

Optimization for extraction on total phenolic content and radical scavenging capacity of Henna (*Lawsonia inermis*) stems using response surface methodology

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Abstract

This study aimed to optimise potential extraction conditions using response surface methodology (RSM) for yielding maximum levels of total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) scavenging capacity of henna (*Lawsonia inermis*) stems. The ranges for selected independent variables, namely acetone concentration (20–90%, v/v), extraction time (10–90 min), and extraction temperature (25–45°C) were identified by screening tests. Optimum conditions obtained for extraction of TPC were 47.0% acetone, extraction time of 47.6 min and extraction temperature of 37.3°C. The result also showed that 75.8% acetone, extraction time of 26.2 min and extraction temperature of 41°C yielded the highest DPPH[•] scavenging capacity. The optimized extraction conditions have resulted in TPC and DPPH[•] scavenging capacity of 5232.4 mg GAE/100 g DW and 6085.7 mg TE/100 g DW, respectively which similar to the predicted values. Therefore, RSM has successfully optimized the extraction conditions for TPC and radical scavenging capacity of henna stems.

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Introduction

Henna (*Lawsonia inermis*) is an Indian medicinal plant and it is native to India, North Africa, Asia and Australia (Wyk and Wink, 2004). It has been recorded that different parts of henna plant are a rich source of various bioactive principles and has been used in traditional medicine (Dasgupta *et al.*, 2003). Recent study on phytochemical content in henna has shown that it is rich in phenolic antioxidants such as phenolic acids, flavonoids, tannins, and coumarins (Khare, 2007).

Extraction is the first important step in the recovery and purification of active ingredients from plant materials. The purpose of extraction is to give a maximum extract yield obtained from plant and of the highest quality which consist of high concentration of the target compounds and antioxidant power of the extract (Spigno *et al.*, 2007). Many techniques have been developed to extract phenolics, such as conventional solvent extraction, microwave-assisted, ultrasound-assisted and supercritical fluid extraction, among which solvent extraction (solid-liquid and liquid-liquid extraction techniques) is

the most commonly used, and has proven to be a reliable and efficient method (Banik and Pandey, 2008). The efficacy of solvent extraction is affected by many factors such as the type of solvent, solvent concentration, time, temperature, pH, number of steps, liquid-to-solid ratio and particle size of the plant material (Cacace and Mazza, 2003).

Response surface methodology (RSM) accounts for possible interaction effects between variables (Banik and Pandey, 2008). Optimization of extraction process using RSM by establishing a mathematical model would not only being a visual aid for a clearer picture about the effects of various factors on extraction but also help in terms of locating the region where the extraction is optimized (Bezerra *et al.*, 2008). RSM has been successfully used to optimize biochemical process including extraction of phenolic compounds (Liyana-Pathirana and Shahidi, 2005). To the best of our knowledge, optimization of extraction conditions for antioxidants in henna stems using RSM has not been reported yet. Therefore, this study aimed to determine the best extraction conditions for henna stems using RSM to obtain optimal levels of total phenolic content (TPC) and DPPH[•] scavenging

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capacity.

Materials and Methods

Chemicals and reagents

All chemicals and solvents used were of analytical reagent (AR) grade. Distilled water used in this study was purified using Millipore water purification system (Millipore Corporation, Billerica, Massachusetts, USA).

Sample preparation

Stems of Henna (*Lawsonia inermis*) were collected from the main city campus of UCSI University, Kuala Lumpur, Malaysia. The species has been identified and confirmed by the Forest Research Institute Malaysia, Kuala Lumpur. The stems were thoroughly washed upon arrival at the laboratory. Approximately 400 g of the stems were cut into pieces of 0.5×2.0 cm. The samples were then oven-dried for 24 h at 40°C. The dried stems were ground into a fine powder (0.5 mm) using MF 10 basic miller (IKA®Werke, Staufen, Germany) before analysis.

