Optimization of Tobacco Waste Extraction using Ultrasonic-Assisted Extraction (UAE) to Produce Biopesticide

Mega Desy Safitri*, W. Wagiman, J. Jumeri

Department of Agro-Industrial Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia
*Corresponding author: Mega Desy Safitri, Email: megadesysafitri1199@mail.ugm.ac.id

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ABSTRACT

Tobacco waste is composed of the same bioactive compounds discovered in tobacco leaves but with lower concentrations, such as nicotine, phenolic compounds, aromatic substances, and solanesols. Optimal processing of this by-product can lead to the production of bioactive compounds with potential environmental benefits. Specifically, tobacco waste can be converted into biopesticides containing nicotine and solanesol which function as antifungals and antioxidants. This study adopted the ultrasonic-assisted extraction (UAE) maceration method, varying parameters such as temperature (40-60 °C), time (15 – 30 minutes), and type of solvent (acetone, methanol, and 96% ethanol). Data were analyzed using Response Surface Methodology (RSM) to determine optimal yield, antifungal bioactivity, and antioxidant activity. The results showed that the optimal UAE maceration conditions were 52.47 °C, 29.86 minutes, and 96% ethanol solvent. This led to a yield of 9.90%, 97.62% antifungal, and antioxidant activity (IC50) 16.72 ppm.

Keywords: Response surface methodology; tobacco waste; Ultrasonic-assisted extraction

INTRODUCTION

Tobacco is a leading commodity in Indonesia, which significantly influences the agricultural and economic sectors. The leaves are predominantly used to process cigarettes, with certain grades, such as nicotine content and size, contributing to the quality of the final product. Those not meeting the standard due to size, damage, defects, unsuitable texture, and position on the stem were classified as waste (Wardhono et al., 2019). Leftover tobacco is often burned, releasing smoke that poses health risks to nearby communities because of the nicotine content present in the stems and roots (Zou et al., 2021). Tobacco waste contains the same bioactive compounds as the leaves but in lower concentrations, such as nicotine, phenolic compounds, aromatic substances, and solanesols (Banožić et al., 2019, 2020).

Tobacco waste or residue should be processed into products offering added value to farmers and producers. For example, pesticides can be derived, serving as substances to control pests and diseases in plants. Nicotine, a component of tobacco waste functions as a contact poison to control several types of leaf-destroying caterpillars, soft-bodied sucking insects, and fungi (Khalalia, 2016; Tuti et al., 2017). Converting tobacco waste into biopesticides presents an effective treatment option for controlling diseases in cayenne pepper. Traditionally, dithiocarbamate pesticides, namely mancozeb, are adopted in managing these diseases. Fungicides work in contact with multiple targets (Meokasan et al., 2014). The application of

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mancozeb fungicide was discovered to be effective with the addition of a systemic fungicide (Paramita et al., 2014). It has the potential to pollute the environment due to the Mn^{2+} and Zn^{2+} ions content, which can increase the concentration of reactive oxygen species in organisms (Sasmita, 2021).

Cayenne pepper (*Capsicum frutescens* L.) is a leading national horticultural commodity, with demand increasing yearly. In 2021, the price of this commodity increased from 2.32 USD/kg to 5.03 USD /kg at a percentage of 117.13%. However, in Indonesia, the production of cayenne pepper in 2021 reached 1.39 million tons, which decreased by 8.09% from the previous year (Badan Pusat Statistika, 2022; Kementrian Perdagangan, 2021). The increase in the price of this commodity was due to high rainfall, which led to reduced farmer yields and attacks by pests and diseases. Based on these problems, tobacco waste can be used as a fungicide-type biopesticide to control fungus on cayenne pepper. A significant problem faced by this plant is anthracnose fruit rot disease, caused by the fungus *Colletotrichum gloeosporioides*. The early symptoms are characterized by appearances such as slightly shiny, slightly sunken, and watery patches that are black, orange, and brown. Anthracnose disease caused by the fungus *Colletotrichum Sp.* can reduce the production of cayenne pepper by 50 – 90% (Meilin, 2014).

Tobacco extract has been shown to inhibit the growth of *Colletotrichum sp.,* with nicotine, flavonoids, and osmotic compounds, contributing to the antifungal properties. Nicotine, in particular, can also inhibit enzyme performance (Duila, 2017). The tobacco extract used in a study conducted by Duila (2017) was a 70% alcohol solvent, leading to 33.78% inhibition at 100% concentration. This study utilized three types of solvents, namely methanol, 96% ethanol, and acetone, to produce varying levels of inhibitions. The extraction of tobacco waste is conducted to harness the nicotine and solanesol content. Therefore, this study aimed to reduce environmental risks posed by the waste and to identify the biopesticide properties of the resulting extracts, to ensure the stability of cayenne pepper production, which is inhibited by pests and diseases.

