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Effect of microwave-assisted extraction (MAE) process on % extraction yield, phenolic compounds and antioxidants activity of natural extract from edible fiddleheads and MAE process optimization by using response surface methodology (RSM)

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ABSTRACT

This study was conducted to explore the effect of Microwave-assisted extraction (MAE) process on % extraction yield, phenolic compounds and antioxidants activity of natural extract from edible fiddleheads. Microwave assisted extraction was done for the extraction of antioxidants rich extract from edible fiddleheads. Response Surface Methodology was used for designing the experiment. Seventeen experimental runs were conferred by Box-Benkhen model. Three independent variables (process parameters), i.e. Microwave power (Watt), time (min) and solid to solvent ratio were selected to see their effect on the three responses, i.e. % extraction yield, TPC and IC50. MAE process parameters; 320 watts (microwave power), 2 min (time) and 48.582 (solid to solvent ratio) were selected as the optimal condition. Results showed that the % extraction yield, TPC and IC50 value ranged from 9.69 to 15.85, 14.1 to 22.5 mg GAE/g and 3.57 to 10.35 mg/g.

Keywords: *Fiddleheads; Microwave assisted extraction; Total phenolic compounds; IC50;extraction yield.*

1. INTRODUCTION

Fiddleheads or fiddlehead greens are new growth shoots that occur as tightly furled fronds of a young fern [1]. In India it is popular by some local names like lingri, lungdu, Kasrod, Limbra. Globally it is known as Ostrich fern (Matteucciastruthiopteris), Western sword fern, (Polystichummunitum), Bracken (Pteridiumaquilinum), Cinnamon fern (Osmundacinnamomea), Midin (Stenochlaenapalustris), Vegetable fern (Athyriumesculentum), Zenmai(Osmunda japonica), Lady fern (Athyriumfilix-femina) and Royal fern (Osmundaregalis) [2,3,4]. Edible fern, Stenochlaenapalustris (Burm. F.) Bedd is native to India [5] and belongs to Blechnaceae family. This fern spreads throughout South-East Asia; especially Malaysia to Ploynesia and till Australia [6, 7]. "Ulam" is the traditional vegetable salad of Malaysia. S. palustris is very popularly used by Malaysian community to prepare Ulam [8].

Fiddleheads are also found to be rich in omega-3 and omega-6 fatty acids along with a good composition of dietary fiber, minerals and vitamins. They are also reported to be enriched with antioxidants [9, 10] mainly phenolic compounds and flavonoids [11, 12]. This fern also shows its healthy potential by having high potassium and low sodium content.

Due to this property it becomes suitable for people having low-sodium diet [13]. Since ancient times, people from South Pacific region and Asian countries (India, Malaysia, Phillippines and Thailand) had been consuming fiddleheads as a vegetable [14, 15, 5, 10]. Studies have also reported that with the evolution of human life, these ferns have been effectively used as food and medicines [16, 10].

De Long *et al*. [9] in their study of fiddlehead ferns in Canada showed that in the edible ostrich fern (Matteuccia struthiopteris); eicosapentanoeic acids, dihomo-γ-linolenic, arachidonic acid and γ-linolenic acid have been found in fiddlehead tissue. It contains antioxidant compounds such as a- and ß-carotene, a- and γ-tocopherol and ascorbic acid. Stenochlaena palustris [17] and Diplaziumesculentum were demonstrated to have alpha glucosidase inhibition hence have antidiabetic activities [18]. Some crude extracts obtained from ferns showed powerful antioxidant activity with respect to free radical scavenging activities as compared with its vitamin C content [19, 20, 10].

Several extraction processes such as enzyme-assisted extraction, ultrasound-assisted extraction, soxhlet extraction, maceration and microwave-assisted extraction (MAE) have been reported for effective extraction of the bioactive compounds. Among these process MAE is a very effective and green extraction process to extract bioactive compounds [21, 22, 23, 24]. As compared with other conventional extraction processes, MAE has several merits such as, lower energy requirement, low temperature, relatively higher extraction efficiency and shorter extraction time and [25, 26, 27].

Response surface methodology (RSM) has been selected here as it is the most efficient statistical tool to design mathematical model for the development, improvement and optimization of process parameters. The major advantages of using RSM are reduction in number of experimental trials that are needed for the evaluation of multiple variables and their co-relation and interactions [28].

Studies related to extraction of natural antioxidants from Fiddleheads fern by using microwave –assisted extraction (MAE) needs a basic approach as no such work has been done. Therefore, the present study has been planned to explore the effects of MAE process on the percentage of extraction yield, TPC and antioxidant activity of natural antioxidants from Fiddleheads fern and the optimization of the extraction process using RSM.

2. MATERIALS AND METHODS

Fresh edible fiddleheads purchased from local market of Dehradun, Uttarakhand. Briefly, 2 kg fiddleheads were purchased; stems were separated; fiddleheads washed with fresh water 2-3 times to remove dust and any other kind of adhering materials. Tray drier was used for the drying of the fiddleheads. A temperature of 50°C was selected after preliminary trials for drying the fiddleheads for 3-4 hrs. Then, the samples were grinded to make it as fine powder. All the chemicals used in this study were purchased from Hi-media, India.