Experimental design

A three-level factorial central composite design (CCD) was used to determine optimize extraction conditions for extraction of antioxidants in Henna stems. Three uncoded independent variables were identified: percentage of acetone (X_1 : 20–90%), extraction time (X_2 : 10–90 min) and extraction temperature (X_3 : 25–45°C) on TPC (Y_1) and DPPH· scavenging capacity (Y_2). Graphical and numerical optimizations were performed to acquire optimum extraction conditions and predicted values for the response variables. Acetone was chosen as the extraction solvent for RSM optimization because it was the best solvent for extraction of phenolic compounds as identified by a set of single factor experiments.

Complete design consisted eight factorial points, six axial points (two axial points on the axis of each design variable at a distance of 1.68 from the design center) and six center points, leading to 20 sets of experiments as tabulated in Table 1. The ranges and the center point values of the three independent variables were based on the results of preliminary experiments. Six replicate runs at the center points of the design were performed to allow the estimation of pure error. All the experiments were performed randomly to minimize the effects of an unexplained variability in the observed responses due to systematic errors.

Verification of model

Optimal conditions for the extraction of phenolic compounds and antioxidant capacity from henna stems depending on solvent concentration, extraction time and extraction temperature were obtained using the second-order polynomial model of RSM, in which the numerical optimization method was adopted to find the points that maximize the responses. A series of solutions were generated, and the solution to be employed for the verification would be selected based on its desirability and suitability. The experimental and predicted values of TPC and DPPH· scavenging capacity were compared in order to determine the validity and adequacy of the model.

Determination of total phenolic content

TPC of henna stem extracts was determined based on Folin-Ciocalteu reagent method described by Lim *et al.* (2007) with slight modification. Crude extract of Henna stems (0.3 ml) was added to 1.5 ml of 10-fold diluted Folin-Ciocalteu reagent and 1.2 ml of 7.5% (w/v) sodium carbonate solution. The mixture was allowed to stand in the dark for 30 min at room temperature. Absorbance of the reaction mixture was read against a blank at 765 nm a spectrophotometer (Model XTD 5, Secomam, Ales Cedex, France). A calibration curve of gallic acid was plotted with an equation of $y = 10.422x + 0.0042$ ($R^2 = 0.9977$). TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of dry weight (DW).

Determination of DPPH radical scavenging capacity

DPPH radical scavenging capacity was determined based on a method described by Chan *et al.* (2007) with slight modification. The crude extract (1 ml) was added to 2 ml of DPPH solution (5.9 mg/100 ml ethanol) and allowed to stand for 30 min. Absorbance of the reaction mixture was measured against blank at 517 nm. DPPH radical scavenging capacity of the sample was calculated based on the following equation:

$$\% \text{ DPPH radical scavenging capacity} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100 \quad (1)$$

Where A_0 is the absorbance at 517 nm of the control (containing ethanolic DPPH solution without the plant extract), A_1 is the absorbance at 517 nm in the presence of the plant extract in ethanolic DPPH solution.

Trolox equivalent (TE) of the sample was

obtained based on the equation of the standard curve as follows: $y = 0.6714x - 8.5048$ ($R^2 = 0.9968$), and expressed as gram of trolox equivalents (TE) per 100 g of DW.

Statistical analysis

Data were expressed as mean values \pm standard deviation (SD) of six measurements ($n = 6$). RSM was adapted to design the CCRD using the Design-Expert Version 7.1.4 (Stat-Ease Inc., MN, USA). Regression analysis was performed based on the experimental data and was fitted into an empiric second-order polynomial model. ANOVA tables were generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. Significant value was set at $p < 0.05$.

Results and Discussion

Fitting the models

Table 1 summarizes the experimental values of the CCRD for this optimization process, along with their predicted values obtained from the model equations. The quadratic models in terms of coded variables are shown in Equations 2 and 3, where (Y_1) represents TPC and (Y_2) represents DPPH' scavenging capacity, as a function of acetone concentration (X_1 , %, v/v), extraction time (X_2 , min) and extraction temperature (X_3 , °C).

$$Y_1 = 5162.27 - 261.21X_1 - 13.20X_2 + 97.09X_3 - 341.15X_1^2 - 80.13X_2^2 - 123.82X_3^2 + 15.86X_1X_2 + 2.27X_1X_3 + 7.81X_2X_3 \quad (2)$$

$$Y_2 = 5955.53 + 51.54X_1 - 19.64X_2 + 3.80X_3 - 11.22X_1^2 + 10.44X_2^2 - 123.82X_3^2 - 16.24X_1X_2 + 46.75X_1X_3 - 23.06X_2X_3 \quad (3)$$