Processing of tobacco waste by maceration or soaking can inhibit nematodes on plant roots (Wiratno et al., 2016). In the extraction of cayenne pepper biopesticides, this waste is processed using ultrasonic-assisted extraction (UAE) maceration, which is expected to effectively inhibit pests. UAE is a modern method that utilizes ultrasonic pressure waves passing through a solvent, producing cavitation. This method can achieve higher extraction efficiency with lower solvent usage (Duan et al., 2016; Shirsath et al., 2012). The optimization of UAE is critical for determining the best process to produce optimal biopesticides. This is achieved using Response Surface Methodology (RSM), a mathematical tool for predicting future responses and determining the value of variables that optimize the desired outcomes (Natabirwa et al., 2018). The UAE maceration extraction method was used in this study to determine the optimum conditions for biopesticide from tobacco waste extract, identify the bioactive compounds extracted under these conditions, and analyze the effectiveness of the compounds in inhibiting cayenne pepper fungi.

METHODS

Materials

The tools used in this study include an ultrasonic processor (UP200St) for homogenizing tobacco waste powder with solvent, a rotary evaporator (IKA RV 10 digital V), a membrane vacuum pump (IKA MVP 10 basic), and a temperature regulator (IKA RC 2 liter). These devices were adopted to remove the solvent present in the tobacco waste solution, leaving only the filtrate.

The materials used consisted of castor-type tobacco waste, specifically damaged leaves sourced from Jember (Indonesia) and *Collectrotichum gloeosporioides* obtained from cayenne pepper of the gongga type in Yogyakarta (Indonesia). The test materials included distilled water, methanol, 96% ethanol, acetone, methanol for analysis (Merck), Potato Dextrose Agar (Merck) media, 10% DMSO, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH).

Time and Place

The study was conducted from March to July 2023 at the Bioindustry Laboratory and Industrial Design and By-product Control Laboratory, Agricultural Industrial Technology, as well as the Laboratory of Chemistry and Biochemistry of Agricultural Food Products, Agricultural Technology and Agricultural Products, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia.

Research Design

The study adopted RSM optimization with a Central Composite Design. The factors considered were temperature, time, and type of solvent.

The factors used in the extraction of UAE maceration were temperatures of 40-60 °C, 15-30 minutes, as well as solvent types 1 (methanol), 2 (ethanol 96%), and 3 (acetone).

Table 1. Research design of responding surface method and central composite design

	X_1 : Temp (°C)	X_2 : Time (min)	X_3 : Solvent (mL)		X_1 : Temp (°C)	X_2 : Time (min)	X_3 : Solvent (mL)
1	40	15	1	11	50	9.89	2
2	60	15	1	12	50	35.11	2
3	40	30	1	13	50	22.5	1
4	60	30	1	14	50	22.5	3
5	40	15	3	15	50	22.5	2
6	60	15	3	16	50	22.5	2
7	40	30	3	17	50	22.5	2
8	60	30	3	18	50	22.5	2
9	33.18	22.5	2	19	50	22.5	2
10	66.82	22.5	2	20	50	22.5	2

Source: Banožić et al. (2020), Duan et al. (2016), Gudeta et al. (2021) and Al-Lahham et al. (2020) modified in temperature, time, and solvent

Preparation Sample

The extraction process began with sorting and drying leaf waste at a temperature of 50 °C. The material was reduced in size and sieved to achieve a uniform particle size of 40 mesh. A solvent was added to the tobacco powder in a 1:10 ratio (40 g powder with 400 mL solvent). The mixture was then stirred using an ultrasonic processor with UAE to break down the bioactive content (Duan et al., 2016; Gudeta et al., 2021). Following ultrasonic processing, the solution was macerated for 24 hours to obtain a more concentrated extract. The mixture was filtered, and the supernatant was soaked again with the same amount of solvent until the desired extract was obtained. Subsequently, the filtered extract was evaporated using a rotary vacuum evaporator at a temperature of 40 °C for 40 minutes. The concentrated extract was dried using a cabinet dryer at 50 °C until a dry extract was obtained. The final step includes calculating the extraction yield, performing an antifungal bioactivity test, and conducting an IC_{50} antioxidant test (Al-Lahham et al., 2020; Banožić et al., 2020).

