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ORIGINAL RESEARCH ARTICLE

Optimised extraction of antioxidant components from *Calophyllum inophyllum* L. seeds using response surface methodology

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ABSTRACT

BACKGROUND AND OBJECTIVES: *Calophyllum inophyllum* (*C. inophyllum*), or Nyamplung, seeds contain various active compounds. Using *C. inophyllum* seeds as a source of flavonoids for natural antioxidants can increase their economic value and provide alternative compounds for cosmetics, including lotions. This study applied maceration and ultrasonic methods using ethanol to extract the active compounds from the *C. inophyllum* seeds. The study optimised extracting the antioxidant components from *C. inophyllum* seeds using response surface methodology.

METHODS: The experimental design used in this study was response surface methodology with a Box–Behnken design to model the influence of variables on the response of the yield and antioxidant activity of extracts obtained through maceration and ultrasonic extraction and to model lotion formulation. The extraction methods were designed with three variables (extraction time, solvent concentration, and sample–solvent ratio) and three levels (low, medium, and high), and the compounds in the extracts were analysed. Lotion formulation was designed with three variables (*C. inophyllum* seed extract, Tween 80, and carbomer) and three levels (low, medium, and high), and the quality of the lotion product (antioxidant activity and viscosity) was analysed.

FINDINGS: The *C. inophyllum* seed extract obtained through maceration had stronger antioxidant activity than that obtained using the ultrasonic method, with 50 per cent inhibition concentration values of 13.154 and 16.343 part per million, respectively. Characterisation with gas chromatography-mass spectroscopy revealed ten compounds with major percentage values, among them 2′-(trimethylsilyl)oxy-3,4,4′,5- tetramethoxychalcone (49.70 per cent). This compound played an important role in enhancing antioxidant activity in *C. inophyllum* seeds extracted through maceration, whereas butylated hydroxytoluene (9.16 per cent) was important in the extract obtained using the ultrasonic method. The lotion produced from the *C. inophyllum* seed extract contained high antioxidant activity with a 50 per cent inhibition concentration of 4.621 part per million; the toxicity text showed it was safe to be used (50 per cent lethal concentration of 789 grams per millilitre).

CONCLUSION: The results showed the effectiveness of this approach in determining the optimal conditions to maximise antioxidant content. The maceration method better ability enhanced the antioxidant activity capacity of *C. inophyllum* seeds compared to the ultrasonic method, as indicated by the response surface method. Both extraction methods produced the same secondary metabolite compounds with a promising reservoir of antioxidant compounds. In addition, the findings of this study showed the high antioxidant activity of *C. inophyllum* seed extract lotion, which could be developed for pharmaceutical, nutraceutical, and other applications.

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INTRODUCTION

Calophyllum inophyllum is one Indonesian plant with the potential to be used as a traditional medicine and a raw material for cosmetic products. *C. inophyllum* is called "Nyamplung" in Indonesian, "tamanu" in English, and "Kamami" in Hawaiian (Susanto *et al.,* 2017). This plant grows well in the hot coastal areas along the Aceh coast (Sugianto *et al.,* 2021). *C. inophyllum* is a mangrove plant that thrives on sandy soils and can thrive at altitudes up to 800 metres above sea level in ecosystems that include mountains, swamps, and forests (Fitriyana *et al.,* 2023). Previous studies have reported that the *C. inophyllum* plant has numerous benefits, particularly its seeds, which contain various active compounds, including calophyllic acid, tacamahin, essential oil resins, glycerol, bitter compounds, tannin, tocopherol, lipid, fibre, protein, and carotenoids (Fitriyana *et al*., 2023). According to Thy *et al*. (2020), *C. inophyllum* seeds have various biological activities such as antiviral, anti-inflammatory, ultraviolet (UV) protection, wound healing, and antioxidant. The utilisation of *C. inophyllum* seeds as a source of flavonoids for natural antioxidants can increase their economic value and provide an alternative source of cosmetic ingredients. *C. inophyllum* seeds contain significant amounts of antioxidant compounds, including tocopherol and delta-tocotrienol, which can increase the sun protection factor (SPF) of cosmetic products (Saechan *et al*., 2021; Aravind *et al.,* 2015). Lotion is a cosmetic product that contains antioxidants to avoid the negative effects of free radicals on the skin (Addor *et al*., 2022). Antioxidant activity is typically stated in terms of inhibition concentration (IC_{50}), the concentrations of the test chemical required to reduce free radicals by 50 per cent (%). The lower the IC_{50}, the greater the antioxidant activity (Martinez-Morales *et al*., 2020). The amounts and kinds of compounds that contribute to antioxidant activity in an extract depend on the solvent and extraction procedure used. Polar solvents, such as ethanol, draw more active polar compounds from natural materials. This solvent can penetrate the cell wall through diffusion and remove the bioactive substances (Gorgani *et al*., 2017). This study used *C. inophyllum* seeds from the Aceh coastal area as a source of antioxidant active compounds, and optimisation design was applied to maximise the amount of extracted active compounds through two different extraction processes: maceration and ultrasonic. The Box–Behnken design (BBD) from response surface methodology (RSM) was used in this study (Tekindal *et al*., 2012). RSM is a statistical and mathematical technique for maximising response rates in experiments by examining the links between one or more variables. The experiments in this study were planned and performed using RSM to obtain as much data as possible with the fewest procedures (Nurman *et al*., 2021). RSM has three design methods which are commonly used, and BBD is superior to the others. BBD provides good prediction accuracy using simpler experiments compared to the complete factorial design (Ni'mah *et al*., 2022). This model often achieves targets by reducing variability in experiments, reducing waste, and increasing production yields (Chadha *et al*., 2020). The factors studied significantly influence the optimisation of the extraction of antioxidant components from *C. inophyllum* seeds; it can increase the yield of antioxidant extraction. Thus, this study was conducted to optimise the extraction of antioxidant components from *C. inophyllum* seeds as a source of active compounds for antioxidant lotion formulations using RSM. This study was conducted in the Research Laboratory of the Chemistry Department, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia, in 2022.

