IJPSR (2015), Vol. 6, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 December, 2014; received in revised form, 13 February, 2015; accepted, 05 May, 2015; published 01 August, 2015

EFFECT OF PROCESS PARAMETERS OF MICROWAVE ASSISTED EXTRACTION (MAE) ON NATURAL PRODUCT YIELD FROM ONION PEEL

Shaileyee Das* and Subhash C Mandal

Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India

Keywords:

Onion peel, Plackett–Burman design, Box–Behnken design

Correspondence to Author:
Shaileyee Das,

Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700 032, India

E-mail: scmandal1963@gmail.com

ABSTRACT: Globally, more than 30 % of the onion peels arises at the retail and purchaser levels, of which the post-harvest and dealing out level wastages report for the major share. So, studies on the characterization of unutilized fractions of the onion peels specify their potential candidature for reprocessing. Maximum preservation of these phytochemicals during extraction requires optimised process parameter conditions. A microwave-assisted extraction (MAE) method was considered for extraction of total phenolics, total flavonoids and DPPH scavenging activity from onion peels. The total phenolic capacity (TPC), total flavonoids content (TFC) and antioxidant activity percentage of extracts at optimised MAE conditions. The influence of six main extraction parameters on the extraction was modelled by using a second-order regression equation. The optimal MAE conditions were 210W microwave power, 15min irradiation time and 40 mL/g solvent to material ratio. Under the MAE optimised conditions, the recovery of TPC was 94.34 mg gallic acid equivalent/g dry weight (DW), TFC was 45.61(mg/g) and free radical scavenging activity 92.25%.When bioactive phytochemicals extracted from onion skin using MAE compared with UAE and CSE, it was also observed that the yield values in MAE extracts were higher than the other two extracts.

INTRODUCTION: Onions (*Allium cepa L.*) are the second most important horticultural crop worldwide, subsequent to tomatoes with present annual production around 66 million tonnes large amount of onion peels are produced by consumption of onion both domestically and industrially, making it necessary to search for their utilization. The major onion peels contain onion skins, two outer fleshy scales and roots generated during industrial peeling and undersized malformed or damaged bulbs. More than 500,000 tonnes of onion peels are thrown away in the European Union each year.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(8).3260-75

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(8).3260-75

Every day, our country India produces between 300kg and 500kg of onion peel. These peels get decayed and add themselves to the soil causing odour and in some cases causing harm to the environment.

The objective of this work is to optimize the extraction from these parts of onions that constitute for their phenolics, flavonoids and antioxidant component isolation.

Traditionally, for the extraction, heat reflux and Soxhlet extraction techniques have been the first line of choice, but they are often detrimental owing to the time taken and the organic solvent consumed. In contrast, microwave-assisted extraction (MAE) is known for its high extraction efficiency, and low consumption of organic solvent and time. In the MAE procedure, many extraction

variables and the interactions among them are known to involve the final yield or outcome [1]. When such circumstances are look forward to, response surface methodology (RSM) is a useful tool for optimising the process, which was described originally [2]. Moreover RSM has been applied productively to various optimisation procedures in extraction processes and pharmaceutical research. The main intention of RSM is to use a series of designed experiments to obtain an optimal outcome or response. In RSM, an approximate relation between a single response and multiple variables is modelled as a polynomial (quadratic) equation obtained through regression analysis. The equation is called a response surface [3, 4, 5].

As a part of the preliminary experimental trial, single factor analysis of different ranges of microwave power, irradiation time, sample: solvent ratio, pre-leaching time, particle size of the raw material and various solvent concentration with the aid of a Plackett–Burman design were performed to find the most significant extraction variable(s), which were further optimised by Box–Behnken design by using their higher ranges.

It is scientifically confirmed that onion peels are rich in flavonoid glycosides. But most important material that is found in onion peels is Quercetin (flavonoid) as huge quantities. The onion peels under a microscope Quercetin is observed as sharp, needle –like crystals. In this type of Quercetin is the most valuable substance [6, 7, 8]. So, onion peels can reduce blood pressure and prevent arteries from clogging according to weight loss. It has also anti-inflammatory, anticancer and cardiovascular effects [9].

Experimental methods:

Sample material and chemicals:

Chemicals:

Methanol, Acetone, ethyl acetate and hexane used in the experimental work was analytical reagent grade chemical (Merck, Germany). Folin–Ciocalteu phenol reagent was purchased from Loba Chemie (Mumbai, India) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Loba Chemie (Mumbai, India).

MATERIALS:

The outer dry and semi-dry layers and the apical trimmings of brown-skin onion bulbs (*Allium cepa*) were collected immediately after processing from household cooking (Kolkata, India). The tissues were dried in hot air oven and ground with a mixture grinder. The ground tissue was sieved by 10, 20, 30, 40, 60, 80,100mm mesh sized sieve and stored before the analysis.

Apparatus and condition:

The extraction system is a microwave extractor (CATA R) manufactured by Catalyst Systems (Pune, India) equipped with a magatron of 2450MHz with a maximum power of 700W (100%), a reflux unit, 10 power levels (140W (20%) to 700W (100%)), time controller, temperature sensor, exhaust system, beam reflector and a stirring device.

Experimental procedures:

Extraction process:

At the start as a part of initial experimental trials using a Plackett–Burman design, an amount of 1 g ground waste sample was placed into a 250mL flask and then extraction was carried out by using different ranges of microwave power (20–50% of 700 W), extraction or irradiation time (1–3min), solvent: sample ratio loading (10:1–60:1mL/g), pre-leaching time (5–10 min), particle size of the sample (10–100 mesh) and methanol concentration (10–100% v/v). After extraction, all the extracts were centrifuged for 15 min at 4˚C and 4000 rpm (R-8C, REMI, Mumbai, India) and the supernatants were evaporated under reduced pressure. Then the extracts are used for analysis the responses total polyphenols contents, total flavonoid contents and antioxidant activity. All experiments were conducted in duplicate and the average value was used for statistical analysis. From Plackett–Burman design analysis, we were got the significant parameters for this experiment.

Higher values of the significant variables indentified were further explored to confirm whether any high ranges of the selected variables showed an increasing trend of polyphenol, flavonoids and antioxidant yield or not and if found statistically significant, then those higher ranges of the significant variables would be further optimised

through a Box–Behnken design RSM to predict and locate regions with optimum yield.

Experimental design:

A Plackett–Burman experiment design was used to identify the relationship existing between the response functions and process parameters as well as to determine those conditions that optimised the extraction process [10, 11]. The six independent variables or factors studied were microwave power (X1, varying between 20–50% of 700 W), extraction or irradiation time (X2, varying between 1–3min), solvent: sample ratio loading (X3, varying between 10:1–60:1ml/g), pre-leaching time (X4, varying between 5–10 min), particle size of the sample (X5, varying between 10–100 mesh) and methanol concentration (X6, varying between 10–100% v/v).

TABLE 1: INITIAL LEVEL OF THE EXTRACTION VARIABLES FOR EXTRACTION ONION WASTE SAMPLE BY USING PLACKETT–BURMAN DESIGN CRITERION

Extraction code	Extraction condition	Low level (-)	High level (+)
X1	microwave power	20% (of 700 W)	50% (of 700 W)
X2	extraction or irradiation time	1 min	3 min
X3	solvent: sample ratio loading	10:1	60:1
X4	pre-leaching time	5 min	10 min
X5	particle size of the sample	10 mesh	100 mesh
X6	methanol concentration	10% v/v	100% v/v

After the identification of significant extraction parameter from Plackett–Burman experiment design based on P values, higher values of the significant variables indentified were further explored to confirm whether any high ranges of the selected parameters showed an increasing trend of polyphenols, flavonoids and antioxidant yield or not and if found statistically significant then those higher ranges of the significant variables would be further optimised through a Box–Behnken design RSM to predict and locate regions with optimum yield. The steps of the design are shown at **Fig.2** by a detail flowchart.

TABLE 2: INDEPENDENT VARIABLES AND THEIR LEVELS IN EXPERIMENTAL DESIGN FOR BOX–BEHNKEN DESIGN

Independent variables	Symbols		Factor's levels of total polyphenol content			Factor's levels of total flavonoids content			Factor's levels of DPPH % value		
	Actual	Coded	-1	0	+1	-1	0	+1	-1	0	+1
microwave power (% of 700 W)	X1	x1	140	210	245	140	210	245	140	210	245
irradiation time (min)	X2	x2	10	15	20	10	15	20	10	15	20
solvent:sample ratio loading (ml/g)	X3	x3	30	40	50	30	40	50	30	40	50

Data analysis:

The design of experiment procedure of Design-expert software (dx9-trial) and Matlab version 6.5 was used to design and analyse both the Plackett-Burman design and BBD. Minitab Pro version 16.1.0.0 (trial version) was used to generate interaction plots. Graph Pad Prism 5 (trial version) was applied for determining the level of significance of various factors involved by using one-way ANOVA test. P values<0.05 were considered significant.

