenges that involve balancing several conflicting objectives simultaneously. In practice, MOGWO mimics the hierarchical arrangement observed in wolf packs by classifying potential solutions into groups analogous to alpha, beta, delta, and omega wolves (Premkumar *et al.*, 2022). At the outset, the algorithm generates a diverse set of random solutions, each symbolizing a wolf's position in the search space. The highest-quality solutions are designated as alpha wolves, while the second- and third-best are labelled beta and delta wolves, respectively; all other solutions are considered omega wolves. As the optimization process unfolds, the positions of these "wolves" are iteratively adjusted in relation to the positions of the alpha, beta, and delta wolves. This iterative updating ensures that the algorithm effectively balances the exploration of new regions with the exploitation of promising areas in the search space (Mirjalilie *et al.*, 2016).

The mathematical formulation of the MOGWO algorithm includes the encircling mechanism, where wolves adjust their positions towards the prey using the equations:

$$\mathbf{D} = |\mathbf{C} * \mathbf{X}_{\text{prey}} - \mathbf{X}| \quad (22)$$

$$\mathbf{X}(t + 1) = \mathbf{X}_{\text{prey}} - \mathbf{A} * \mathbf{D} \quad (23)$$

In this context, $\mathbf{X}_{\text{prey}}$ denotes the vector representing the prey's location, while $\mathbf{X}$ signifies the positional vector of an individual grey wolf. Additionally, $\mathbf{A}$ and $\mathbf{C}$ are coefficient vectors that influence the algorithm's behaviour, and t denotes the current iteration (Premkumar *et al.*, 2022).

The coefficients $\mathbf{A}$ and $\mathbf{C}$ are calculated as follows:

$$\mathbf{A} = 2\mathbf{a} * \mathbf{r}_1 - \mathbf{a} \quad (24)$$

$$\mathbf{C} = 2 * \mathbf{r}_2 \quad (25)$$

In this formulation, the parameter $\mathbf{a}$ is steadily reduced linearly from an initial value of 2 to 0 as the iterations progress. Meanwhile, the vectors $\mathbf{r}_1$ and $\mathbf{r}_2$ are generated randomly, with their elements uniformly distributed within the range [0, 1].

For multi-objective optimization, MOGWO maintains an archive of non-dominated solutions

based on Pareto optimality. Each wolf's position is evaluated across multiple objectives, and the archive is updated to retain the best non-dominated solutions found so far. This approach ensures a diverse set of solutions that represent the trade-offs between conflicting objectives (Makhadmeh *et al.*, 2022).

2.6.2 Implementation of Multi-Objective Grey Wolf Optimization

The MOGWO-TOPSIS was performed on the objective functions of TPC, EY and AA in MATLAB 2017 environment. The primary goal was to maximize simultaneously the TPC, EY and AA of *A. aspera* leaves and determine the process variables of OT, S/L and ET that achieve the purpose. The final codes integrated CCD-RSM models (being better predictors) with the MOGWO-TOPSIS for objective functions' prediction in the cause of optimal solution search. The parameters, description and their set values are displayed in Table 1.

Table 1: Parameter settings for MOGWO implementation

Hyper parameter	Description	Value
numAgents	Number of grey wolves (agents)	30
maxIter	Maximum number of iterations	100
Dim	Number of decision variables (A, B, C)	3
Lb	Lower bounds for the decision variables (A, B, C)	[30, 1:60, 60]
Ub	Upper bounds for the decision variables (A, B, C)	[70, 1:10, 240]
A	Coefficient adjusting exploration-exploitation balance	$a = 2 - t * (2 / \text{maxIter})$
r1, r2	Random numbers in the range [0, 1] used in position update	Randomly generated during each iteration

2.6.3 Experimental validation

To validate the predicted optimal conditions for HAE obtained using MOGWO, a series of meticulously planned experiments was systematically executed in the laboratory. The observed experimental results were evaluated against the predicted values using the relative standard deviation (RSD). The RSD was computed according to the formula presented in Eq. (26):

$$\% \text{ RSD} = \frac{SD_{1-2}}{M_v} * 100 \quad (26)$$

In this context, the RSD represents the measure of the standard deviation (SD_{1-2}). This metric is used to assess the level of consistency or discrepancy between the predicted values and the experimental results by comparing the mean (M_v) obtained from each dataset.

2.7 Determination Total Phenolic Content

The TPC of *A. aspera* leaf extract was determined using the Folin-Ciocalteu (FC) method, which relies on the redox behaviour of phenolic compounds. The procedure, adapted from Alara *et al.* (2018) with minor modifications, began with

diluting the extract at a 1:10 ratio. From this diluted sample, 0.1 mL was mixed with 1.8 mL of the FC reagent. This mixture was allowed to react for 5 minutes to initiate the interaction between the reagent and the phenolic compounds. Subsequently, 1.2 mL of a 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added to create an alkaline environment essential for colour development. The solution was then incubated for an additional 2 hours to ensure complete reaction and full colour formation. Absorbance was measured at 765 nm using a Hitachi U-1800 UV-vis spectrophotometer. A calibration curve was constructed using gallic acid as the standard, described by the equation $y = 0.037x + 0.0025$ with an R^2 value of 0.997, indicating excellent linearity. Finally, the phenolic content was quantified and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g d.w.), with all experiments performed in triplicate.

$$\text{TPC} = \frac{C \cdot V}{m} \quad (27)$$

In this equation, the symbol C (mg/mL) represents the concentration of gallic acid, which is determined from the calibration curve. The term V (mL) refers to the volume of the solvent used during the analysis. Meanwhile, m (g) indicates the mass of the dried *A. aspera* leaves that were employed in the laboratory procedure.

2.8 Determination of Antioxidant Activity

The antioxidant potential of the extracts was assessed using the ferric reducing antioxidant power (FRAP) assay, following the procedure outlined by Uddin et al. (2016). This assay evaluates the capacity of antioxidants to convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}), reflecting the reducing strength of the extracts. To prepare the FRAP reagent, three solutions were combined in a specific ratio of 10:1:1, consisting of 0.1 M acetate buffer, 0.01 M TPTZ (2,4,6-tripyridyl-s-triazine) solution, and 0.02 M ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) solution. For the assay, 50 μL of the extract, previously diluted tenfold with distilled water, was mixed with 1.5 mL of the freshly prepared FRAP reagent to form the reaction mixture. This mixture was allowed to incubate at

ambient temperature for 30 minutes. Following incubation, the absorbance of the solution was measured at 593 nm using a UV-Visible spectrophotometer. The AA of the extracts was quantified based on a standard calibration curve generated using ascorbic acid. The results were expressed as micromoles of ascorbic acid equivalents per gram of dry weight ($\mu\text{M AAE/g d.w.}$). The calibration curve adhered to the linear equation $y = 0.0051x + 0.0015$, with a high correlation coefficient ($R^2 = 0.995$), ensuring the reliability and precision of the antioxidant capacity measurements.

2.9 Determination of Extract Yield

The extract yield (EY) was determined following the procedure outlined by Alara et al. (2021). First, after separating the *A. aspera* leaf fibers as detailed in Section 2.3, the liquid extracts were carefully decanted. These collected extracts were then concentrated and dried using a convective oven dryer (Stangas SG-90526, Stangas Italy). The drying process continued until a constant weight was achieved, ensuring that the solvent (water) was completely removed. Finally, the percentage of extract yield was calculated precisely using Eq. (28).

$$\% \text{ EY} = \frac{W_1}{W_2} * 100 \quad (28)$$

Here, EY denotes the percentage yield of *A. aspera* leaf extracts. W_1 represents the weight of the extracted *A. aspera* leaves (in grams), and W_2 signifies the weight of the *A. aspera* leaves (in grams) utilized in the HAE process.

2.10 High Performance Liquid Chromatography Profiling of Extract

The HPLC analysis was carried out to thoroughly characterize the extract obtained from *A. aspera* leaves, following the methodology detailed by Krishna and Manohar (2014). Under optimized conditions, the bioactive extract was carefully prepared prior to its examination by HPLC. Initially, the sample was passed through a 0.45 μm membrane filter to remove particulate matter, such as fibers and other impurities. After filtration, the

extract was analyzed using an advanced chromatography system equipped with a UV diode array detector (UV-DAD) that scans wavelengths from 190 to 800 nm. Approximately 10 μ L of the filtered extract was injected into a Shimadzu Corporation HPLC instrument from Kyoto, Japan. The system featured a VP-ODS column (150 $\times$ 4.6 mm, 5 μ m particle size), which efficiently separated the bioactive compounds in the sample. Detection was performed at a wavelength of 220 nm, while a mobile phase consisting of 0.2% v/v formic acid and acetonitrile was used to facilitate the elution of the compounds from the column. The resulting chromatogram was meticulously recorded, and the data were processed using specialized Windows LC solution software. This software enabled the identification and quantification of the active constituents by comparing their elution times and signal intensities with those of known standards. In summary, the analysis yielded a comprehensive chromatographic profile that plotted elution times against signal intensities, providing detailed insight into the bioactive components present in the *A. aspera* leaf extract.

3.0 Discussion of Results

3.1 Effect of Extraction Process Parameters on Total Phenolic Compound, Antioxidant Activity, and Extract Yield of *A. aspera* Leaves

The CCD-RSM was employed for the experimental investigation and modelling of bioactive

antioxidant extraction parameters, as well as the key responses in the heat-assisted extraction of *Achyranthes aspera* leaves. CCD-RSM was chosen for its ability to minimize the number of required experimental runs, while maintaining adaptability to various experimental settings and its effectiveness in constructing second-order (quadratic) models (Myers and Montgomery, 2002). A distinguishing feature of the CCD approach is the inclusion of axial (star) points, which are strategically placed outside the typical range of the process variables. These points enhance the model's accuracy, extend the explored modelling process ranges, and enable rotatable designs, all of which are vital for robust response surface (Montgomery, 2017). For this study, the key process variables selected for bioactive recovery from *A. aspera* leaves included OT (35–55°C), ET (100–200 min), and S/L (1:20–1:60), while the experimental responses measured were EY, TPC, and AA. The selection of these variables and their respective ranges aligns with previous studies on the extraction of bioactive compounds (Adeyi *et al.*, 2023; Olalere *et al.*, 2019). Table 1 provides the experimental runs based on CCD, the corresponding extraction conditions (OT (°C), S/L (g/mL), and ET (min)), and the laboratory-measured responses for EY (w/w %), TPC (mg GAE/g dw), and AA (μ M AAE/g dw).