MATERIALS AND METHOD

Materials and Instruments

C. inophyllum seeds were obtained from White Coastal Beach, Krueng Raya, Aceh Besar Sub-district, Indonesia and harvested from 0 to 10 m above sea level. Ethanol was used as the solvent; other chemicals used were distilled water, 1 M sulfuric acid (H_2SO_4), Dragendorff's reagent, magnesium (Mg) powder, concentrated hydrogen chloride (HCl), 10% sodium chloride (NaCl), ferric chloride ($FeCl_3$), chloroform ($CHCl_3$), Liebermann–Burchard reagent, and 2N HCl as an analytical reagent. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), vitamin C, and methanol were used as antioxidant test materials. Each material was of pro analysis (PA) quality from several manufacturers, including Sigma-Aldrich and Merck. The instruments used in this study were an ultrasonic cleaning bath (Bransonic 8510),

rotary vacuum evaporator (Yamato RE 200), and a gas chromatography-mass spectrometer (GC-MS) (SHIMADZU QP 2010 SE) with an RTX-5MS (5% diphenyl/95% dimethyl polysiloxane) column and helium as the mobile phase.

Experimental design

This study consisted of two steps: the extraction of active compounds from *C. inophyllum* seeds and the use of the extract in skin lotion formulations.

Extraction of active compounds

The extraction treatment process designed using RSM-BBD was configured. This design used Design Expert Version 10.0.3.0 software with three factors (x_1= extraction time, x_2= solvent concentration, and x_3= sample/solvent ratio) and three levels (low, medium, and high), as presented in Table 1. The selection of levels was based on the results of preliminary research that extracted *C. inophyllum* seeds using several methods and solvents, namely: pressed method (with/without degumming process), maceration method (with ethanol), and ultrasonic method (with ethanol). The maceration and ultrasonic methods (with ethanol) produced seed extracts with high antioxidant activities with IC_{50} values of 14.68 and 17.94 part per million (ppm) (Fitriyana *et al.*, 2023). Analysis of variance (ANOVA) results were also obtained under RSM-BBD (Nurman *et al.*, 2020).

The current research used maceration and ultrasonic methods with ethanol as the solvent to extract active compounds from *C. inophyllum* seeds. Eq. 1 has been used to determine the RSM model (Carrera *et al.*, 2023):

$$y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2 + \beta_5X_2X_3 + \beta_6X_1X_3 + \beta_7X_1^2 + \beta_8X_2^2 + \beta_9X_3^2 \quad (1)$$

Where, y is the value of the measured response, β_0–β_9 are the regression coefficients and X_1, X_2, and X_3 are the independent factors. ANOVA, lack of fit, and coefficient of determination (R^2) were used to measure model goodness of fit.

The extraction of *C. inophyllum* seeds was performed according to the treatment design. In the maceration method, the crushed *C. inophyllum* seeds were weighed and soaked in ethanol (Khazaai *et al.*, 2023). In the ultrasonic method, the crushed seeds were weighed, ethanol was added, and ultrasonic wave-assisted extraction was performed. Ultrasonic wave-assisted extraction was conducted using an ultrasonic cleaning bath (Bransonic 8510) at a frequency of 42 kHz (Yulianti *et al.*, 2022). The yield of the extract was calculated, and the extract was analysed (antioxidant activity, phytochemical, and GC-MS analyses). The extract yield in each treatment was calculated using Eq. 2 (Manandhar *et al.*, 2019).

$$yield\,(\%) = \frac{dry\ weight\ of\ extract}{dry\ weight\ of\ plant\ material} x100 \quad (2)$$