Analytical methods: Total polyphenol content, total flavonoids content and antioxidant activity of the extracts were determined for every sample [12,13,14].

Determination of total polyphenol content:

The Folin–Ciocalteu method with a slight modification was used to determine the total polyphenol content. Briefly, 100 ml of extract with concentration of 1 mg/ml was stirred in the test tube together with 500 ml of Folin–Ciocalteu reagent and 6 ml of distilled water. The contents of the test tubes were strongly mixed and after that 2 ml of 15% Na_2CO_3 solution and 1.4 ml of distilled water was added. Absorbance was measured at 750 nm after 2 h with blank which was prepared in the same way at the same time only with distilled water

instead extract sample. The results were expressed as Gallic acid equivalents (mg Gallic acid/g of extract dry matter) through the calibration curve of Gallic acid (1–1500 mg/ml).

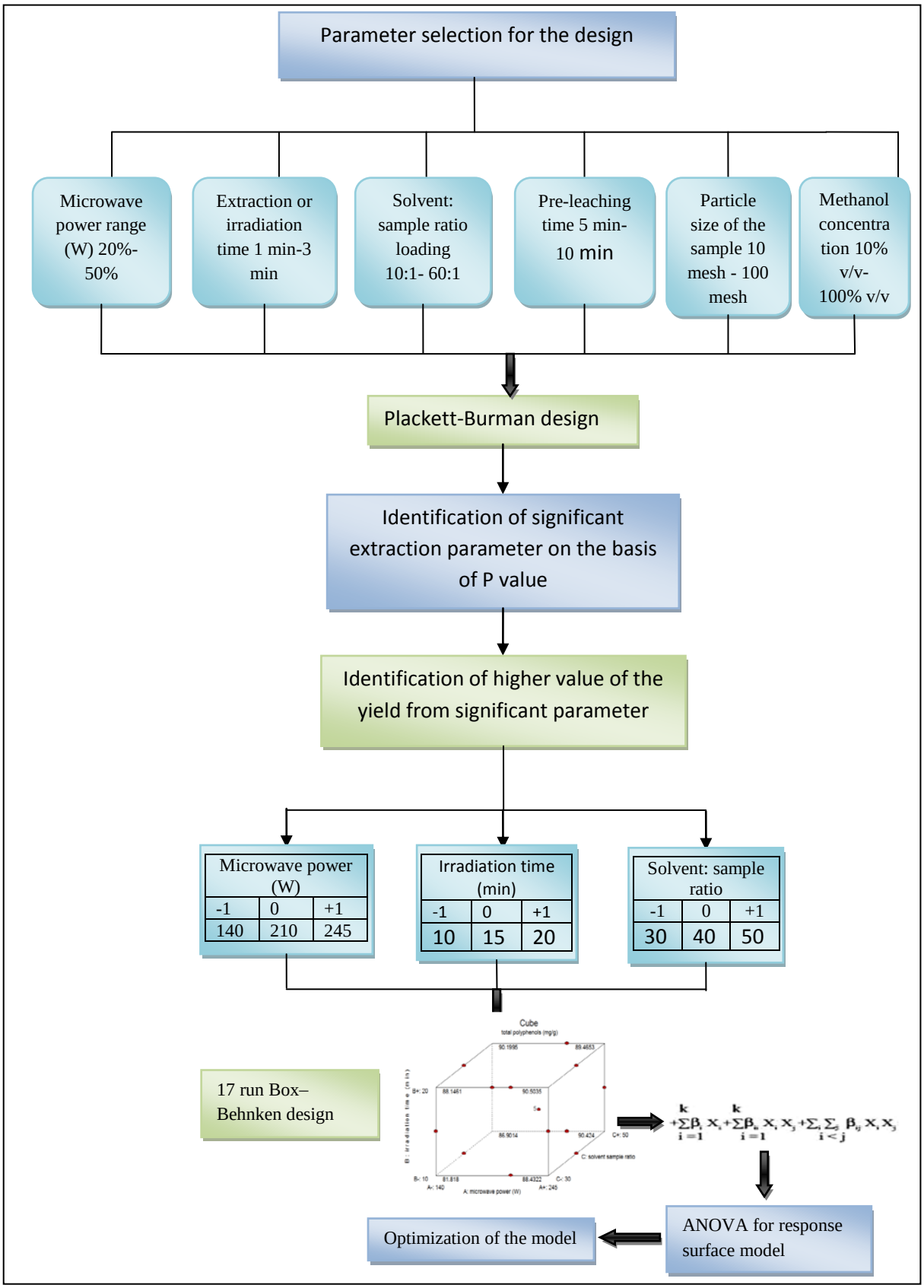


FIG.2: DESIGN OF EXPERIMENT APPROACH DEPICTING THE PROCEDURE OF EXPERIMENTAL DESIGN.

Determination of total flavonoids content:
The total flavonoid content of onion was estimated by aluminium chloride ($AlCl_3$) colorimetric method [15, 16, 17].

a) Preparation of Standards:
To quantify the total flavonoid content, Quercetin was used as the standard, which was expressed as Quercetin equivalent (QE). A standard curve of known concentrations of Quercetin was produced by preparing and testing five concentrations of Quercetin standard solution, which were 0, 25, 50, 75, and 100 mg/L. A stock Quercetin solution was prepared by dissolving 25 mg of Quercetin in 100ml of 80% methanol. Then, the standard working solutions were made up by pipetting 0, 1, 2, 3, 4 and 5ml aliquots of the stock solution (250 mg/L) into 10ml-volumetric flasks and adjusting the volume with 80% methanol. By using test tubes, 1ml of each standard solution was reacted with 1ml of 2% ethanolic dilution of $AlCl_3$ reagent. The mixture was mixed thoroughly by vortex mixer for about 30s. It was allowed standing at incubator for 30-60 min. Absorbance readings were taken by a UV/Visible Spectrophotometer at 415 nm.

Preparation of Samples:
The content of flavonoids in the examined each plant extract was determined using spectrophotometric method. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in ethanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at $\lambda max = 415$ nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.
Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of Quercetin equivalent (mg/g of extract).

DPPH Free Radical Scavenging Activity:
The anti-oxidant potential of any compound can be determined on the basis of its scavenging activity of the stable 1, 1 –diphenyl-2-picrylhydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually make use of detection of the radical scavenging activity in chemical analysis. Stable DPPH radical in methanol is at 517nm [14, 15, 16]. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules anti radical progresses, which outcomes in the scavenging of the radical by hydrogen donation [18, 19, 20, 21, 22].

Preparation of DPPH solution:
Solution of DPPH (0.1 mM) in methanol is prepared by dissolving 1.9mg of DPPH in methanol and volume is made up to 100ml with methanol. The solution is kept in darkness for 30 minutes to complete the reaction. After that the solution is kept in refrigerator at 4°C for further work.

Protocol for estimation of DPPH scavenging activity:
Different concentrations of test sample are taken at 3 ml each. Mixed with 5 ml of methanolic solution of DPPH (0.1mM) and allowed to stand at room temperature for 30mins. After 30mins, the absorbance is recorded at 517nm. Similarly 3 ml of different concentration of L-ascorbic acid are added to 5 ml of DPPH solution and the absorbance is measured at same nm in a spectrophotometer. Decrease in the absorbance in the presence of test sample solution and standard at different concentration is noted. A blank reading is taken using methanol instead of test sample solution. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical is calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \frac{A_{control} - A_{sample}}{A_{sample}} \times 100$$

Where;
$A_{control}$ = absorbance of DPPH along with L-ascorbic acid,
A_{sample} = Absorbance of DPPH along with different concentrations of test samples.

RESULTS AND DISCUSSION:
Fitting the process variables:
The Plackett–Burman experimental design and corresponding responses for the obtaining of onion peel extracts are presented in **Table 1**. Model presented the total of 12 experiments. The response values at different experimental combination for

coded variables were listed in **Table 3**. The adequacy of the model was calculated, and the variables showing statistically significant effects were screened via regression analysis. Among six extraction variables (microwave power, irradiation/extraction time, solvent: sample ratio loading, particle size, solvent concentration and pre-leaching time) studied, three variables (microwave power, irradiation/extraction time and solvent: sample ratio loading) were found to have significant influence on phenols, flavonoids and antioxidants extraction in three cases combination.