Table 2. Central Composite Design and Observed Responses^d

Run	HAE conditions		Experimental values			
	Temperature (°C)	Solid to liquid ratio (g/mL)	Time (min)	EYs (w/w %)	TPC (mg GAE/g dw)	AA (μM AAE/g dw)
1	45.00	1:35	150.00	17.13	26.03	4.10
2	45.00	1:60.23	150.00	16.34	36.14	9.18
3	55.00	1:20	200.00	18.77	18.32	2.11
4	45.00	1:35	65.91	20.03	20.51	5.02
5	45.00	1:35	150.00	17.11	25.67	3.78
6	55.00	1:50	200.00	16.55	25.30	5.84
7	28.18	1:35	150.00	16.73	18.37	4.64
8	35.00	1:50	200.00	18.07	33.18	7.07
9	45.00	1:35	234.09	19.44	18.67	3.72
10	35.00	1:20	100.00	18.14	11.89	2.48
11	45.00	1:35	150.00	17.33	25.16	4.53
12	35.00	1:50	100.00	16.99	27.19	6.90
13	45.00	1:35	150.00	17.21	24.94	4.28
14	55.00	1:20	100.00	20.04	21.21	3.33
15	61.82	1:35	150.00	17.28	23.57	4.64
16	45.00	1:35	150.00	17.05	27.66	4.21
17	45.00	1:35	150.00	17.38	25.45	4.39
18	45.00	1:9.77	150.00	19.48	13.54	1.63
19	55.00	1:50	100.00	17.67	23.82	8.96
20	35.00	1:20	200.00	18.93	10.21	3.33

^d OT is operating temperature; S:L refers to solid to liquid ratio; ET represents extraction time; EY is extract yield; TPC refers to total phenolic content ; AA represents antioxidant activity

The CCD-RSM produced 20 experimental runs, and each row in the table corresponds to a particular experimental trial, outlining the combination of extraction parameters and the corresponding responses. Analysis of Table 2 shows that higher temperatures (e.g., 55°C) generally enhance yield but may reduce TPC and AA due to phenolic degradation, as seen in Run 3. Moderate temperatures, such as 45°C in Run 2, balance outcomes with the highest TPC (36.14 mg GAE/g dw) and AA (9.18 μM AAE/g dw). Lower temperatures (e.g., 28.18°C in Run 7) underperform in all metrics. S/L ratios also significantly affect results. High ratios (e.g., 1:60.23 in Run 2) maximize TPC and AA but slightly lower yields due to dilution effects. Low ratios (e.g., 1:9.77 in Run 18) yield the highest EYs (19.48%) but compromise TPC and AA, highlighting the need for solvent sufficiency for phenolic recovery. Intermediate ratios (e.g., 1:35) provide balanced outcomes, as seen in Runs 1, 11, and 16. In the same vein, shorter extraction times (e.g., 65.91 min in Run 4) maintain competitive yields and stability,

while extended durations (e.g., 234.09 min in Run 9) show diminishing returns on TPC and AA. Therefore, the laboratory observed results presented Table 1 suggest that moderate extraction conditions—OT, S/L ratio, and ET—are key to maximizing bioactive recovery while maintaining process efficiency (Bouafia *et al.*, 2021; Boutoub *et al.*, 2022).

3.2 CCD-RSM Modelling, Evaluation of Model Adequacies and Statistical Analysis

The CCD approach was employed to establish the relationships between the process variables (OT, S/L, and ET) and the responses (TPC, EY, and AA). The coded quadratic predictive models for TPC, EY, and AA in terms of the process variables are given by Eq. (28), (29), and (30). In these models, A, B, and C represent OT, S/L, and ET, respectively. The positive and negative signs in Eq. (29), (30), and (31) indicate the contributions that increase or decrease the responses. Therefore, the factors OT, S/L, and ET, along with their

interactions, demonstrated varying effects on the models for TPC, EY, and AA.

$$\text{TPC} = +25.82 + 1.09 * A + 6.29 * B - 0.014 * C - 1.75 * A^2 - 0.38 * B^2 - 2.24 * C^2 - 3.59 * A * B - 0.71 * A * C + 1.50 * B * C \quad (29)$$

$$\text{EY} = +17.21 + 0.13 * A - 0.87 * B - 0.11 * C - 0.098 * A^2 + 0.22 * B^2 + 0.87 * C^2 - 0.32 * A * B - 0.53 * A * C + 0.055 * B * C \quad (30)$$

$$\text{AA} = +4.21 + 0.034 * A + 2.21 * B - 0.40 * C + 0.18 * A^2 + 0.45 * B^2 + 0.089 * C^2 + 0.15 * A * B + 0.15 * A * C - 0.67 * B * C \quad (31)$$

Table 2 presents a detailed Analysis of Variance (ANOVA) for the quadratic models describing TPC, EY, and AA in relation to three process variables: OT, S/L, and ET. The results indicate that the overall models for all three responses are statistically significant, with P-values < 0.0001, highlighting that the process variables and their interactions explain a large portion of the variation in TPC, EY, and AA. The R² values for TPC, EY, and AA are 0.9840, 0.9910, and 0.9919, respectively, indicating that the models explain a substantial proportion of the variability in the data. These high R² values are further supported by the adjusted R² values of 0.9697, 0.9829, and 0.9846, confirming that the models fit the data well after accounting for the number of predictors. Among the individual factors, S/L has the most significant impact on all three response variables, with a P-value of less than

0.0001, indicating that the solvent-to-solid ratio is a critical factor in the extraction process. This is especially evident in TPC, where the sum of squares for factor B (539.90) is much larger than the other factors, with an F-value of 422.8. The OT also significantly affects TPC, EY, and AA, though its influence on AA is less pronounced, with a P-value of 0.6306 for AA. This indicates that temperature plays an essential role in the extraction of phenolic compounds, increasing their yield at higher temperatures but potentially degrading some of their antioxidant activity. On the other hand, ET has a minimal effect on TPC (P = 0.9638) but significantly influences both EY (P = 0.0228) and AA (P = 0.0001), suggesting that longer extraction times can enhance yield and antioxidant activity without significantly increasing phenolic content.

Table 3. Analysis of Variance (ANOVA) for TPC, EYs and AA Quadratic Models*

Source	TPC (mg GAE/g d.w)				EYs (%)				AA (μ M AAE/g d.w)			
	Sum of squares	df	F-value	P-value P>F	Sum of squares	df	F-value	P-value P>F	Sum of squares	df	F-value	P-value P>F
Model	787.32	9	68.50	< 0.0001	25.55	9	122.3	< 0.0001	76.98	9	135.8	<0.0001
A	16.31	1	12.77	0.0051	0.24	1	10.51	0.0088	0.015	1	0.25	0.6306
B	539.90	1	422.8	< 0.0001	10.34	1	445.3	< 0.0001	66.86	1	1061.	<0.0001
C	0.00277	1	0.002	0.9638	0.17	1	7.22	0.0228	2.22	1	35.26	0.0001
A ²	44.10	1	34.53	0.0002	0.14	1	5.92	0.0352	0.49	1	7.78	0.0191
B ²	2.09	1	1.64	0.2295	0.71	1	30.69	0.0002	2.98	1	47.35	<0.0001
C ²	72.12	1	56.48	< 0.0001	10.85	1	467.3	< 0.0001	0.11	1	1.81	0.2082
AB	102.82	1	80.52	< 0.0001	0.83	1	35.85	0.0001	0.18	1	2.86	0.1218
AC	4.09	1	3.20	0.1038	2.27	1	97.75	< 0.0001	3.59	1	57.03	<0.0001
BC	18.12	1	14.19	0.0037	0.024	1	1.04	0.3312	0.83	1	13.21	0.0046
Residual	12.77	10			0.23	10			0.63	10		
Lack of fit	7.97	5	1.66	0.2956	0.15	5	1.73	0.2802	0.29	5	0.87	0.5584
Pure error	4.80	5			0.085	5			0.34	5		
Cor.	800.09	19			25.78	19			0.34	19		
Total												
CV%	4.85				0.85				5.33			
PRESS	69.60				1.24				3.00			
Adeq	32.080				35.748				41.944			
Precision												
n												
R ²	0.9840				0.9910				0.9919			
Adj R ²	0.9697				0.9829				0.9846			
Pred R ²	0.9130				0.9519				0.9614			

* OT stands for operating temperature; S:L refers to the solid-to-liquid ratio; ET represents extraction time; EY is the extract yield; TPC indicates total phenolic content; AA denotes antioxidant activity; df stands for degree of freedom; CV refers to the coefficient of variation; Adeq Precision measures the adequacy of precision; Adj R² is the adjusted R-squared value; and Pred R² is the predicted R-squared value.

The interaction terms between the variables also reveal important insights. The interaction between OT and S/L (AB) is highly significant for all three responses, with a P-value less than 0.0001, indicating that the combined effect of these two factors is crucial for optimizing the extraction process, particularly in maximizing phenolic content. Similarly, the BC interaction significantly impacts both EY and AA, with P-values of less than 0.0001, suggesting that the interaction between S/L and ET plays a major role in improving EY and AA. The AC interaction also affects EY and AA, with

highly significant P-values ($P < 0.0001$), but has a less notable impact on TPC ($P = 0.1038$), implying that OT and ET interact in a way that is more relevant for optimizing EY and AA than for improving phenolic content alone. The residual sum of squares is small, indicating a good fit of the models, and the lack of fit tests for all models show no significant deviation from the predicted values, with P-values greater than 0.05 ($P = 0.2956$ for TPC, $P = 0.2802$ for EY, and $P = 0.5584$ for AA), further supporting the adequacy of the models. The pure error term is also minimal, suggesting that there are

no substantial experimental variations that affect the results. The adequacy of the models is confirmed by the adequate precision values, which are 32.080 for TPC, 35.748 for EY, and 41.944 for AA, indicating that the models provide a sufficient signal-to-noise ratio and are reliable for further analysis. The predicted R^2 values for TPC (0.9130), EY (0.9519), and AA (0.9614) are close to the adjusted R^2 values, reinforcing the accuracy and predictive power of the models.