To fit the response function and experimental data, the multiple regression coefficients of intercept, linear, quadratic, and interaction terms in the experimental model were calculated, and their levels of significance were determined using the ANOVA. Gan *et al.* (2007) suggested that for a good fit of a model should have R^2 of at least 0.80. The adjusted R^2 is a corrected value for R^2 after the elimination of the unnecessary model terms. If there were many non-significant terms have been included in the model, the adjusted R^2 would be remarkably smaller than the R^2 . In the present work, good fits were achieved and most of the responses' variability was explained by the model, the R^2 being 0.9648 and 0.9665 for TPC and DPPH' scavenging capacity models, respectively. The R^2 values for these response

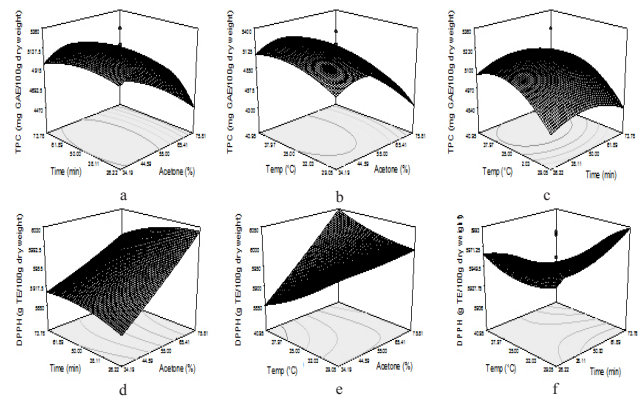


Figure 1. Response surface plots correspond to total phenolic content (TPC) and DPPH radical scavenging capacity of henna stems as a function of (a, d) acetone concentration and extraction time; (b, e) acetone concentration and extraction temperature; and (c, f) extraction time and extraction temperature, by keeping one of the variables at a middle level.

variables were remarkably close to 1, denoting that the regression models provide excellent explanations of the relationship between the independent factors and the responses.

Analysis of response surfaces of total phenolic content

By considering two variables at one time while keeping the third one at the middle level, the response surface plots of the solvent concentration (X_1), extraction time (X_2) and extraction temperature (X_3) on the TPC were generated to aid in visualization (Figure 1a–c). Acetone concentration was shown to be the most significant factor in the regression model for TPC, where both its linear and quadratic terms had a negative effect on TPC. On the other hand, the effect of extraction time was quadratic ($p < 0.05$) regardless of the proportion of acetone in the medium, while its linear term exhibited non-significant effect on TPC. The ANOVA revealed no significant ($p \geq 0.05$) synergism was observed between acetone concentration and extraction time. The response surface plot indicates that a constant extraction temperature of 35°C has caused an increase in the acetone concentration and extraction time as TPC was gradually mounted up. However, the TPC yield gradually after they reached their peaks at about 50% (v/v) and 50 min.

Both acetone concentration and extraction temperature demonstrated appreciable linear and quadratic effects on the TPC. However, the interaction effect between acetone concentration and extraction time was not profound. Significantly negative linear and quadratic effects of acetone concentration ($p < 0.05$) were obtained indicating that there is a maximum in the TPC extraction at a certain acetone

Table 1. Central composite rotatable design (CCRD) criterion of extraction parameters with the experimental data (Expt.) and their predicted value (Pred.) under different conditions