The antioxidant analysis of *C. inophyllum* seed extract followed adapted procedures from several sources, including the preparation of DPPH solution

Table 1: The level design of extraction process with three factors (x) and three levels

Factor	Parameters	Levels		
		Low	Medium	High
		(−)	0	(+)
	Maceration process			
x_1	Extraction time (hour)	12	24	36
x_2	Solvent concentration (%)	50	70	90
x_3	Sample:solvent ratio (g/mL)	1:3	1:5	1:7
	Ultrasonic process			
x_1	Extraction time (hour)	2.5	5.0	7.5
x_2	Solvent concentration (%)	50	70	90
x_3	Sample-solvent ratio (g/mL)	1:3	1:5	1:7

and various extract solutions with vitamin C, the analysis of a blank solution, the antioxidant analysis of the extracts and vitamin C, and the formula for calculating IC_{50} using Eq. 3 (Ginting *et al.,* 2017).

$$inhibitory\ concentration(\%) = \frac{blank\ absorbance - sample\ absorbance}{blank\ absorbance} x100 \quad (3)$$

The phytochemical analysis aimed to determine the secondary metabolism compounds in *C. inophyllum* seed extracts, including alkaloids, flavonoids, tannins, steroids, and saponins, using the methods described by (Ahmad *et al*., 2017).

GC-MS was used to identify the components of the *C. inophyllum* seed extract. A 1 microlitre (µL) aliquot of sample was injected into the GC at a He carrier gas flow of 12 cubic centimetres per minute (cc/min), and separation occurred on a 30 m × 0.25 mm × 0.25 micrometre (µm). The stationary phase was 5% diphenyl/95% dimethyl polysiloxane with an inlet temperature of 250°C. The abundance (%) and compound type were determined from the GC-MS data. These peaks appear at a retention time, and the fragmentation pattern is obtained from the retention time and matched with the fragmentation pattern of standard compounds in the database (Ismail *et al*., 2017).

The use of the extract in skin lotion formulation

The lotion formulation was designed using RSM-BBD with three components (*C. inophyllum* seed extract, Tween 80, and carbomer) and three levels (low, medium, and high), as presented in Table 2. The oil phase ingredients (2% cera alba, 5% stearic acid, 0.18% nipagin) were put in a glass beaker, homogenised, and heated on a hot plate at 75°C. The aqueous phase (Tween 80 and 0.02% nipasol) was then placed in a glass beaker and heated at 75°C. The *C. inophyllum* seed extract was then slowly added to the water phase under continuous stirring with an electric stirrer in an intermittent shaking manner (2 minutes stirring with a 20-second pause). The carbomer was mixed with 0.2% NaOH and distilled water until the composition was completely homogenous (Muthukumarasamy *et al*., 2016). The quality of the lotion product (antioxidant activity and viscosity) was analysed.

RESULTS AND DISCUSSION

C. inophyllum is a member of the Clusiaceae family that contains many active compounds (Fitriyana *et al*., 2023). *C. inophyllum* seed extract is a potential source of antioxidants, as indicated by multiple prior studies, which indicate that the antioxidant activity of *C. inophyllum* seeds is very strong, with IC_{50} <50 microgram per millilitre (µg/mL) (Mai *et al*., 2020).

Extraction

C. inophyllum seeds were extracted using several procedures, including maceration and ultrasonic extraction with ethanol. The yield from maceration was higher than that from the ultrasonic method. The higher the yield is, the greater the content of active chemical compounds obtained from the extraction procedure. This agreed with the antioxidant analysis, which showed that the antioxidant activity of *C. inophyllum* seed extract obtained by maceration was superior to that from the ultrasonic method. The IC_{50} values of *C. inophyllum* seed extract obtained using the maceration and ultrasonic techniques were 13.557 and 16.736 ppm, respectively. The lower the IC_{50} is, the stronger the antioxidant activity of an extract (Fidrianny *et al*., 2015). The yields of *C. inophyllum* seeds extracted using maceration and ultrasonic techniques at varied concentrations are presented in Table 3. Maceration generated better yields. The presence of antioxidant activity in *C. inophyllum* seeds in this investigation was demonstrated by the DPPH radical inhibition activity, as shown in Table 3.

Table 2: Level design of lotion formulation with three factors (x) and three levels

Factors	Parameters	Levels		
		Low (−)	Medium (0)	High (+)
x_1	*C. inophyllum* seed extract (%)	2.5	5	7.5
x_2	Tween 80 (emulsifier) (%)	2	4	6
x_3	Carbomer (thickener) (%)	0.25	0.5	0.75

Table 3: Optimum conditions for extraction of *C. inophyllum* seeds using maceration and ultrasonic methods

Factor		Value	
		Maceration	Ultrasonic
Yield (%)		14.002	9.154
Extraction time (hour)		23.841	5.032
Solvent concentration (%)		88.250	68.064
Sample solvent ratio (g/mL)		1:3	1:7
Desirability		0.877	0.793
Response			
Theoretical result	Yield (%)	13.958	9.366
	Antioxidant (IC_{50})	13.785	16.876
Experimental result	Yield (%)	14.002	9.154
	Antioxidant (IC_{50})	13.557	16.736

The *C. inophyllum* seed extract from the maceration process also had stronger antioxidant activity (lower IC_{50}) than that generated using the ultrasonic method.