Extract Analysis

Yield

The yield calculation was performed to determine the percentage of extract produced from tobacco waste powder (Equation 1) (Gudeta et al., 2021; Hasnaeni et al., 2019).

$$Yield = \frac{Extract\ weight}{Raw\ material\ weight} \times 100\% \quad (1)$$

Antifungal bioactivity

The antifungal bioactivity test was conducted by isolating the fungus that caused anthracnose in cayenne pepper using a direct plating method. Fungal hyphae growing on this plant were transferred to new Potato Dextrose Agar (PDA) media to produce pure cultures (Oo et al., 2018). Subsequently, PDA was put into a sterile petri dish, and the tobacco extract was added to 10% DMSO. The inoculum of the fungus *Colletotrichum sp* was inoculated on compact media and incubated at 25 °C for 7 days. Observations were conducted every 1x24 hours by measuring the area of the fungal colonies growing on the media (Equation 2) (Sinclair & Dhingra, 2019).

$$D = \frac{DK-DP}{DK} \times 100\% \quad (2)$$

Description:
D = antifungal activity (%)
DK = The area of fungal colonies that grow on the negative control (cm^2)
DP = The area of fungal colonies that grew in the treatment (cm^2)

Antioxidant (IC_{50})

A 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared by dissolving 1.98 mg of DPPH powder dissolved in 50 mL of methanol p.a. Subsequently, 2 mL of this solution was put into a test tube, followed by the addition of 2 mL of methanol p.a, and covered with aluminum foil. The mixture was homogenized using a vortex and incubated in the dark for 30 minutes. Finally, the absorption of the blank

solution was measured at the maximum wavelength of 517 nm using a UV-Vis spectrophotometer (Haveni et al., 2019; Tristantini et al., 2016).

A 2.5 mg tobacco waste extract was weighed into a volumetric flask and 50 mL of methanol was added until the sample concentration was 500 ppm (stock solution). The stock solution was diluted to obtain concentrations of 50 ppm, 75 ppm, 100 ppm, and 125 ppm. Subsequently, 1 mL of each concentration was collected, mixed with 0.6 mL of DPPH (0.1 mM), and methanol was added to the mark/volume of 3 mL. The mixture was homogenized with a vortex, incubated for 30 minutes in a dark room, and tested for absorption values using a UV-Vis spectrophotometer at a wavelength of 517 nm (Do et al., 2014; Docheva et al., 2014). Antioxidant activity was interpreted using the DPPH method with an efficient concentration value (EC_{50}), commonly known as $IC_{50,}$ a value calculated based on % inhibition 50% using y = ax+b, as shown in Equation 3.

$$\%\ Inhibiton = \frac{Absorbance\ of\ the\ blank - Sample\ absorbance}{Blank\ absorbance} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Waste Extract using Ultrasonic-Assisted Extraction Maceration

Tobacco waste processed by UAE maceration produced relatively stable test values, as shown in the results in Table 2. The extraction process utilized methanol, 96% ethanol, and acetone solvents to produce antifungal compounds, polyphenols, flavonoids, and chlorogenic acid (Docheva et al., 2014; Duan et al., 2016). The water content of tobacco waste was 12.3%, while the powdered form had a water content of 8.43%.

Table 2. Analysis of tobacco waste extract using response surface method

Temp (°C)	Time (min)	Solvent	Yield (%)	Antifungal (%)	Antioxidant IC_{50} (ppm)
40	15	Methanol	8.73	92.93 ± 0.05	21.71 ± 0.56
60	15	Methanol	8.93	93.27 ± 0.04	20.70 ± 1.66
40	30	Methanol	9.78	93.83 ± 0.06	19.77 ± 0.95
60	30	Methanol	9.88	94.78 ± 0.01	19.79 ± 2.22
40	15	Acetone	8.58	92.70 ± 0.04	21.66 ± 1.83
60	15	Acetone	8.85	93.50 ± 0.04	20.22 ± 0.20
40	30	Acetone	8.13	93.27 ± 0.04	17.89 ± 0.81
60	30	Acetone	8.53	94.57 ± 0.01	18.59 ± 1.15
33.18	22.5	96% ethanol	9.45	96.51 ± 0.03	18.26 ± 0.48
66.82	22.5	96% ethanol	9.90	96.83 ± 0.01	19.20 ± 1.59
50	9.89	96% ethanol	9.08	94.26 ± 0.04	19.41 ± 0.69
50	35.11	96% ethanol	10.28	98.89 ± 0.02	19.57 ± 0.99
50	22.5	Methanol	8.45	95.91 ± 0.05	18.34 ± 1.70
50	22.5	Acetone	8.75	92.70 ± 0.03	18.78 ± 1.82
50	22.5	96% ethanol	9.53	96.76 ± 0.03	13.84 ± 1.84
50	22.5	96% ethanol	10.03	97.83 ± 0.01	15.59 ± 0.79
50	22.5	96% ethanol	9.20	95.07 ± 0.02	16.42 ± 1.76
50	22.5	96% ethanol	9.60	97.50 ± 0.02	15.75 ± 0.53
50	22.5	96% ethanol	9.35	95.36 ± 0.04	18.29 ± 0.77
50	22.5	96% ethanol	9.40	97.57 ± 0.06	14.65 ± 1.23