Standard Order ^a	Independent variables			Dependent variables (Responses)			
	X ₁ ^b	X ₂ ^c	X ₃ ^d	Y ₁ , Total phenolic content (TPC) (mg GAE/100 g DW)		Y ₂ , DPPH radical scavenging capacity (mg TE/100 g DW)	
				Expt.	Pred.	Expt.	Pred.
1	34.19	26.22	29.05	4845.39	4871.89	5966.43	5971.39
2	75.81	26.22	29.05	4357.96	4313.22	6021.75	6013.46
3	34.19	73.78	29.05	4842.83	4798.15	6037.56	6038.21
4	75.81	73.78	29.05	4357.32	4302.93	6005.95	6015.30
5	34.19	26.22	40.95	5024.50	5045.92	5901.16	5884.70
6	75.81	26.22	40.95	4484.62	4496.32	6121.55	6113.79
7	34.19	73.78	40.95	4991.64	5003.41	5858.11	5859.30
8	75.81	73.78	40.95	4576.73	4517.25	6035.48	6023.41
9	55.00	50.00	35.00	5174.82	5213.73	5984.38	5986.92
10	55.00	50.00	35.00	5359.69	5213.73	5986.21	5986.92
11	55.00	50.00	35.00	5188.26	5213.73	5988.17	5986.92
12	55.00	50.00	35.00	5000.27	5213.73	5960.51	5986.92
13	20.00	50.00	35.00	4609.99	4585.17	5845.90	5848.21
14	90.00	50.00	35.00	3635.13	3706.58	6013.85	6021.59
15	55.00	10.00	35.00	4931.11	4906.37	5889.37	5902.33
16	55.00	90.00	35.00	4790.59	4861.97	5885.42	5882.52
17	55.00	50.00	25.00	4543.47	4597.33	5994.09	5986.70
18	55.00	50.00	45.00	4931.11	4923.89	5903.19	5920.63
19	55.00	50.00	35.00	5180.76	5110.81	5939.22	5924.15
20	55.00	50.00	35.00	5180.76	5110.81	5939.22	5924.15

^a Non-randomized^bX₁: Acetone concentration (% v/v)^cX₂: Extraction time (min)^dX₃: Extraction temperature (°C)

Table 2. Optimum conditions, predicted and experimental values of responses on extraction of henna stems

Dependent responses	Independent variables			Optimum value		
	Acetone (%)	Extraction time (min)	Extraction temperature (°C)	Experimental ^a	Predicted	% Differences
Total phenolic content (TPC) (mg GAE/100 g DW)	47.02	47.56	37.30	5232.39 ± 16.62	5231.79	0.01
DPPH radical scavenging capacity (mg TE/100 g DW)	75.81	26.22	40.95	6085.70 ± 35.89	6082.4	0.05

^a Mean ± standard deviation (SD) of six determinations (n = 6) from two extract replications

concentration followed by a decline with further increase in acetone concentration. With respect to extraction temperature, significant positive linear and negative quadratic effects on the response within a 99.0% confidence interval ($p < 0.05$) are displayed. Similarly, at lower and upper levels of acetone concentration, the increase in extraction temperature led to a gradual increase in the TPC and yielded maximum in the region of extraction temperature between 36°C and 38°C. Further increase in extraction temperature led to a marked deceleration in the extraction of TPC. Indeed, mild heat treatment can induce the formation of compounds with antioxidant properties or improve the antioxidant capacity of naturally occurring antioxidants, yet gentle enough to avoid heat degradation of the phenolic compounds.

TPC is noticed to be significantly affected by the linear and quadratic effects of the extraction temperature at 99.0% confidence level. It was found that the linear term of extraction temperature had a positive effect on TPC while showing a negative effect in the second-order term, contributing to a saddled shape. As for extraction time, it only influenced the response in a quadratic manner ($p < 0.05$) and hence TPC increased with increasing extraction

time to a certain level (approximately 50 min), but became flattened and showed a tendency to decline with further increase in extraction time. Therefore, excessively lengthening extraction time is not useful to extract more phenolics. The interaction between extraction time and extraction temperature had no significant ($p \geq 0.05$) effect on TPC. In other terms, extraction temperature influenced TPC independently of the extraction time.

Analysis of response surfaces of DPPH radical scavenging capacity

To determine optimal levels of the variables (acetone concentration, extraction time and extraction temperature) for the extraction of DPPH scavenging capacity from henna stem extracts, three-dimensional response surfaces (Figure 1d–f) were constructed by keeping one of the variables constant. The constant was equal to the natural value of zero level. With regard to acetone concentration, the linear effect indicating that the DPPH scavenging capacity extraction increases with the increase in acetone proportion theoretically up to 100% as shown in the response surface plot within the tested range.