The process used in this study to obtain the extraction conditions (treatment conditions) that produce higher amounts of antioxidants does not differ from traditional methods; however, the use of supporting technology, such as ultra-sonication, can help facilitate better mass transfer between components, which increases the extraction yield by combining the crude and solvent. Therefore, optimising treatment conditions can be crucial to boost the concentration of antioxidant components in the extract, even if the procedure remains the same. In order to increase extraction efficiency, this method uses the physicochemical characteristics of the antioxidant components and their interactions with solvents (Debiasi et al., 2021). The considerable difference in antioxidant activity between extracts produced using the maceration and ultrasonic methods was attributed to the phenolic content: the longer the extraction period, the more metabolite compounds were extracted (Mandal *et al*., 2007). Using ultrasonic equipment for a long time can produce heat, and this heat exposure can damage or reduce the total phenol content (Osorio-Tobón *et al*., 2021). Ultrasonication can also damage plant cells due to cavitation, resulting in decreased permeability for some insoluble chemicals, which are subsequently reabsorbed by the plant matrix, explaining the decreased extract yield following 120 minutes of sonication. In contrast, the absence of mechanical impulses in maceration allows the compounds to remain dissolved in the medium (Farahani, 2021).

ANOVA was used to assess the fit and appropriateness of the generated model. The F-value of the model for *C. inophyllum* seed extract had a "probability>F" value less than 0.05, indicating that the model was significant. Linear model analysis was chosen because it has a high R^2 value and low predicted residual sum of squares (PRESS) for yield response compared to other models. ANOVA analysis for the linear model of the yield response of *C. inophyllum* seed extract obtained using the maceration method had an F-value of 115.52, implying the model was significant; there was only a 0.01% chance that an F-value of this magnitude could occur due to noise. A "Probability > F" value of less than 0.0500 indicated a significant model term. In this case, A (Extraction time in hours) and B (Solvent concentration in %) were significant model factors. The F-value of lack of fit of 0.69 implied nominal values relative to pure (Tekindal *et al*., 2012). The linear model of *C. inophyllum* seed extract yield response with the maceration method showed a predicted R^2 value of 0.9453 following the adjusted R^2 value of 0.9555, with a difference of less than 0.2. The adequacy precision value measured the signal-to-noise ratio as 33.013, which is considered an adequate signal because it is greater than 4. This model could be used to navigate the design

space (Nurman *et al.*, 2021). The quadratic model data analysis of the antioxidant response of *C. inophyllum* seed extract obtained using the maceration method showed a predicted R^2 value of 0.8566 following the adjusted R^2 value of 0.9561 with a difference of less than 0.2. The Adeq Precision value, which measures the signal-to-noise ratio, was greater than 4, with a ratio value of 18.941, indicating an adequate signal (Nurman *et al.*, 2021). ANOVA analysis for the quadratic model of the yield response of *C. inophyllum* seed extracted with the ultrasonic method showed that the model F value was 5.64 and the "Prob > F" value was less than 0.0500, implying the model was significant, while a value greater than 0.1000 indicates the model term was insignificant. In addition, the F-value of the lack of fit, 0.38, implied insignificant values relative to pure (Tekindal *et al.*, 2012). Data analysis of the quadratic model of the *C. inophyllum* seed extract yield response produced using the ultrasonic method showed a predicted R^2 value of 0.4195 corresponding to the adjusted R^2 value of 0.7231 with a difference greater than 0.2. The Adeq Precision value measured the signal-to-noise ratio; at 7.412, it showed an adequate signal because it was greater than 4. The relationship between the factor and the resulting response can depicted in a three-dimensional (3D) plot. Peak formation was strongly influenced by the B factor, solvent concentration (%). 3D response surface plots and two-dimensional culture plots were generated using RSM based on BBD to demonstrate the influence of components, such as antioxidant activity (IC_{50}), on the response (Deng *et al.*, 2017). Fig. 1 shows the relationship between the factors and response of *C. inophyllum* seed extract produced using the maceration method, whereas Fig. 2 shows the relationship between the factors and response of *C. inophyllum* seeds extracted using the ultrasonic method.

