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Optimization and modelling of microwave-assisted aqueous extraction for enhanced recovery of phenolic and tannin compounds from tulsi leaves

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Abstract

The present study investigates the effectiveness of aqueous extraction using microwaves (MAAE) for isolating phenolic compounds from tulsi leaves (*Ocimum sanctum*). Independent factors, including microwave power (280–580 W), treatment duration (20–60 seconds), and leaf-to-water ratio (1:40–1:60), were modelled and optimized using Box–Behnken design and response surface methodology (RSM). The optimization process identified the ideal conditions as 1:60 leaf-to-water ratio, 580 W microwave power and 40 seconds of extraction time, resulting in significant yields of total phenolic(T_{PL}) and tannin (T_{TN}) contents. The reliability and accuracy of the regression model were confirmed through statistical analyses, including ANOVA and various error metrics. The study highlights the advantages of MAAE of polyphenols from tulsi leaves demonstrating its potential as a sustainable substitute forconventional extraction techniques. Tulsi leaves were identified as a rich supply of bioactive components, contributing to by-product valorization and promoting environmental sustainability. These findings underscore the applicability of this method in producing value-added products, with promising prospects for scale-up and integration into the pharmaceutical and food sectors.

Keywords: Microwave-assisted extraction, Phenolic compounds, Response surface methodology, Tannin content, Tulsi leaves

Introduction

In regions where traditional medicine systems like Ayurveda are heavily practiced, medicinal plants play a significant role in daily healthcare for treating various diseases. Of all the medicinal plants used in Ayurveda, *Ocimum sanctum* (commonly known as "*tulsi*" which is the Sanskrit for 'the incomparable one') is the most revered (Mallikarjun et al., 2016). Tulsi has been shown to offer health advantages over the centuries, with studies highlighting the therapeutic potential of several portions of the plant, including its roots, leaves, seeds, and flowers (Cohen, 2014). Among these, tulsi leaves are the most widely utilized, as they are rich in bioactive components. Notably, polyphenolic compounds, including flavonoids, tannins and phenolic acids are the primary bioactive constituents responsible for a number of biological functions, including antimicrobial, anticancer, and antioxidant effects (Priya and Peddha, 2023). The growing recognition of these compound's importance has spurred significant scientific interest in their extraction and characterization.

Traditional solvent-based methods for phenolic content extraction are limited by economic feasibility, efficiency, and time, prompting the exploration of cutting-edge method like enzyme-assisted, microwave-assisted, and ultrasound-assisted extraction (Patra et al., 2022). Among these, microwave-assisted aqueous extraction (MAAE) offers distinct benefits, including significantly shorter processing times, non-toxic solvent usage, higher extraction efficiency, reduced costs and superior selectivity (Manoj et al., 2023; Loganathan et al., 2024).

For instance, Shijarath et al.(2024) successfully extracted tannins and polyphenols compounds from banana and pomegranate peels with higher yield and quality using MAAE. Similarly, Manoj et al.(2023) demonstrated that MAAE significantly enhanced the phenolic compound yields from jackfruit, jamun, and papaya seeds compared to traditional techniques. Kaderides et al.(2019) also reported a 1.7-fold increase in phenolic yield using MAAE over conventional methods. All of these research emphasise how crucial process parameters are, such as water-to-solid ratio, treatment time (T_{time}), and microwave power (M_{power}), in determining extraction efficiency. Additionally, they underscore water as a sustainable alternative to conventional solvents due to its eco-friendly properties (Vu et al., 2019). Given the demonstrated effectiveness of MAAE in enhancing

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the recovery of bioactive compounds, this technique presents a promising opportunity for optimizing extraction protocols. Thus, the present study focuses on developing efficient process parameters for the isolation of bioactive compounds, specifically polyphenols and tannins from tulsi leaves using MAAE.

The specific purposes of this study are to (a) assess the influence of MAAE on the yields of total phenolic content (T_{PL}) and total tannin (T_{TN}) content from tulsi leaves, (b) analyse the individual and combined impacts of key variables-M_{power}, T_{time}, and leaf-to-water ratio (LW_{ratio})-on the extraction yields of T_{PL} and T_{TN} contents and (c) optimize the extraction protocol to maximize the recovery of T_{PL} and T_{TN} contents from tulsi leaves using response surface methodology (RSM).

