Hybrid drying of ultrasound assisted osmotic dehydration (UOAD) and hot air drying of *Eucalyptus deglupta*

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Abstract: *Eucalyptus deglupta* is one of the promising medicinal plants from *Myraceae* family consisting of bioactive compounds that are to be used in medications. The bioactive compounds present in *Eucalyptus deglupta* were extracted at the best yield using a hybrid drying method consisting of ultrasound assisted osmotic dehydration (UOAD) and hot air drying in this study. The drying conditions of UAOD were optimised with response surface methodology (RSM) to attain the highest antioxidant activity via DPPH radical scavenging assay. Four parameters were optimised with response surface methodology, namely concentration of sucrose (v/w), temperature (°C), duration of drying and intensity of ultrasound (%) ranged from 30% to 50%, 20°C to 60°C, 40 min to 100 min and 60% to 100%, respectively. A series of 27 combinations of the UAOD drying conditions were performed and followed by hot air drying performed at 60°C until a constant weight was achieved. A moderate scavenging activity of DPPH assay (56.12%) was achieved at a concentration of sucrose, temperature, duration of drying and intensity of ultrasound of 50%, 40°C, 100 min and 264 W, respectively.

1. Introduction

*Eucalyptus deglupta* or known as rainbow eucalyptus, originating from the *Myraceae* family is widely known for its distinct feature of revealing multi-colours like orange, yellow, blue, red and grey as a result of its peeling bark [1]. *Eucalyptus deglupta* being a source of timber and cellulose associated product has gained attention as a source of medication due to its biological properties such as antioxidant, antifungal and antibacterial. Based on each species of *Eucalyptus*, the morphology would vary from each other especially on the leaves which produces the most essential oil [2].

Antioxidants have a significant effect in protecting the cells of the human body against free radicals that can form cardiovascular diseases and cancerous cells. Research has shown that the antioxidant activity is related to the existence of α-pinene and the *Eucalyptus deglupta* has been good and commercially exploited source for these antioxidants [3]. Industries are shifting to replace synthetically antioxidant produced medicine with herbal medicines as to reduce the hazard associated with synthetically produced antioxidants.

Drying techniques have been a significant pre-treatment process for extraction processes. Conventional drying techniques such as infrared drying, freeze drying, osmotic dehydration and hot air drying are the common drying methods utilized, yet the combination of drying technique, namely hybrid
drying method removes a greater moisture content [4]. The impact of ultrasound-assisted osmotic dehydration (UOAD) combined with hot air drying of *Eucalyptus deglupta* leaves on antioxidant activity was evaluated in this study.

Drying is a process that involves mass transfer where the moisture content is removed from another matter. Unfortunately, conventional drying methods do possess some downsides such as low retention of bioactive compounds, costly, energy-intensive and longer time periods required. Therefore, in this study, the preliminary drying, UAOD was used as a preliminary drying of *Eucalyptus deglupta* leaves and its drying conditions such as the ratio of sucrose solution to experimented sample (v/w), temperature (°C), duration of drying and concentration of sucrose(g.mol⁻¹) were optimized based on the antioxidant activity of *Eucalyptus deglupta* leaves using response surface methodology (RSM). The complete drying of *Eucalyptus deglupta* leaves was then finished by hot air drying

2. Methodology

2.1. Experimental setup
The leaves of *Eucalyptus deglupta* were cleaned prior to the experiment with distilled water to wash dirt off the leave. The methanol liquid chromatography grade with a concentration of 99.9%, anhydrous sucrose crystal and DPPH with a purity of 99% and ultrapure water were used for analytical purposes.

2.1.1. Experimental design
Response surface methodology (RSM) optimization as shown in table 1 was conducted with central composite design approach [5]. This enables estimation on incurred errors to be determined with the offset in fit.

| Variables | Defined measurements | Parameters |
|-----------|----------------------|------------|
| Independent | Temperature of pre-drying, $U_T$ | 20°C – 60°C |
| | Time taken for pre-drying, $U_s$ | 60 min – 100 min |
| | Ultrasound intensity, $U_I$ | 330 kW |
| | Concentration of osmotic solution, $O_c$ | 30% - 50% |
| Dependant | Scavenging activity for 2,2-diphenylpicrylhydrazyl | $P_I$ (%) |
| Fixed | The extraction in terms of liquid to solid ratio | 0.2 g/ml |
| | Centrifugation speed | 5000 rpm |

2.1.2. Preliminary drying (pre-drying) of *Eucalyptus deglupta* (UOAD)
Initially, the sucrose solutions were prepared using anhydrous sucrose crystal ultrapure water. The concentration of osmotic solution prepared for the experiment ranged from 30% (w/v) to 50% (w/v). The sample leaves were placed in an osmotic solution and placed under the exposure of ultrasound
(ELMA P120H, Germany) bath where the leaves were exposed to ultrasonic waves for a time period of 60 min to 100 min. The studied temperature was ranged from 20°C to 60°C. The intensity was set at 80 kW for all the experiments.