As for the extraction periods, the response

variable exhibited significant ($p < 0.05$) negative quadratic effect. However, the quadratic effect of acetone concentration was not significant ($p \geq 0.05$). Interestingly, the interaction between acetone concentration and extraction time (X_1X_2) was found to be significantly ($p < 0.05$) negative for DPPH[•] scavenging capacity, which means that the factor extraction time depends on the level of acetone proportion being used. It was predicted that high DPPH[•] scavenging capacity tended generally to occur at higher levels of acetone concentration ($\geq 75.81\%$) and low extraction time (≤ 26.22 min).

The results showed a very significant positive and linear effect for the factor acetone concentration on the DPPH[•] scavenging capacity of henna stems. On the other hand, DPPH[•] scavenging capacity was found to be a function of the negative linear effect and positive quadratic effect ($p < 0.05$) of extraction temperature suggesting that an increase or decrease of the extraction temperature from the optimum temperature (approximately 40°C) may improve the DPPH[•] scavenging capacity. This implicates that the extraction is largely favoured in two cases, such as low extraction temperature in the presence of low acetone concentration or elevated temperature in the presence of high acetone concentration.

The results revealed that DPPH[•] scavenging capacity had a negative quadratic effect with extraction time ($p < 0.05$), elucidating that the increase of extraction time may enhance the yield of DPPH[•] scavenging capacity from henna stems. After this point, the yield of the DPPH[•] scavenging capacity started to decrease with increasing the extraction time. Whereas concerning the extraction temperature, DPPH[•] scavenging capacity had both negative linear and positive quadratic ($p < 0.05$) effects with extraction temperature as previously described. Interaction between the two variables had a significant negative effect on DPPH[•] scavenging capacity giving an overall saddle nature to the response surface. In other words, higher DPPH[•] scavenging capacity may be obtained either with long extraction time and low temperature or with high extraction temperature and short extraction time as indicated by their negative interaction coefficient ($\beta_{23} = -23.06$).

In this study, the high DPPH[•] scavenging capacity was mainly contributed by the antioxidants extracted by the aqueous acetone. The results showed that when the percentage of acetone increases, there is an increase in DPPH[•] scavenging capacity. However, Figure 1a–c shows that the extracted TPC reached the optimal point at the percentage of acetone of about 55%. Increasing the percentage of acetone showed no increment in the extracted TPC. Thus the increasing

values for the DPPH[•] scavenging capacity were possibly contributed by other phytochemicals besides phenolic compounds. The result is in agreement with Prior *et al.* (1998) on the poor recovery of total phenolics in *Vaccinium* species by aqueous acetone. On the other hand, an increase in the extraction time showed an increase in the DPPH[•] scavenging capacity. Similarly, an increase in extraction temperature to 41°C showed a minor increment in the DPPH[•] scavenging capacity. Therefore, high level of acetone will not improve the extraction of total phenolics, but it contributes to the increment of DPPH[•] scavenging capacity.

Verification of the predictive model

In order to validate the adequacy and suitability of the model equations for predicting the optimum response value, verification experiments were carried out under the optimal conditions as given in Table 2. To ensure the predicted results were not biased toward the practical values, verification experimental was performed using these deduced optimal conditions ($n = 6$). Verification of the experiments were accomplished by using the recommended optimized conditions, the mean values of 5232.39 mg GAE/100 g DW for TPC and 6085.70 mg TE/100 g DW for DPPH[•] scavenging capacity were obtained from real experiments, demonstrating the validation of the RSM model. The percentage errors between the actual and predicted values for the TPC and DPPH[•] scavenging capacity were calculated, and were observed to be 0.01% and 0.05% from Table 2. The excellent correlation between these results confirmed that the response models were satisfactory, accurate and adequate for reflecting the expected optimization.