Materials and methods

Collection and preparation of tulsi leaves

Tulsi leaves were sourced locally from the locality of TKM Institute of Technology, Kerala, India. The leaves were separated from their stems, cleaned with fresh water to remove any impurities, and subsequently dried at 45 ± 1°C for 48 hours in a controlled environment. The achieved tulsi leaf powder was then stored in an airtight container at -30 ± 1°C to preserve its quality and prevent degradation until further use (Patra et al., 2021a; Shijarath et al., 2024).

MAAE

A microwave oven (Morphy Richards, 25 CG, India) with a maximum operating power of 1400 W and 2450 MHz of constant frequency was employed for the extraction process. The device allowed a maximum runtime of 30 minutes. Tulsi leaf powder (1 g) served as the substrate for all experiments, with distilled water as the solvent. The extraction procedures were conducted under varying conditions of M_{power}(280–580 W), T_{time}(20–60 seconds), and LW_{ratio}(1:40–1:60). These parameters were chosen based on initial experiments to maximize extraction efficiency.The range of the independent parameters were decided based on previous literatures and preliminary experiments (Patra et al., 2021a; Shijarath et al., 2024; Manoj et al., 2023).

The experimental design and combinations of independent variables were determined using a Box–Behnken Design (BBD) coupled with the RSM. This approach enabled the modelling of the impacts of the variables on T_{PL} and T_{TN} contents. Table 1 presents the 17 experimental runs, with five replicates at the centre points to ensure the robustness of the model.

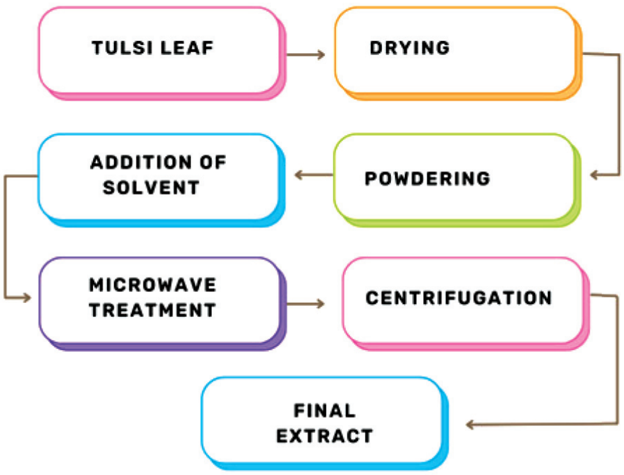

Figure 1. Diagrammatic Illustration of the Experimental Workflow

Following the treatment in microwave, the solutions were centrifuged at 5000 rpm for 15 minutes, as reported by Manoj et al. (2023). The supernatant was separated and stored for the determination of T_{PL} and T_{TN} contents. A schematic representation of the process is provided in Figure 1.

Untreated samples were prepared for the control extract following the procedure outlined by Patra et al.(2021b). Using this approach, one gram of tulsi leaf powder was mixed with distilled water (60 mL) for one hour, after which the sample underwent centrifugation at 5000 rpm for 15 minutes. The supernatant was obtained and analyzed to compare extraction yields with the MAAE technique.

Determination of T_{PL} content

The T_{PL} of tulsi leaf extractwas measured using the Folin-Ciocalteu method, as outlined by Patel et al. (2022) and Patra et al. (2021a). 0.1 mL of supernatant from tulsi extract was combined with 1.9 mL of distilled water , then 0.5mL of FC reagent was added and the mixture incubated for three minutes and 2mL Na_2CO_3 was added to it and the mixture is stirred well and kept in dark for 30 minutes. The solutions absorbance was measured later.

Determination of T_{TN} content

The method described by Abdullah et al. (2022) and Abdullah et al. (2020)was employed toestimate the T_{TN} content of tulsi leaf extract. For this, 1mL of supernatant from tulsi extract was combined with 5 mL of Folin Denis reagent and 10mL of Na_2CO_3 solution and made up to 100mL with distilled water. The solution is stirred well, kept for 30 minutes incubation and absorbance was measured later.

Process modelling

The analysis of the experimental values was performed using RSM via Design-Expert software. The average values of T_{PL}

contentand T_{TN} content, obtained from triplicate measurements of tulsi leaf extracts, were employed in the regression study.