TABLE 3: YIELD OF TOTAL POLYPHENOLS, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY FROM SOLID WASTES ONION PEELS USING THE DIFFERENT LEVELS OF EXTRACTION VARIABLES OF THE PLACKETT–BURMAN DESIGN CRITERION

Standard order	Run order	Point type	Block	X1	X2	X3	X4	X5	X6	TPC	TFC	DPPH %
5	1	1	1	140	1	60	10	100	10	87.59	35.87	88.96
8	2	1	1	455	3	10	10	10	10	88.12	36.34	89.38
6	3	1	1	140	1	10	100	10	10	87.09	35.48	88.30
2	4	1	1	140	3	60	10	100	10	87.65	35.89	88.98
4	5	1	1	140	3	10	100	100	5	87.71	35.95	88.07
10	6	1	1	140	3	60	100	10	5	87.88	36.1	89.13
12	7	1	1	140	1	10	10	10	5	87.14	35.52	88.37
1	8	1	1	455	3	10	100	100	10	88.01	36.31	89.46
3	9	1	1	455	1	60	100	10	10	88.03	36.32	89.40
9	10	1	1	455	3	60	10	10	5	88.38	36.65	89.65
7	11	1	1	455	1	10	10	100	5	87.54	35.79	88.90
11	12	1	1	455	1	60	100	100	5	88.11	36.48	89.58

The significance of each coefficient was determined using the *F*-test and *p*-values (**Table 4, 5** and **6**) of each response. The corresponding variables are more significant if the absolute *F*-value becomes greater and the *p*-value becomes smaller. It can be seen that the variables with the largest effect were the linear terms of microwave power ($x1$), extraction time ($x2$) and the quadratic term of liquid: solid ratio ($x3$). The results suggest that the change of microwave power, extraction time and liquid: solid ratio had highly significant effects on the yield of polyphenols, flavonoids and antioxidants ($p < 0.0001$) from the onion peels.

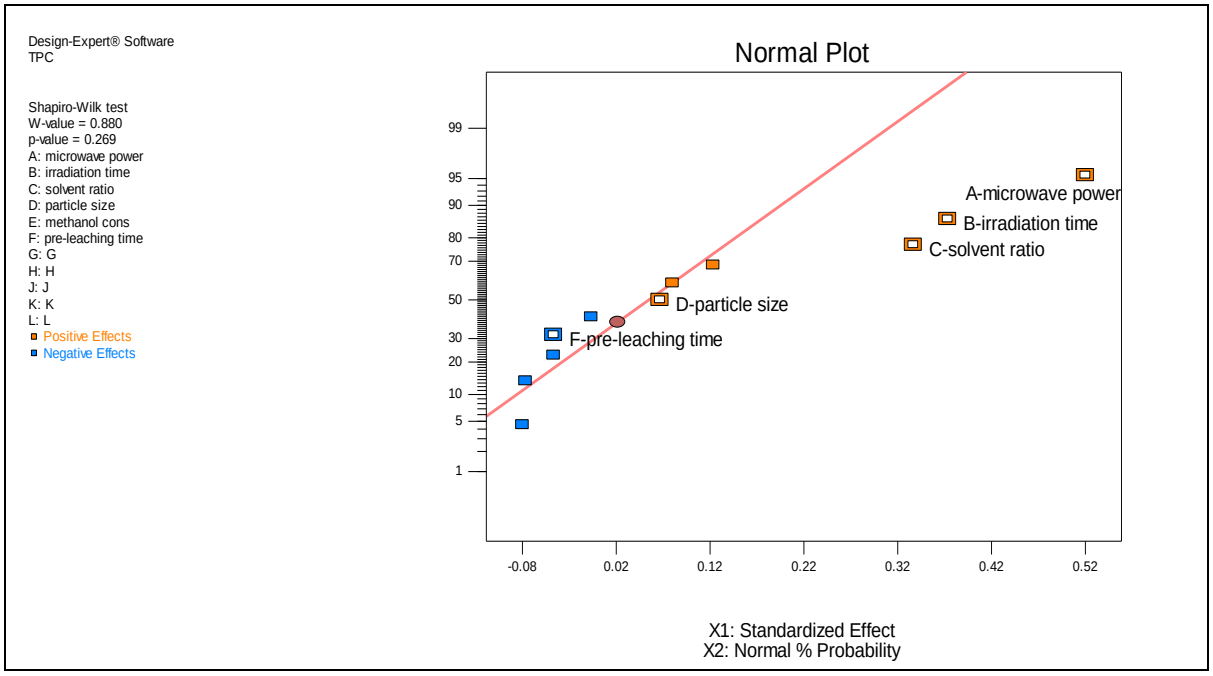


FIG. 3: NORMAL PLOT OF POLYPHENOLS SHOWING SIGNIFICANT VARIABLES OBTAINED IN PLACKETT–BURMAN DESIGN (MICROWAVE POWER (%), P=0.0016; SOLVENT: SAMPLE RATIO, P = 0.0029 AND IRRADIATION TIME, P = 0.0005 WERE SIGNIFICANT EXTRACTION VARIABLES).

TABLE 4: ANOVA AND REGRESSION ANALYSIS OF PLACKETT–BURMAN DESIGN CRITERION DATA FOR THE PREDICTION OF SIGNIFICANT EXTRACTION VARIABLES

Source of variation	Degrees of freedom	Sum of squares (partial)	Mean squares (partial)	F ratio	P value	Inference
Model	5	1.60	0.32	19.81	0.0006	significance
Main Effects	5	1.60	0.32	19.81	0.0006	significance
Residual	6	0.11	0.018			
Lack of Fit	6	0.11	0.018			
Total	11	1.71				

(b) Regression data Term	Effect	Coefficient	Standard error	Low CI	High CI	F value	P value	Inference
Intercept		87.77	0.039	87.68	87.87			significance
A: microwave power (%)	**1**	0.26	0.039	0.17	0.36	45.22	0.0016	significance
B: irradiation time	1	0.19	0.039	0.093	0.28	23.37	0.0005	significance
C: solvent:sample ratio loading	1	0.17	0.039	0.074	0.26	19.02	0.0029	significance
D: particle size	1	0.034	0.039	-0.061	0.13	0.78	0.0048	
E: methanol concentration	1	-0.022	0.039	-0.12	0.072	0.34	0.4123	
F: pre-leaching time	1	87.77	0.039	87.68	87.87	45.22	0.5830	