2.1.3. Full moisture removal under hot air drying
Hot air drying at 60°C using an oven (Memmert, Germany) was utilised for the second stage drying. The complete drying of the leaf was achieved after repetitive weighing of the leaf until a constant weight was achieved using mass balance (Pioneer Ohaus PA4102, USA). The leaves were then powdered and sieved. The powdered leaves were chilled before the essential oil extraction process.

2.1.4. Extraction of essential oil
The essential oil was extracted as conducted in [6] with minor changes. The dried powder of the leaf of 1 g was placed with liquid chromatography grade of methanol of 99% concentration in a centrifugal tube. The ratio opted for the solution in the centrifugal tube was 1:5 (g/ml basis) of solid to concentrated solvent and was placed in a centrifuge machine for a period of 30 min at 5000 rpm and at 25°C to extract the essential oil. The centrifuged liquid extract was then separated with a syringe and filtered with HPLC polypropene filter of size 25 mm. The filtered extract was then stored in a centrifuge tube.

2.1.5. Antioxidant activity
The DPPH radical scavenging assay as described in [7] was performed similarly with minor modifications using a microplate reader (BioTek Epoch 2) with a wavelength of 520 nm. It is crucial to perform these experiments in a dark room as the DPPH solution is photosensitive and a change in colour is observed. The scavenging activity was calculated in equation (1) [8].

\[
\text{DPPH scavenging effect (\%) } = \frac{a_o - a_i}{a_o} \times 100\%
\]  

where \(a_o\) is the absorbance of control and \(a_i\) is the absorbance of standard.

2.1.6. Statistical analysis of ANOVA
The statistical analysis performed for the collected data from RSM using analysis of variance (ANOVA). The model suitability was validated based on the root mean square (RSME) and coefficient of determination \(R^2\) statistical parameters [19]. \(R^2\) and RSME were calculated based on equations (2) and (3), respectively.

\[
R^2 = 1 - \frac{(\bar{y} - \bar{y})^2}{(y - \bar{y})^2}
\]

\[
RSME = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (y_i - \bar{y})^2}
\]

where \(y_i\) is the predicted value, \(\bar{y}\) is the actual value and \(\bar{y}\) is the mean value.

2.1.7. Validation of optimised conditions
Based on central composite design (CCD), the parameters concentration of osmotic solution, intensity of the ultrasound source, pre-drying duration and pre-drying temperature were optimized with response surface methodology (RSM). The RSM method in optimizing the desired parameters was conducted with central composite design (CCD) approach.

3. Results and discussion
3.1. Control assay
The control assay was performed with three replicates. 5 g leaves were dried at 60°C with hot air drying. The complete drying of the leaves with hot air drying was achieved once a constant weight was achieved. The control assay consisting of hot air drying method was conducted to compare the antioxidant activity of *Eucalyptus deglupta* leaves dried with UAOD hybrid drying.

3.2. Response surface methodology optimization (RSM)
The optimization of pre-treatment UOAD conditions with the aid of ultrasound was studied in this research using RSM optimization with central composite design (CCD) design to achieve the highest radical scavenging activity of DPPH [9]. The concentration of osmotic solution (Oc), intensity of the ultrasound source (Ui), pre-drying duration (Us), and pre-drying temperature (Ut) were the independent variables chosen to optimize the scavenging activity of DPPH (%), P1.

Table 2 shows the response obtained from the experimental values of CCD for radical scavenging activity of DPPH. The design of experiment of CCD method was achieved with Design Expert 8.0.6 software. Based on table 2, the radical scavenging activity ranged from 15.54 % to 56.12%. The statistical analysis approach used to study the response was analysis of variance (ANOVA). The coefficient of determination and lack of fit between the parameters and respective interactions were determined.