2.1. Microwave assisted extraction.

2.5 gram of previously prepared dried sample was taken into extraction vessel. Ethanol (60%) was used as a solvent for extraction purpose of the study. The level of MAE parameters i.e., microwave power (Watt), time (min) and solid to solvent ratio were selected as per the experimental design shown in Table 1. Solid and liquid phase of extract were separated through whatman filter paper No. 41 and concentrated under vacuum at 40°C to obtain the dry extract.

2.2. Analytical procedures.

The procedure for the determination of Extraction yield and DPPH assay (free radical scavenging activity) was performed according to [29]. Extraction yield was expressed as percentage and free radical scavenging activity was expressed as IC50. TPC was determined by the standard protocol of [30] and expressed as mg GAE/gm. The following correlations were used to determine % extraction yield as Eq. (1) and DPPH radical scavenging activity (IA %) as Eq. (2).

$$\%\ \text{Extraction yield} = \frac{\text{Dried wt.of extract}}{\text{Total wt.of the peel powder}} \times 100 \qquad \ldots\ldots\ldots 1$$

$$\text{DPPH radical scavenging activity (IA \%)} = \frac{AC - AS}{AC} \times 100 \qquad \ldots\ldots 2$$

Where, AC= Absorbance with Control

AS= Absorbance with Sample

2.3. Statistical analysis.

The analysis of the experimental data and their optimization was done by Design expert 10.0.1. To evaluate the effect of MAE parameters i.e. microwave power (Watt), time (min) and solid to solvent ratio on the responses, i.e., %extraction yield, TPC and IC50 a second order response function was implemented for three independent variables having following general form Eq. (3).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \qquad \ldots .3$$

Where,

Y= Response

β0, βii, βij = Coefficients

Xi and Xj = Independent Variables

ANOVA was used for determining the statistical significance of the independent parameters and their relative interactions. The adequacy of the model was explained in terms of R^2 (coefficient of determination), F-value (Fisher's value) and LOF (lack-of-fit).

Table 1. Experimental design.

Independent variables		Coded Levels		
Name	Code	-1	0	1
		Actual Levels		
Microwave power (Watt)	X_1	320	480	640
Time (min)	X_2	2	3	4
Solid to Solvent ratio	X_3	10	30	50

Table 2. Experimental results of microwave assisted extraction (MAE) of natural antioxidant of fiddlehead fern.

Exp. No.	Coded values X_1 Power (Watt)	X_2 Time (min)	X_3 Soild to Sovent ratio	% Extraction Yield	TPC mgGAE/gm	DPPH IC_{50} mg/gm
1	320	2	30	14.85	18.7	5.77
2	640	2	30	15.85**	17.1	5.83
3	320	4	30	13.28	19.4	4.46
4	640	4	30	12.49	18.7	6.55
5	320	3	10	10.63	15.6	5.35
6	640	3	10	14.59	17.8	3.89
7	320	3	50	15.12	17.2	3.57*
8	640	3	50	12.24	17.9	5.43
9	480	2	10	13.76	18.6	6.08
10	480	4	10	14.51	22.5**	5.25
11	480	2	50	15.1	20.8	4.32
12	480	4	50	12.38	19.9	4.91
13	480	3	30	9.69*	14.1*	10.1
14	480	3	30	10.62	14.1	10.35**
15	480	3	30	9.69	15.2	9.23
16	480	3	30	9.69	14.1	9.78
17	480	3	30	9.69	15.8	9.23

***minimum, **maximum**

Table 3. Coefficients for different responses by design Expert 10.0.1

Factor	Coefficients		
	% Extraction Yield	TPC mg GAE/gm	DPPH IC_{50} mg/gm
Intercept	9.876	14.66	9.738
X_1 Power (Watt)	0.16125	0.075	0.31875
X_2 Time (min)	-0.8625^{***}	0.6625	-0.10375
X_3 Solid to solvent ratio	0.16875	0.1625	-0.2925
X_1X_2	-0.4475	0.225	0.5075
X_1X_3	-1.71^{***}	-0.375	0.83^{**}
X_2X_3	-0.8675^{**}	-1.2^{**}	0.355
X_1^2	1.7245^{***}	0.245	-2.33275^{***}
X_2^2	2.517^{***}	3.57^{***}	-1.75275^{***}
X_3^2	1.5445^{***}	2.22^{***}	-2.84525^{***}

*****, ** Significant at 1, 5 % level of significance respectively**

Table 4. Analysis of variance (ANOVA).