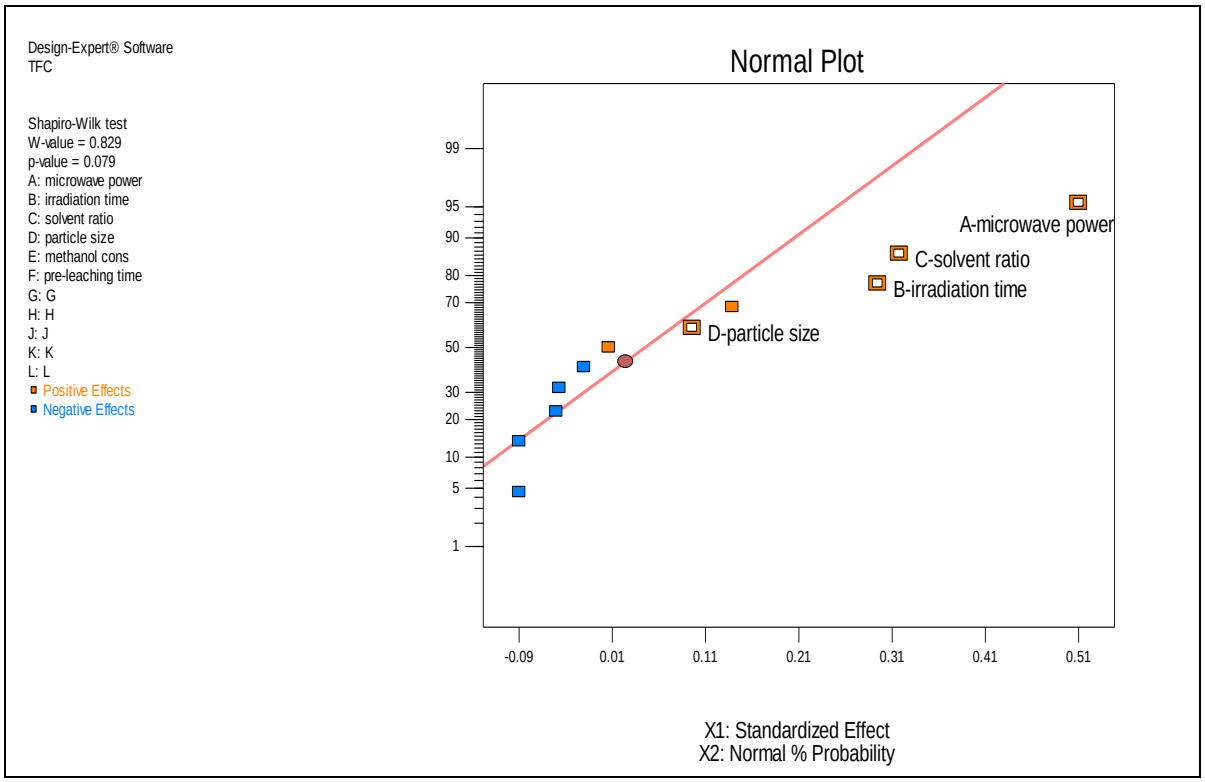


FIG. 4: NORMAL PLOT OF FLAVONOIDS SHOWING SIGNIFICANT VARIABLES OBTAINED IN PLACKETT–BURMAN DESIGN (MICROWAVE POWER (%), P=0.0016; SOLVENT: SAMPLE RATIO, P = 0.0029 AND IRRADIATION TIME, P = 0.0005 WERE SIGNIFICANT EXTRACTION VARIABLES).

TABLE 5: ANOVA AND REGRESSION ANALYSIS OF PLACKETT–BURMAN DESIGN CRITERION DATA FOR THE PREDICTION OF SIGNIFICANT EXTRACTION VARIABLES

Source of variation	Degrees of freedom	Sum of squares (partial)	Mean squares (partial)	F ratio	P value	Inference
Model	4	1.39	0.35	19.81	0.0006	significance
Main Effects	4	1.39	0.35	19.81	0.0006	significance

Residual	**5**	0.12	0.023					
Lack of Fit	**5**	0.12	0.023					
Total	**9**	1.51	0.373					
(b) Regression data Term	**Effect**	**Coefficient**	**Standard error**	**Low CI**	**High CI**	**F value**	**P value**	**Inference**
Intercept		36.06	0.044	35.95	36.17			significance
A: microwave power (%)	**1**	0.26	0.044	0.14	0.37	34.36	0.0020	significance
B: irradiation time	1	0.15	0.044	0.036	0.26	11.48	0.0195	significance
C: solvent:sample ratio loading	1	0.16	0.044	0.047	0.27	13.35	0.0147	significance
D: particle size	1	0.048	0.044	-0.064	0.16	1.22	0.3199	
E: methanol concentration	1	-0.010	0.044	-0.12	0.10	0.052	0.8284	
F: pre-leaching time	1	-0.023	0.044	-0.14	0.089	0.28	0.6169	

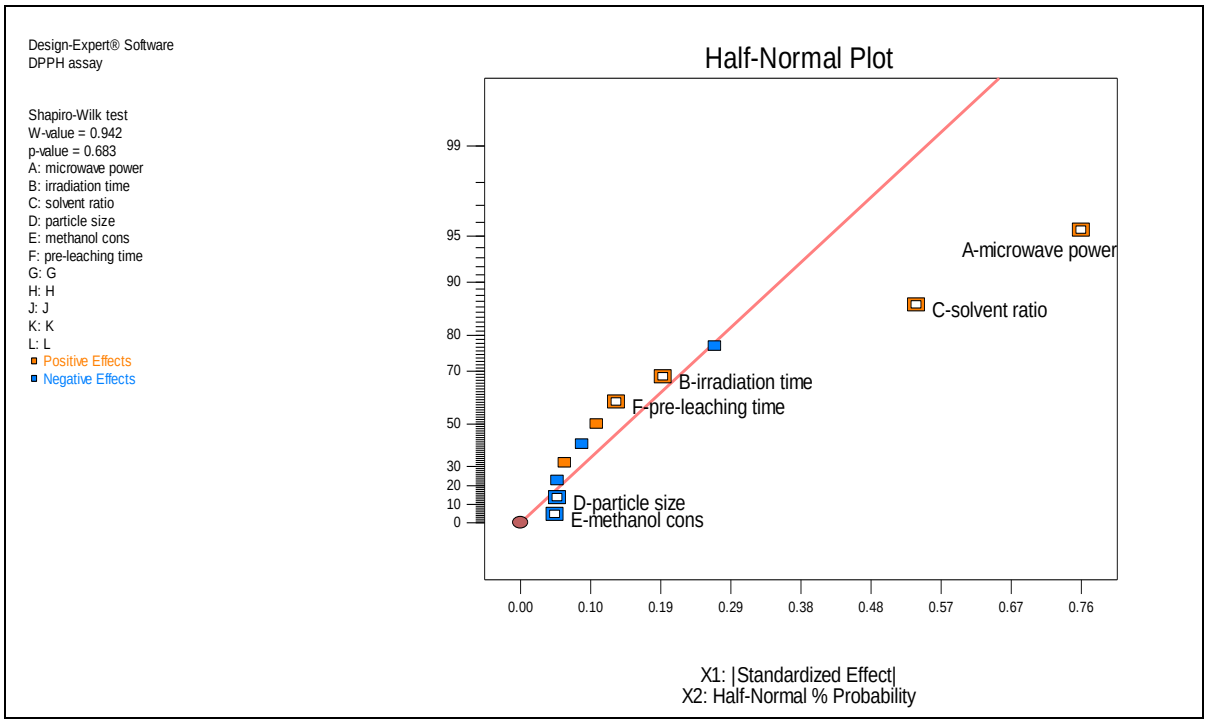


FIG. 5: HALF NORMAL PLOT OF DPPH ASSAY SHOWING SIGNIFICANT VARIABLES OBTAINED IN PLACKETT–BURMAN DESIGN (MICROWAVE POWER (%), P=0.0016; SOLVENT: SAMPLE RATIO, P = 0.0029 AND IRRADIATION TIME, P = 0.0005 WERE SIGNIFICANT EXTRACTION VARIABLES).

TABLE 6: ANOVA AND REGRESSION ANALYSIS OF PLACKETT–BURMAN DESIGN CRITERION DATA FOR THE PREDICTION OF SIGNIFICANT EXTRACTION VARIABLES

Source of variation	**Degrees of freedom**	**Sum of squares (partial)**	**Mean squares (partial)**	**F ratio**	**P value**	**Inference**		
Model	6	2.77	0.46	19.81	0.0006	significance		
Main Effects	6	2.77	0.46	19.81	0.0006	significance		
Residual	5	0.28	0.056					
Lack of Fit	5	0.28	0.056					
Total	11	3.05	0.373					
(b) Regression data Term	**Effect**	**Coefficient**	**Standard error**	**Low CI**	**High CI**	**F value**	**P value**	**Inference**
Intercept		89.02	0.068	88.84	89.19			significance
A: microwave power (%)	1	0.38	0.068	0.20	0.56	31.03	0.0026	significance

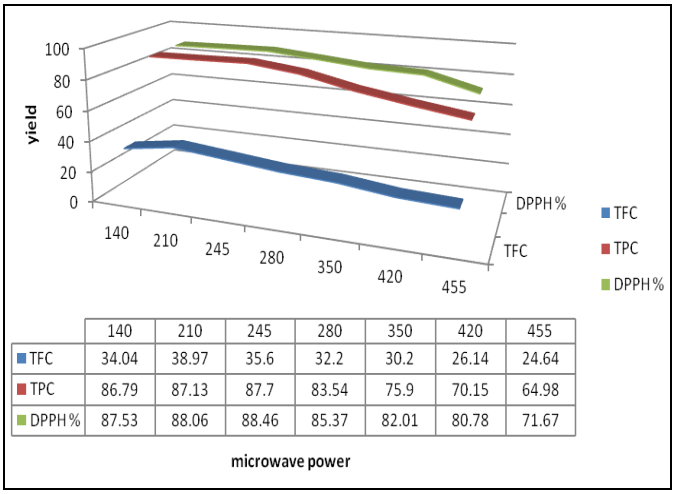
B: irradiation time	1	0.097	0.068	-0.079	0.27	2.01	0.2156	significance
C: solvent:sample ratio loading	1	0.27	0.068	0.093	0.44	15.47	0.0110	significance
D: particle size	1	-0.025	0.068	-0.20	0.15	0.13	0.7290	
E: methanol concentration	1	-0.023	0.068	-0.20	0.15	0.12	0.7462	
F: pre-leaching time	1	0.065	0.068	-0.11	0.24	0.91	0.3844	

Level determination for the three selected significant extraction variables to be used for optimization:

The three significant parameters microwave power (X1), irradiation time (X2) and solvent sample ratio (X3) was analysed further for getting the higher values of yield.