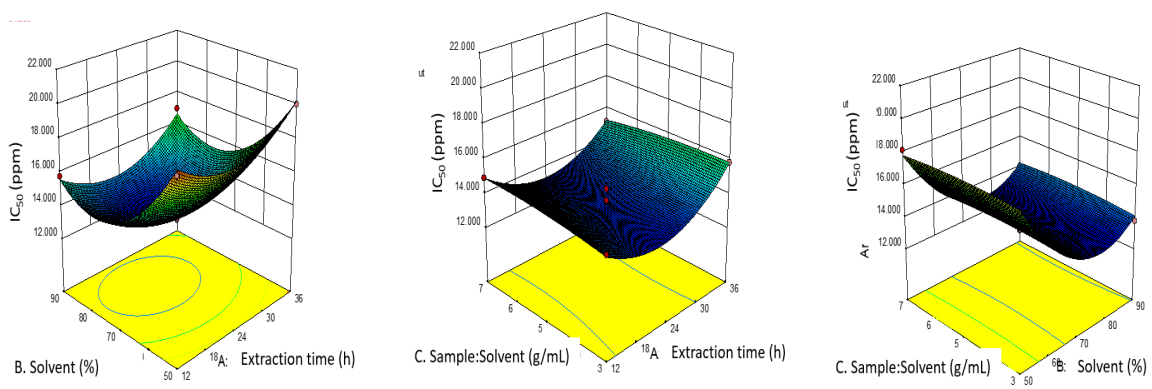


Fig. 1: 3D plot of the effect of each factor on the antioxidant activity of *C. inophyllum* seed extracted using maceration

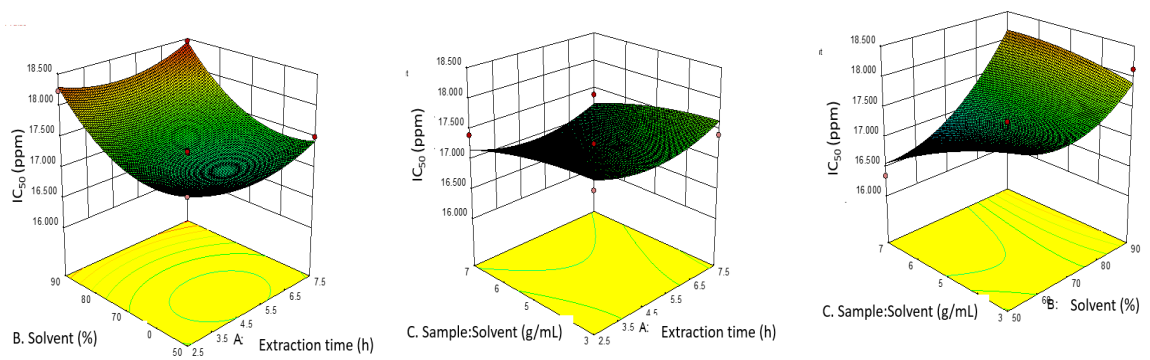
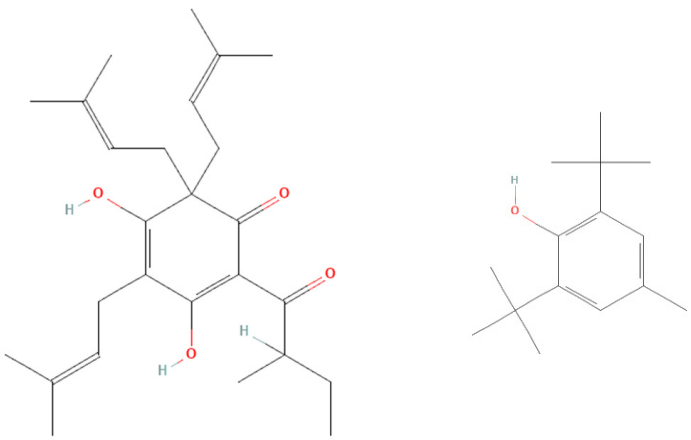


Fig. 2: 3D plot of RSM-BBD design

Table 4: Phytochemical Screening of *C. inophyllum* Seed Extracts

Phytochemical screening	Maceration	Ultrasonic
Phenolics	+	+
Tannins	-	-
Flavonoids	+	+
Terpenoids	+	+
Steroids	+	+
Saponins	+	+
Alkaloids:		
- Dragendorff	-	-
- Mayer	-	-
- Wagner	-	-



(a) (b)

Fig. 4: (a): Adlupulone (PubChem CID: 9909740) and (b): Butylated hydroxytoluene (PubChem CID: 31404

Characterisation of C. inophyllum seed extracts

The characterisation of the *C. inophyllum* seed extracts began with phytochemical screening. Phytochemical analysis was performed to identify the active compounds contained in the natural materials. The results of the phytochemical analysis of the two extraction methods used in this study indicated the presence of the same active components in both extracts; specifically, phenolics, flavonoids, terpenoids, steroids, and saponins were detected (Table 4). No alkaloid compounds were detected. This result agreed with previous work that has found similar effects (Fitriyana *et al.*, 2023).

