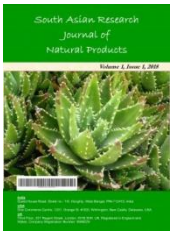

South Asian Research Journal of Natural Products

1(2): 77-85, 2018; Article no.SARJNP.43322

Optimizing the Extraction of Bioactive Compounds from Leaves of *Gymnema Lactiferum*, an Edible Green Leafy Vegetable

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Authors' contributions

This work was carried out in collaboration between all authors. Author KDPPG designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors KKDSR and HPVR managed the analyses of the study. Author RMTRKR gave the technical supports. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SARJNP/2018/43322
Editor(s):
(1) Dildar Ahmed, Professor, Department of Chemistry, Forman Christian College University Lahore, Pakistan.
Reviewers:
(1) Ramaiah Maddi, Hindu College of Pharmacy, India.
(2) Roshan Keshari, Delhi Pharmaceutical Sciences and Research University, India.
(3) Ogunwa Tomisin Happy, Adekunle Ajasin University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/25805

Original Research Article

Received 25th May 2018
Accepted 3rd August 2018
Published 7th August 2018

ABSTRACT

Gymnema lactiferum is a leafy vegetable which contains phenolics and carotenoids and also possesses many health benefits such as antidiabetic properties. Response surface methodology (RSM) has been used to optimize the extraction parameters for the recovery of total phenolic compounds and carotenoids from leaves of *Gymnema lactiferum*. Solvent concentration (30-100%), extraction temperature (30-60°C) and extraction time (30-90 min) were used as the independent variables. A three-factor inscribed central composite design (CCD) was used to identify the relationship existing between the response functions (total phenolics and carotenoids) and the

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process variables, as well as to determine those conditions that optimised the extraction process of total phenolics and carotenoids contents of the extracts. A second order polynomial model produced a satisfactory fitting of the experimental data with regard to total phenolics (R^2 = 86.75%, $p < 0.002$) and carotenoid (R^2 = 84.74, $p < 0.017$) contents. The optimum extraction conditions of ethanol concentration, extraction temperature and extraction time for phenolics, were 19.2%, 70.2°C and 98.2 min for phenolics and 100%, 70.20°C and 110.5 min for carotenoids. The experimental values for total phenolics were 4.01±0.74 mg gallic acid equivalent (GAE) g extract and 3.56±0.19 mg/g dry weight (DW) carotenoids and no significant difference ($p < 0.05$) was found between the experimental and predicted values of the extractable phenolics and carotenoids

Keywords: Gymnema lactiferum leaves; phenolics; carotenoids; response surface methodology.

1. INTRODUCTION

Phytochemicals such as phenolics and carotenoids from numerous vegetables exert several health-promoting functions including reducing the risks of many chronic diseases such as cancer, diabetics and heart and neurodegenerative diseases [1]. Most of these preventive effects of these phytochemicals are associated with their antioxidant activity by protecting cells and tissues from oxidative damage which can be by various free radicals and reactive oxygen species [2,3]. *Gymnema lactiferum* (Linn.) is an edible plant and the leaves of *G. lactiferum* are used as salads, curries and in herbal gruels. According to Gunathilake and Ranaweera [4] and Gunathilake et al. [5], leaves of this plant contain high levels of total phenolics and carotenoids and having good antioxidant properties. *G. lactiferum* leaves possess anti-inflammatory properties [6] and also it has been used as a supportive treatment for diabetes in Sri Lanka for several decades [7,8, 9]. Therapeutic properties of this plant extracts may possibly be attributed to the phenolic and carotenoids compounds present. There is a current trend for natural dietary sources of antioxidants for the formulation of the functional foods and nutraceutical ingredients. In this course, extraction is the initial and most vital step in the recovery and purification of bioactive compounds from plant sources [10] and many factors such as solvent concentration, extraction temperature, solvent-to-solid ratio and extraction duration may significantly influence the extraction efficiency and concentration of bioactive compounds [11]. Optimization of these extraction conditions is therefore needed to achieve the higher bioactive recovery. Response surface methodology (RSM), nowadays, is one of the most popular optimisation techniques in the area of food science and technology and has been applied for the extraction of antioxidant bio actives from a number of dietary sources. However, there are no reports in the literature on the optimisation of the extraction conditions for polyphenols and carotenoids from leaves of *G. lactiferum.* Therefore, the objective of this study was to investigate the optimum extraction conditions for *G. lactiferum* leaves for higher total phenolics and carotenoids recovery.

2. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

G. lactiferum leaves were collected from home gardens in Kottawa area of Sri Lanka. All the chemicals used were of analytical grade.

2.2 Preparation of Extracts

One gram of air-dried and ground leaf sample was placed in a conical flask with 20 mL aqueous ethanol (1:20 solid/liquid ratio) at desired concentrations and extraction was carried out using a rotary shaker (Unimax 1010, Heidolph, Kelheim, Germany) at 400 rpm, under specified temperature according to the experimental design as described in Gunathilake et al. [12].

2.3 Determination of Total Phenolic Content

The total phenolic content was determined using the Folin–Ciocalteu assay [13] with some modification, as described by Gunathilake [14]. Briefly, 0.5 mL of extract and 0.1 mL of Folin–Ciocalteu reagent (0.5N) were mixed and incubated at room temperature for 15 min in the dark. Then 2.5 mL 7.5% sodium carbonate were added to the mixture and further incubated for 2 hours in the dark at room temperature and then the absorbance was measured at 760 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). The concentration of total polyphenols was expressed as mmol gallic acid equivalents (GAE) per g dry weight (FW) of the leaves.

2.4 Total Carotenoids Content

The carotenoid content was analysed according to the method described by Sükran et al. [15] with slight modifications. The homogenate was filtered through a filter paper (No: 42 Whatman) and centrifuged using the centrifuge (EBA20) for 10 min at 245 g. The supernatant was separated, and the absorbance was read at 470, 653, 666 nm on UV/VIS spectrometer (SP-3000). The concentration of each pigment was calculated according to the following formulas and the carotenoid contents were reported as mg/g DW

Chlorophyl a = 11.75 (A662) − 2.350(A645).
Chlorophyl b = 18.61 (A645) − 3.960 (A662).
Carotene = 1000 (A470) − 2.270 (C_a) − 81.4 (C_b)/227.

Where, C_a, Chlorophyll a and C_b, chlorophyll b;

2.5 Experimental Design

The effect of three independent variables, ethanol concentration, extraction temperature, and extraction time; and the response variables were total phenolic and total carotenoid contents were studied. The response surface optimisation procedure was designed based on a three-factor inscribed central composite design (CCD) consisting of aqueous ethanol (30–100%), extraction temperature (30–60°C) and extraction time (30-90 min) as shown in Table 1. During the study, solid to liquid ratio were maintained at 1:20. The selection and range of these three factors were based on previous studies. Each variable to be optimized was coded at three levels 1, 0, +1 (Table 1) and twenty randomised experiments including six replicates as the center points were assigned based on CCD and measured response variables were total phenolic content and carotenoid content, as given in Table 2.

2.6 Statistical Design

The assumptions of normality and constant variance were checked and confirmed. A response surface analysis and analysis of variance (ANOVA) were employed to determine the regression coefficients, the statistical significance of the model terms and to fit the mathematical models of the experimental data that aimed to optimize the overall region for both response variables. A second-order polynomial model was applied to predict the response variables. The adequacy of the model was predicted through the regression analysis (R^2) and the ANOVA analysis. Optimum extraction conditions were computed using the MINITAB 15 software to achieve the maximum recovery of polyphenols and carotenoids. For the verification of predicted extraction conditions, experimental data for the contents of phenolics and carotenoids in *G. lactiferum* leaf samples were determined according to the best extractions conditions obtained with RSM.

3. RESULTS AND DISCUSSION

The efficiency and effectiveness of the phenolics and carotenoids extraction process are generally manipulated by multiple variables, including solid to solvent ratio, extraction time, temperature and solvent composition [16]. RSM is accepted as a powerful tool in optimizing experimental conditions to maximize various responses [17]. The obtained data were used for the prediction of an optimum set of extraction parameters from leaf extract with higher phenolics and carotenoids contents. The amount of phenolics and carotenoids in the extracts were employed in a multiple regression analysis, performed using RSM to fit the second-order polynomial equations as shown in Table 3 and 4 for phenolics and carotenoids, respectively. The ‘‘fitness’’ of the model was studied through the lack-of-fit test ($p > 0.05$) and the quality of fit to the second-order polynomial models for *G. lactiferum* leaf extracts was established. The software generated the estimated regressions coefficients for quadratic equations for both phenolics and carotenoids as appeared in Table 3.