The correlation between the independent variables and the T_{PL} and T_{TN}contents was described using the second degree equation proposed by RSM (Equation 1):

$$N = a_0 + a_1M_1 + a_2M_2 + a_3M_3 + a_{1,2}M_1M_2 + a_{1,3}M_1M_3 + a_{2,3}M_2M_3 + a_{1,1}M_1^2 + a_{2,2}M_2^2 + a_{3,3}M_3^2 \quad (1)$$

Where N = Value of responses (predicted)
a_0 ,a_1 , a_2 , a_3 = Coefficients of regression
M_1 ,M_2 , M_3 : Independent variables

The reliability and importance of the developed models were assessed through analysis of variance (ANOVA). This included statistical measures such as the lack of fit (LoF), p-value, overall coefficient of determination (R^2), adjusted coefficient of determination (adj. R^2), and predicted coefficient of determination (Pred. R^2)(A Patra et al., 2022)

Numerical optimization

The modelled data's process optimization was done using the Design-Expert software's numerical optimization feature. The procedure involved maximizing the response variables (T_{PL} and T_{TN} contents) while ensuring that the process parameters remained within the defined range, as outlined by Patra et al.(2021a).

Statistical examination

All experiments were carried outthree times, and the results were expressed as average values accompanied by their respective standard deviations (SD).

The model's effectiveness was assessed using several statistical metrics, including normalized mean square error (NMSE), mean percentage error (MPE), root mean square error (RMSE), average absolute deviation (AAD), normalized root mean square error (NRMSE), mean square error (MSE), and coefficient of determination (R^2) (Manoj et al., 2023). These metrics were computed using the subsequent equations (Eqns. 2–8):

$$MSE = \frac{\sum_{i=1}^{n}(a_{Pred} - a_{expe})^2}{n} \quad (2)$$

$$NMSE = \frac{MSE}{a_{mean}} \quad (3)$$

$$AAD = \frac{\sum_{i=1}^{n}|a_{pred} - a_{expe}|}{n} \quad (4)$$

$$MPE = \frac{100}{n}\sum \left|\frac{(a_{Pred} - a_{expe})}{a_{Pred}}\right| \quad (5)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(a_{Pred} - a_{expe})^2}{\sum_{i=1}^{n}(a_{Pred} - a_{mean})^2} \quad (6)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(a_{Pred} - a_{expe})^2}{n}} \quad (7)$$

$$NRMSE = \frac{RMSE}{a_{mean}} \quad (8)$$

Where, a_{expe}:experimental data, a_{pred}:predicted data, n: number of experiments and a_{mean}: mean of the experimental data.

The optimal model for describing the responses was determined based on achieving the lowest values for MSE, NMSE, AAD, MPE, RMSE, and NRMSE, along with the highest R_2 value.

Results and discussion

Model fitting

The effects of process parameters on the yield of T_{PL}content and T_{TN}content from tulsi leaves are summarized in Table 1. The regression coefficients along with statistical indicators, including pred. R^2, adj. R^2, lack of fit, AAD, RMSE, NMSE, MSE, MPE and NRMSEfor the developed second-order equations are provided in Table 2. The statistical analysis confirms the reliability and accuracy of these models.

Jaddu et al. (2022b) and Navya et al. (2024) have suggested that regression models are deemed reliable when the R^2 value exceeds 0.8 and the difference between pred. and adj. R^2 is less than 0.2. In the present work, the R^2 values of all models are>0.9, and the pred.-adj. R^2 gap consistently remained within the 0.2 threshold, thereby affirming the robustness of the models developed. Moreover, all models demonstrated statistical significance (p<0.05) with a non-significant lack of fit (p>0.05). The minimal values of AAD, NMSE, RMSE, MSE, MPE and further corroborate the strong agreement between the observed and predicted data (Saranya et al., 2024). Additionally, the second-degree polynomial models derived from nonlinear multiple regression analysis for the tulsi leaf dataset, are presented in Equations 9, and 10. Furthermore, the T_{PL} and T_{TN}values of the extract were predicted with the help of these equations and are presented in Table 1.

$$T_{PL}\text{content (mg/100 g)} = 59.24 + 3.13 X_1 + 10.01X_2 + 0.83 X_3 - 5.12 X_1X_2 + 7.33 X_1X_3 - 4.91X_2X_3 + 5.18 X_1^2 - 14.13 X_2^2 + 8.70 X_3^2 \quad (9)$$

Table 1. Predicted and experimental T_{PL} and T_{TN} contents of tulsi leaf extract at various process parameter combinations