The active ingredients in the *C. inophyllum* seed extracts were identified using GC-MS. The chemical analysis revealed that they contained 30–40 compounds. Table 5 shows only the ten compounds with the highest percentage. Based on these results, the compound that increases the antioxidant activity in *C. inophyllum* was identified as adlupulone ($C_{26}H_{38}O_4$). The ultrasonic extract of the *C. Inophyllum* also contained butylated hydroxytoluene (BHT), an antioxidant often used in foods. Both are phenolic compounds, in agreement with previous research showing that terpenoid, phenolic, and fatty acid

Table 5: Chemical Compound Components and Retention Times of *C. inophyllum* Seed Extracts from Maceration and Ultrasonic Processes

Extraction process	Compound name	Secondary Metabolite	Retention time (min)	Relative area (%)
Maceration	2'-(Trimethylsilyl)oxy-3,4,4',5- tetramethoxychalcone	Flavonoid	52.430	49.70
	Adlupulone	Benzoquinone	46.798	18.57
	4,6,8(14)-Cholestatriene	Steroid	54.365	4.58
	3'H-Chycloprop(1,2)-5a-cholest-1-en-3-one	Steroid	48.301	4.06
	Ethyl iso-allocholate	Terpenoid	54.250	3.44
	4,6,8(14)-Cholestatriene	Steroid	54.076	3.07
	Kolavenic acid trimethylsilyl ester	Carboxylic acid Methyl Ester	46.652	2.59
	2'-(Trimethylsilyl)oxy-3,4,4',5- tetramethoxychalcone	Flavonoid	53.141	1.95
	R)-6ß,11ß,21-Trihydroxy-16a,17apropylmethylenedioxypregna-1,4-diene3,20-dione	Steroid	53.454	1.78
	10-Octadecanoic acid, methyl ester	Fatty Acid, Methyl Ester	35.265	1.73
Ultrasonic	4H-Pyran-4-one, 2,3-dihydro-3,5-6-methyl-dihydroxy-	Piranon derivatives	13.176	30.64
	d-Glycero-d-ido-heptose	Sugar derivatives	17.451	21.59
	I-Gala-I-ido-octose	Sugar derivatives	22.029	13.82
	Butylated Hydroxytoluene	Phenylpropane	23.032	9.16
	6-Acetyl-d-mannose	Sugar derivatives	15.781	5.07
	Hexadecanoic acid, methyl ester	Fatty Acid	31.943	4.31
	10-Octadecenoic acid, methyl ester	Fatty Acid	35.253	2.75
	1,4-Diacetyl-3-acetoxymethyl-2,5-methylene-I-rhamnitol	Sugar derivatives	20.138	1.92
	L-Glucose	Sugar derivatives	29.501	1.71
	Cyclopentanol, 2-methyl	Alcohol Derivates	6.296	1.46

groups comprise most of the bioactive chemicals found in seed oil (Hien *et al*., 2011).

The maceration extraction method produced significant antioxidant activity (IC_{50} 13.557 ppm), which surpassed traditional maceration under optimum conditions (13.785 ppm). The compound predicted to have high antioxidant activity was 2'-(trimethylsilyl)oxy-3,4,4',5-tetramethoxychalcone, a flavonoid. This compound had the highest percentage (49.70%). Another compound in the extract was adlupulone, which is essential to the antioxidant activity. This compound is a natural product identified in *Humulus* and *Humulus lupulus* with β-bitter acid properties, with the acyl group described as 2-methylbutanoyl (Lyu *et al*., 2022). Although the ultrasonic method required shorter extraction times than maceration, the extract produced using the ultrasonic method showed lower antioxidant activity (16.736 ppm). Butylated hydroxytoluene (BHT), an antioxidant commonly used in foods, was also identified in ultrasonic extracts of *C. inophyllum*.

Characterisation of lotions

The use of *C. inophyllum* seed extract in the formulation of lotion products affected the antioxidant value of the lotion. A low IC_{50} value for the lotion indicates the potential role of the lotion in deterring free radicals (Ginting *et al*., 2017). Other studies have indicated that *C. inophyllum* seeds have

Table 6: Combination design and response of antioxidant and viscosity of *C. inophyllum* seed extract lotion using response surface methodology with Box–Behnken design

Run	Factor 1 A: *C. inophyllum* seed extract (%)	Factor 2 B: Tween 80 (%)	Factor 3 C: Carbomer (%)	Respond 1 Antioxidant IC_{50} (ppm)	Respond 2 Viscosity (cP)
1	5	4	0.5	9.8	2402.63
2	5	4	0.5	9.8	3353.37
3	5	4	0.5	8.8	2591.93
4	5	6	0.25	8.2	2313.59
5	2.5	2	0.5	13.1	2493.56
6	7.5	4	0.75	4.5	3779.51
7	5	6	0.75	8.7	3652.93
8	2.5	6	0.5	13.2	3108.07
9	2.5	4	0.75	12.5	4592.57
10	2.5	4	0.25	12.7	2669.94
11	5	2	0.75	9.6	2573.18
12	5	4	0.5	8.9	2248.34
13	5	2	0.25	8.4	2357.49
14	7.5	6	0.5	5.0	3393.32
15	7.5	2	0.5	4.6	2544.42
16	7.5	4	0.25	4.8	2491.63
17	5	4	0.5	8.1	2151.05