Conclusions

In a nutshell, the experimental optimum conditions that allow fast, quantitative and maximum extractions of phenolic antioxidants from henna stems were obtained through the application of RSM with CCRD. The best combination of response functions for maximum TPC was found to be acetone concentration 47.02% (v/v), extraction time 47.56 min and extraction temperature 37.30°C whereas the optimum conditions for highest DPPH[•] scavenging capacity were acetone concentration 75.81% (v/v), extraction time 26.22 min and extraction temperature 40.95°C. Under these optimized conditions, the experimental values clearly agreed with the predicted values. The optimum conditions can be useful for the development of a large-scale industrial extraction processes of phenolic antioxidants from henna

stems as they satisfy the constraints of operating at a moderate extraction temperature at which all phenolic compounds are stable, an extraction time sufficient to overcome diffusion limitations and a solvent composition capable of extracting both the lipophilic and hydrophilic phenolic compounds.

References

- Andjelkovic, M., Camp, J.V., Meulenaer, B.D., Depaemelaere, G., Socaciu, C., Verloo, M. and Verhe R. 2006. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chemistry* 98 (1): 23–31.
- Balasundram, N., Ai, T.Y., Sambanthamurthi, R., Sundram, K. and Samman, S. 2005. Antioxidant properties of palm fruit extracts. *Asia Pacific Journal of Clinical Nutrition* 4 (4): 319–324.
- Banik, R.M. and Pandey, D.K. 2008. Optimizing conditions for oleanolic acid extraction from *Lantana camara* roots using response surface methodology. *Industrial Crops and Products* 27 (3): 241–248.
- Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S. and Escaleira, L.A. 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* 76 (5): 965–977.
- Cacace, J.E. and Mazza, G. 2003. Optimization of extraction of anthocyanins from black currants with aqueous ethanol. *Journal of Food Science* 68 (1): 209–215.
- Chan, E.W.C., Lim, Y.Y. and Chew, Y.L. 2007. Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. *Food Chemistry* 102 (4): 1214–1222.
- Dasgupta, T., Rao, A.R. and Yadava, P.K. 2003. Modulatory effect of Henna Leaf (*Lawsonia inermis*) on drug metabolizing phase I and phase II enzymes, antioxidant enzymes, lipid peroxidation and chemically induced skin and forestomach papillomagenesis in mice. *Molecular and Cellular Biochemistry* 245 (1–2): 11–22.
- Fan, G.J., Han, Y.B., Gu, Z.X. and Chen, D.M. 2008. Optimizing conditions for anthocyanins extraction from purple sweet potato using response surface methodology (RSM). *LWT-Food Science and Technology* 41 (1): 155–160.
- Gan, H.E., Karim, R., Muhammad, S.K.S., Bakar, J.A., Hashim, D.M. and Rahman, R.A. 2007. Optimization of the basic formulation of a traditional baked cassava cake using response surface methodology. *Food Science and Technology* 40 (4): 611–618.
- Kasankala, L.M., Xue, Y., Weilong, Y., Hong, S.D. and He, Q. 2007. Optimization of gelatine extraction from grass carp (*Ctenopharyngodon idella*) fish skin by response surface methodology. *Bioresource Technology* 98 (17): 3338–3343.
- Khare C.P. 2007. *Indian Medicinal Plants—An Illustrated Dictionary*. New York: Springer.
- Lim, Y.Y., Lim, T.T. and Tee, J.J. 2007. Antioxidant properties of several tropical fruits: A comparative studies. *Food Chemistry* 103 (3): 1003–1008.
- Liyana-Pathirana, C.M. and Shahidi, F. 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry* 93 (1): 47–56.
- Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G. and Mainland, C.M. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry* 46 (7): 2686–2693.
- Rice-Evans, C., Miller, N. and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* 20 (7): 933–956.
- Spigno, G., Tramelli, L. and De Faveri, D.M. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering* 81 (1): 200–208.
- Stratil, P., Klejdus, B., Kudylo, A., Oszmianski, J. and Czemerzys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry* 105 (3): 940–949.
- Tabart, J., Kevers, C., Sipel, A., Pincemail, J., Defraigne, J.O. and Dommès, J. 2007. Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. *Food Chemistry* 105 (3): 1268–1275.
- Wyk, B.E.V. and Wink, M. 2004. *Medicinal Plants of the World: An Illustrated Scientific Guide to Important Medicinal Plants and Their Uses*. Singapore: Timber Press.