Optimization of bioactive compound extraction from tobacco stem (*Nicotiana tabacum* L.)

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Abstract. Tobacco stem represents an underutilized agricultural by-product rich in bioactive secondary metabolites such as phenolics, flavonoids, alkaloids, terpenoids, and saponins. Efficient recovery of these compounds depends on appropriate extraction conditions. The aim of this study was to determine the optimal solvent volume and extraction temperature for maximizing the flavonoid content, phenolic concentration, and antioxidant activity of tobacco stem extract using RSM. A Central Composite Design was applied to evaluate the influence of solvent volume and heating temperature on total flavonoid content, total phenolic content, and antioxidant activity of the ethanol extract. The developed linear regression models demonstrated good predictive accuracy and non-significant lack-of-fit values, indicating model suitability. The optimization analysis predicted the optimal extraction condition at a solvent volume of 80 mL and a heating temperature of 65°C. This prediction was validated experimentally, yielding a total flavonoid content of 2.178 mg QE/mL, total phenolic content of 18.355 mg GAE/mL, and antioxidant activity of 71.159% inhibition. These results confirm that response surface optimization effectively enhances the extraction efficiency of bioactive compounds from tobacco stem, supporting its potential valorization as a natural antioxidant source.

Keywords : extraction, optimization, response surface method, tobacco stem

1 Introduction

Tobacco (*Nicotiana tabacum* L.) contains bioactive compounds such as phenolics, flavonoids, saponins, terpenoids, and alkaloids, supporting its potential use in pharmaceuticals, cosmetics, and natural preservatives [1–3]. Although tobacco leaves are commonly used, stems remain underutilized despite their high secondary metabolite content [4], and large quantities are discarded as waste. Proper utilization of tobacco stems may add economic value by converting waste into functional products.

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Extraction is commonly used to isolate secondary metabolites, and maceration with organic or mixed solvents has been reported as effective for phytochemical recovery [5]. Extraction efficiency depends on factors such as solvent type, solvent-to-material ratio, and temperature [6]. Ethanol was selected because it effectively dissolves phenolics, flavonoids, and alkaloids [2], while heating improves compound diffusion and cell permeability.

Optimizing these conditions requires an approach capable of evaluating variable interactions. Response Surface Methodology (RSM) enables efficient modeling and prediction while minimizing experimental runs [7,8]. Therefore, the aim of this study was to determine the optimal solvent volume and extraction temperature for maximizing the flavonoid content, phenolic concentration, and antioxidant activity of tobacco stem extract using RSM.

2 Materials and Methods

2.1 Sample

The samples used were dried Kasturi tobacco stems (*N. tabacum* L.) obtained from Langsepan Village, Kranjingan, Sumbersari, Jember. The stems were collected and cleaned, then dried in the sun for three days to ensure complete drying. The stems were then ground using a disk mill until they became coarse flakes.

2.2 Tobacco extraction

The preparation of the extract begins with weighing 10 grams of coarse powder from tobacco stems dissolved in ethanol with a volume according to the treatment combination in Table 1. The mixture was then heated using a water bath for 1 hour at a heating temperature according to Table 1. The sample was then cooled to room temperature [9].

Then the sample was macerated for 24 hours in an incubator shaker at a speed of 85 rpm. The extract was then filtered using filter paper. The filtrate was put into a dark glass bottle and can be stored at 4ºC. The extract obtained was then analyzed for total flavonoid content, total phenolic content, and antioxidant activity.

Table 1. Optimized combination of solvent volume and heating temperature treatment

No.	Solution volume (mL)	Temperature (ºC)
1	80	45
2	120	45
3	80	65
4	120	65
5	71,7157	55
6	128,284	55
7	100	40,85
8	100	69,14
9	100	55
10	100	55
11	100	55
12	100	55
13	100	55

2.3 Total Flavonoid Content Testing [10]

One milliliter of extract was diluted to 10 mL with ethanol. A 1 mL aliquot was mixed with 1 mL of 2% $AlCl_3$ and 1 mL of 120 mM potassium acetate, then incubated at room temperature for 1 hour. Absorbance was measured at 435 nm using a UV–Vis spectrophotometer. Analyses were performed in triplicate, and TFC was calculated using a standard calibration curve.

2.4 Total Phenolic Content Testing [10]

Ten milliliters of extract were diluted to 100 mL with distilled water. A 1 mL aliquot was mixed with 5 mL of 10% Folin–Ciocalteu reagent, allowed to stand for 3–8 minutes, then combined with 4 mL of 7.5% sodium carbonate. After incubation in the dark for 2 hours, absorbance was recorded at 740 nm. Triplicate measurements were used to calculate TPC based on a calibration curve.