Table 3. Model Determination for Yield, Antifungal, and Antioxidant

Respon	Model Order	Significant (*p*-value<0,05)	Lack of fit (*p*-value>0,05)	Adjusted R^2 model	Predicted R^2	Adequate precision
Yield	Quadratic	0.0060	0.2884	0.6888	0.3216	6.5169
Antifungal	Quadratic	0.0151	0.5041	0.6167	0.1520	9.0748
Antioxidant	Quadratic	0.0145	0.7353	0.6199	0.1547	10.8309

Following UAE maceration, the processed waste had an average water content of 11.24% in the form of dry crystals. In the study, tobacco waste extraction had a yield ranging from 8.13 – 10.28%, with antifungal activity between 92-98%, and antioxidant activity (IC_{50}) ranging from 13.84 – 21.71 ppm.

Statistical Analysis and Model Determination

The yield, antifungal value, and antioxidant activity produced in this study were insignificant or relatively stable. The variables used in the UAE treatment consisted of time, temperature, and type of solvent. To determine the optimal value, an ANOVA test was conducted with the stages of the Sum of Square test, Lack of Fit test, and R^2 test. Modeling was performed by RSM using 20 treatment combinations with 6 replications at the center point to obtain a good estimate of experimental error (Moradi et al., 2016; Virgine, 2021). Table 3 shows the results of the analysis test from Design Expert 12 and the outcomes were by the RSM significance value.

Yield Extract

The yield response of tobacco waste extract ranged from 8.13 – 10.28%. The results of the variance analysis were significant ($p < 0.05$), implying a meaningful difference in the outcomes across the 20 treatments at a significance level 5% (Virgine, 2021). The less fit value showed a *p*-value > 0.05, suggesting that the model was good and in line with the response provided. The values for both adjusted and predicted R^2 were close to 1, and this confirmed the reliability and accuracy of the model in reflecting the predetermined model. The adequacy of the model is further supported by an adequate precision value greater than 4, which is necessary to achieve the desired optimization (Moradi et al., 2016). Based on the modeling analysis, the following equation was obtained (Equation 4).

$$Y_1 = 9.49 + 0.1268\ X_1 + 0.2375\ X_2 - 0.2920\ X_3 + 0.0031\ X_1X_2 + 0.0469\ X_1X_3 - 0.3469\ X_2X_3 + 0,0891\ X_1^2 + 0.0819\ X_2^2 - 0.7604\ X_3^2 \quad (4)$$

Description:
X_1 : Temperature
X_2 : Time
X_3 : Type of solvent

The yield is the value obtained from the comparison of the extraction results of the materials used. The size of the yield value showed the effectiveness of the extraction process which was influenced by the solvent used, particle size, extraction method, and duration. The yield of tobacco waste extract using UAE was 14–17% with a time of 4–6 hours (Gudeta et al., 2021), while in this study, it was 8.13 – 10.28% with a time of 30 – 60 minutes. The differences in values were influenced by the addition of NaOH to bioinsecticide processing, causing higher yields. Furthermore, the ratio between powder and solvent has a direct proportionality effect on the yield value (Putra et al., 2020). The longer the extraction time and the higher the extraction temperature, the higher the yield value (Margaretta et al., 2011; Yuliantari et al., 2017).

Differences in yield were influenced by several factors, namely the stirring process during maceration, the type of solvent, as well as time and temperature during the extraction process (Zlotek et al., 2016). Stirring aims to ensure that the solvent binds all the polar components contained in the leaves and that the heat is evenly distributed. Furthermore, it can affect the yield during the process of extraction. The longer the stirring time, the higher the extract yield and phenolic content. This is in accordance with a study conducted by Shirsath et al., (2012), where the application of UAE bound bioactive compounds in the extract and produced a higher yield.