Total phenolics content of leaf extracts varied from 1.12 to 4.26 mg GAE/g dry sample and the total carotenoids contents varied from 1.08 to 2.82 mg/g DW. The ANOVA of the second order polynomial models for the phenolics extractions from *G. lactiferum* leaves show that the models were significant ($p < 0.05$) with R^2 and p-values of 0.86 and 0.02, respectively (Table 3). There was no significance in the lack of fit ($p = 0.58$) in the model indicating that the model could be used to predict the responses. For carotenoids extraction, the model was significant ($p < 0.05$) and the R^2 and p-values were 0.81 and 0.01, respectively (Table 3). The lack of fit ($p = 0.69$) in the model was not significance ($p<0.05$) and this indicated that the model could be used to predict responses. To visualize the relationship between the response and experimental levels of the

independent variables for the total phenolics and carotenoids extraction, three-dimensional (3D) surface plots were constructed as in Figs. 1 and 2.

Response surfaces were used to illustrate the effects of solvent concentration, extraction time and the temperature on the responses (Figs. 1–2). Extraction of phenolic compounds depends greatly affect on the extraction of phenolics and carotenoids from *G. lactiferum* leaves (Figs. 1 and 2). The use of ethanol is relatively cheap, reusable and non-toxic, an environmentally friendly preparation of potentially bioactive extracts for food uses can be achieved.

Extraction of phenolic compounds depends significantly on the polarity of the solvents used in the extraction process. Use of a pure ethanol may not be effective for the separation of phenolics from plant materials [10]. According to Fig. 1, an ethanol-based extraction system with lower ethanol % is preferred for phenolic extraction from *G. lactiferum* as the highest level of extract yield of phenolics was found when compared with the extract with pure ethanol (100%). Many earlier findings also stated that phenolics are more extractable in polar solvents as compared to non-polar ones [10,3]. Ethanol is also a good solvent that can be used for carotenoids extraction [18] and the extraction is highly influenced by extractions variables including solvent concentration, extraction temperature and time [19]. Influence of three extraction conditions towards total carotenoids extraction was reported in surface plots in Fig. 2 and the regression coefficients of the second-order polynomial regression equation appear in Table 3. Among the extraction variables, extraction solvent showed a greater influence on the carotenoids extraction. The extraction and separation of carotenoids depend largely on the nature of the polarity of the solvents [19]. For *G. lactiferum*, higher carotenoids extractions were observed when 100% ethanol was used (Fig. 2) compared with aqueous ethanol solvent system. When ethanol concentration increased from 30% to 100% while keeping extraction temperature and time at 30°C and 30 min, respectively, increase in the carotenoids content from 1.71 to 2.38 mg /g DW was observed (Table 2).

In terms of extraction temperature on total phenolics, the recovery of phenolics was increased considerably when the extraction temperature was increased to 60°C, while the % ethanol maintained at a low level (Figs. 1 c & d). Results showed that at a lower solvent concentration (30%) and extraction duration (30 min), increasing the extraction temperature from 30°C to 60°C and extraction time (30 min), increased the extractable phenolics from 3.64 to 4.02 mg GAE/g DW. Extractable carotenoid concentration increased when used pure ethanol (100%) and extraction time of 30 min from 2.38 to 2.60 mg/g DW. This could be due to the increase in the solubility of these bioactive compounds, diffusion rate, mass transfer rate, extraction rate and reduced solvent viscosity and surface tension at higher temperatures and solvent polarities which could improve the phenolic extractability [20]. However, extractable carotenoids content decrease from 1.71 to 1.08 mg/g DW when the extraction temperature increased from 30 to 60 while keeping solvent concentration and 30% and extraction time of 30 min. The extraction time was another important parameter in the extraction procedure for bioactive in many previous studies. However, the results showed that extraction time did not have a significant effect on the phenolics and carotenoids extraction from *G. lactiferum* leaves at $P < 0.05$ level.