Sl. No.	M_{power} (X_1) W	T_{time} (X_2) sec	LW_{ratio}(X_3)	Tulsi leaf: Total Phenolic Content mg GAE/100 g		Tulsi leaf: Total Tannin Contentmg Tannic acid/100 g	
				Experimental value	RSM predicted value	Experimental value	RSM predicted value
1	280	40	60	61.33±0.12	63.50	47.38±0.27	47.37
2	280	20	50	34.61±0.21	32.02	25.88±0.13	25.37
3	580	20	50	46.91±0.23	48.51	33.72±0.17	34.30
4	280	40	40	74.45±0.64	76.48	53.52±0.41	54.62
5	280	60	50	63.89±0.34	62.29	42.5±0.26	41.92
6	430	40	50	64.83±0.29	59.24	46.61±0.28	43.19
7	430	40	50	59.24±0.09	59.24	42.59±0.21	43.19
8	430	60	60	60.31±0.36	59.75	43.36±0.32	43.95
9	430	40	50	63.16±0.60	59.24	46.41±0.29	43.19
10	430	40	50	55.62±0.46	59.24	39.99±0.31	43.19
11	430	20	60	49.12±0.43	49.54	37.32±0.17	37.84
12	580	40	60	86.43±0.71	84.41	61.14±0.41	60.04
13	580	40	40	70.25±0.59	68.08	50.51±0.33	50.52
14	580	60	50	55.71±0.26	58.30	41.05±0.27	41.56
15	430	40	50	53.34±0.17	59.24	40.35±0.31	43.19
16	430	20	40	37.48±0.11	38.05	31.51±0.16	30.91
17	430	60	40	68.32±0.52	67.90	49.12±0.19	48.60

Where, M_{power}: Microwave power, T_{time}: Treatment time, LW_{ratio}: Leaf to water ratio

$$T_{TN}\text{ content (mg/100 g)} = 43.19 + 2.14 X_1 + 5.95 X_2 + 0.57 X_3 - 2.32 X_1X_2 + 4.19 X_1X_3 - 2.89 X_2X_3 + 2.70 X_1^2 - 10.10 X_2^2 + 7.24 X_3^2 \quad (10)$$

Impact of independent parameters on T_{PL} and T_{TN} contents

The findings presented in Table 2 highlight that the linear effect of T_{time} ($p < 0.001$), the quadratic effects of M_{power} ($p < 0.05$) and LW_{ratio} ($p < 0.01$), and the interaction between M_{power} and LW_{ratio} ($p < 0.05$) significantly and positively influenced the T_{PL} yield of the extract. Conversely, the quadratic effect of T_{time} ($p < 0.001$) and the interaction between M_{power} and T_{time} ($p < 0.05$) exhibited a significant negative impact. The model demonstrated an R^2 of 0.9502, indicating that it explains more than 95 per cent data variability.