Temperature can also affect the amount of yield obtained and the extraction of bioactive compounds. Therefore, an optimal temperature was needed to extract bioactive compounds to obtain a high yield and the best extract quality. The type of solvent was also an influencing factor due to the polarity. The yield of acetone extract from tobacco waste has a lower value than 96% ethanol and methanol extracts (Banožić et al., 2020). This is in accordance with a study by (Do et al., 2014)

where acetone extract was lower compared to 96% ethanol and methanol extracts. Acetone was a polar-protic solvent and could not produce OH- ions, while methanol and 96% ethanol were polar-protic solvents that produced OH- ions and interacted more easily with polar functional groups (Marnoto et al., 2012).

Antifungal Bioactivity Extract

The antifungal response test for tobacco waste extract was between 92.70 – 98.89%. The results of the variance analysis were significant with a *p*-value < 0.05. This implied a meaningful difference in outcomes across the 20 treatments at a significance level of 5% (Virgine, 2021). The less fit value shows a *p*-value > 0.05, suggesting that the model is good and was in line with the response provided. The values for both customized and predicted R^2 were close to 1, ensuring the correlation of the model with the predetermined model. This is in accordance with a study where a value close to 1 and adequate precision greater than 4, were necessary to obtain the expected optimization value (Moradi et al., 2016). Based on the modeling analysis, the following equation was obtained:

$$Y_2 = 96.77 + 0.2882\ X_1 + 0.8670\ X_2 - 0.3990\ X_3 + 0.1359\ X_1X_2 + 0.1021\ X_1X_3 - 0.0939\ X_2X_3 - 0.0852\ X_1^2 - 0.1192X_2^2 - 2.86\ X_3^2$$

Description:
X_1 : Temperature
X_2 : Time
X_3 : Type of solvent

Antifungal activity has 5 levels of inhibition, namely >75%, 50 – 75%, 25 – 50%, 0 – 25%, and 0%, implying very strong, strong, moderate, weak, and inactive, respectively (Diana et al., 2014). The antifungal inhibitory power of each solvent was 70%, 50 – 70%, and 60%, for methanol, 96% ethanol, and acetone (Duan et al., 2016). This is in accordance with the result of literature where the antifungal inhibitory power ranges between 92 – 98% with a very strong fungal inhibitory activity, influenced by the time and temperature used to extract tobacco waste. Extended extraction time and the higher the temperature, increase resistance value. However, the value can decrease due to the cavitation power associated with the UAE. The study by Ardianti & Kusnadi (2014) showed that at a higher temperature and longer time, fungal inhibition decreases.

Antimicrobials can function as either fungistatic or fungicidal. Fungistatic is a condition that describes the action of a substance (fungicide) to inhibit the growth of fungi, often due to suboptimal antimicrobial concentration. In contrast, fungicidal is a condition that describes the action of a substance (fungicide) to stop fungal growth. The inhibition of the growth of *Colletotrichum sp*. by tobacco extract is influenced by the presence of nicotine, flavonoids, and osmotic compounds which act as antifungals. Nicotine, in particular, plays a crucial role in inhibiting fungal growth by interfering with enzymatic activity (Ariyanti et al., 2012; Yan et al., 2019; Zou et al., 2021). Tobacco typically contains 1–3% nicotine, sufficient to impede fungal growth (Wardhono et al., 2019). This study showed a content of 1.29%, effectively inhibiting the growth of *Colletotrichum sp*. The compound acts by inhibiting the enzyme acetylcholinesterase enzyme, thereby preventing the breakdown of acetylcholine (ACh) into choline and acetyl-CoA. The subsequent accumulation of acetylcholine at the receptor sites leads to the inhibition of nerve cell function (Suprayitno et al., 2020).

The extract obtained from the UAE method contained complex compounds, namely phenolic compounds, and chlorogenic acid. It also contains routines that are of high value in the pharmaceutical and agricultural industries. The stirring that occurs increases the extraction of compounds containing high levels of solanesol and nicotine. This compound contains antifungals capable of preventing fungal growth (Banožić et al., 2020; Yan et al., 2019). According to a study, tobacco waste extract can inhibit the growth of cayenne pepper fungus by up to 89%.