The software generated predicted optimal ethanol concentration, extraction temperature, extraction time were developed for maximizing both responses and they were 19.2%, 70.2°C and 98.2 min for phenolics and 100%, 70.20°C and 110.5 min for carotenoids, respectively. The values obtained experimentally for both response variables are near to the predicted values, indicating a satisfactory model. The experimental

Table 1. Levels of extraction variables for experimental designs

Independent variables	Level total phenol content/ carotene content				
	+1	**0**	**-1**	**+1.682**	**-1.682**
X1: Ethanol (%)	100	65	30	123.86	6.137
X2: Temperature (°C)	60	45	30	70.23	19.773
X3: Time (min)	90	60	30	110.45	9.546

Table 2. Central composite design arrangement for extraction of phenolics and carotenoids from *Gymnema lactiferum*

Run order	Ethanol %	Temperature (°C)	Time (min)	Phenolic mg/g GAE	Carotenoids mg/g
1	65.00	19.77	60.00	3.49	2.42
2	65.00	45.00	60.00	3.19	2.09
3	65.00	45.00	110.45	3.94	2.41
4	6.14	45.00	60.00	3.75	1.17
5	30.00	30.00	90.00	3.68	1.95
6	100.00	60.00	90.00	1.62	2.82
7	65.00	45.00	60.00	3.38	2.11
8	100.00	30.00	90.00	1.63	2.15
9	30.00	60.00	30.00	4.02	1.08
10	65.00	45.00	60.00	2.71	1.60
11	65.00	70.23	60.00	3.81	2.55
12	100.00	60.00	30.00	1.66	2.60
13	65.000	45.00	9.55	3.16	2.06
14	123.86	45.00	60.00	1.12	1.89
15	30.00	30.00	30.00	3.64	1.71
16	65.00	45.00	60.00	3.59	2.48
17	30.00	60.00	90.00	4.26	1.32
18	100.00	30.00	30.00	1.57	2.38
19	65.00	45.00	60.00	3.88	2.45
20	65.00	45.00	60.00	4.22	2.07

values for total phenolics were 4.01±0.74 mg GAE g extract and 3.56±0.19 mg/g DW carotenoids and no significant difference ($p < 0.05$) was found between the experimental and predicted values of the extractable phenolics and carotenoids.

Table 3. Estimated Regression Coefficients for phenolics and carotenoids using data in uncoded units

Terms	Phenolics	Carotenoids
Constant	1.88493	3.18114
Ethanol %	0.0324392	0.0154882
Temperature (°C)	0.0343011	-0.0845285
Time (min)	0.0142711	-0.00180295
Ethanol %*Ethanol %	-3.81259E-04	-1.87407E-04
Temperature *Temperature (°C)	-1.65574E-04	0.000477953
Time (min)*Time (min)	-8.06772E-05	2.12791E-05
Ethanol %*Temperature (°C)	-2.08333E-04	0.000511905
Ethanol %*Time (min)	-3.12500E-05	-5.83333E-05
Temperature (°C)*Time (min	3.12500E-05	0.000125000
R^2	86.87%	81.34%
P values for regression	0.002	0.011
P values for lack of fit	0.576	0.686

Table 4. Predicted values and experimental values of total phenolics and carotenoids at the optimum extraction conditions for *Gymnema lactiferum*

Optimum extraction conditions		Predicted values (mg/g)		Experimental values (mg/g)	
Phenolics	**Carotenoids**	**Phenolics**	**Carotenoids**	**Phenolics**	**Carotenoids**
ETOH:19.2% Temp:70.2 °C Time:98.2 min	ETOH:100% Temp:70.2°C Time:110.5 min	4.46	3.33	4.01±0.74	3.56±0.19