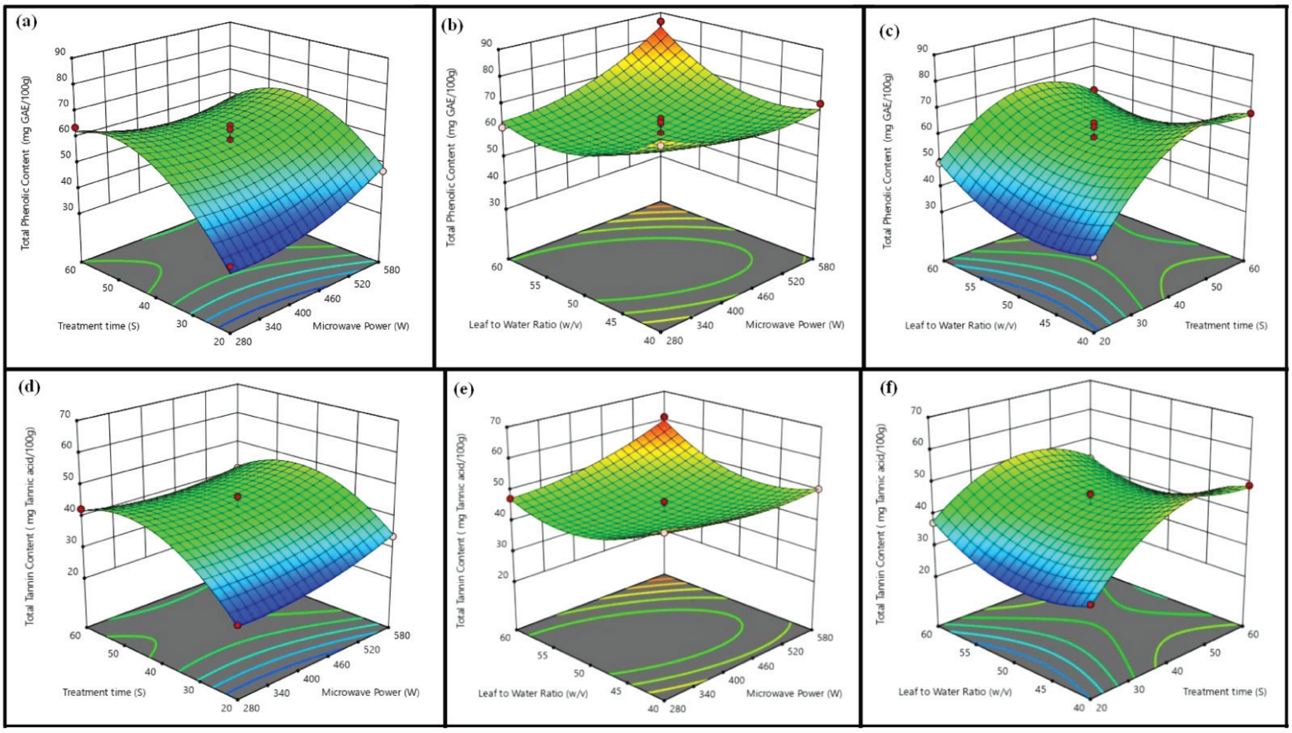

Figure 2:Tulsi leaf extract's response surface diagram for–T_{PL} content as a function of (a) T_{time} and M_{power}; (b) LW_{ratio} and M_{power}; (c) LW_{ratio} and T_{time} and T_{TN} content as a function of (d) T_{time} and M_{power}; (e) LW_{ratio} and M_{power}; (f) LW_{ratio} and T_{time}.

Fig. 2 (a) to (c) demonstrates that the phenolic yield rises proportionally with T_{time} until 55 seconds, beyond which it plateaus. The T_{PL} content shows a steady increase with M_{power} until 400 W, succeeded by a sharper rise. Additionally, the figures indicate a reduction in T_{PL} yield as the LW_{ratio} decreases to 1:50, after which the content begins to rise again.

Table 2. further reveals that the linear effects of M_{power} ($p < 0.05$) and T_{time} ($p < 0.001$), the quadratic effect of the LW_{ratio} ($p < 0.001$), and the interaction between M_{power} and LW_{ratio} ($p < 0.05$) significantly enhanced the T_{TN} content in the tulsi leaf extract. However, a significant negative correlation ($p < 0.001$) was observed for the quadratic effect of T_{time}. An R^2 of 0.9601 was achieved by the model, accounting for over 96 per cent data variability.

In the same way, Figure 2 (d) to (f) illustrates a steep early rise in T_{TN} content with longer T_{time}, which eventually stabilizes. The T_{TN} yield also shows a consistent rise with increasing M_{power}. Moreover, the figures depict an early decline in T_{TN} content as the LW_{ratio} increases, followed by a subsequent rise. The findings highlight the pivotal role of M_{power} in how well phenolic compounds are extracted from leaves. During MAAE, the M_{power} generates electromagnetic radiation, causing breakage of leaf cell walls and facilitating the leakage of intracellular compounds into water (Alara et al., 2021). As M_{power} surges, the magnitude of the cell wall rupture enhances, improving extraction recovery (Alara and Abdurahman, 2019; Sharma and Dash, 2022). Nevertheless, excessive M_{power} can result in thermal degradation of various polyphenols, potentially lowering the extraction efficiency (Sahin et al., 2017). Notably, here, thermal degradation was not witnessed at greaterM_{power} evels, most likely attributable to the optimized process parameters that prevented excessive heating (Patra et al., 2022).