Antioxidant Activity (IC_{50}) Extract

The antioxidant activity response test (IC_{50}) of tobacco waste extract was between 13.84 and 21.71 ppm. The results of the variance analysis were significant because of a *p*-value <0.05. This implied a meaningful difference in outcomes across 20 treatments at a significance level of 5% (Virgine, 2021). The mismatch value showed a *p*-value> 0.05 which was stated as a good model according to the response provided. The adjusted and predicted R^2 and values were close to 1, suggesting that the model was in line with the predetermined framework. This is in accordance with a study where a value close to 1 and adequate precision, greater than 4, were essential for achieving the desired optimization (Moradi et al., 2016). Based on modeling analysis, the following equation was obtained (Equation 5).

$$Y_3 = 15.88 - 0.0105\ X_1 - 0.5845\ X_2 - 0.3169\ X_3 + 0.3952\ X_1X_2 + 0.0313\ X_1X_3 - 0.3188\ X_2X_3 + 0.9413\ X_1^2 + 1.21\ X_2^2 + 2.14\ X_3^2 \quad (5)$$

Description:
X_1 : Temperature
X_2 : Time
X_3 : Type of solvent

Antioxidant activity is obtained by using the IC_{50} value or inhibitory concentration. This includes the concentration of antioxidant substances that provide an inhibitory power of 50%. Substances with high antioxidant activity produce low IC_{50} values. According to (Molyneux, 2004), antioxidants were divided into very strong, strong, medium, weak, and very weak categories with IC_{50} values of less than 50 ppm, 50 ppm to 100 ppm, 100 ppm to 150 ppm, 150 ppm to 200 ppm, and more than 200 ppm, respectively. The antioxidant activity of methanol extract from tobacco waste was 21 ± 9.6 ppm (Al-Lahham et al., 2020). This is in accordance with a study where 15.60 – 21.71 ppm was reported and signified very strong activity. The antioxidant activity of tobacco waste 96% ethanol extract was 66 – 230 ppm or had an inhibitory power of 20% (Prommaban et al., 2022). In this study, values from 13.84 – 20.22 ppm were reported, which showed strong activity. The antioxidant activity of tobacco waste acetone extract was 6 ± 0.1 ppm. This is in accordance with a study where IC_{50} of 14.65 – 21.66 ppm was recorded, reflecting strong activity (Al-Lahham et al., 2020).

Extracts with acetone solvents have the highest antioxidant activity compared to methanol, 96% ethanol, water, and isopropanol solvents (Suryani et al., 2016). This is because the bioactive compounds contained in the acetone solvent extract play a more active role as antioxidants in reducing DPPH free radicals. The result is in line with a study where the solvent had higher antioxidant activity than methanol and 96% ethanol.

Interaction Effect of Parameters

Tobacco waste extract parameters affected the yield, antifungal, and antioxidant properties, with a significant value (*p*-value) of less than 0.05.

Interaction effect of temperature and extraction time

The response of tobacco waste extract was significantly influenced by both temperature and extraction time, and these factors were interdependent with continuous effect. A temperature and longer extraction time, lead to greater yield and enhanced antifungal activity, but are inversely proportional to antioxidant activity. Furthermore, a lower IC_{50} value signifies a higher antioxidant content within the strong category. Based on Figure 1 (a), high yield was observed at a high temperature and longer time. Figure 1 (d) shows maximum antifungal activity at a high temperature and a relatively long time. In Figure 1 (g), a strong antioxidant activity was observed at a low temperature and short time. Increasing the temperature and the extraction time can cause the loss of compounds due to evaporation, leading to decreased antioxidant activity. Additionally, excessive temperature and insufficient extraction time can reduce antifungal inhibition in the extract, a phenomenon influenced by cavitation power in the UAE (Ardianti & Kusnadi, 2014). Therefore, temperature and time were relatively influential according to the target to be obtained (Gudeta et al., 2021; Ibrahim et al., 2015; Yuliantari et al., 2017).

Interaction effect of temperature and type of solvent

The type of solvent was determined based on its polarity, which affected the temperature used in the extraction. The solubility of a solution can determine the yield value, antifungal activity, and antioxidant activity. Acetone, a polar aprotic solvent did not produce OH ions, while methanol and 96% ethanol were protic solvents that generate OH ions and produce maximum extract. Based on Figure 1 (b), high yield was achieved with high temperature and polar solvent. Figure 1(e) showed that antifungal activity had high values at high temperatures and polar solvents. According to Figure 1 (h), strong antioxidant activity was observed at low temperatures with slightly polar solvents, as evidenced by a low IC_{50} value. The polarity present in acetone contributed to higher antioxidant activity. The presence of free hydrogen in acetone enhanced the antioxidant activity of phenolic compounds (Rachmawati et al., 2020).