Effect of microwave power on the yield of TFC, TPC and DPPH %

Fig.6 highlights the typical yield – microwave power plot for the extraction of TFC, TPC and DPPH %. All the experiments were carried out in duplicate and the mean value of yields were taken for statistical analysis. In general it was noticed that there was a significant improvement in extraction yield with increase in microwave power from 140 to 245 W ($P<0.05$) when the extraction was carried out with 10min, 15 min and 20min initially. A significant change ($P<0.05$) in the extraction yield plot was not observed between 280 to 455 W microwave power, when compared with lower microwave powers, as shown in **Fig. 6**.



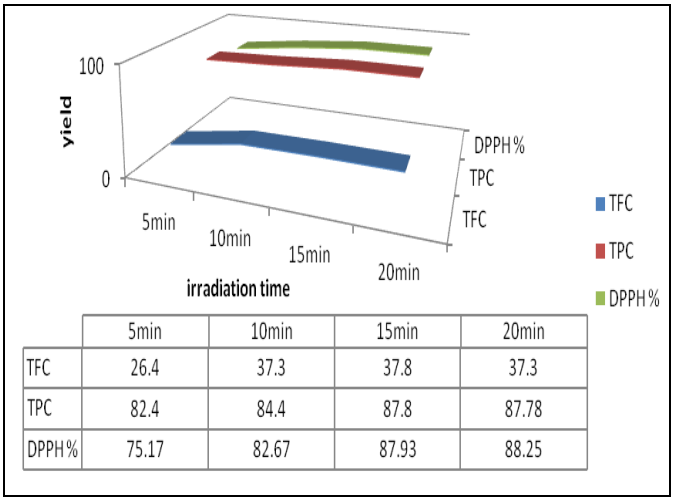
	140	210	245	280	350	420	455
TFC	34.04	38.97	35.6	32.2	30.2	26.14	24.64
TPC	86.79	87.13	87.7	83.54	75.9	70.15	64.98
DPPH%	87.53	88.06	88.46	85.37	82.01	80.78	71.67

FIG.6: MICROWAVE POWER VSTFC, TPC AND DPPH %YIELD

Therefore a microwave power range of 140-245 (of 700 W) was selected for the optimisation study. It can be assumed that more microwave energy was transferred to the extraction system quickly. The process improved the extraction efficiency when the microwave power increased from 140W to 245W.

Effect of irradiation (extraction) time on TFC, TPC and DPPH %:

Figure 7 highlights the effect of irradiation time of 5, 10, 15 and 20min at 210W microwave power on the extraction yield of poly-phenols, flavonoids and antioxidants. All the experiments were carried out in duplicate and the mean value of poly-phenols, flavonoids and DPPH %yield was taken for statistical analysis. Therefore an irradiation time range of 10min to 20min was considered for the optimisation study as an obvious increase in yield was noticed within this range. Other extraction conditions were preliminary loading ratio of 30:1ml/g, 10min pre-leaching time for each run and particles were screened through sieve number 40.



	5min	10min	15min	20min
TFC	26.4	37.3	37.8	37.3
TPC	82.4	84.4	87.8	87.78
DPPH%	75.17	82.67	87.93	88.25

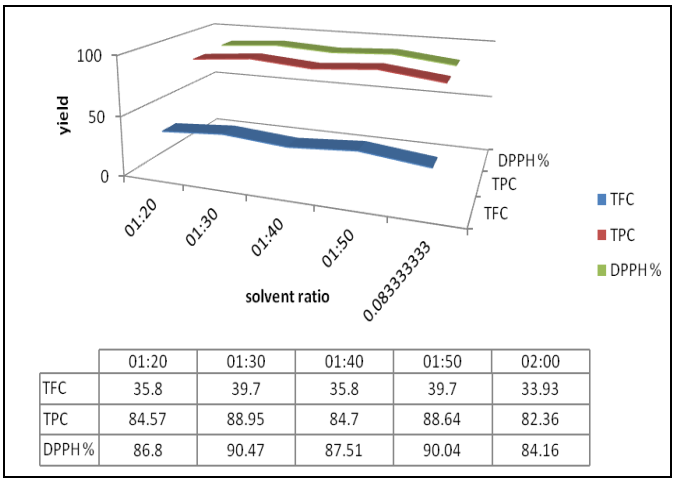
FIG.7: IRRADIATION TIME VS TFC TPC AND DPPH% YIELD

Effect of solvent: sample ratio loading on TFC, TPC and DPPH %:

The effect of different solvent to sample ratios in their higher ranges (20:1, 30:1, 40:1, 50:1 and 60:1 ml/g) at 210W microwave power and 15 min of irradiation time on the yield of poly-phenols,

flavonoids and DPPH % is shown in **Fig. 8**. All the experiments were carried out in duplicate and the mean value of poly-phenols, flavonoids and DPPH % yield was taken. The results show that a significant extraction yield (P<0.05) was seen in the range of 30:1 ml/g to 50:1 ml/g. However, the increase in yield was not found to be significant at loading ratios lower 30:1 ml/g and above 50:1 ml/g, which were also considered costlier in terms of energy and money.

This is because, as the loading ratio decreases, the solvent volume is increased. Moreover, a large volume of solvent causes more absorption of microwave energy and thus sufficient microwave energy may not be available for cell breakage, which is considered important for effective leaching out of the target analytes.



	01:20	01:30	01:40	01:50	02:00
TFC	35.8	39.7	35.8	39.7	33.93
TPC	84.57	88.95	84.7	88.64	82.36
DPPH%	86.8	90.47	87.51	90.04	84.16

FIG.8: SOLVENT RATIO VS TFC TPC AND DPPH% YIELD

BBD model fitting and statistical analysis:

Fitting the model and checking model adequacy:

So, from figure 6, 7 and 8 we got the highest yield value condition using three significant parameters. Those are: microwave power [140, 210 and 245], irradiation time [10, 15, and 20] and solvent ratio [1:30, 1:40 and 1:50]. It's shown at Table 2. By this higher value Box–Behnken design was carried out. The whole design consisted of 17 experimental runs carried out in random order to minimise the effects of uncontrolled factors (instrumental and operative errors) that could have introduced bias into the measurements.

Five replicates (run numbers 1, 5, 7, 14 and 17) at the centre of the design were used to allow for estimation of a pure error sum of squares. Data from the Box–Behnken design (BBD) were analysed by multiple regressions to fit into the following non-linear computer generated quadratic (second order) polynomial model:

$$X = b_{\circ} + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=2>j}^{n} b_{ij} X_i X_j + E$$

Where X is yield, b_0, b_i, b_{ii}, and b_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms respectively, and Yi, and Yj are the independent variables. The regression coefficients of individual linear, quadratic and interaction terms were determined according to the analysis of variance (ANOVA). The regression coefficients were then used to make statistical calculation to generate three-dimensional and two-dimensional contour maps from the regression model.

The P values of<0.05 were considered to be statistically significant at **Table 7**. All the experiments were carried out in duplicate and the mean value of TFC, TPC and DPPH % yield was taken for statistical analysis. It can also be seen that the variables having significant contribution were the linear terms of microwave power (*X1*),irradiation time (*X2*), solvent: sample/loading ratio (*X3*) and the quadratic term of irradiation time ($X2^2$), microwave power ($X1^2$), solvent: sample/loading ratio($X3^2$) and followed by the interaction effects of microwave power (*X1*) × irradiation time (*X2*), irradiation time (*X2*) × solvent: sample ratio loading (*X3*), microwave power (*X1*) × solvent: sample ratio loading (*X3*).