Table 2. Thestatistical indicators and regression coefficients of the T_{PL} and T_{TN} content of tulsi leaf under differentcombinations of process parameters

Coefficients	Tulsi leaf	
	Total Phenolic Content	Total Tannin Content
b_0	59.238***	43.19***
b_1	3.1275	2.1425*
b_2	10.01375***	5.95***
b_3	0.83625	0.5675
b_{12}	-5.12*	-2.3225
b_{13}	7.325*	4.1925*
b_{23}	-4.9125	-2.8925
b_{11}	5.17475*	2.70375
b_{22}	-14.1328***	-10.1063***
b_{33}	8.70225**	7.24375***
R^2	0.9502	0.9601
Adj. R^2	0.8861	0.9087
Pred. R^2	0.7190	0.8762
Lack of fit (p-value)	0.5245	0.9187
MSE	7.7480	2.6813
NMSE	0.1311	0.0622
RMSE	2.7835	1.6375
NRMSE	0.0471	0.0380
MPE	3.8871	2.7413
AAD	2.2228	1.1706

1: M_{power},2:T_{time}, and 3LW_{ratio}; b_0:constant term; b_{12}, b_{13}, b_{23}: interaction terms; b_{11}, b_{22}, b_{33}: quadratic terms and b_1, b_2, b_3: linear terms.
***: significance at p d" 0.001. *: significanceat p d" 0.05.**: significance at p d" 0.01.

The T_{time} also significantly affects the extraction yield. Prolonged exposure leads to greater cell wall rupture, enabling more phenolic compounds release into the solvent(Shijarath et al., 2024). Similarly, the T_{time} was found to significantly influence phenolic yields during the recovery of phenolic compounds from black carrot pomace using the microwave-assisted extraction method, as demonstrated by (Kumar et al., 2019). This observation supports with the study of Chaari et al. (2024), Manoj et al. (2023) and Patra et al. (2021b), who observed a direct relationship between T_{time} and phenolic compound recovery.

The LW_{ratio} is also an important parameter that affects the extraction efficiency. Increasing this ratio alters the concentration gradient across the cell membranes, improving the extraction of phenolic components into the medium. This occurrence has been noted in previous reports, where higher aqueous concentration improved bioactive compound extraction (Vázquez-Espinosa et al., 2019; Patra et al., 2022; Loganathan et al., 2024). However, the current work identified an optimal LW_{ratio}, beyond which the phenolic recovery plateaued or decreased, likely due to excessive dilution reducing the mass transfer driving force.

A similar tendency was observed for tannin content, where both M_{power} and T_{time} had a positive correlation with tannin recovery. The increase was followed by stabilization, emphasizing the importance of maintaining optimal conditions to prevent excessive tannin degradation. The quadratic terms in the analysis further underline this need for balance. These findings align with prior study by Chaari et al. (2024), Fonteles et al. (2017) and Simsek et al. (2012), which highlighted the critical role of optimizing M_{power} and T_{time} to maximize the recovery of bioactive compounds.

Numerical optimization

The process parameters were carefully kept within specified ranges, each allotted a prominence level of '5', while the targets for T_{TN} and T_{PL} contents were set to maximize their extraction.

The highest desirability value of 0.965 was achieved under the optimal conditions: a T_{time} of 40 seconds, M_{power} of 580 W, and a LW_{ratio} of 1:60. Under these parameters, the pred.

T_{TN} and T_{PL} yield were 60.05 mg tannic acid/100 g and 84.40 mg GAE/100 g, respectively.

The predictions were confirmedby experiments performed at the optimum settings. The experimental outcome were statistically analysed, and confirmed no significantdifference between the predicted and actual values of T_{PL} and T_{TN} contents, using the Duncan Homogeneity Test.

A comparative analysis was also performed using controlled sample and MAAE. T_{TN} and T_{PL} yield were 46.45 mg tannic acid/100 g and 73.50 mg GAE/100 g, respectively. There is an increase in 13.6 % in total tannin content and 10.9% increase in total phenolic content.

Conclusion

This research established the efficacy of MAAE as an efficient and sustainable technique for extracting phenolic components from tulsi leaves. The optimization of independent variables such as M_{power}, T_{time}, and LW_{ratio} yielded considerable amounts of T_{PL} and T_{TN} contents. The regression model's reliability was established through RSM analysis, identifying optimal conditions of 580 W M_{power}, 40 seconds of T_{time}, and aLW_{ratio} of 1:60. The MAAE provides several advantages, including lower energy consumption and reduced extraction time, making it an environmentally friendly alternative. The results emphasize the possibility of tulsi leaves as rich reservoirs of biologically active components, causative to by-product valorization and ecological preservation. Subsequent studies could emphasize optimizing this method and investigating its potential uses in the pharmaceutical and food industries, thereby advancing the utilization of tulsi leaves in value-added product.

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