2.5 Antioxidant [11]

Two milliliters of extract were mixed with 2 mL of 0.1 mM DPPH and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm to determine inhibition percentage.

2.6. Statistical Analysis

TFC, TPC, and antioxidant activity data were processed using Microsoft Excel. Optimization was performed using Design Expert 13, and prediction accuracy was evaluated using a one-sample *t*-test in IBM SPSS Statistics 27.

3. Results and Discussion

The ethanol extract of tobacco stems exhibited a total flavonoid content (TFC) of 1.098–2.559 mg QE/mL, a total phenolic content (TPC) of 11.738–22.636 mg GAE/mL, and antioxidant activity of 64.234–76.590% (Table 2). The linear regression models accurately predicted these responses, as indicated by high R^2 values. In addition, the non-significant lack-of-fit values ($p > 0.05$) confirmed that the models were statistically adequate for predicting phytochemical content and antioxidant activity (Table 3).

Contour and three-dimensional surface plots showed that both solvent volume and extraction temperature significantly affected TFC. The lowest TFC (1.098 mg QE/mL) was observed at 128.28 mL of solvent and 55°C, whereas the highest value (2.559 mg QE/mL) occurred at 71.71 mL and 55°C (Fig. 1–2). The higher TFC at a lower solvent volume corresponds to increased solvent concentration and improved mass-transfer efficiency until saturation is reached, beyond which no further extraction improvement occurs [12]. The optimal extraction temperature of 55°C is consistent with findings by Antony and Farid [13], indicating sufficient thermal energy for solute diffusion without causing degradation.

A similar trend was observed for TPC, which ranged from 11.738 to 22.636 mg GAE/mL under comparable conditions (Fig. 3–4). This parallel relationship suggests that flavonoids and other phenolics share similar extraction mechanisms, with 55°C supporting compound solubility and stability [12].

Antioxidant activity followed the same pattern, increasing from 64.23% to 76.59% inhibition at 71.71 mL and 55°C (Fig. 5–6). The strong correlation between TFC, TPC, and antioxidant activity indicates that polyphenolic constituents are the primary contributors to radical-scavenging potential. Overall, extraction at 71.71 mL solvent volume and 55°C represents the optimal condition for maximizing phytochemical yield and antioxidant capacity in tobacco stem extracts.

Table 2. Results of analysis of total flavonoid content, total phenolic content, and antioxidant activity based on central composite design

No.	Solution Volume (g/mL) (X_1)	Temperature (ºC) (X_2)	Total Flavonoid (mg QE/ml)	Total Phenolic (mg GAE/ml)	Antioxidant Activity (% Inhibition)
1	80	45	2,221	19,303	73,699
2	120	45	1,138	12,896	65,39
3	80	65	2,042	17,53	71,387
4	120	65	1,243	14,125	67,413
5	71,7157	55	2,559	22,636	76,59
6	128,284	55	1,098	11,738	64,234
7	100	40,85	1,587	15,875	69,003
8	100	69,14	1,774	17,719	71,315
9	100	55	1,655	16.537	69,364
10	100	55	1,71	17,033	70,665
11	100	55	1,475	14,314	67,775
12	100	55	1,44	15,165	68,28
13	100	55	1,471	15,686	68,786

Table 3. Equation Model (R^2 and *lack of fit*)

Response	Equation Model	R2	Lack of fit
Flavonoid Total	$Y = +3,983 – 0,025\ X_1 + 0,002\ X_2$	0,901	0,316
Phenolic Total	$Y = +30,543 – 0,158\ X_1 + 0,026\ X_2$	0,833	0,334
Antioxidant Activity	$Y = +86,079 – 0,186X_1 + 0,037\ X_2$	0,857	0,296

The relationship between solvent volume and heating temperature on the response of total flavonoid content, total phenolic content, and antioxidant activity was displayed in the form of a contour plot and a 3D graph. The X-axis (A: solvent volume) ranges from 80 ml to 120 ml, and the Y-axis (B: heating temperature) ranges from 45ºC to 65ºC. The background color of the contour indicates total flavonoid content, total phenolic content, and antioxidant activity.