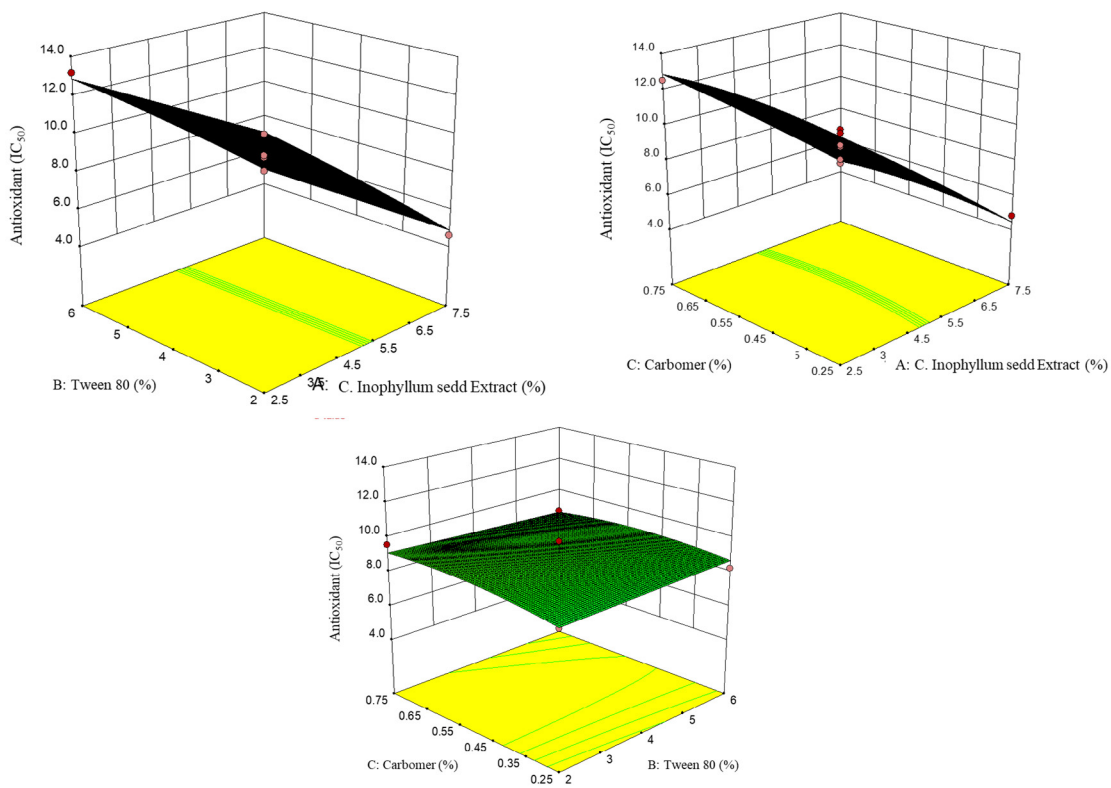


Fig. 3: 3D plot of the effect of each factor on antioxidant activity of *C. inophyllum* seed extract lotion

Table 7: Optimum condition solution of *C. inophyllum* seed extraction lotion

Factors		Value
C. inophyllum Seed Extract (%)		7.484
Tween 80 (%)		3.174
Carbomer (%)		0.272
Desirability		1.000
Responses		Value
Theoretical Result	Antioxidant (IC_{50})	4.487
	Viscosity (cP)	2112.233
Experimental Result	Antioxidant (IC_{50})	4.621
	Viscosity (cP)	2302.982

high antioxidant value and good benefits for the skin, such as the anti-ageing activity of polyphenol-rich *C. inophyllum* fruit extract in *Saccharomyces cerevisiae* BY611 yeast cells (Kavilasha and Sasidharan, 2021). It has also been used as an antioxidant in cosmetic products (Saechan *et al.*, 2021). The highest antioxidant activity in the lotion with an IC_{50} of 4.5 ppm was obtained by adding 7.5% *C. inophyllum* seed extract (run 16). This lotion had a characteristic viscosity value of 3779.51 centipoises (cP) (Table 6). This was also reinforced by the relationship between factors and the resulting response in the 3D plot in Fig 3, in which the A factor strongly influenced the antioxidant response (IC_{50}); the higher the A factor is, the lower the IC_{50} value.