Interaction effect of time and type of solvent

Short extraction times provided low results because not all components were extracted (Irawan & Jos, 2010), while long extraction times increased solvent penetration into the material. The solubility of the component in the material was proportional to the extraction time. However, at the optimal time, the solubility of the component was decreased (Yulianti et al., 2014). Based on Figure 1 (c), a long time and a polar solvent produced a high yield. Figure 1 (f) shows that high antifungal activity was influenced by longer extraction times and polar solvents. In Figure 1 (i), a relatively short time with a slightly polar solvent led to higher antioxidant activity at a low IC_{50} value. This is because high extraction temperatures affected the destruction of bioactive compounds, which reduced free radical inhibitory activity (Hwang & Thi, 2014).

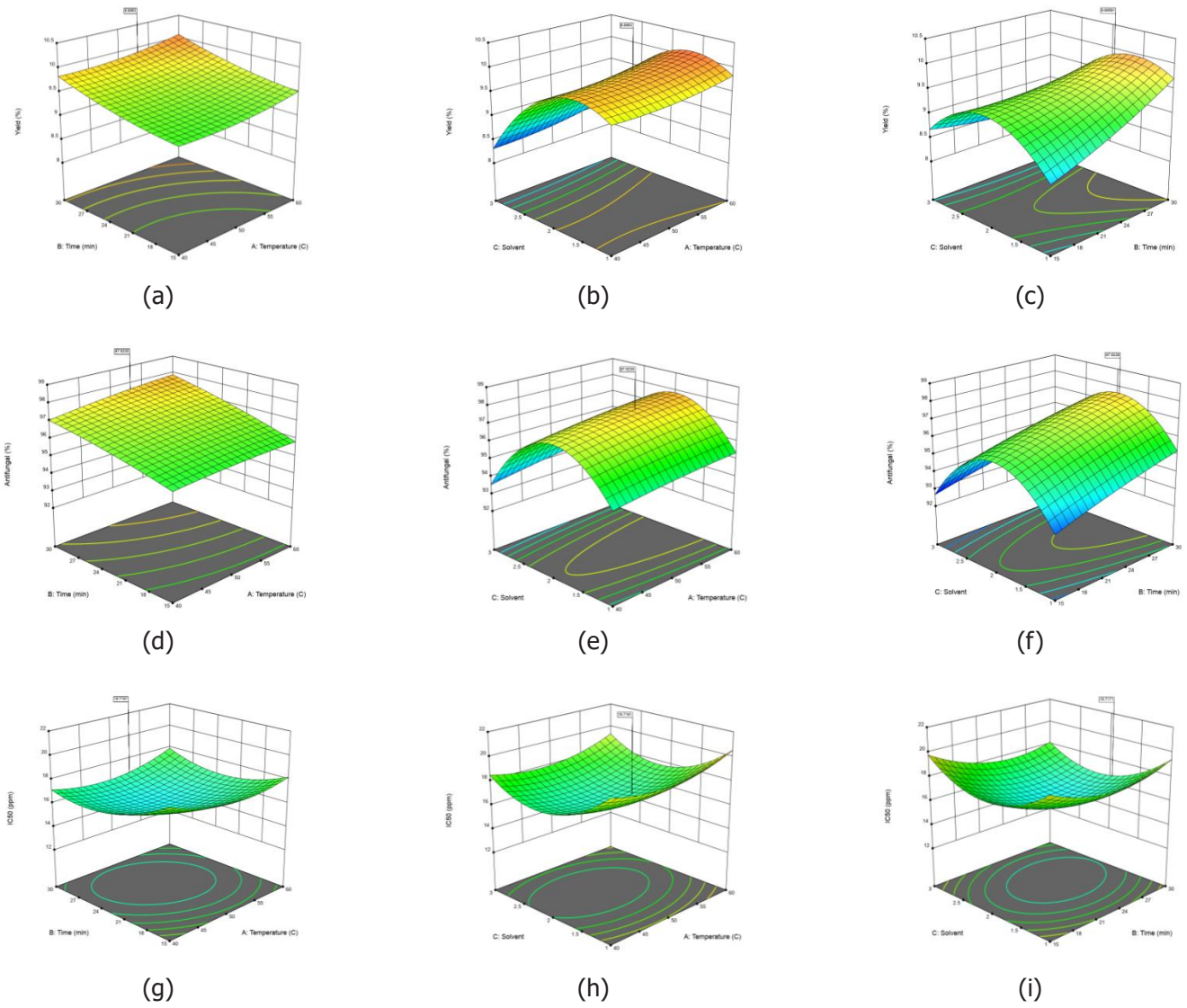

Figure 1. Yield response; (a) the effect of temperature and time, (b) the effect of temperature and type of solvent, and (c) the effect of time and type of solvent; Antifungal response; (d) the effect of temperature and time, (e) the effect of temperature and type of solvent, and (f) the effect of time and type of solvent; Antioxidant response; (g) the effect of temperature and time, (h) the effect of temperature and type of solvent, and (i) the effect of time and type of solvent.

Optimization

RSM is run in the Expert 12.0 Design program to provide solutions in the form of several selected conditions that comply with the criteria and limits on predetermined variables (Virgine, 2021). The desirability was a value close to 1 which was selected as the optimum condition in the process. A total of 5 process conditions are sorted based on the desired value.

In obtaining the optimal formula, variables and responses influenced the desirability value. The increasing number of components and responses contributed to a low desirability value, leading to

Table 4. Optimization Results

Temperature (°C)	52.47
Time (min)	29.86
Type of solvent	2 (ethanol 96%)
Yield	9.90%
Antifungal	97.62%
IC_{50}	16.72 ppm
Desirability	0.746
Note	Selected

Table 5. Response optimization results verification

Respon	Predict mean	Research mean data	95% CI*	95% PI*
Y_1	9.90	9.92 ± 0.520	9.558 – 10.232	9.352 – 10.439
Y_2	97.62	97.89 ± 1.074	96.414 – 98.835	95.673 – 99.575
Y_3	16.72	16.75 ± 0.399	15.343 – 18.093	14.503 – 18.933

* Y_1 = yield
Y_2 = antifungal
Y_3 = antioxidant

unachieved optimal conditions. Optimal formulas with high desirability were difficult to obtain due to the increasing importance of a component or response (Natabirwa et al., 2018).

Based on Table 4, Design Expert 12.0 showed 5 process conditions that offer appropriate optimization for each process. The yield value, with an importance rating of 5, presents stable results, producing both high and low yields based on UAE process conditions. The antifungal value, also rated 5, reflects the presence of strong bioactive compounds in the biopesticide. Antioxidant activity, with an important value of 5, signifies the presence of bioactive compounds effective in scavenging free radicals.