Figure 1 presents a comparison between the predicted values derived from the model and the

actual experimental values for TPC, AA, and EY. The x-axis represents the actual experimental values, while the y-axis shows the predicted values generated by the model. As illustrated in Figure 1, the data points closely align with the diagonal line ($y = x$), indicating a strong agreement between the predicted and observed values. This alignment suggests that the developed CCD-RSM models are accurate and effective in predicting the experimental outcomes (Adeyi *et al.*, 2022; Olalere *et al.*, 2022).

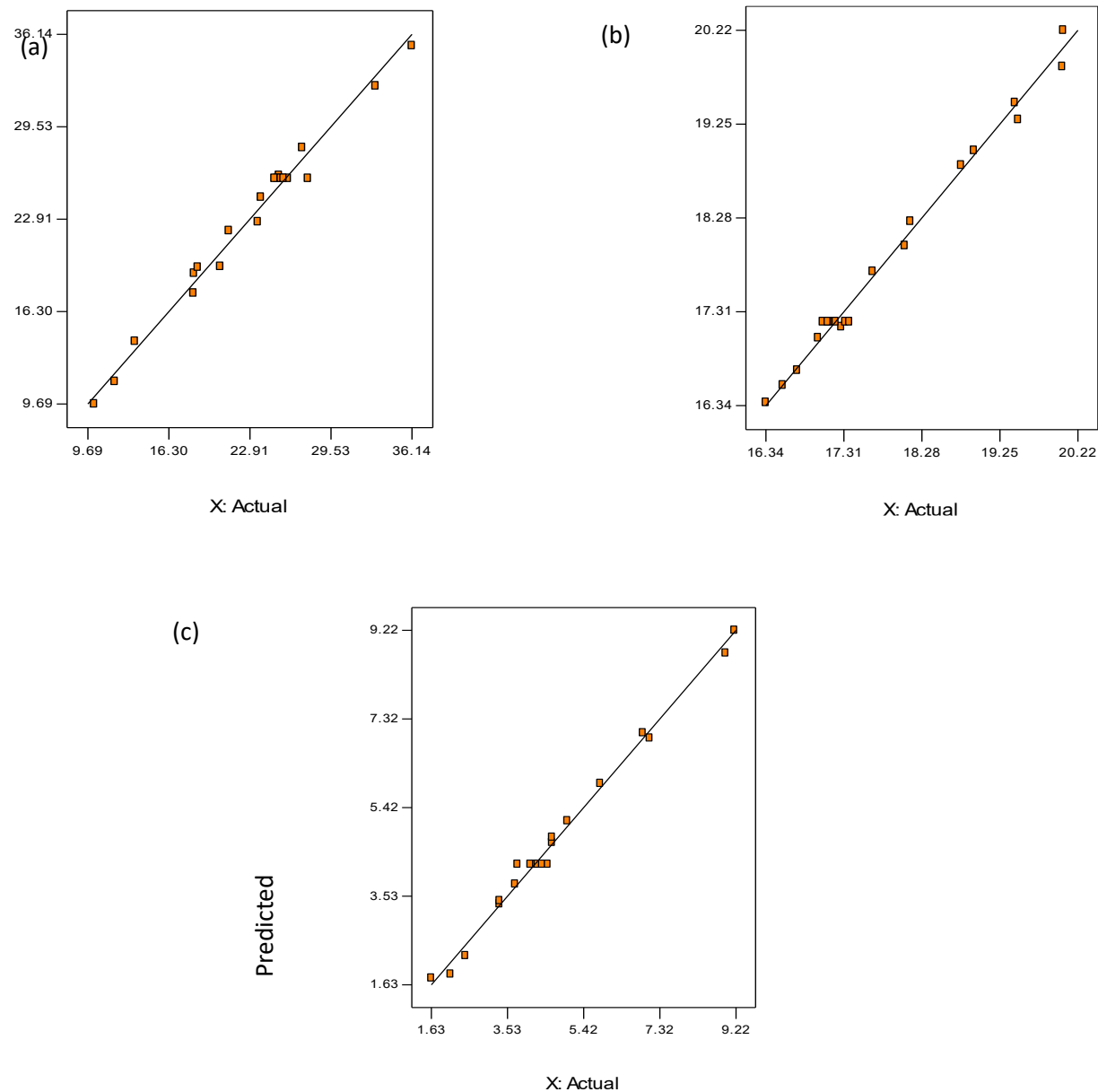


Fig. 1. Parity graphs of experimental and predicted data for (a) TPC (b) EY and (c) AA

3.3 Data Statistics and Gaussian process Regression-Based Modelling

The dataset provides comprehensive descriptive statistics for key process variables—OT, S:L, and ET—and response variables—EY, TPC, and AA. Each variable is characterized by several statistical parameters, including mean, standard error, median, mode, standard deviation, sample variance, kurtosis, skewness, range, minimum, maximum,

sum, and count. These statistics are instrumental in modelling and optimizing extraction processes to enhance the efficiency and quality of bioactive compound recovery. The total data used the Gaussian process regression-based modelling was 120 data points with each variable comprises 20 observations. Table 3 presents an overview of the descriptive statistics for the dataset utilized in the GPR-based modelling

Table 4: Summary of Process and Response Variables Statistics^d

Statistical parameters	OT (°C)	S:L (mL/g)	ET (min)	EY (w/w %)	TPC (mg GAE/g d.w)	AA (μM AAE/g)
Mean	45	35	150	17.8835	22.8415	4.707
Standard error	1.8958576	2.843786	9.478821	0.26049	1.451034	0.451916
Median	45	35	150	17.355	24.38	4.335
Mode	45	35	150	N/A	N/A	4.64
Standard Deviation	8.4785326	12.7178	42.39058	1.164941	6.489223	2.021029
Sample Variance	71.885516	161.7424	1796.961	1.357087	42.11001	4.084559
Kurtosis	-0.1830346	-0.18303	-0.18344	-0.7954	0.224248	0.584184
Skewness	-2.883E-15	-2.1E-16	0	0.703801	-0.1377	0.89624
Range	33.64	50.46	168.18	3.7	25.93	7.55
Minimum	28.18	9.77	65.91	16.34	10.21	1.63
Maximum	61.82	60.23	234.09	20.04	36.14	9.18
Sum	900	700	3000	357.67	456.83	94.14
Count	20	20	20	20	20	20

The mean values for OT, S:L, ET, EY, TPC, and AA are 45°C, 35 mL/g, 150 minutes, 17.88%, 22.84 mg GAE/g d.w, and 4.707 μM AAE/g, respectively. These means provide a central value for each variable, offering insight into the typical observations within the dataset. The medians closely align with the means, indicating a relatively symmetrical distribution for most variables. However, the mode is only available for OT, S:L, and ET, matching their respective means and medians, suggesting a unimodal distribution for these variables. Standard deviation and sample variance measure the dispersion of data around the mean. For instance, OT has a standard deviation of 8.48°C, indicating that most temperature observations deviate from the mean by this amount. Similarly, S:L and ET exhibit standard deviations of 12.72 g/mL and 42.39 min, respectively, reflecting variability in these parameters. EY, TPC, and AA have standard deviations of 1.16%, 6.49 mg GAE/g d.w, and 2.02 μM AAE/g, respectively, indicating

varying degrees of dispersion in extraction outcomes. Skewness and kurtosis provide insights into the shape of the data distribution. Skewness values near zero suggest symmetrical distributions for OT, S:L, and ET. EY and AA exhibit positive skewness (0.7038 and 0.8962, respectively), indicating a longer right tail, while TPC shows a slight negative skewness (-0.1377), suggesting a marginally longer left tail. Kurtosis values close to zero for OT, S:L, ET, and EY suggest mesokurtic distributions, similar to a normal distribution. TPC and AA have positive kurtosis (0.2242 and 0.5842, respectively), indicating a tendency toward heavier tails or a more peaked distribution compared to the normal distribution. The range, defined as the difference between maximum and minimum values, highlights the span of data. OT ranges from 28.18°C to 61.82°C, a span of 33.64°C. S:L varies between 9.77 g/mL and 60.23 g/mL, covering 50.46 g/mL. ET spans 65.91 to 234.09 min, a range of 168.18 min. EY ranges from 16.34% to 20.04%, TPC from

10.21 to 36.14 mg GAE/g d.w, and AA from 1.63 to 9.18 μM AAE/g, indicating varying degrees of variability across different parameters.

The GPR models utilizing GPR-SE, GPR-RQ, GPR-M-5/2, and GPR-Ex kernels were used to train and predict the extraction data from *A. aspera* leaves. The models were constructed to investigate how essential process variables correlate with the observed outcomes. Specifically, EY, TPC and AA were modelled as functions of the process parameters—OT, S/L, and ET. The GPR models were then trained to capture the complex interactions between these inputs and their

corresponding responses, thereby establishing a robust framework for predicting extraction outcomes. Table 4 summarizes the performance metrics of all the GPR models, illustrating their effectiveness in modelling and predicting EY, TPC, and AA based on the selected process variables. The models' performance was assessed using several critical metrics, including RMSE, R^2 , MAE, prediction speed (obs/ s), and training time (s). These metrics provide a comprehensive understanding of the models' accuracy, computational efficiency, and applicability for modelling extraction processes.

Table 5. Predictability Parameters for GPR-Based Models of TPC, EY, and AA

GPR-based type	RMSE	R^2	MAE	Prediction speed (obs/s)	Training Time (s)
TPC (mg GAE/g)					
GPR-RQ	2.28	0.88	1.76	630	169.20
GPR-SE	2.28	0.88	1.76	240	169.13
GPR-M 5/2	2.74	0.83	2.05	230	168.51
GPR-Ex	4.04	0.62	2.81	650	170.05
EY (%)					
GPR-RQ	0.47	0.84	0.35	780	177.91
GPR-SE	0.47	0.84	0.35	280	178.42
GPR-M 5/2	0.61	0.72	0.46	230	177.94
GPR-Ex	0.88	0.43	0.66	700	178.79
AA (μM AAE/g d.w.)					
GPR-RQ	0.48	0.94	0.37	670	105.40
GPR-SE	0.48	0.94	0.37	210	102.44
GPR-M 5/2	0.52	0.93	0.38	130	101.26
GPR-Ex	0.86	0.82	0.61	780	105.50

GPR-RQ refers to a Gaussian Process Regression model utilizing a Rational Quadratic kernel; GPR-SE denotes a model employing a Squared-Exponential kernel; GPR-Ex indicates a model based on an Exponential kernel; and GPR-5/2 signifies a model configured with a Marten 5/2 kernel.