TABLE 7: BOX–BEHNKEN EXPERIMENTAL DESIGN MATRIX WITH OBSERVED RESPONSES AND PREDICTED VALUES FOR MAE YIELD OF TPC, TFC AND DPPH %

Experiment number	actual			Observed yield TPC	Software predicted yield	Observed yield TFC	Software predicted yield	Observed yield DPPH %	Software predicted yield
	-1	0	1						
1	210	15	40	94.51	94.31	48.55	48.37	95.8	95.54
2	210	10	30	87.74	87.74	41.99	41.90	88.16	88.32
3	210	20	50	91.24	91.23	45.55	45.63	92.43	92.26

4	140	15	50	90.84	90.94	44.89	45.14	91.28	91.5
5	210	15	40	94.36	94.31	48.59	48.37	95.74	95.54
6	210	10	50	91	90.77	45.55	45.38	92.55	92.24
7	210	15	40	94.42	94.31	48.5	48.37	95.99	95.54
8	140	20	40	90.94	90.83	45.49	45.17	91.59	93.68
9	245	15	50	92.21	92.34	46.79	46.61	93.43	91.48
10	140	10	40	85.98	86.03	40.16	40.01	86.06	86.04
11	245	20	40	91.77	91.65	45.81	45.87	92.79	92.76
12	140	15	30	87.43	87.37	41.17	41.37	88.34	88.24
13	245	15	30	92.05	91.86	46.25	45.97	93.48	93.09
14	210	15	40	94.19	94.31	48.61	48.37	95.16	95.54
15	210	20	30	91.01	91.24	45.59	45.75	92.9	93.2
16	245	10	40	90.92	91.09	44.99	45.38	91.64	91.8
17	210	15	40	94.07	94.31	47.6	48.37	95.02	95.54

TABLE 8: ANALYSES OF VARIANCE OF THE EXPERIMENTAL RESULTS FOR 17 RUN BOX–BEHNKEN DESIGN

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Interference
Model	102.79	9	11.42	61.09	< 0.0001	significant
X1-microwave power	18.39	1	18.39	98.38	< 0.0001	significant
X2-irradiation time	15.19	1	15.19	81.23	< 0.0001	significant
X3-solvent sample ratio	9.24	1	9.24	49.45	0.0002	significant
X1X2	5.77	1	5.77	30.85	0.0009	significant
X1X3	2.58	1	2.58	13.82	0.0075	significant
X2X3	3.24	1	3.24	17.33	0.0042	significant
X1^2	9.82	1	9.82	52.50	0.0002	significant
X2^2	20.03	1	20.03	107.16	< 0.0001	significant
X3^2	9.71	1	9.71	51.95	0.0002	significant
Residual	1.31	7	0.19			
Lack of Fit	0.56	3	0.19	1.00	0.4794	*not significant*
Pure Error	0.75	4	0.19			
Cor Total	104.09	16				

TABLE 9: ANOVA OF THE FITTED QUADRATIC REGRESSION MODEL

Item	Standard deviation	Mean	CV (%)	PRESS	R2	R2 adj	Adequate precision	Predicted R2
value	0.43	45.65	0.95	11.86	0.9874	0.9713	25.198	0.8860

Analysis of response surface generated:

The regression equation was graphically represented by a three-dimensional response surface and two-dimensional contour plots. From the three-dimensional response surface and normal plots shown in **Fig. 9–11**, the effect of the independent variables and their mutual interaction on yield from onion peels can be seen.

Fig. 9 shows the interaction between microwave power (W; X1) and irradiation time (X2) on the yield of TPC, TFC and DPPH % activity. An increase in microwave power from 140 to 245 W with irradiation time from 10 to 20 min depicts an enhanced extraction yield, but with an increase in microwave power and irradiation time to approximately over 245W and 20min respectively, there was a gradual decline in the response with no obvious effect on the extraction yield. This could be explained by the fact that increased extraction time caused chemical decomposition resulting in a diminished or lower extraction yield.

Fig. 10 shows the interaction between microwave power (W; X1) and solvent: sample ratio loading (X3) on yield of TPC, TFC and DPPH % activity, where it is seen that varying microwave power from 140 to 245W and with an increase in loading

ratio from 30:1 to 50:1ml/g, the extraction yield of TPC, TFC and DPPH % activity increased. With an increase in microwave power and loading ratio over 245W and 50:1ml/g respectively, the response decreased gradually and did not show any prominent effect on TPC, TFC and DPPH % activity yield.

As seen from **Table 8**, the interactive effect of microwave power and loading ratio on the TPC, TFC and DPPH % activity yield was not highly significant (model F value = 13.82, P = 0.0075) compared to the interactive effect of microwave power and irradiation time(model F value = 30.85, P = 0.0009).

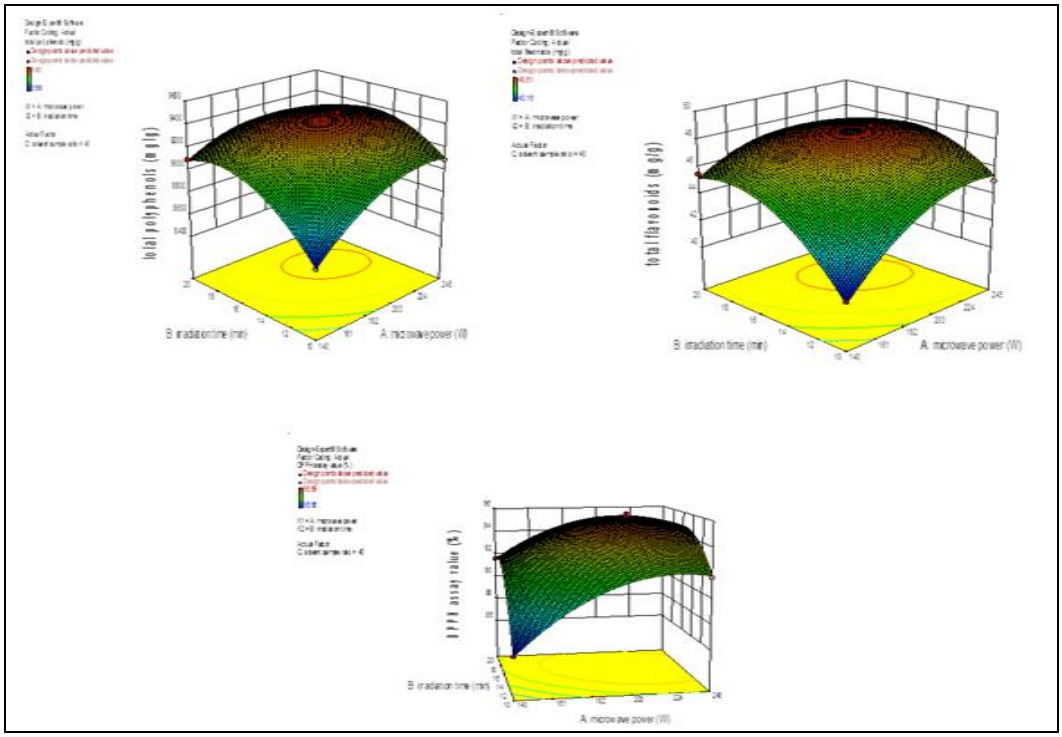


FIG. 9: 3D GRAPH PLOTS FOR THE EFFECT OF MICROWAVE POWER (W), IRRADIATION TIME (MIN) ON THE TOTAL PHENOLIC CONTENT, TOTAL FLAVONOIDS CONTENT AND DPPH SCAVENGING ACTIVITY IN ONION WASTE MATERIAL.

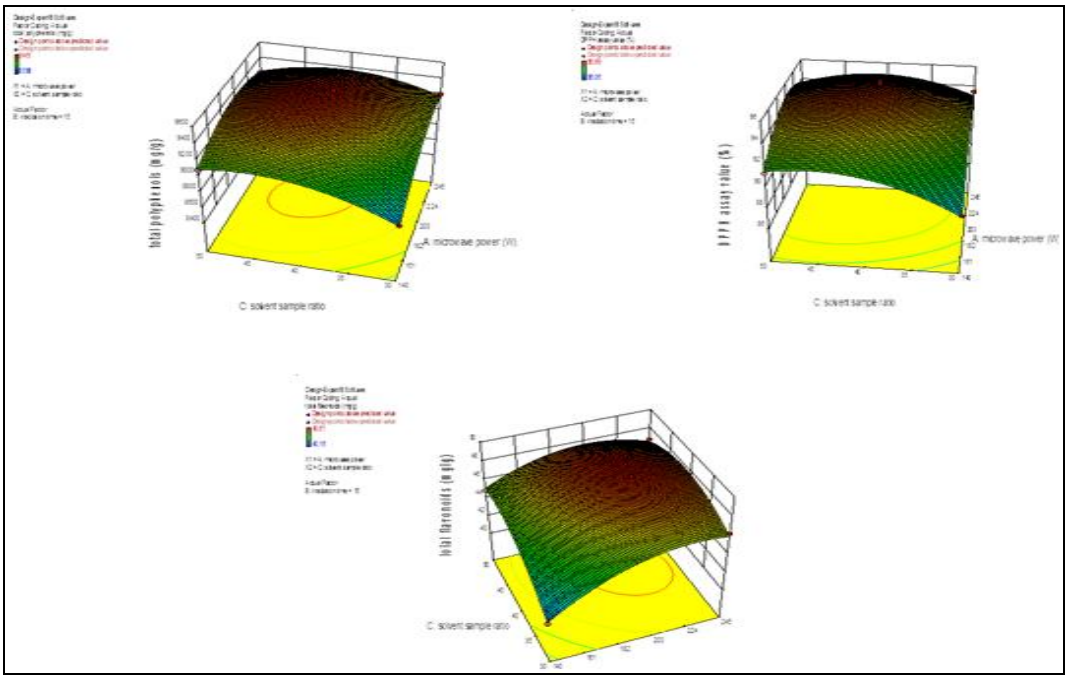


FIG.10: 3D GRAPH PLOTS FOR THE EFFECT OF MICROWAVE POWER (% OF 700 W) AND LOADING RATIO ON THE TOTAL PHENOLIC CONTENT, TOTAL FLAVONOIDS CONTENT AND DPPH SCAVENGING ACTIVITY IN ONION WASTE MATERIAL.