Source	Degree of freedom	% Extraction Yield		TPC mg GAE/gm		DPPH IC_{50} (mg/gm)	
		F value	P Value Prob>F	F value	P Value Prob>F	F value	P Value Prob>F
Model	9	20.15	0.0003	10.70	0.0025	35.08	< 0.0001
X_1	1	0.49	0.5054	0.048	0.8324	3.07	0.1233
X_2	1	14.10	0.0071	3.77	0.0934	0.33	0.5864
X_3	1	0.54	0.4864	0.23	0.6485	2.58	0.1520
$X_1 X_2$	1	1.90	0.2108	0.22	0.6553	3.89	0.0892
$X_1 X_3$	1	27.71	0.0012	0.60	0.4627	10.40	0.0145
$X_2 X_3$	1	7.13	0.0320	6.18	0.0418	1.90	0.2102
X_1^2	1	29.66	0.0010	0.27	0.6186	86.51	< 0.0001
X_2^2	1	63.19	< 0.0001	57.57	0.0001	48.84	0.0002
X_3^2	1	23.79	0.0018	22.26	0.0022	128.69	< 0.0001
Lack-of- fit	3	NS	NS	NS	NS	NS	NS
R^2		0.96		0.93		0.97	

Table 5. Constrains for optimization of microwave assisted extraction (MAE) of natural antioxidant of fiddlehead fern

Name	Lower Limit	Upper Limit	Goals		Predicted	Experimental
			Optimum Conditions *	Economic Conditions**		
A:Power	320	640	minimize	minimize	320	320
B:Time	2	4	minimize	minimize	2	2
C: Solid to solvent ratio	10	50	is in range	minimize	48.582	48.582
%Yield	9.69	15.85	maximize	maximize	18.256	18.185
Total Phenolic Content (TPC)	14.1	22.5	is in range	maximize	21.493	20.984
IC_{50} (mg/gm)	3.57	10.35	minimize	minimize	2.116	2.235

*maximization of % extraction yield with minimizes IC 50

**maximization of % extraction yield with minimization of IC 50, power and time to reduce final costs.

3. RESULTS

Total 17 experiments were designed by using Box-Benkhen design of RSM. These runs were performed to see the effect of MAE parameters, i.e., microwave power (Watt), time (mins) and solid to solvent ratio on the said responses, i.e., %extraction yield, TPC and IC50. The results were statistically analyzed for being either significant or non-significant. The results of the experiment are given in Table 2. ANOVA and regression analysis was performed to check the adequacy of the model as shown in Table 3 and Table 4, respectively.

3.1. Effect of MAE process parameters on the responses.

Table 2 shows the analyzed effect of MAE process parameters on all the three responses, i.e., % extraction yield, TPC and IC50. The % extraction yield, TPC and IC50 value ranged from 9.69 to 15.85, 14.1 to 22.5 mg GAE/gm and 3.57 to 10.35 mg/gm, respectively. F-value for % extraction yield (20.15), TPC (10.70) and IC50 (35.08) were found to be significant at 5% ($p<0.05$), 5% ($p<0.05$) and 1% ($p<0.01$) level of significance, respectively (Table 4). However, lack of fit i.e., LOF was found to be insignificant. Hence, the second order model was chosen for explaining the responses. The value of R2 for % extraction yield, TPC and IC50 were 0.96, 0.93 and 0.97, respectively. The attained R2 value was greater than 0.90. Hence, it is shown that the regression model explained the reaction well. Table 3 presented here shows the effect of individual parameters on all the three responses at linear, quadratic and interactive levels.

3.1.1. Effect of MAE process on % extraction yield.

The linear term of time, quadratic term of microwave power, time and solid to solvent ratio and interactive term of microwave power and solid to the solvent ratio found significant at ($p<0.01$) i.e., 1% significant level. However, the interactive term of time and solid to solvent ratio came significant at ($p<0.05$) i.e., 5% significant level and all the other interactive effects were insignificant.

Fig. 1 (a, b, c) represents the 3D response surface graph. It had been observed from Fig. 1(a) that with increasing time and microwave power, the % extraction yield got decreased. However,

Fig. 1(b) showed that with the increase in solid to solvent ratio and microwave power, the % extraction yield got increased. Fig. 1(c) represented the increasing pattern in % extraction yield with increasing time and solid to solvent ratio.

Our findings were in accordance with the results given by [31]. They reported that when phenolic compounds were extracted from cherry laurel (Prunuslaurocerasus) leaves; a similar trend of increase in % extraction yield was observed with increasing time and solid to solvent ratio. Cheng, *et al*., and Xie *et al*. [32, 33] had also reported the similar findings when the extraction of polysaccharides was done from Gentianascabra and Crotalaria sessiliflora was selected for the extraction of flavonoids.

3.1.2. Effect of MAE process on TPC.

The quadratic term of time and solid to solvent ratio was technically found significant at ($p<0.01$), i.e. 1% significant level. However, the interactive term of time and solid to solvent ratio was significant at 5% level of significance ($p<0.05$) and all the other effects were observed to be insignificant.

The three dimensional response surfaces are represented by Fig. 2 (a, b, c). Fig. 2(a) showed that the TPC decreased slightly with increasing time and microwave power. Zhang [34] had reported for the decrease in TPC in case of buckwheat flour (roasted as well as microwave heated). According to Randhir [35] when the heat-susceptible phenolic compound gets degraded thermally; it results in a decrease in the total phenolic content. As reported by Altan [36], the reason for the decrease in TPC lies in the alteration of the molecular structure of phenolic compounds. Due to this the efficiency of extractability gets decreased, because of the change in degree of polymerization (DP).