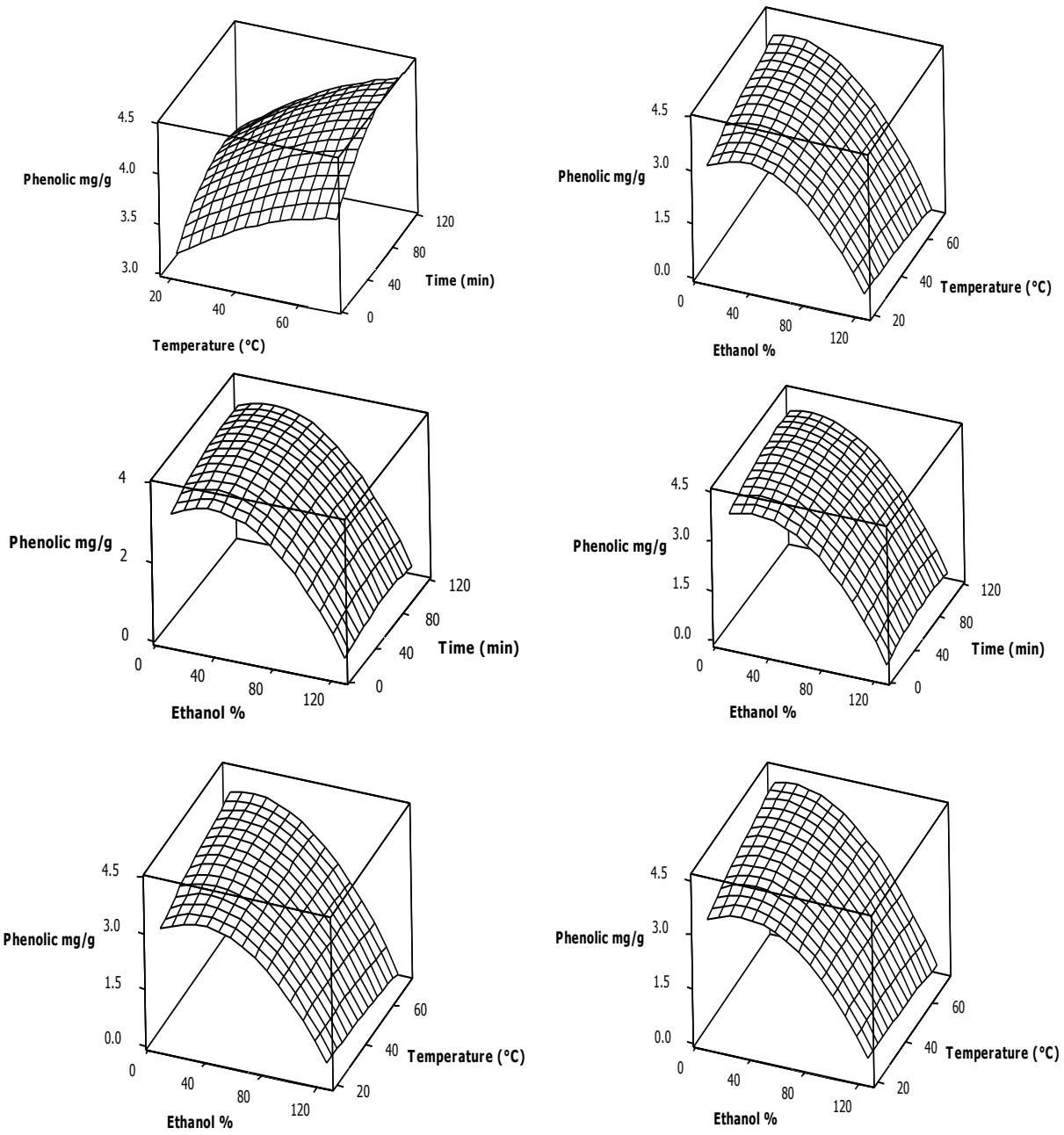

Fig. 1. Pair-wise response surface plots of the phenolics (mg GAE/g DW) extraction from *Gymnema lactiferum* leaves as a function of ethanol %, extraction temperature and time: ethanol % was kept constant at 30% (a) and 100% (b); temperature of extraction was kept constant at 30 °C (c) and 60 °C (d); the time of extraction was kept constant at 30 min (e) and 90 min (f)