The toxicity tests of the *C. inophyllum* seed extract lotion showed an LC_{50} value of 789 µg/mL, indicating that the *C. inophyllum* extract lotion has slightly toxic properties. Based on Meyer *et al.* (1982), LC_{50} over 1000 µg/mL are toxic, while LC_{50} under 1000 µg/mL are non-toxic. As shown in Table 7, both the antioxidant activity (IC_{50} 4.487 ppm) and the viscosity (2112.233 cP) of the theoretical result, as well as those of the experimental results (IC_{50} 4.621 ppm and 2302.982 cP), provided a solution to the formulation of the *C. inophyllum* seed extract lotion. This lotion formula could be used as a reference for *C. inophyllum* seed extract in the lotion because it has a desirability value close to 1.000 (Zhao *et al.*, 2020). This lotion meets Indonesian National Standard (SNI) #16-4399-1996, which states that the viscosity ranges from 2000 to 50000 cP.

CONCLUSION

In this study, *C. inophyllum* seed extract had great potential as a source of antioxidants, especially when obtained through maceration and ultrasonic extraction methods with ethanol. The three factors tested using response surface methodology (RSM) with Box–Behnken design, extraction time, solvent concentration, and sample ratio, showed interconnected contributions to maceration and ultrasonic extraction processes. The optimisation results showed the extraction of *C. inophyllum* seeds was performed under optimal conditions using maceration and ultrasonic methods. Both theoretical and experimental data indicated that the yields from the maceration extraction method were higher than those from the ultrasonic method. The extract obtained from the maceration extraction method also had higher antioxidant activity than the ultrasonic extraction method. This was also evidenced by statistical analysis using linear and quadratic models that showed the extraction time and solvent concentration significantly affected the yield and antioxidant activity. Although the ultrasonic method required less time and solvent, the resulting extract had lower antioxidant activity than the maceration method. Phytochemical analysis revealed that secondary metabolites from both methods produced the same compounds, namely phenolics, flavonoids, terpenoids, steroids, and saponins. Using GC-MS, adlupulone was identified as the compound with potential antioxidant activity in *C. inophyllum* seed extract. The application of *C. inophyllum* seed extract in lotions showed that adding 7.5% *C. inophyllum* seed extract produced the lotion with the highest antioxidant activity (IC_{50} 4.5 ppm) and a characteristic viscosity of 3779.51 cP. The toxicity test showed that the lotion was safe to use on the skin, with an LC_{50} value of 789 µg/mL. The best lotion formulation (7.484% *C. inophyllum* seed extract, 3.174% Tween 80, 0.272% Carbomer) was identified, and the theoretical results (IC_{50}

4.487 ppm and viscosity 2112.233 cP) and experimental results (IC_{50} 4.621 ppm and viscosity 2302.982 cP) support the lotion quality. This lotion formulation can be used as a reference for using *C. inophyllum* seed extract in skin care products, as it meets SNI standards while providing significant antioxidant benefits. Overall, this study contributes to understanding the antioxidant potential of *C. inophyllum* seed extract and its application in safe and effective lotion formulations.

AUTHOR CONTRIBUTIONS

L. Fitriyana conceived the study and performed the experimental design, data acquisition, analysis and interpretation of data, drafting of the manuscript, and statistical analysis. M.D. Supardan conceived the study and performed the design and critical revision of the manuscript for important intellectual content, obtained funding, administrative, technical, or material support, and provided supervision. Y. Aisyah performed the critical revision of the manuscript for important intellectual content, administrative, technical, or material support, and provided supervision. Irfan performed the critical revision of the manuscript for important intellectual content, administrative, technical, or material support, and provided supervision.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. The ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy were observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

ABBREVIATIONS	Definition
%	Per cent
°	Degree
°C	Degrees Celsius
µL	Microlitres
µm	Micrometre
3D	Three-dimensional
Adeq Precision	Adequate precision
Adj R-Squared	Adjusted r-squared
AICc	Akaike information criterion
ANOVA	Analysis of variance
BBD	Box–Behnken design
BHT	Butylated hydroxytoluene
BIC	Bayesian information criterion
cc/min	Cubic centimetres per minute
$CHCl_3$	Chloroform
cP	Centipoise

CV	Coefficient of variation
DPPH	2,2-Diphenyl-1-picrylhydrazyl
$FeCl_3$	Ferric chloride
g/mL	Gram per millilitres
GC-MS	Gas chromatography-mass spectrometry
H_2SO_4	Sulfuric acid
HCl	Hydrogen chloride
IC_{50}	Inhibition concentration 50%
LC_{50}	Lethal concentration 50%
Masl	Metres above sea level
Mg	Magnesium
mL	Millilitre
NaCl	Sodium chloride
PA	Pro analysis
ppm	Part per million
Pred R-Squared	Predicted r-squared
RSM-BBD	Response surface methodology with Box–Behnken Design
SD	Standard deviation
SNI	Indonesian National Standard
SPF	Sun protection factor

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