Fig. 2(b) showed that as the solid to solvent ratio got increased, the TPC increased slightly with increasing microwave power. This result was in accordance with [37] who also reported that the yield of caffeine content in tea increased with the increasing microwave power and the liquid/solid ratio. When the microwave power was kept constant, Fig. 2(c) represented that with increasing the time and solvent to solid ratio, TPC increased significantly. Our finding favors the finding of [38] who also reported a similar pattern in their study of characterization of phenolic compounds from Phyllanthusemblica fruits.

3.1.3. Effect of MAE process on IC50.

The quadratic term of microwave power, time and solid to solvent ratio had been found significant at ($p<0.01$), i.e. 1% significant level while, the interactive term of microwave power and solid to solvent ratio had been found significant at ($p<0.05$), i.e. 5% significant level and all the other effects were observed to be insignificant.

The three dimensional response surfaces are represented by Fig. 3(a, b, c). IC50 value represents the concentration of the extract at which radical scavenging activity gets reduced by 50 % in 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay. The lowest is the IC50; the higher is the antioxidant activity.

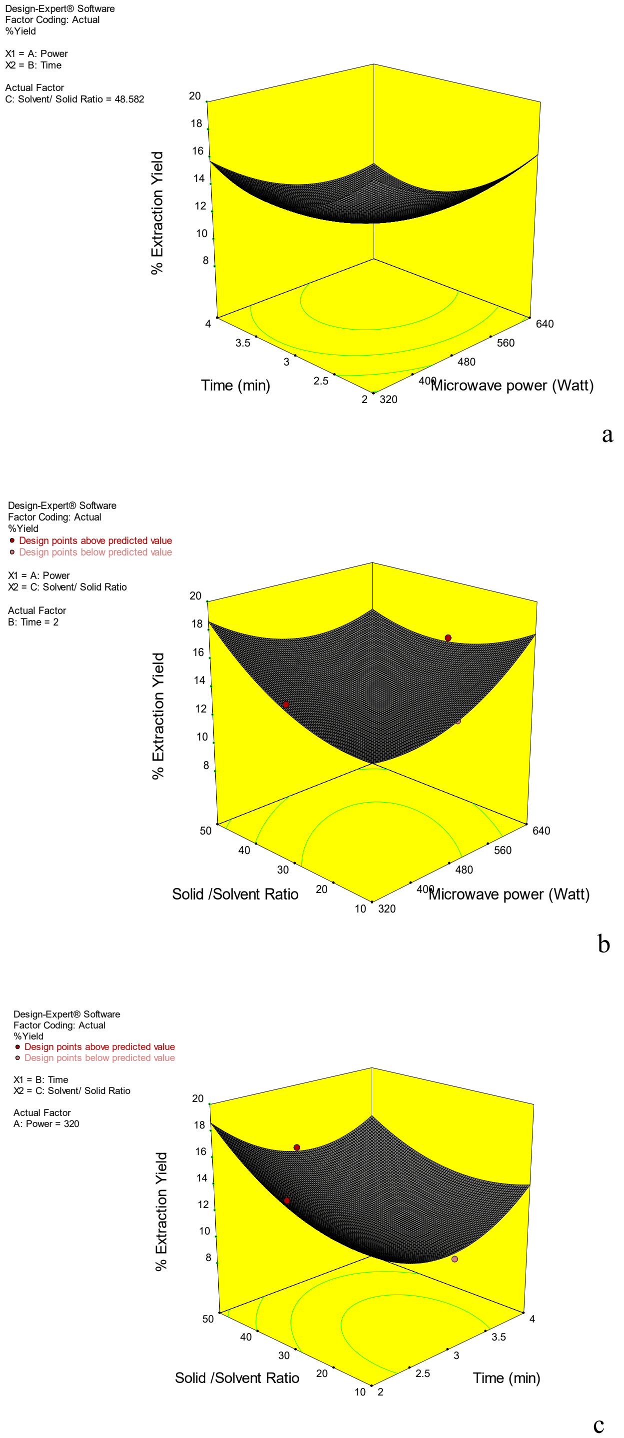

Figure 1. Response surfaces showing interaction effect of MAE process parameters on % extraction yield a) time and microwave power ii) solid/ solvent ratio and microwave power iii) solid/ solvent ratio and time.

Fig.3 (a), (b) and (c) showed that the IC50 value of the extract decreased with increasing microwave power, time and solid to solvent ratio. The minimum IC50 (3.57mg/ml) was observed at the following process parameters, i.e. microwave power (320 watt), time (3 min) and solid to solvent ratio (50). Khizar *et al*. and Kim *et al*. [39, 40] had also reported the similar findings that when citrus mandarin pomace was given microwave treatment and the citrus pomace was treated with electron beam irradiation, the antioxidant activity increased, respectively. Our findings are also in lieu of the findings of [41] that in case of tomato (SolanumLycopersicum) during microwave heating, IC50 decreased significantly.