The performance evaluation of Gaussian Process Regression (GPR) models employing GPR-RQ, GPR-SE, GPR-M 5/2, and GPR-Ex kernels for predicting TPC, EY, and AA of *A. aspera* leaves revealed notable variations in their effectiveness. GPR-RQ consistently demonstrated superior predictive accuracy, achieving the RMSE and MAE, along with the highest R^2 values across all responses. For TPC and EY, GPR-RQ and GPR-SE performed similarly in terms of RMSE and R^2 values (TPC: RMSE = 2.28, R^2 = 0.88; EY: RMSE = 0.47, R^2 = 0.84), with GPR-RQ offering faster prediction speeds. GPR-M 5/2 displayed moderate performance for both variables, while GPR-Ex

exhibited the weakest accuracy (TPC: RMSE = 4.04, R^2 = 0.62; EY: RMSE = 0.88, R^2 = 0.43). For AA, GPR-RQ and GPR-SE again led with high accuracy (RMSE = 0.48, R^2 = 0.94), whereas GPR-M 5/2 was slightly less precise (RMSE = 0.52, R^2 = 0.93), and GPR-Ex trailed (RMSE = 0.86, R^2 = 0.82). Training times were comparable among models, but GPR-RQ consistently outperformed others in prediction speed. While GPR-SE was a reliable alternative, GPR-M 5/2 and GPR-Ex were less suitable, particularly for high-precision tasks. Thus, GPR-RQ is the most robust model, offering the best balance of accuracy, reliability, and

computational efficiency for modelling extraction processes.

Fig. 2(a), (b), and (c) present the response plots of the GPR-RQ model's predicted data for TPC, EY, and AA as functions of ET, S/L, and OT, based on the experimental data provided in Table 1. As shown in Fig. 2(a), (b), and (c), predictions for all 20 experimental runs in Table 1, each conducted under specific conditions, were made using the best-performing GPR-RQ model. The blue dots on the graphs represent the experimental values of the responses, while the orange dots denote the

corresponding predicted values. The orange solid lines connecting the blue and orange dots in the figure illustrates the difference (error) between the laboratory-observed data and the GPR-RQ model's predicted data. This error was quantified for each data point (experimental run), as shown in the figure. As seen in Fig. 2, the disparity between the experimental and predicted values varies, which can also be observed in Fig. 3. Fig. 3(a), (b), and (c) present a set of residual plots for TPC, EY, and AA, respectively, as predicted by the GPR-RQ model, highlighting the differences between the observed and predicted values.

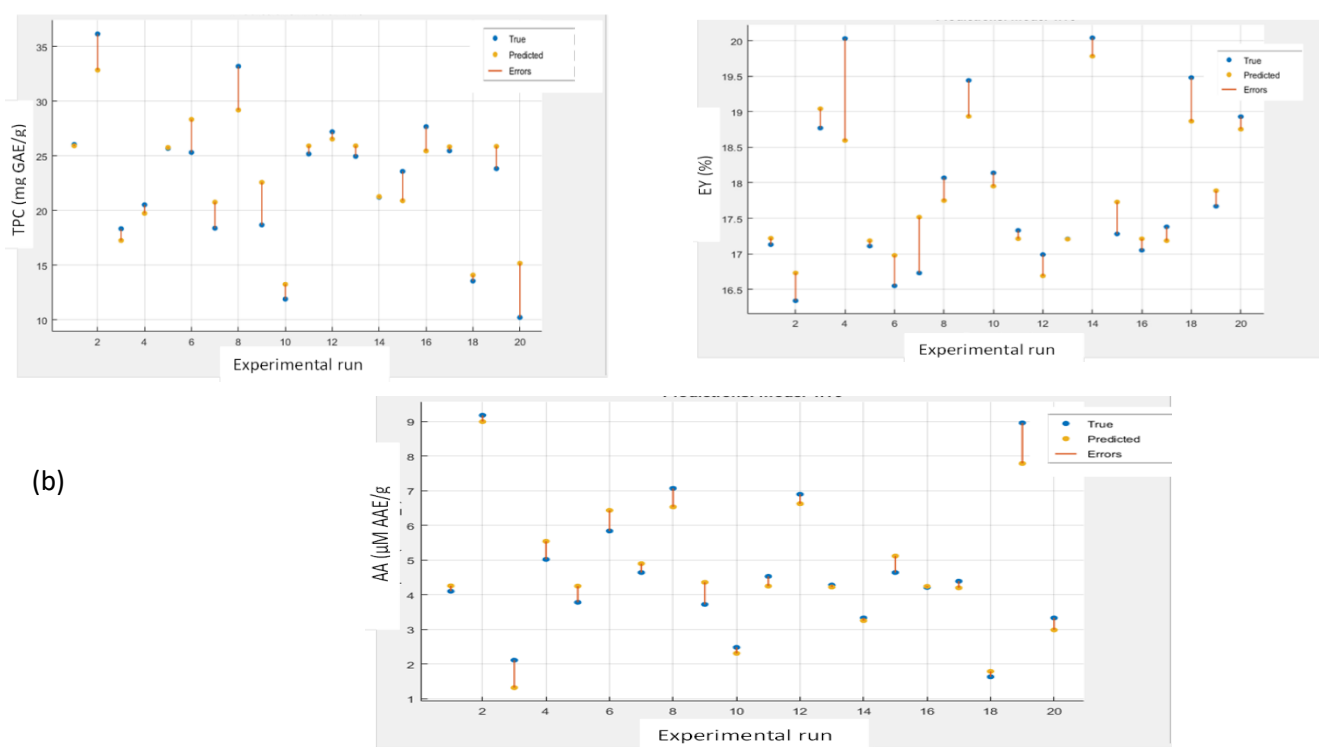
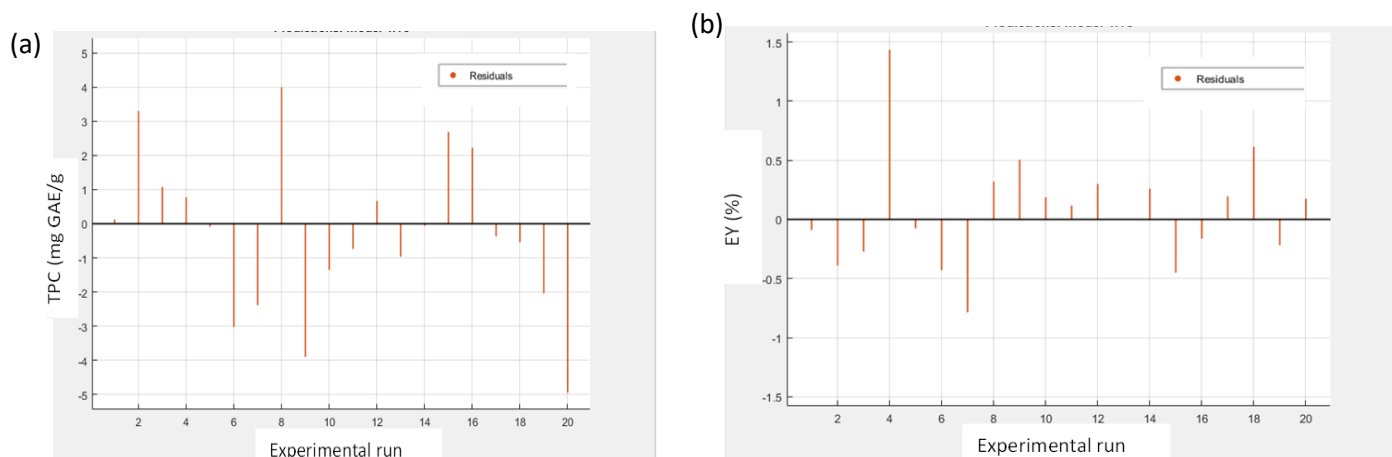


Fig. 2 Response plot for GPR-RQ predicted data for (a) TPC (mg GAE/g) (b) EY (%) (c) AA (μM AAE/g d.w.)



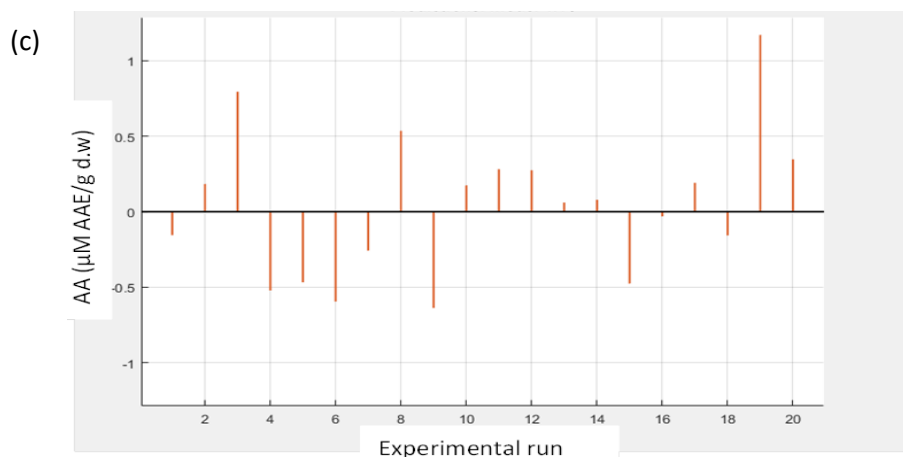


Fig. 3. Residuals of GPR-RQ predicted data and experimental for (a) TPC (mg GAE/g) (b) EY (%) (c) AA ($\mu\text{M AAE/g d.w.}$)