| Std | Run | Concentration, Oc (%) | Intensity, Ui (%) | Time, Us (min) | Temperature, Ut (°C) | Response P1 (%) |
|-----|-----|----------------------|------------------|--------------|------------------|----------------|
| 26  | 1   | 40                   | 80               | 80           | 40               | 0.389          |
| 17  | 2   | 40                   | 80               | 80           | 20               | 0.414          |
| 2   | 3   | 30                   | 80               | 60           | 60               | 0.483          |
| 9   | 4   | 30                   | 60               | 100          | 20               | 0.420          |
| 6   | 5   | 30                   | 100              | 60           | 60               | 0.439          |
| 22  | 6   | 40                   | 100              | 80           | 40               | 0.445          |
| 14  | 7   | 30                   | 100              | 100          | 60               | 0.381          |
| 21  | 8   | 40                   | 60               | 80           | 40               | 0.470          |
| 24  | 9   | 40                   | 80               | 100          | 40               | 0.441          |
| 20  | 10  | 50                   | 80               | 80           | 40               | 0.319          |
| 3   | 11  | 50                   | 60               | 60           | 20               | 0.479          |
| 4   | 12  | 50                   | 60               | 60           | 60               | 0.470          |
| 7   | 13  | 50                   | 100              | 60           | 20               | 0.452          |
| 27  | 14  | 40                   | 80               | 80           | 40               | 0.419          |
| 16  | 15  | 50                   | 100              | 100          | 60               | 0.315          |
| 8   | 16  | 50                   | 100              | 60           | 60               | 0.373          |
| 25  | 17  | 40                   | 80               | 80           | 40               | 0.407          |
| 13  | 18  | 30                   | 100              | 100          | 20               | 0.409          |
| 5   | 19  | 30                   | 100              | 60           | 20               | 0.394          |
| 23  | 20  | 40                   | 80               | 60           | 40               | 0.455          |
| 19  | 21  | 30                   | 80               | 80           | 40               | 0.338          |
| 11  | 22  | 50                   | 60               | 100          | 20               | 0.432          |
| 10  | 23  | 30                   | 60               | 100          | 60               | 0.419          |
3.3. Quadratic modelling of analysis of variance (ANOVA)

The F-test in regression analysis was conducted to test the hypothesis of the corresponding parameters. As a rule of thumb, the null hypothesis will be rejected if \( F > 2.5 \) [10]. The level of significance was determined in this study using F-test and p-test. Table 3 shows the ANOVA analysis on the quadratic model developed based on the experimental results.

| Source            | Sum of Fit | df  | Mean Square | F-Value | p-Value |
|-------------------|------------|-----|-------------|---------|---------|
| Model             | 26.213     | 14  | 1.872       | 33.203  | < 0.0001 significant |
| \( U_T \) -Temperature | 0.969   | 1   | 0.969       | 17.178  | 0.0014 |
| \( U_S \) -Time   | 0.079      | 1   | 0.079       | 1.398   | 0.2600 |
| \( U_I \) -intensity | 0.157   | 1   | 0.157       | 2.779   | 0.1214 |
| \( O_c \) -concentration | 0.049   | 1   | 0.049       | 0.870   | 0.3694 |
| \( U_T U_S \)      | 2.769      | 1   | 2.769       | 49.102  | < 0.0001 |
| \( U_T U_I \)      | 2.470      | 1   | 2.470       | 43.808  | < 0.0001 |
| \( U_T O_c \)      | 2.792      | 1   | 2.792       | 49.520  | < 0.0001 |
| \( U_S U_I \)      | 0.257      | 1   | 0.257       | 4.554   | 0.0542 |
| \( U_S O_c \)      | 4.211      | 1   | 4.211       | 74.672  | < 0.0001 |
| \( U_I O_c \)      | 0.517      | 1   | 0.517       | 9.165   | 0.0105 |
| \( U_T^2 \)        | 1.099      | 1   | 1.099       | 19.486  | 0.0008 |
| \( U_S^2 \)        | 6.747      | 1   | 6.747       | 119.652 | < 0.0001 |
| \( U_I^2 \)        | 0.192      | 1   | 0.192       | 3.412   | 0.0895 |
| \( O_c^2 \)        | 0.534      | 1   | 0.534       | 9.476   | 0.0096 |
| Residual           | 0.677      | 12  | 0.056       |         |         |
| Lack of Fit        | 0.639      | 10  | 0.064       | 3.398   | 0.2487 not significant |
| Pure Error         | 0.038      | 2   | 0.019       |         |         |
| Cor Total          | 26.889     | 26  |             |         |         |

The p-value determined was less than 0.0001 and the F-value determined was 33.203 from the quadratic modelling indicated that this model was significant. Therefore, low value of p-test and high F-test value based on ANOVA concluded the quadratic model for DPPH assay was well fitted. The lack of p-value determined was 0.0838, thus it was not significant to the pure error.