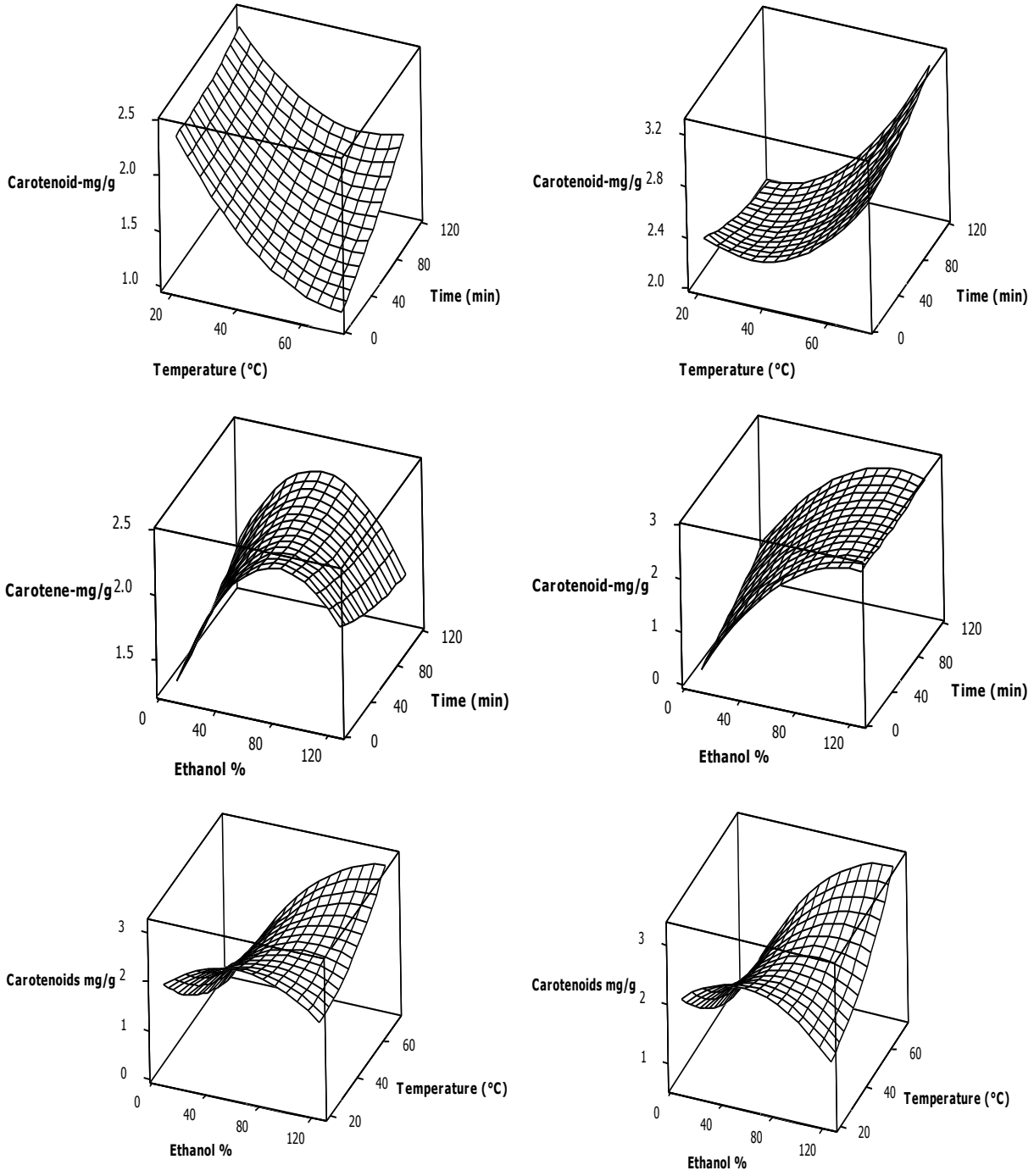

Fig. 2. Pair-wise response surface plots of the carotenoids (mg/g DW) extraction from *Gymnema lactiferum* leaves as a function of ethanol %, extraction temperature and time: ethanol % was kept constant at 30% (a) and 100% (b); temperature of extraction was kept constant at 30 °C (c) and 60 °C (d); the time of extraction was kept constant at 30 min (e) and 90 min (f).

4. CONCLUSIONS

An ethanol-based extraction technique was applied for the extraction of phenolic and carotenoids compounds from *G. lactiferum* leaves and optimised by response surface methodology. The results showed that the solvent concentration had a greater influence on extraction yields of total phenolics and total carotenoids from *G. lactiferum* than extraction

temperature and time. It was confirmed that the predicted total phenolics and carotenoids content were not significantly different from those of experimented values.