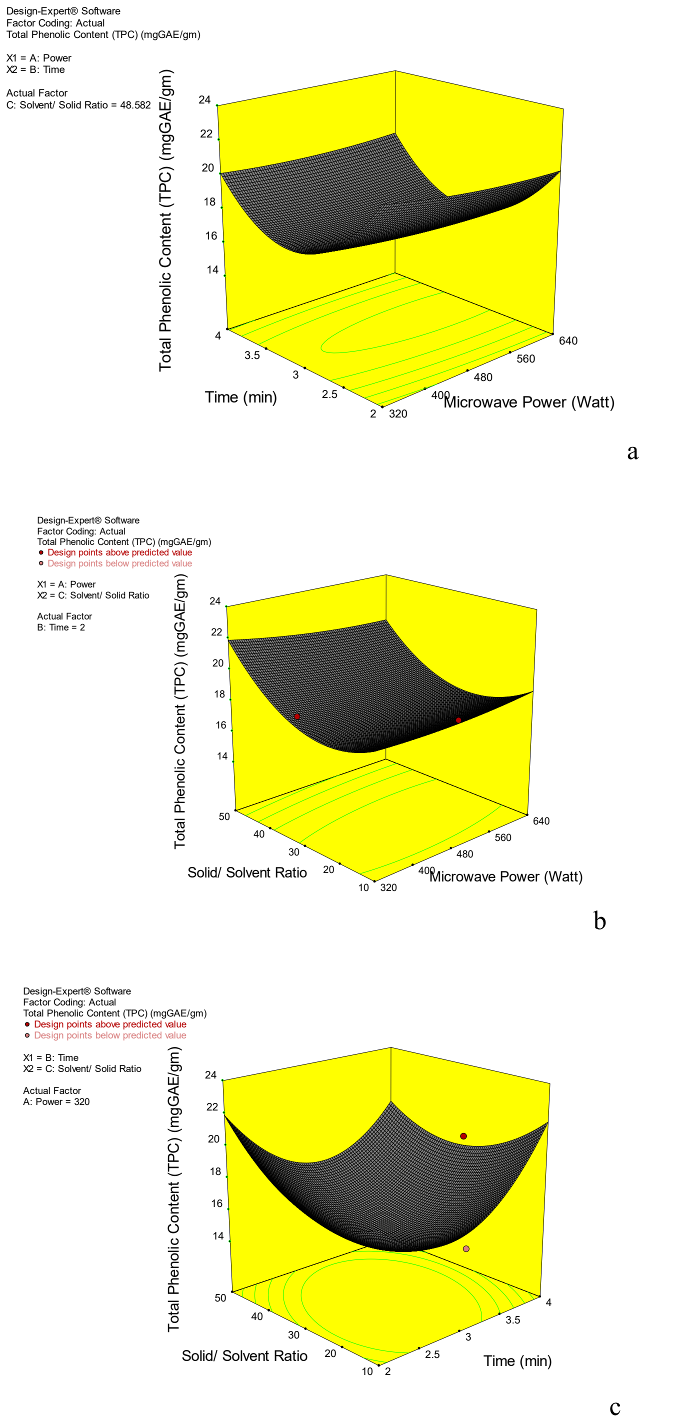

Figure 2. Response surfaces showing interaction effect of MAE process parameters on Total Phenolic Content a) time and microwave power ii) solid/ solvent ratio and microwave power iii) solid/ solvent ratio and time.

3.2. Numerical Optimization and validation.

According to the criteria obtained in Table 5, the numerical optimization was performed. Total 83 solutions were obtained after the optimization and one of the best solutions with the highest desirability was selected. The optimized values of MAE process parameters were found to be 320 watts (microwave power), 2 min (time) and 48.582 (solid to solvent ratio). To validate the optimized values, experiments were conducted at optimum values and responses were studied. From Table 5, it was found that the predicted values obtained were in close agreement with the experimental result.