As shown in **Fig. 11** and **Table 8**, the interaction of irradiation time and solvent: sample ratio loading had a significant effect on the extraction yield (model F value= 17.33, P = 0.0042). **Fig. 11** shows that the highest extraction yield was achieved when irradiation time and loading ratio was slightly above 20min and 40ml/g respectively. However, the extraction yield gradually became decreased at a loading ratio above 50ml/g. This is because, as the loading ratio proceeds above 50mL/g, the solvent volume increases. The lacks of significant increase in yields were probably due to inadequate stirring of the solvent when microwaves were applied at larger solvent volumes. Moreover, large solvent volume might have caused more absorption of microwave energy and thus sufficient microwave energy was not available for cell rupture, which is considered important for effective leaching out of yields.

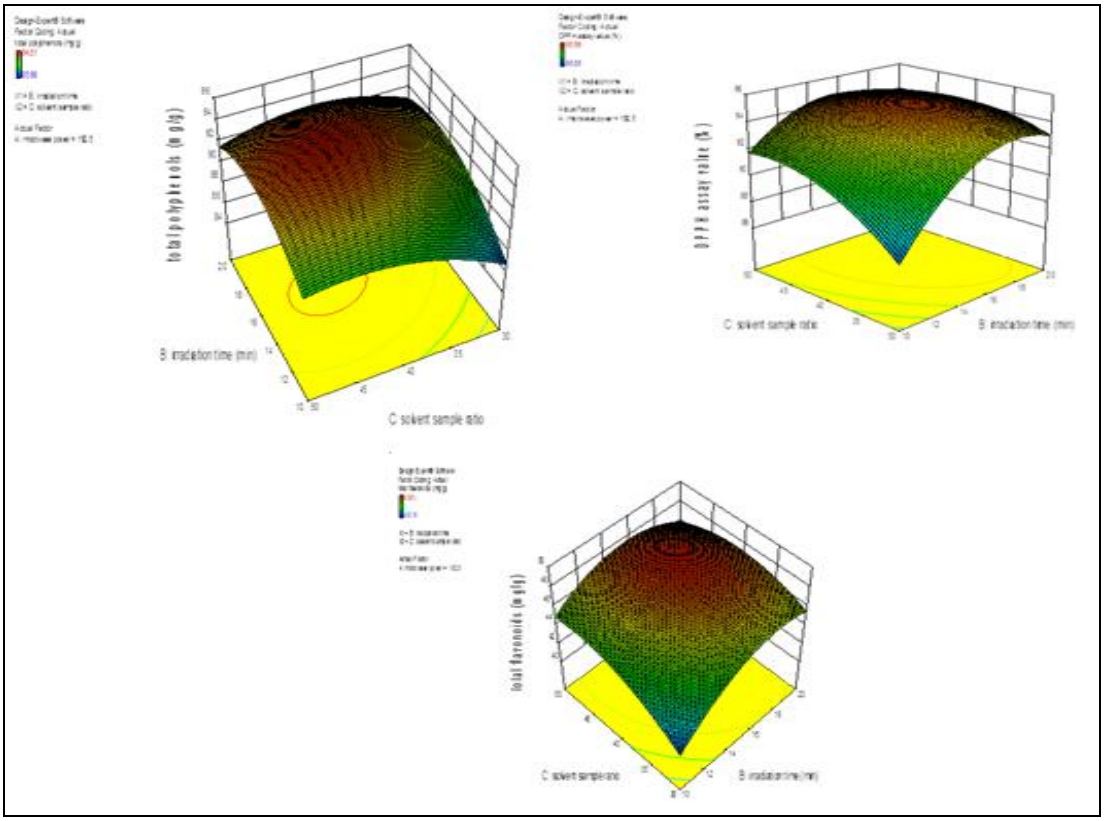


FIG. 11: 3D GRAPH PLOTS FOR THE EFFECT OF IRRADIATION TIME (MIN) AND LOADING RATIO ON THE TOTAL PHENOLIC CONTENT, TOTAL FLAVONOIDS CONTENT AND DPPH SCAVENGING ACTIVITY IN ONION WASTE MATERIAL.

Optimisation of the MAE process by RSM:

The aim of our optimisation study using various phases of RSM was to find the conditions that would produce the maximum extraction yield. The software predicted that the optimum microwave power, irradiation time and loading ratio to be 210W, 15min and 40mL/g respectively, and the theoretical polyphenols yield that was predicted by the software under the aforesaid conditions was 94.31 mg/g, flavonoids yield that was predicted by the software under the aforesaid conditions was 45.63 mg/g and antioxidant activity that was predicted by the software under the aforesaid conditions was 92.26% of dried peels. It was observed that a number of different variable combinations are possible that could give a maximum yield.

The path of improvement (path of steepest ascent) in achieving maximum yield and the operability region, or region of interest that symbolises the probable zone of maximum yield, as obtained from the software prediction is shown is **Fig. 12.** A validation of the MAE process was carried out subsequently by slightly modifying the optimal extraction conditions thus obtained from the software, to check the precision and acceptability of the process.