In Fig 3, the thick black line represents the zero reference line, while the residuals plotted above and below this line indicate instances where the GPR-RQ model predicted values either exceed or fall short of the experimental data, respectively. The closer a residual is to the zero line, the more accurate the prediction by the GPR-RQ model. As observed in Fig. 3(a), (b), and (c), the discrepancies between the experimental and predicted values are minimal. The highest residuals above the zero line are less than 4 mg GAE/g, 1.45%, and 1.2 $\mu\text{M AAE/g d.w.}$, while the lowest residuals below the zero line are less than -5 mg GAE/g, -0.8%, and -0.7 $\mu\text{M AAE/g d.w.}$ for TPC, EY, and AA, respectively. These small deviations demonstrate an excellent agreement between the experimental data and the predictions made by the GPR-RQ model, indicating that the GPR-RQ model effectively captured the nonlinear relationships between the process parameters (ET, S/L and ET) and the response variables (TPC, EY, and AA). This is consistent with the literature, which highlights GPR models as reliable tools for process optimization and prediction (Morita *et al.*, 2022; Shi *et al.*, 2022). The

GPR-RQ was evaluated for its robustness by predicting TPC, EY, and AA data. Figure 4 presents parity plots comparing the experimental and predicted values across all data points, illustrating the model's predictive accuracy. The parity plots in Fig. 4 collectively demonstrated the predictive performance of GPR-RQ kernel model for TPC, EY, and AA in *A. aspera* leaf extracts. Each plot compares the model's predicted values against the experimentally observed data, with the diagonal line representing perfect prediction. The close clustering of data points around this line across all three subfigures indicated that the GPR-RQ model effectively captured the underlying relationships between the extraction parameters and the respective responses. This high degree of alignment suggests minimal prediction errors, underscoring the model's robustness and reliability in modelling complex, nonlinear processes inherent in phytochemical extraction. Such performance is consistent with findings in related studies, where GPR-based models have been successfully

(a) oyed for similar predictive tasks (El-Adawy *et al.* 024; Rui *et al.*, 2020).

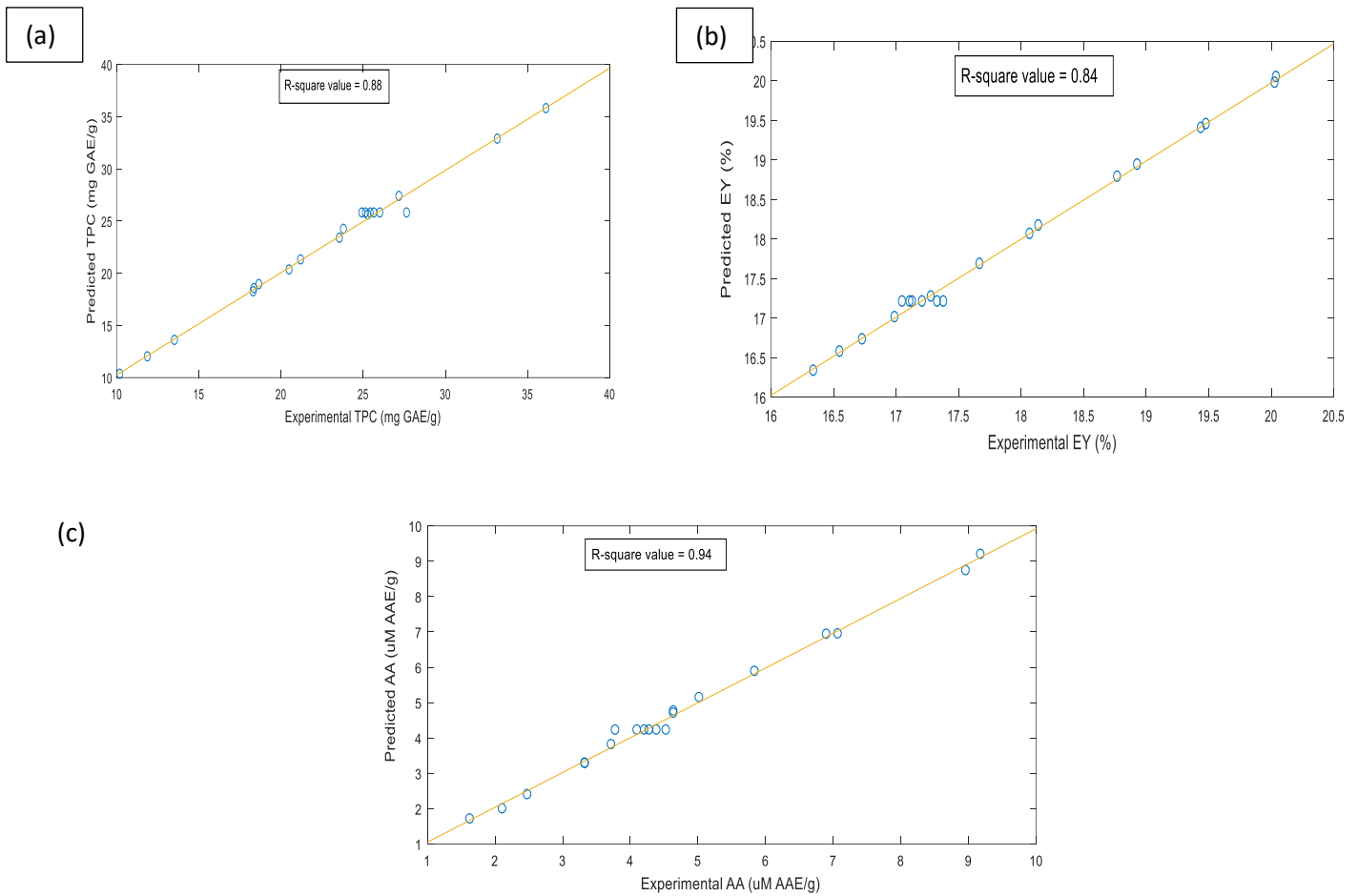


Fig. 4 Comparison between experimental and GPR- RQ predicted (a) TPC (b) EY (c) AA of *A. aspera* leaves

3.4 Evaluation of Predictive Performance: CCD-RSM vs. GPR-RQ

The comparative analysis of statistical metrics between the CCD-RSM and GPR-RQ models reveals that CCD-RSM demonstrated superior predictive accuracy for TPC, EY, and AA as shown in Table 5. Specifically, CCD-RSM achieved higher coefficients of determination (R^2)—0.9840 for TPC, 0.9910 for EY, and 0.9919 for AA—indicating a stronger correlation between predicted and experimental values. Additionally, CCD-RSM exhibits lower Root Mean Square Error (RMSE)

values, suggesting minimal prediction errors. In contrast, GPR-RQ shows comparatively lower R^2 values and higher RMSEs, reflecting less precise predictions. These findings align with existing literature, where CCD-RSM has been effectively utilized for process optimization due to its robust predictive capabilities (Huang *et al.*, 2024; Ruslan *et al.*, 2021). However, the performance of predictive models can vary depending on the specific application and dataset characteristics.

Table 5: Statistical Performance Metrics of CCD-RSM and GPR-RQ Models

Statistics	CCD-RSM			GPR-RQ		
	TPC	EY	AA	TPC	EY	AA
R^2	0.9840	0.9910	0.9919	0.8800	0.8400	0.9400
RMSE	0.1149	0.0387	0.0170	2.2800	0.4700	0.4800

The visually demonstration of the performances of these models (CCD-RSM and GPR-RQ) in predicting the experimental laboratory observed data as functions of process parameters of OT, S/L

and ET for all the 20 runs in Table 1 is presented in Fig. 5. It is clear from the Fig. 5 that both CCD-RSM and GPR-RQ demonstrated excellent ability in the modelling and prediction of observed laboratory data of TPC, EY and AA as functions of OT, S/L and ET. The juxtaposition of CCD-RSM and GPR-RQ in the figure, however, underscores the strengths

and limitations of each modeling approach. CCD-RSM offers a structured framework for understanding variable interactions and optimizing responses within a defined experimental space.

(a) However, its reliance on predefined polynomial equations may limit its applicability in modeling highly complex or non-linear systems.

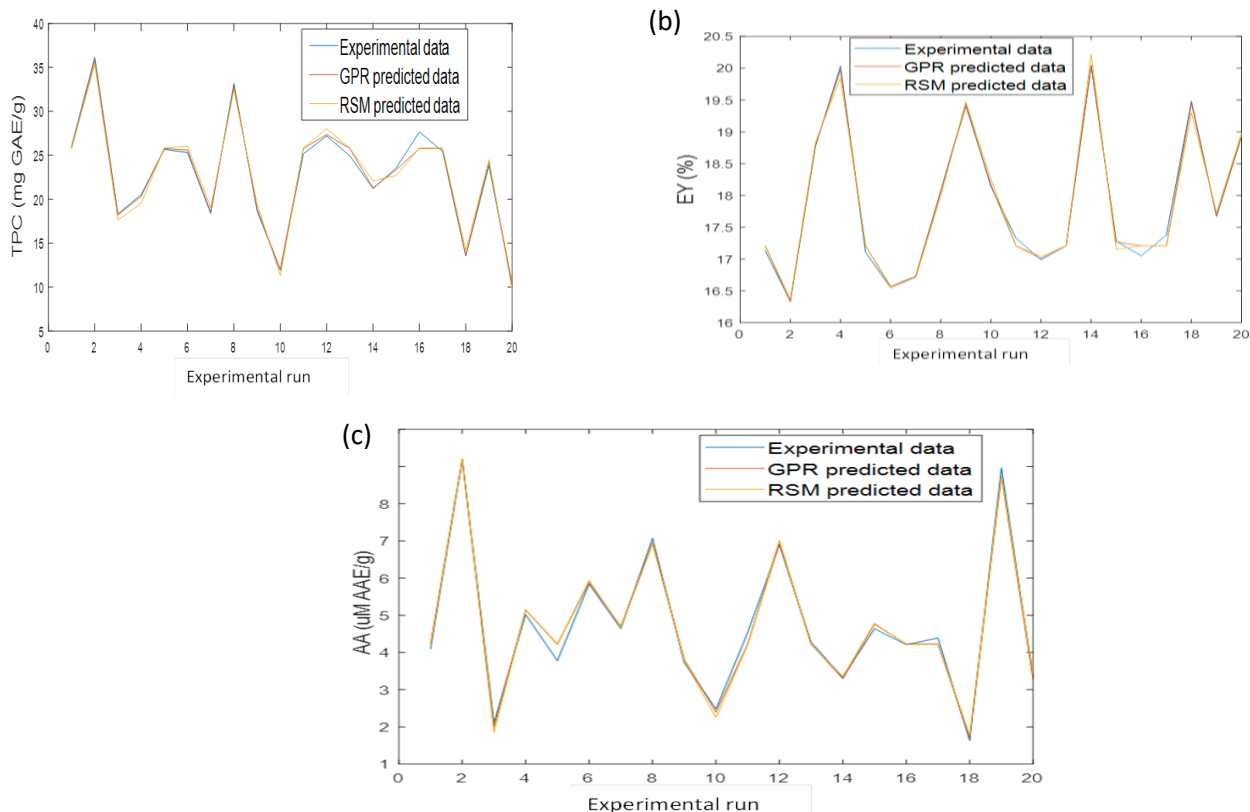


Fig 5: Practical modelling of CCD-RSM and GPR-RQ for (a) TPC (mg GAE/g) (b) EY (%) and (c) AA(μ M AAE/g d.w.)