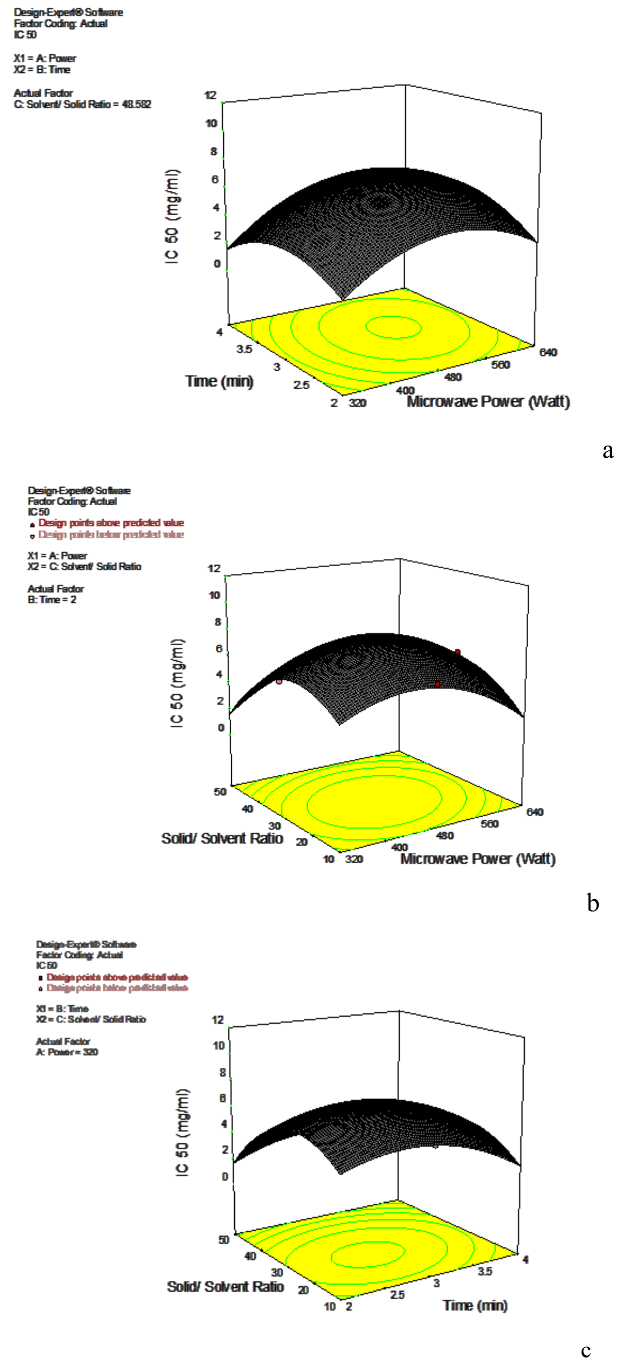

Figure 3. Response surfaces showing interaction effect of MAE process parameters on IC50 a) time and microwave power ii) solid/ solvent ratio and microwave power iii) solid/ solvent ratio and time.

4. CONCLUSIONS

A microwave assisted extraction method along with response surface methodology as the design tool has been used to conduct the present study. The present work was to extract natural antioxidants from Fiddleheads fern and then, to optimize the MAE process parameters. A second order regression model was obtained and the optimum process parameters that were obtained are- 320 watts (microwave power), 2 min (time) and 48.582 (solid to solvent ratio). The high R^2 value of 0.96, 0.93 and 0.97 was obtained for % extraction yield, TPC and IC50, showing that the model is highly significant.

Therefore, this study positively corresponds to the utilization of microwave assisted dried Fiddleheads for making positive human health impacts. Furthermore, studies could be conducted by incorporating MA extract of Fiddleheads in a variety of food products and studying their mechanisms.