3.4. Polynomial model derivation and response surface optimisation

A mathematical model was derived for DPPH antioxidant activity, \( P_1 \) (%) as a function of concentration of osmotic solution \( (O_c) \), intensity of the ultrasound source \( (U_I) \), pre-drying duration \( (U_S) \), and pre-drying temperature \( (U_T) \) as shown in equation (4).
\[ P_1 = 40.46 - 1.03 U_T + 0.2278 U_S - 0.7472 U_I - 1.06 O_c + 0.9537 U_T U_S + 1.8 U_T U_I - 1.64 U_T O_c - 5.41 U_S U_I - 0.0925 U_S O_c - 0.7472 U_I - 1.06 O_c + 0.9537 U_T U_S + 1.8 U_T U_I - 1.64 U_T O_c + 0.5389 O_c^2 \]  

(4)

Based on the model polynomial equation (4), it was notable that the factors with coefficients possessing a positive magnitude indicated a greater effect on response. In this case, the response of high pre-drying temperature and pre-drying concentration showed a very significant effect among other variables for optimizing the yield of radical scavenging activity of DPPH assay \((P_1)\). Response of yield of DPPH assay, \(P_1\) was directly proportional for coefficients with positive magnitude and inverse proportional for coefficients with negative magnitude. Based on the linear variable of DPPH assay, all the coefficients were positive. Thus, with a positive coefficient linear variable, an increase in pre-drying temperature, osmotic concentration, intensity of ultrasound source and pre-drying duration for respective extents maximized the yield of radical scavenging activity of DPPH assay. The optimized conditions were obtained using Design Expert 8.0.6 software in maximizing the \(P_1\). The optimum pretreatment conditions were 50\%, 264 W, 100 min and 40\(^\circ\)C for the concentration of osmotic solution \((O_c)\), intensity of the ultrasound source \((U_I)\), pre-drying duration \((U_S)\), and pre-drying temperature \((U_T)\), respectively resulting with a yield of 56.12\% \((P_1)\).

3.5. Respective analysis of variance(ANOVA) for fitting of DPPH assay quadratic model

The evaluation on the fitting and the significance of the DPPH assay quadratic model was measured with ANOVA to study the linear variable interaction effects for respective response. The corresponding significance for equation (4) was verified with the analysis of variance(ANOVA) which is tabulated in the table 4.

| Item                          | Values   |
|-------------------------------|----------|
| DPPH Assay \((P_1)\)          |          |
| Standard Deviation            | 3.53     |
| Mean                          | 35.04    |
| Coefficient Variation, %      | 12.83    |
| Press                         | 1877.98  |
| \(R^2\)                       | 0.9730   |
| Adjusted \(R^2\)              | 0.8931   |
| Predicted \(R^2\)             | 0.5893   |
| Adequate Precision            | 11.294   |

The coefficient of determination \((R^2)\) was used to verify the adequacy of the model to check on the fitting of the respective polynomial model on its response. The value of \(R^2\) obtained for model of DPPH assay was 0.9730 and it was close to unity of 1 where only a total variation of 7.99\% was neglected in the model. The value of \(R^2\) that inferred more 97.030\% of experimental data of DPPH assay response was feasible with data predicted for the model. Therefore, the DPPH assay response in this study had a good correlation of values for predicted and actual, resulting in a good fit of the DPPH assay quadratic model.

The adjusted coefficient of determination (Adjusted \(R^2\)) showed a value of 0.8931 for DPPH assay model. This showed a greater degree of correlation for the DPPH assay responses between theoretical
value and experimental data. Similarly, the adequate precision that measured the degree of error for the predicted response and any ratio that is more than 4 is considered desirable [11]. The model’s adequate precision is this study had a value more than 4 where DPPH assay had a value of 11.294.

The high coefficient of variation signifies poor reliability of respective experiment [12]. Typically, the coefficient of variation less than 10% indicates a good satisfactorily response model, a coefficient of variation between 10% and 20% is an acceptable satisfactorily response model and a coefficient of variation more than 20% indicates model not satisfied and rejected [12][13]. For this study, DPPH assay model had a coefficient of variation between 10% and 20%. Even though, the value of DPPH assay was more than 10%, it was very close to good response model. This established the DPPH assay model as acceptable, and the model reliably met an adequate response model where there was little margin of error from predicted data.

4. Conclusion
As conclusion, the parameters of UOAD pre-treatment drying conditions of *Eucalyptus deglupta* leaves were optimized to attain the desired highest yield antioxidant activity. Pre-treatment conditions of concentration of osmotic solution (*O*), intensity of the ultrasound source (*U*), pre-drying duration (*U*), and pre-drying temperature (*U*), were 50%, 264 W, 100 min and 40°C, respectively resulting with a yield of 56.12% (*P*).

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