The optimum desirability of 0.746 shows that the resulting product had a value of 74.6%, meaning it is close to perfect. This suggested that further refinement of each variable and response is necessary. The attainment of an optimal formula is influenced by the variables and responses, which impact the desirability value. A low desirability often results from an increasing number of components and responses, hindering the achievement of optimal conditions. Meanwhile, high desirability values were difficult to obtain due to the increased importance of a component or response (Natabirwa et al., 2018).

Verification of Optimization

The verification stage is a further stage of optimal data suggested by the program and executed according to the process. The predicted optimal response value was verified with response data, to identify a significant comparison between predicted and run values.

Based on the verification results in Table 5, the antifungal yield and response values correspond with confidence interval (CI) values, while the antioxidant activity response (IC_{50}) corresponds with Prediction Interval (PI) values. The estimated average measurement at a significance level of 5% was shown by the CI parameter. At a significance level of 5%, the expected outcome of subsequent response measurements under the same conditions was signified by the PI parameter (Lins et al., 2015). The optimization results with the factors, such as temperature, time, and type of solvent in the extraction of tobacco waste have been verified.

The verification and prediction results had a difference of less than 5%, namely the yield, antifungal bioactivity, and antioxidant activity, were 0.2%, 0.27%, and 0.17%, respectively. This showed the suitability of the model for the process, and the result is in line with a study by (Indrayani, 2018) where the difference between predicted and verified values did not exceed 5%.

CONCLUSION

In conclusion, tobacco waste was processed into products beneficial for the environment, such as biopesticide for cayenne pepper mushrooms. Biopesticides were macerated using the UAE method to obtain more bioactive compounds. This method was optimized by determining the optimal production process, with temperature, time, and solvent type of 52.47 °C, 29.8 minutes, and 96% ethanol. The results of the optimization program and study data showed minimal differences and remained within the expected value interval. In this study, the yield value, antifungal, and antioxidant activity obtained were 9.90%, 97.62%, and 16.72 ppm, respectively.

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CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest in connection with the study, authorship, and/ or publication of this article.

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