Conversely, GPR-RQ's flexibility allows it to model complex relationships without assuming a specific functional form, providing a probabilistic measure of prediction uncertainty. This characteristic is advantageous in applications where understanding

the confidence of predictions is crucial. Therefore, the choice between these methods should be guided by the specific objectives of the study, the nature of the data, and the desired balance between interpretability and flexibility.

3.5 Multi-Objective Grey Wolf Optimization and Validation

The optimization of bioactive compound extraction from *A.aspera* leaves was conducted using a combination of MOGWO and the Technique for Order Preference by Similarity to Ideal Solution (TOPSIS). The CCD-RSM predictive models for TPC, EY and AA, being more superior to GPR-RQ model counterparts were integrated with the

MOGWO-TOPSIS for the purpose of optimization. This methodological approach aimed to determine the optimal extraction conditions by evaluating the effects of three process variables: OT, S/L, and ET. The optimization goals were to maximize TPC, EY and AA simultaneously in the *A.aspera* leaves extract.

Fig. 6 presents the relationship between decision variables and responses. The scatter plot of decision

variables is fundamental for understanding the influence of the process parameters. The distribution of data points reveals that higher S/L and longer ET are associated with improved extraction responses, particularly TPC and AA. However, these improvements plateau at specific thresholds, suggesting diminishing returns beyond certain levels of S/L and ET. The OT demonstrated a non-linear effect on responses, where moderate temperatures around 35°C optimize the process by enhancing phenolic compound solubility without risking thermal degradation. This observation highlights the delicate balance required in setting extraction conditions, consistent with prior studies emphasizing the role of temperature in maintaining compound stability (Vergara-Salinas *et al.*, 2012; Hossain *et al.*, 2011).

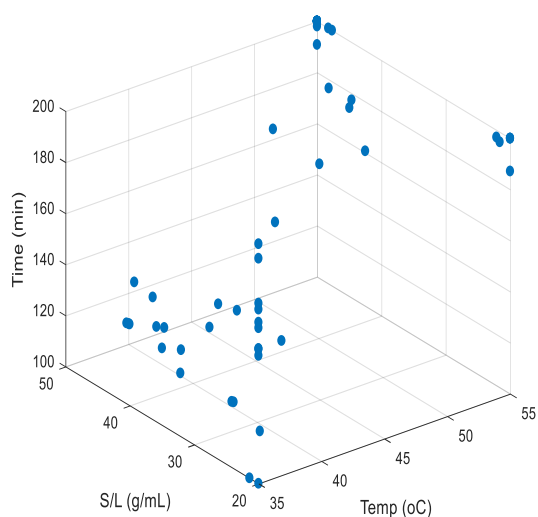


Fig. 6. Decision variable interactions in the optimization of bioactive compound extraction from *A. aspera* leaves

Fig.7 provides a 3D Pareto front that represents the trade-offs among the three responses (TPC, EY, and AA). The Pareto front analysis offers a multi-objective perspective, showcasing the trade-offs between TPC, EY, and AA. The data points along the Pareto front illustrate the inherent compromises in maximizing one response at the expense of others. For instance, while TPC and AA exhibit a positive correlation—suggesting that conditions conducive to phenolic extraction also enhance antioxidant activity—EY behaves more independently, necessitating careful optimization. This visualization underscores the complexity of

balancing multiple objectives in bioactive compound extraction and validates the efficacy of using MOGWO-TOPSIS for this purpose.

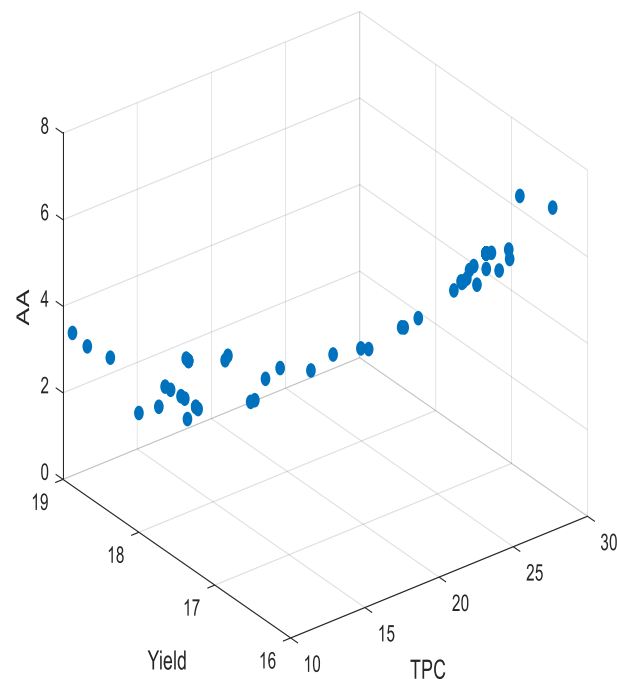


Fig. 7 Pareto Front depicting trade-offs among TPC, EY, and AA

MOGWO-TOPSIS is a powerful tool for multi-objective optimization. Rao *et al.* (2024) optimized Thyristor Controlled Series Capacitors (TCSC) placement in power systems, minimizing losses, while Mazraeh *et al.* (2022) applied it to stock portfolio optimization. Both studies highlight its effectiveness in balancing conflicting objectives, ensuring optimal trade-offs across diverse applications.

The iterative convergence plot (Fig. 8) demonstrates the efficiency of the MOGWO algorithm in optimizing the extraction process. TPC, EY, and AA all stabilize after a few iterations, with TPC reaching a peak of approximately 32.49 mg GAE/g, EY at 17.94%, and AA at 6.91 μ M AAE/g d.w. The rapid convergence to these optimal values underscores the robustness of the algorithm in navigating the multi-dimensional optimization space and achieving a balance among competing objectives. The stability observed across iterations also highlights the reliability of the GWO approach in identifying consistent optimal solutions.

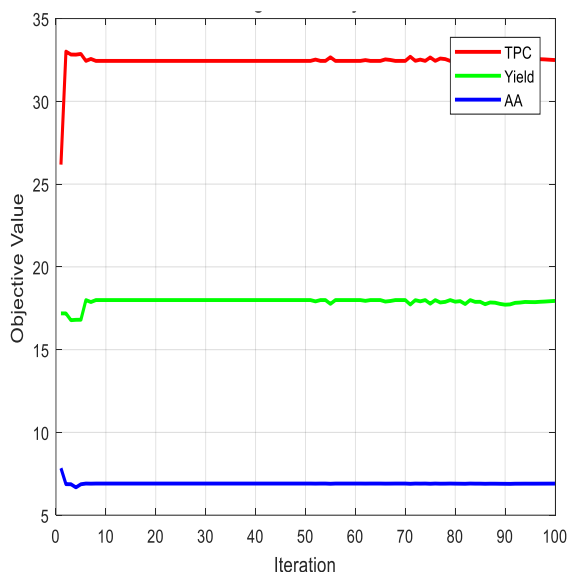


Fig.8 Iterative convergence plot for TPC, EY and AA

The combined insights from these figures identified the optimal extraction conditions as an operating temperature of 35°C, a liquid-to-solid ratio of 1:50 g/mL, and an extraction time of 198.87 minutes. Under these conditions, the extraction achieves a TPC of 32.49 mg GAE/g, an EY of 17.94%, and an AA of 6.91 μ M AAE/g d.w.

To confirm the predictions made by the MOGWO-TOPSIS model, a validation experiment was carried out in the laboratory under the specified operating conditions. The HAE of bioactive antioxidants from *A. aspera* leaves was performed at 35°C, a S/L of 50 mL/g, and an ET of 198.87 min. The % RSD was utilized to evaluate the consistency between the predicted and experimentally validated results. The experimentally validated outcomes yielded a TPC of 36.2023 mg GAE/g dry basis (db), an EY of 18.2291%, and AA of 6.2178 μ M AAE/g dry weight (dw). When compared with the predicted values, the % RSDs for TPC, EY, and AA were calculated as 7.64%, 1.13%, and 7.46%, respectively. Since the % RSD for all responses was below 10%, the experimental results were deemed consistent with the model's predictions (Domínguez *et al.*, 2020). These findings validate the effectiveness of GWO-TOPSIS in optimizing the extraction process, demonstrating its reliability in

providing a practical solution that ensures both efficiency and quality.

3.6 Elucidation of Bioactive Contents of *A. aspera* Leaves Extracts

HPLC fingerprinting is a critical tool for identifying bioactive compounds that contribute to the functional and therapeutic properties of plants, making it indispensable for drug development and product formulation. In this study, the extract of *A. aspera* leaves, obtained under optimized extraction conditions (OT of 35°C, S/L ratio of 50 mL/g, and ET of 198.87 min), was analyzed using HPLC to profile its bioactive constituents. Eight phenolic acids with well-documented therapeutic potential were used as reference standards, and their retention times (RT) were compared with those of the compounds present in the *A. aspera* extract. The resulting chromatogram, shown in Figure 8, reveals a diverse array of phenolic compounds with significant variations in peak areas, reflecting differences in their relative concentrations.