5. REFERENCES

1. Hogan, C. M. *Fern. Encyclopedia of Earth. National council for Science and the Environment Archived*. 2011**.**
2. Alverson, E.R. Peter, Z. *Ferns and friends in the Wallowa Mountains, oregon*. 2008**;** pp. 15.
3. Churchill, E. Enjoy your midin without fear—Professor. *The Borneo Post* **2018**.
4. Chai, P.P.K. Midin (*Stenochlaena palustris*), the popular wild vegetable of Sarawak. *Agriculture Science Journal* **2006,** *2,* 18–20.
5. Giesen, W.; Wulffraat, S.; Zieren, M.; Scholten, L. *Mangrove Guidebook for Southeast Asia.* RAP Publications, FAO and Wet- lands International, Thailand 2006.
6. Zuraini, Z.; Sasidharan, S.; Kaur, S.R.; Nithiyayini, M. Antimicrobial and Antifungal Activities of Local Edible Fern *Stenochlaena Palustris* (Burm. F.) Bedd. *Pharmacologyonline* **2010,** *1,* 233-237.
7. Arullappan, S.; Sawai, S., Chee, L., Mahandan, M., Shanmugavelan, R. Phytochemical Screening and Evaluation of Cytotoxic Effect and Antioxidant Activity of Fractions Isolated from Stenochlaena palustri (Burm.f.) Bedd. Leaves. *Indian J Pharm. Educ* **2017,** *51,* S735-740, https://doi.org/10.5530/ijper.51.4s.106.
8. Dash, G.K.; Hashim, Z.S.B.M. Pharmacognostic studies on *Stenochlaena palustris* (Burm. f) Bedd. *Der Pharmacia Lettre* **2016,** *8*, 132-137.
9. DeLong, J.M.; Hodges, D.M.; Prange, R.K.; Forney, C.F.; Toivenon, P.M.A.; Bishop, M.C.; Elliot, M.L.; Jordan, M.A. The unique fatty acid and antioxidant composition of ostrich fern (*Matteuccia struthiopteris*) fiddleheads. *Can. J. Plant Sci.* **2011,** *91,* 919-930, https://doi.org/10.1139/CJPS2010-042.
10. Lee, C.H.; Shin, S.L. Functional activities of ferns for human health. In: *Working with ferns: Issues and applications.* Fernández, H.; Revilla, M.A.; Kumar, A. (eds.), Springer, New York, 2011; pp. 347-359, https://doi.org/10.1007/978-1-4419-7162-3_24.
11. Dai, J.; Mumper, R.J. Plant Phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* **2010,** *15*, 7313-7352, https://doi.org/10.3390/molecules15107313.
12. Procházková, D.; Boušová, I.; Wilhelmová, N. Anti- oxidant and prooxidant properties of flavonoids. *Fitoterapia* **2011,** *82,* 513-523, https://doi.org/10.1016/j.fitote.2011.01.018.
13. Bushway, A.A.; Wilson, A.M.; McGann, D.F.; Bushway, R.J. The Nutrient Composition of Fresh Fiddlehead Greens. *Journal of Food Science* **1982,** *47*, 666–667, https://doi.org/10.1111/j.1365-2621.1982.tb10147.x.
14. Ahmad, F.B.; Holdsworth, D.K. Medicinal Plants of Sabah, Malaysia, Part II. The Muruts. *Int. J. Pharmacogn*. **1994,** *32*, 384-387, https://doi.org/10.3109/13880209409083019.
15. Antonio, M.A.; Utrera, R.T.; Agustin, E.O.; Jamias, D.L.; Badar, A.J.; Pascua, M.E. Survey and characterization of indigenous food plants in Ilocos Norte, Philippines. *South-east Asian Regional Centre for Graduate Study and Research in Agriculture (SEARCA), Los Banos, Philippines* **2011**.
16. Nonato, F.R.; Barros, T.A.; Lucchese, A.M.; Oliveira, C.E.; dos Santos, R.R.; Soares, M.B.; Villarreal, C.F. Anti-inflammatory and antinociceptive activities of Blechnum occidentale L. extract. *J. Ethnopharmacol* **2009,** *125,* 102-107, https://doi.org/10.1016/j.jep.2009.06.005.
17. Chai, T.T.; Kwek, M.T.; Ong, H.C.; Wong, F.C. Water fraction of edible medicinal fern Stenochlaena palustris is a potent α-glucosidase inhibitor with concurrent antioxidant activity. *Food Chemistry* **2015a,** *186,* 26-31, https://doi.org/10.1016/j.foodchem.2014.12.099.
18. Chai, T.T.; Yeoh, L.Y.; Ismail, N.M.; Ong, H.C.; Manan, F.A.; Wong, F.C. Evaluation of glucosidase inhibitory and cytotoxic potential of five selected edible and medicinal ferns. *Tropical Journal of Pharmaceutical Research* **2015b,** *14,* 449-454, http://dx.doi.org/10.4314/tjpr.v14i3.13.
19. Chen, Y.H.; Chang, F.R.; Lin, Y.J.; Wang, L.; Chen, J.F.; Wu, Y.C.; Wu, M.J. Identification of phenolic antioxidants from Sword Brake fern (*Pteris ensiformis Burm*.). *Food Chemistry* **2007,** *105,* 48–56, https://doi.org/10.1016/j.foodchem.2007.03.055.
20. Hort, M.A.; DalBó, S.; Brighente, I.M.; Pizzolatti, M.G.; Pedrosa, R.C.; Ribeiro-do-Valle, R.M. Antioxidant and hepatoprotective effects of *Cyathea phalerata Mart*. (Cyatheaceae). *Basic and Clinical Pharmacology and Toxicology* **2008,** *103*, 17-24, https://doi.org/10.1111/j.1742-7843.2008.00214.x.
21. Xu, D.P.; Li, Y.; Meng, X.; Zhou, T.; Zhou, Y.; Zheng, J.; Zhang, J.J.; Li, H.B. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *Int. J. Mol. Sci* **2017a,** *18*, 96, https://doi.org/10.3390/ijms18010096.
22. Zhou, Y.; Zheng, J.; Gan, R.; Zhou, T.; Xu, D.; Li, H. Optimization of ultrasound-assisted extraction of antioxidants from the mung bean coat. *Molecules* **2017,** *22,* 638, https://doi.org/10.3390/molecules22040638.
23. Xu, D.P.; Zheng, J.; Zhou, Y.; Li, Y.; Li, S.; Li, H.B. Ultrasound-assisted extraction of natural antioxidants from the flower of Limonium sinuatum: Optimization and comparison with conventional methods. *Food Chem* **2017b,** *217*, 552–559, https://doi.org/10.1016/j.foodchem.2016.09.013.
24. Li, A.N.; Li, S.; Li, Y.; Xu, D.P.; Li, H.B. Optimization of ultrasound-assisted extraction of natural antioxidants from the *Osmanthus fragrans* flower. *Molecules* **2016,** *21*, 218, https://doi.org/10.3390/molecules21020218.
25. Ballard, T.S.; Mallikarjunan, P.; Zhou, K.Q.; O'Keefe, S. Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. *Food Chem* **2010,** *120,* 1185–1192, https://doi.org/10.1016/j.foodchem.2009.11.063.
26. Buldini, P.L.; Ricci, L.; Sharma, J.L. Recent applications of sample preparation in food analysis. *J. Chromatography A* **2002,** *975*, 47–70, https://doi.org/10.1016/S0021-9673(02)01335-3.
27. Eskilsson, C.S.; Bjorklund, E. Analytical-scale microwave-assisted extraction. *J. Chromatography A* **2002,** *902*, 227–250, https://doi.org/10.1016/S0021-9673(00)00921-3.
28. Dean, A.; Voss, D. *Design and Analysis of Experiments.* Springer, New York Berlin Heidelberg. 1999; pp. 561–564.
29. Singh, S.; Immanuel, G. Extraction of Antioxidants from Fruit Peels and its Utilization in Paneer. *J Food Process Technol* **2014,** *5*, 1-5, https://doi.org/10.4172/2157-7110.1000349.
30. Makkar, H.P.S.; Bluemmel, M.; Borowy, N.K.; Becker, K. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J Sci Food Agric* **1963,** *61*, 161-165, https://doi.org/10.1002/jsfa.2740610205.
31. Karabegović, I.T.; Stojičević, S.S.; Veličković, D.T.; Todorović, Z.B.; Nikolić, N.Č.; Lazić, M.L. The effect of