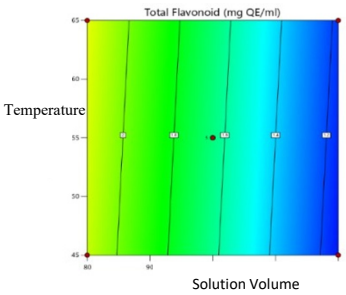

Fig. 1. Plot Graph of Response to Total Flavonoid Levels

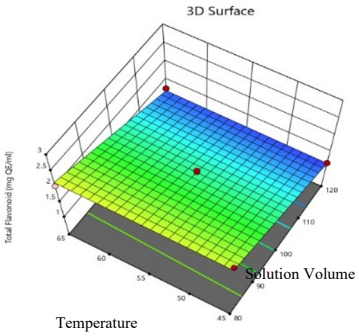

Fig. 2. Total Flavonoids as Graphic 3D Response

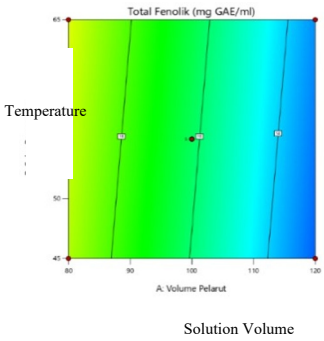

Fig. 3. Plot Graph of Response to Total Phenolic Levels

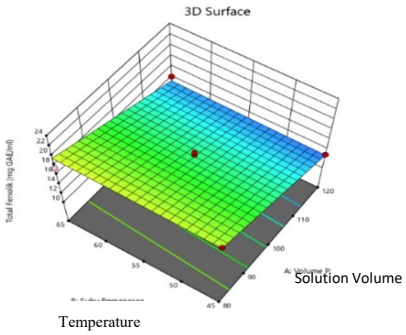

Fig. 4. Total Phenolic as Graphic 3D Response

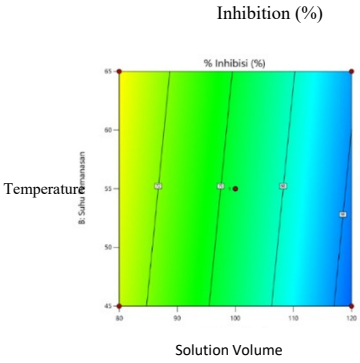

Fig. 5. Plot Graph of Response to Antioxidant Activity

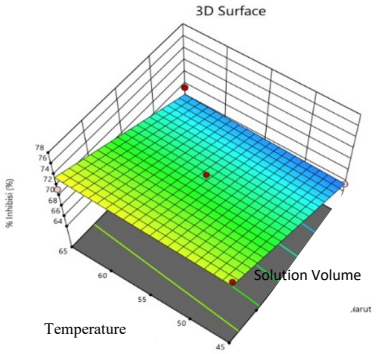

Fig. 6. Antioxidant Activity as Graph 3D Response

The criteria set for solvent volume and heating temperature are within the established upper and lower limit values for the response of total flavonoid content, total phenolic content, and antioxidant activity weare maximized because it is expected to produce an extract with optimal bioactive compound content to provide added value to tobacco stem waste. From several solutions that have been generated, the best combination is selected, marked with the "selected" sign with the maximum desirability value.

Table 4. Optimal Point Solution of Selected Criteria

Solution Volume (mL)	**Temperature (ºC)**	**Total Flavonoid (mg QE/mL)**	**Total Phenolic (mg GAE/mL)**	**Antioxidant Activity (%)**	***Desirability***
80	65	2,164	19,608	73,623	0,737

The optimal point solution of the extraction process with solvent volume and heating temperature factors was shown in Table 4. The desirability value of the solution is 0.737,

which means that 73.7% of the response of total flavonoid content, total phenolic content, and antioxidant activity is influenced by solvent volume and heating temperature. Thus, other factor influenced for 26.3%. The accuracy of the actual values compared to the predicted values for total flavonoid content, total phenolic content, and antioxidant activity (% inhibition) was 100.6%, 93.61%, and 96.65%, respectively These results showed that the prediction model was able to predict the actual value of the response parameters with a high level of accuracy [6].

The optimized ethanol extract of tobacco stems exhibited a total flavonoid content of 2.18 mg QE/mL and a total phenolic content of 18.36 mg GAE/mL, indicating higher phytochemical yield than water extracts and comparable levels to methanolic extracts reported by Zou et al. [13]. Variations in metabolite content among extracts were influenced by solvent polarity, cultivar differences, and environmental conditions [14]. The extract also showed strong antioxidant activity (71.15% inhibition), aligning with findings by Sharma et al. [14]. The high antioxidant potential reflects a positive correlation between phenolic and flavonoid concentrations and radical scavenging capacity.

4. Conclusion

This study successfully optimized the extraction of bioactive compounds from tobacco stem using ethanol, identifying 80 mL solvent volume and 65°C as the optimal extraction conditions, with a desirability score of 73.7%. Validation demonstrated strong alignment between predicted and experimental values, confirming the reliability of the model. The optimized extract contained 2.178 mg QE/mL flavonoids, 18.355 mg GAE/mL phenolics, and 71.159% antioxidant inhibition, demonstrating the potential of tobacco stem extract as a natural antioxidant source.

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