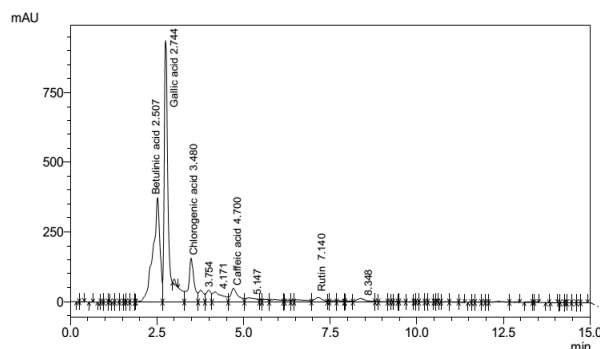


Fig. 9 Chromatogram of *A. aspera* leaves extract

From the chromatogram, distinct peaks corresponding to bioactive compounds such as betulinic acid (retention time 2.507 min), gallic acid (2.744 min), chlorogenic acid (3.480 min), caffeic acid (4.700 min), and rutin (7.140 min) are identified. These compounds are well-documented in the literature for their pharmacological properties. For instance, betulinic acid is known for its anti-inflammatory and anticancer activities (Yogeeswari and Sriram, 2005), while gallic acid exhibits potent antioxidant and antimicrobial properties (Kahkeshani *et al.*, 2019). Similarly, chlorogenic acid is recognized for its role in modulating glucose

metabolism and antioxidative effects (Farah & de Paula Lima, 2019). Caffeic acid and rutin are also widely studied for their antioxidant and anti-inflammatory activities (Mondal *et al.*, 2021). The presence of these phenolic and flavonoid compounds highlights the therapeutic potential of *A. aspera* leaves, supporting their use in traditional medicine for managing oxidative stress-related diseases and inflammation. Moreover, the chromatographic profile suggests that the extraction method used is effective in isolating a wide range of bioactive compounds.

Conclusion

This study establishes HAE as an efficient method for recovering phenolic-rich antioxidants from *A. aspera* leaves, leveraging GWO for multi-objective optimization and GPR alongside CCD-RSM for predictive modeling. Optimized conditions yielded a TPC of 36.2023 mg GAE/g db, EY of 18.2291%, and AA of 6.2178 μ M AAE/g dw, with % RSD values below 10%, validating the optimization framework. CCD-RSM outperformed GPR in predictive accuracy, with higher R^2 values and lower RMSE, making it more reliable for modelling linear and quadratic interactions. However, GPR offered flexibility in capturing complex, nonlinear relationships, emphasizing the complementary strengths of these models. HAE proved to be an energy-efficient and scalable process, ensuring the recovery of high-quality bioactive compounds. This work provides valuable insights into combining optimization tools for robust process design, supporting the industrial-scale production of phenolic antioxidants for diverse applications in food, pharmaceutical, and cosmetic sectors.

References

- Adeyi, O., Adeyi, A. J., Oke, E. O., Okolo, B. I., Olalere, A. O., Otolorin, J. A., ... & Akatobi, K. N. (2022)a. Process integration for food colorant production from Hibiscus sabdariffa calyx: a case of multi-gene genetic programming (MGGP) model and techno-economics. *Alexandria Engineering Journal*, 61(7), 5235–5252.
- Adeyi, O., Oke, E. O., Adeyi, A. J., Okolo, B. I., Olalere, A. O., Otolorin, J. A., ... & Oladunni, S. (2022)b. Drying characteristics of thermally pre-treated COBRA 26 F1 tomato slabs and applicability of Gaussian Process Regression-based models for the prediction of experimental kinetic data. *Korean Journal of Chemical Engineering*, 39(5), 1135–1145.
- Adeyi, O., Adeyi, A. J., Oke, E. O., Okolo, B. I., Olalere, O. A., Taiwo, A. E., ... & Ogunsola, A. D. (2023). Heat-assisted extraction of phenolic-rich bioactive antioxidants from Enantia chlorantha stem bark: multi-objective optimization, integrated process techno-economics and profitability risk assessment. *SN Applied Sciences*, 5(6), 153.
- Alanzi, H., Alenezi, H., Adeyi, O., Adeyi, A. J., Olusola, E., Gan, C. Y., & Olalere, O. A. (2024). Process optimization, multi-gene genetic programming modeling and reliability assessment of bioactive extracts recovery from Phyllanthus emblica. *Journal of Engineering Research*, 12(2), 453 - 471.
- Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escalera, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, 76(5), 965–977.
- Bhuyan, D. J., & Basu, A. (2017). Phenolic compounds potential health benefits and toxicity. In *Utilisation of bioactive compounds from agricultural and food production waste* (27–59). CRC Press.
- Bouafia, M., Colak, N., Ayaz, F. A., Benarfa, A., Harrat, M., Gourine, N., & Yousfi, M. (2021). The optimization of ultrasonic-assisted extraction of Centaurea sp. antioxidative phenolic compounds using response surface methodology. *Journal of Applied Research on Medicinal and Aromatic Plants*, 25, 100330.
- Boutoub, O., Aazza, S., El-Guendouz, S., El Ghadraoui, L., & Miguel, M. (2022). Response surface methodology (RSM) for optimization of Euphorbia resinifera and Euphorbia officinarum extracts with antioxidant and anti-diabetic activities. *Pharmacognosy Magazine*, 18(80), 940–952.

- Cerda-Flores, S. C., Rojas-Punzo, A. A., & Nápoles-Rivera, F. (2022). Applications of multi-objective optimization to industrial processes: a literature review. *Processes*, 10(1), 133.
- Chakraborty, S., Uppaluri, R., & Das, C. (2020). Optimization of ultrasound-assisted extraction (UAE) process for the recovery of bioactive compounds from bitter gourd using response surface methodology (RSM). *Food and Bioproducts Processing*, 120, 114–122.
- Chelladurai, S. J. S., Murugan, K., Ray, A. P., Upadhyaya, M., Narasimharaj, V., & Gnanasekaran, S. (2021). Optimization of process parameters using response surface methodology: A review. *Materials Today: Proceedings*, 37, 1301–1304.
- Chen, H., Wang, B., Li, J., Xu, J., Zeng, J., Gao, W., & Chen, K. (2023). Comparative study on the extraction efficiency, characterization, and bioactivities of *Bletilla striata* polysaccharides using response surface methodology (RSM) and genetic algorithm-artificial neural network (GA-ANN). *International Journal of Biological Macromolecules*, 226, 982–995.
- Dai, Z., Liu, Y., & Zhao, Y. (2018). Optimization of extraction conditions for phenolic compounds from *Achyranthes aspera* leaves using response surface methodology. *Food Science & Nutrition*, 6(7), 1851–1859. <https://doi.org/10.1002/fsn3.749>
- Dahmoune, F., Remini, H., Dairi, S., Aoun, O., Moussi, K., Bouaoudia-Madi, N., ... & Madani, K. (2015). Ultrasound assisted extraction of phenolic compounds from *P. lentiscus* L. leaves: Comparative study of artificial neural network (ANN) versus degree of experiment for prediction ability of phenolic compounds recovery. *Industrial Crops and Products*, 77, 251–261.
- Domínguez, R., Zhang, L., Rocchetti, G., Lucini, L., Pateiro, M., Munekata, P. E., Lorenzo, J. M., 2020. Elderberry (*Sambucus nigra* L.) as potential source of antioxidants. Characterization, optimization of extraction parameters and bioactive properties. *Food Chem.*, 330, 127266.
- El-Adawy, M., Zayed, M. E., Shboul, B., Ashraf, W. M., & Nemitallah, M. A. (2024). Performance improvement of compression ignition engine fueled by second generation biodiesel fuel blends enriched with ZnO nanoparticles: Experimental study and Gaussian process regression AI modelling. *Process Safety and Environmental Protection*, 190, 1372–1385.
- Farah, A., & de Paula Lima, J. (2019). Chlorogenic acids: Daily consumption through coffee, metabolism, and health benefits. In *Coffee: Production, Quality and Chemistry* (161–192).
- Faris, H., Aljarah, I., Al-Betar, M. A., & Mirjalili, S. (2018). Grey wolf optimizer: a review of recent variants and applications. *Neural Computing and Applications*, 30, 413–435.
- Golpour, I., Ferrão, A. C., Gonçalves, F., Correia, P. M., Blanco-Marigorta, A. M., & Guiné, R. P. (2021). Extraction of phenolic compounds with antioxidant activity from strawberries: Modelling with artificial neural networks (ANNs). *Foods*, 10(9), 2228.
- Hossain, M. B., Barry-Ryan, C., Martin-Diana, A. B., & Brunton, N. P. (2011). Optimisation of accelerated solvent extraction of antioxidant compounds from rosemary (*Rosmarinus officinalis* L.), marjoram (*Origanum majorana* L.) and oregano (*Origanum vulgare* L.) using response surface methodology. *Food Chemistry*, 126(1), 339–346.
- Huang, T., Chen, Y., Zhou, Q., Sun, S., Gao, Z., & Zhang, X. (2024). Optimization of the extraction process of Danggui Xiaobai granules based on CCD-RSM. In *Proceedings of the 2024 5th International Symposium on Artificial Intelligence for Medicine Science* (764–769).
- Jankovič, D., Šimic, M., & Herakovič, N. (2024). A data-driven simulation and gaussian process regression model for hydraulic press condition diagnosis. *Advanced Engineering Informatics*, 59, 102276.
- Kahkeshani, N., Farzaei, F., Fotouhi, M., Alavi, S. S., Bahramsoltani, R., Naseri, R., ... & Bishayee, A. (2019). Pharmacological effects of gallic acid in health and diseases:

A mechanistic review. *Iranian Journal of Basic Medical Sciences*, 22(3), 225.