different extraction techniques on the composition and antioxidant activity of cherry laurel (*Prunus laurocerasus*) leaf and fruit extracts. *Industrial Crops and Products* **2014,** *54,* 142-148, https://doi.org/10.1016/j.indcrop.2013.12.047.

32. Cheng, Z.Y.; Song, H.Y.; Cao, X.L.; Shen, Q.H.; Han, D.D.; Zhong, F.L.; Hu, H.B.; Yang, Y.J. Simultaneous extraction and purification of polysaccharides from Gentiana scabra Bunge by microwave-assisted ethanol-salt aqueous two-phase system. *Ind. Crop Prod* **2017,** *102,* 75–87, https://doi.org/10.1016/j.indcrop.2017.03.029.

33. Xie, X.J.; Zhu, D.; Zhang, W.; Huai, W.B.; Wang, K.; Huang, X.W.; Zhou, L.; Fan, H.J. Microwave-assisted aqueous two-phase extraction coupled with high performance liquid chromatography for simultaneous extraction and determination of four flavonoids in *Crotalaria sessiliflora* L. *Industrial Crops and Products* **2017,** *95,* 632–642, https://doi.org/10.1016/j.indcrop.2016.11.032.

34. Zhang, M.; Chen, H.; Li, J.; Pei, Y.; Liang, Y. Antioxidant properties of tartary buckwheat extracts as affected by different thermal processing. *LWT Food Sci. Technol* **2010,** *43*, 181–185, https://doi.org/10.1016/j.lwt.2009.06.020.

35. Randhir, R.; Kwon, Y.I.; Shetty, K. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain and sprouts and seedling. *Innov. Food Sci. Emerg. Technol* **2008,** *9*, 355–364, https://doi.org/10.1016/j.ifset.2007.10.004.

36. Altan, A.; McCarthy, K.L.; Maskan, M. Effect of extrusion process on antioxidant activity, total phenolics and b-glucan content of extrudates developed from barley–fruit and vegetable by-products. *Int. J. Food Sci. Technol* **2009,** *44*, 1263–1271, https://doi.org/10.1111/j.1365-2621.2009.01956.x.

37. Pan, X.; Niu, G.; Liu, H. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem. Eng. Process* **2003,** *42,* 129–133, https://doi.org/10.1016/S0255-2701(02)00037-5.

38. Li, Y.; Guo, B.; Wang, W.; Li, L.; Cao, L.; Yang, C.; Liu, J.; Liang, Q.; Chen, J.; Wu, S.; Zhang, L. Characterization of phenolic compounds from *Phyllanthus emblica* fruits using HPLC-ESI-TOF-MS as affected by an optimized microwave-assisted extraction. *International Journal of Food Properties* **2019,** *22,* 330-342, https://doi.org/10.1080/10942912.2019.1583249.

39. Khizar, H.; Xiaoming, Z.; Umar, F.; Shabbar, A.; Shuqin, X.; Chengsheng, J.; Fang, Z.; Jing, Z. Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. *Food chemistry* **2010,** *123,* 423-429, https://doi.org/10.1016/j.foodchem.2010.04.060.

40. Kim, J.W.; Lee, B.C.; Lee, J.H.; Nam, K.C.; Lee, S.C. Effect of electron-beam irradiation on the antioxidant activity of extracts from Citrus unshiu pomaces. *Radiation Physics and chemistry* **2008,** *77*, 87-91, https://doi.org/10.1016/j.radphyschem.2007.02.082.

41. Mahieddine, B.; Amina, B.; Faouzi, S.M.; Sana, B.; Wided, D. Effects of microwave heating on the antioxidant activities of tomato (*Solanum lycopersicum*). *Annals of Agricultural Sciences* **2018,** *63,* 135-139, https://doi.org/10.1016/j.aoas.2018.09.001.

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