ACKNOWLEDGEMENTS

The authors would like to acknowledge National Science Foundation of Sri Lanka for financial support under the Competitive Research Grant Scheme (Project No: RG/AG/2014/04).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vita JA. Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. American Journal of Clinical Nutrition. 2005;81:292−297.
2. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. American Journal of Clinical Nutrition.1995;62:1315-1321.
3. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L and *Juniperus phoenicea* L. fruit extracts. Food Chemistry. 2007;105:1126–1134.
4. Gunathilake, KDPP, Ranaweera KKDS. Antioxidative properties of 34 green leafy vegetables. Journal of Functional Foods. 2016;26:176-186.
5. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Change of phenolics, carotenoids, and antioxidant capacity following simulated gastrointestinal digestion and dialysis of selected edible green leaves. Food Chemistry. 2018;245: 371–379.
6. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Influence of boiling, steaming and frying of selected leafy vegetables on the *in vitro* anti-inflammation associated biological activities. Plants. 2018;*7*(1):22.
7. Bandara T, Rokeya B, Khan S, Ali L, Ekanayake S, Jansz ER, Balasubramanium K. Effects of *Gymnema lactiferum* leaves on glycemic and lipidemic status in type 2 diabetic subjects. Bangladesh Journal of Pharmacology. 2009;4:92-95.
8. Bandara T, Rokeya B, Khan S, Ali L, Ekanayake S, Jansz ER, Balasubramanium K. Effects of *Gymnema lactiferum* leaf on serum glucose and cholesterol levels of streptozotocin-induced diabetic rats. International Journal of Biol. Chem. Sci. 2010;4(3):815-819.
9. Perera HKI, Handuwalage CS. Detection of protein glycation inhibitory potential of nine antidiabetic plants using a novel method. Asian Journal of Medical Sciences. 2015;6(2).
10. Prasad KN, Hassan FA, Yang B, Kong KW, Ramanan RN, Azlan A, Ismail A. Response surface optimization for the extraction of phenolic compounds and antioxidant capacities of underutilized *Mangifera pajang* Kosterm peels. Food Chemistry. 2011;128(4):1121-1127.
11. Gan C, Latiff AA. Optimisation of the solvent extraction of bioactive compounds from *Parkia speciosa* pod using response surface methodology Food Chemistry. 2011;124:1277–1283.
12. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Optimization of phenolics and carotenoids extraction from leaves of *Passiflora edulis* using response surface methods. Asian Journal of Biotechnology and Bioresource Technology. 2017;1(1):1-11.
13. Singleton VL, Orthofer R, Lamuela-Raventos R. Analysis of total phenols and other oxidation substrates and antioxidants by means of FC reagent. Methods in Enzymology. 1999;29:152–178.
14. Gunathilake KDPP. A fruit-based functional beverage designed to reduce the risk of cardiovascular disease. MSc Thesis. Dalhousie University; Halifax, NS, Canada; 2012.
15. Sükran DERE, Gunes T, Sivaci R. Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. Turkish Journal of Botany. 1998;22(1):13-18.
16. Alothman M, Bhat R, Karim AA. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry. 2009;115:785–788.
17. Hajj YE, Louka N, Nguyen C, Maroun RG. Low-cost process for phenolic compounds extraction from cabernet sauvignon grapes

(*Vitis vinifera* L. cv. cabernet sauvignon). Optimization by response surface methodology. Food Nutr. Science. 2012;3: 89–103.

18. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV, Perera ODAN, Jayaweera HPS. Response surface optimization of extraction of polyphenols and carotenoids from *Sesbania grandiflora* leaves with ethanol-water system. Asian Journal of Biotechnology and Bioresource Technology. 2017;2(1).
19. Wang L, Liu Y. Optimization of solvent extraction conditions for total carotenoids in rapeseed using response surface methodology. Natural Science. 2009;1(01): 23.
20. Richter BE, Jones BA, Ezzell JL, Porter NL, Avdalovic N, Pohl C. Accelerated solvent extraction: A technique for sample preparation. Analytical Chem. 1996;68: 033–1039.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25805