- Krishna Murthy T.P., Manohar B., 2014. Optimization of supercritical carbon dioxide extraction of phenolic compounds from mango ginger rhizome (*Curcuma amada* Roxb.) using response surface methodology. *Biomed. biotechnol.* 2, 14-19.
- Lee, W. C., Yusof, S. A. L. M. A. H., Hamid, N. S. A., & Baharin, B. S. (2006). Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). *Journal of Food Engineering*, 73(1), 55–63.
- Liao, J., Qu, B., & Zheng, N. (2016). Effects of process parameters on the extraction of quercetin and rutin from the stalks of *Euonymus Alatus* (Thumb.) Sieb and predictive model based on least squares support vector machine optimized by an improved fruit fly optimization algorithm. *Applied Sciences*, 6(11), 340.
- Lopez-Hortas, L., Rodriguez, P., Diaz-Reinoso, B., Gaspar, M. C., de Sousa, H. C., Braga, M. E., & Dominguez, H. (2022). Supercritical fluid extraction as a suitable technology to recover bioactive compounds from flowers. *The Journal of Supercritical Fluids*, 188, 105652.
- Makhadmeh, S. N., Alomari, O. A., Mirjalili, S., Al-Betar, M. A., & Elnagar, A. (2022). Recent advances in multi-objective grey wolf optimizer, its versions and applications. *Neural Computing and Applications*, 34(22), 19723–19749.
- Mandefro, B., Fereja, W. M., Fremichael, D., Mereta, S. T., & Ambelu, A. (2024). Analysis of *Achyranthes aspera* leaf extract and acute toxicity study on fingerlings of Nile tilapia, *Oreochromis niloticus*. *Biochemistry and Biophysics Reports*, 37, 101624.
- Mazraeh, N. B., Daneshvar, A., Madanchi zaj, M., & Roodposhti, F. R. (2022). Stock portfolio optimization using a combined approach of multi objective grey wolf optimizer and machine learning preselection methods. *Computational Intelligence and Neuroscience*, 2022(1), 5974842.
- Mirjalili, S., Saremi, S., Mirjalili, S. M., & Coelho, L. D. S. (2016). Multi-objective grey wolf optimizer: a novel algorithm for multi-criterion optimization. *Expert Systems with Applications*, 47, 106–119.
- Mirjalili, S., Mirjalili, S. M., & Lewis, A. (2014). Grey wolf optimizer. *Advances in Engineering Software*, 69, 46–61.
- Mondal, S., Soumya, N. P. P., Mini, S., & Sivan, S. K. (2021). Bioactive compounds in functional food and their role as therapeutics. *Bioactive Compounds in Health and Disease*, 4(3), 24–39.
- Montgomery, D. C. (2017). Design and analysis of experiments (9th ed.). Wiley.
- Morita, Y., Rezaeiravesh, S., Tabatabaei, N., Vinuesa, R., Fukagata, K., & Schlatter, P. (2022). Applying Bayesian optimization with Gaussian process regression to computational fluid dynamics problems. *Journal of Computational Physics*, 449, 110788.
- Myers, R. H., & Montgomery, D. C. (2002). Response surface methodology: Process and product optimization using designed experiments (2nd ed.). Wiley.
- Nargatti, P., Patil, S., & Wadkar, K. (2021). Phytochemical profile and pharmacological aspects of *Achyranthes aspera* Linn-An overview. *Journal of Pharmaceutical Research International*, 33, 187–206.
- Ndhlala, A. R., Ghebrehiwot, H. M., Ncube, B., Aremu, A. O., Gruz, J., Šubrtová, M., ... & Van Staden, J. (2015). Antimicrobial, anthelmintic activities and characterisation of functional phenolic acids of *Achyranthes aspera* Linn.: a medicinal plant used for the treatment of wounds and ringworm in east Africa. *Frontiers in Pharmacology*, 6, 274.
- Oke, E. O., Adeyi, O., Okolo, B. I., Adeyi, J. A., Ayanyemi, J., Osoh, K. A., & Adegoke, T. S. (2020). Phenolic compound extraction from Nigerian *Azadirachta indica* leaves: response surface and neuro-fuzzy modelling performance evaluation with Cuckoo search multi-objective optimization. *Results in Engineering*, 8, 100160.

- Olalere, O. A., Gan, C. Y., Adediji, P. A., Olalere, M. E., & Aljbour, N. (2022). Multi-objective Deng's grey incidence analysis, orthogonal optimization, and artificial neural network modelling in hot-maceration-assisted extraction of African cucumber leaves (*Momordica balsamina*). *The Canadian Journal of Chemical Engineering*, 100(3), 588–597.
- Olalere, O. A., Abdurahman, H. N., & Gan, C. Y. (2019). Microwave-enhanced extraction and mass spectrometry fingerprints of polyphenolic constituents in *Sesamum indicum* leaves. *Industrial Crops and Products*, 131, 151–159.
- Olalere, O. A., Gan, C. Y., Akintomiwa, O. E., Adeyi, O., & Adeyi, A. (2021). Optimisation of microwave-assisted extraction and functional elucidation of bioactive compounds from *Cola nitida* pod. *Phytochemical Analysis*, 32(5), 850–858.
- Pereira, D. T. V., Tarone, A. G., Cazarin, C. B. B., Barbero, G. F., & Martinez, J. (2019). Pressurized liquid extraction of bioactive compounds from grape marc. *Journal of Food Engineering*, 240, 105–113.
- Poyraz, Ç., Küçükyıldız, G., Kırbaşlar, Ş. İ., Cığeroğlu, Z., & Şahin, S. (2021). Valorization of Citrus unshiu biowastes to value-added products: an optimization of ultrasound-assisted extraction method using response surface methodology and particle swarm optimization. *Biomass Conversion and Biorefinery*, 1–11.
- Premkumar, M., Jangir, P., Kumar, B. S., Alqudah, M. A., & Nisar, K. S. (2022). Multi-Objective Grey Wolf Optimization Algorithm for Solving Real-World BLDC Motor Design Problem. *Computers, Materials & Continua*, 70(2).
- Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., ... & Uddin, M. S. (2021). Role of phenolic compounds in human disease: current knowledge and future prospects. *Molecules*, 27(1), 233.
- Rangaiah, G. P., Feng, Z., & Hoadley, A. F. (2020). Multi-objective optimization applications in chemical process engineering: Tutorial and review. *Processes*, 8(5), 508.
- Rasouli, H., Farzaei, M. H., & Khodarahmi, R. (2017). Polyphenols and their benefits: A review. *International Journal of Food Properties*, 20(sup2), 1700–1741.
- Rao, N. T., Kumar, K. K., Kumar, P. P., Nuvvula, R. S., Mutharasan, A., Dhanamjayulu, C., ... & Khan, B. (2024). Multiobjective optimal TCSC placement using multiobjective grey wolf optimizer for power losses reduction. *Scientific Reports*, 14(1), 21857.
- Rehman, R., Melki, D., Shehzad, A., Nadeem, F., & Khalid, T. (2018). Commercial importance, medicinal value and therapeutic potentials of chaff flower (*Achyranthes aspera*) – a review. *International Journal of Chemical and Biochemical Sciences*, 14, 62–70.
- Rui, J., Zhang, H., Ren, Q., Yan, L., Guo, Q., & Zhang, D. (2020). TOC content prediction based on a combined Gaussian process regression model. *Marine and Petroleum Geology*, 118, 104429.
- Sanguri, S., Kapil, S., Gopinathan, P., Pandey, F. K., & Bhatnagar, T. (2011). Comparative screening of antibacterial and antifungal activities of some Weeds and medicinal plants leaf extracts: An in-vitro study. *Environmental Ecology*, 29, 1351–1354.
- Sarma, L., Chakraborty, S., & Duary, R. K. (2018). Solvent-based microwave-assisted extraction and identification of bioactive compounds from *Sesamum indicum* leaves using particle swarm optimization-integrated response surface methodology. *Pharmacognosy Magazine*, 14(57s).
- Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods*, 18, 820–897.
- Shi, X., Jiang, D., Qian, W., & Liang, Y. (2022). Application of the Gaussian process regression method based on a combined kernel function in engine performance prediction. *ACS omega*, 7(45), 41732–41743.
- Shiekh, K. A., Olatunde, O. O., Zhang, B., Huda, N., & Benjakul, S. (2021). Pulsed electric field assisted process for extraction of bioactive

- compounds from custard apple (*Annona squamosa*) leaves. *Food Chemistry*, 359, 129976.
- Shi, X., Jiang, D., Qian, W., & Liang, Y. (2022). Application of the Gaussian process regression method based on a combined kernel function in engine performance prediction. *ACS omega*, 7(45), 41732–41743.
- Tan, M. K., Yusoff, M. M., & Lee, H. K. (2019). A review on the extraction of phenolic compounds from *Achyranthes aspera* and their antioxidant properties. *Journal of Food Science and Technology*, 56(4), 2341–2350. <https://doi.org/10.1007/s11483-019-01864-4>
- Tiwari, B. K., O'donnell, C. P., Patras, A., Brunton, N., & Cullen, P. J. (2009). Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage. *European Food Research and Technology*, 228, 717–724.
- Vergara-Salinas, J. R., Pérez-Jiménez, J., Torres, J. L., Agosin, E., & Pérez-Correa, J. R. (2012). Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (*Thymus vulgaris*). *Journal of Agricultural and Food Chemistry*, 60(44), 10920–10929.
- Verma, K. K., Sharma, A., Raj, H., & Kumar, B. (2021). A comprehensive review on traditional uses, chemical compositions and pharmacology properties of *Achyranthes aspera* (Amaranthaceae). *Journal of Drug Delivery and Therapeutics*, 11(2-S), 143–149.
- Wang, J. (2023). An intuitive tutorial to Gaussian processes regression. *Computing in Science & Engineering*.
- Wang, Y., Yang, Y., Jiao, J., Wu, Z., & Yang, M. (2018). Support vector regression approach to predict the design space for the extraction process of *Pueraria lobata*. *Molecules*, 23(10), 2405.
- Weremfo, A., Abassah-Oppong, S., Adulley, F., Dabie, K., & Seidu-Larry, S. (2023). Response surface methodology as a tool to optimize the extraction of bioactive compounds from plant sources. *Journal of the Science of Food and Agriculture*, 103(1), 26–36.
- Yogeeswari, P., & Sriram, D. (2005). Betulinic acid and its derivatives: a review on their biological properties. *Current Medicinal Chemistry*, 12(6), 657–666.
- Zakaria, F., Talip, B. A., Kahar, E. E. M., Muhammad, N., Abdullah, N., & Basri, H. (2020). Solvent used in extraction process of agarwood: A systematic review. *Food Research*, 4(3), 731–737.
- Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine*, 13, 1–26.
- Zhang, Z., Guo, S., Chen, C., Lin, J., Chen, L., Wang, D., ... & Wang, X. (2022). Optimization of extraction and bioactivity detection of celery leaf flavonoids using BP neural network combined with genetic algorithm and response. *Preparative Biochemistry & Biotechnology*, 52(6), 648–656.