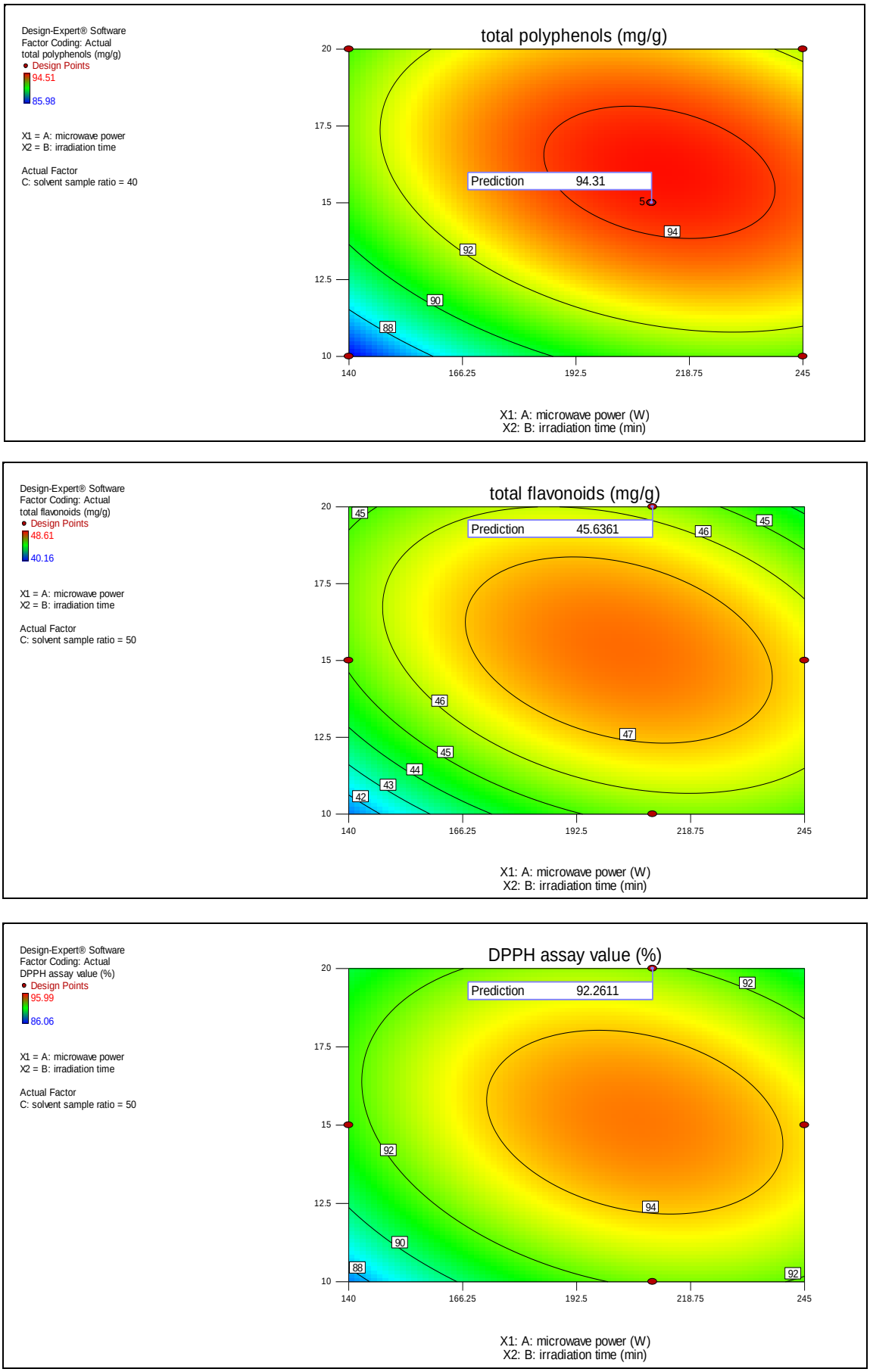


FIG. 12: CONTOUR PLOTS OF THE INTERACTION EFFECTS WITH PATH OF STEEPEST ASCENT TOWARDS THE OPTIMUM REGION FROM CURRENT OPERATING CONDITIONS AFTER OPTIMISATION.

Validation of the microwave assisted extraction process:

Reproducibility of the MAE process:

To determine the reproducibility of the novel extraction strategy of the MAE process, five samples of the same weight (1 g) were processed under the same optimum extraction conditions as obtained from the Box–Behnken design. The mean extraction of yield obtained under the optimised conditions was found to be polyphenols 94.34 (mg/g), flavonoids 45.61(mg/g) and free radical scavenging activity 92.25%. The calculated %RSD (relative standard deviation) value of 0.224971, 0.432381 and 0.42869 respectively shows that the proposed method has an acceptable precision and that the optimisation study was reliable as well.

Comparison of MAE with conventional extraction methods:

In the current study, MAE was compared with the other conventional extraction techniques for the extraction from onion peel based on their yields. The best results were obtained by MAE, which gave significantly higher values when compared with other extraction techniques. With respect to extraction time, MAE was also the fastest extraction method with only 15 min of extraction time. The MAE was found more effective when compared with ultrasound extraction, soxhlet extraction, stirring and maceration. These features along with its ease operation and implementation would position MAE as a valuable and cost-effective technology suitable for today's highly competitive industries, with growing demand for increased productivity, improved efficiency and reduced extraction time.

CONCLUSIONS: In conclusion it can be said that a design of experiment based extraction strategy was introduced to study its effectiveness on MAE techniques for the extraction from onion peel for the first time. Comparing with other research work on extraction, this proposed strategy has reduced the number of necessary experimental trials for saving time and power consumption apart from evaluating the important interactions between the multiple variables involved. The experimental data were fitted to a second-order polynomial equation using multiple regression analysis. The results showed probability value ($P<0.0001$) demonstrated a high significance for the regression model. The optimum condition found was microwave power of 210 W, extraction time of 15 min and loading ratio of 40mL/g. Under these conditions, the mean yield was polyphenols 94.34 (mg/g), flavonoids 45.61(mg/g) and free radical scavenging activity 92.25%.

Thus the design of experiment concept of employing response surface methodology can be applied to all natural products and if explored properly, can prove to be efficient for large-scale industrial application. In addition, the recyclable aspect of the total procedure is a key feature because research be appropriating to new alternative extraction strategies aimed at reducing the various negative forces on the environment and human health is paramount.

ACKNOWLEDGEMENT: We gratefully acknowledge the aid of Jadavpur University for providing facilities to carry out the project work.

REFERENCES:

1. Box GEP, Wilson KB. 1951. On the experimental attainment of optimum conditions. J R Stat Soc Ser. 13: 1–45.
2. Hao J, Han W, Huang S, Xue B, Deng X. 2002. Microwave-assisted extraction of artemisinin from Artemisia annua L. Sep Purif Technol 28: 191–196.
3. Mandal V, Dewanjee S, Mandal SC. 2009.Microwave-assisted extraction of total bioactive saponin fraction from Gymnema sylvestre with reference to gymnemagenin: a potential biomarker. Phytochem Anal 20: 491–497.
4. Pan X, Niu G, Liu H. 2003. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. Chem Eng Process 42: 129–133.
5. Renjie L. 2008.Optimization of extraction process of Glycyrrhiza glabra polysaccharides by response surface methodology. Carbohydr Polym 74: 858–861.
6. ReuVera.Health Benefits of Home Remedies. Miracle of Onion Peels. Health Benefits of Onions. Retrieved from http://reuvera.hubpages.com/hub/Health-Benefits-of-Home-Remedies-Miracle-of-Onion-Peels.
7. Jiyoung M, Hyun JD, Oh Yoen K, Min J S.2013.Antiobesity effects of quercetin-rich onion peel extract on the differentiation of 3T3-L1 preadipocytes and the adipogenesis in high fat-fed rats. Food and Chemical Toxicology 58: 347–354.
8. Seung M L, Jiyoung M, Hyun J D, Ji HC, Kyung HL, Yong JC, Min JS. 2012. Onion peel extract increases hepatic low-density lipoprotein receptor and ATP-binding cassette transporter A1 messenger RNA expressions in Sprague-Dawley rats fed a high-fat diet. Nutrition Research 32: 210–217.
9. Geetha M, Saravanakumar M, Suganyadevi P. 2012.Anticancerous Activity Of Anthocyanin Analyzed Using Different Cell Lines From The Onion Peel (*Allium*

Cepa) Extraction. International Journal of Current Research 4:008-012.
10. Ballard TS, Mallikarjunan P, Zhou K, Keefe SO. 2010. Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. Food Chemistry 120:1185–1192.
11. Banik RM, Pandey DK. 2008. Optimizing conditions for oleanolic acid extraction from Lantana camara roots using response surface methodology. Ind Crops Prod. 27: 241–249.
12. Chemat S, Lagha A, AitAmar H, Bartels PV, Chemat F. 2004. Comparison of conventional and ultrasound-assisted extraction of carvone and limonene from caraway seeds. Flav Frag J 19: 188–195.
13. Guo Z, Jin Q, Fan G, Duan Y, Qin C, Wen, M. 2001. Microwave-assisted extraction of effective constituents from a Chinese herbal medicine Radix puerariae. Anal Chim Acta 436: 41–47.
14. Hemwimon S, Pavasant P, Shotipruk A. 2007. Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia. Sep Purif Technol 54: 44–50.
15. Krishnaswamy K, Orsat V, Gariépy Y, Thangavel K. 2013. Optimization of microwave-assisted extraction of phenolic antioxidants from grape seeds (Vitis vinifera). Food and Bioprocess Technology 6: 441–455.
16. Li Y, Skouroumounis GK, Elsey GM, Taylor DK. 2011. Microwave-assistance provides very rapid and efficient extraction of grape seed polyphenols. Food Chemistry 129: 570–576.
17. Marija D. Pavlovic, Aneta V. Buntic, Slavica S. Siler-Marinkovic, Suzana I. Dimitrijevic-Brankovic. 2013. Ethanol influenced fast microwave-assisted extraction for natural antioxidants obtaining from spent filter coffee. Separation and Purification Technology 118: 503–510.
18. Moreira MM, Morais S, Barros AA, Delerue-Matos C, Guido LF. 2013. A novel application of microwave-assisted extraction of polyphenols from brewer's M.D. Separation and Purification Technology 118: 503–510.
19. Singh A, Sabally K, Kubow S, Donnelly DJ, Gariepy Y, Orsat V, Raghavan GSV. 2011. Microwave-assisted extraction of phenolic antioxidants from potato peels. Molecules 16: 2218–2232.
20. Upadhyay R, Ramalakshmi K, Rao LJM. 2012. Microwave-assisted extraction of chlorogenic acids from green coffee beans. Food Chemistry 130: 184–188.
21. Wang L, Li D, Bao C, You J, Wang Z, Shi Y, Zhang H. 2008. Ultrasonic extraction and separation of anthraquinones from Rheum palmatum L. Ultrason Sonochem 15: 738–746.
22. Xu H, Sun LP, Shi YZ, Wu YH, Zhang B, Zhao DQ. 2008. Optimization of Cultivation Conditions for Extracellular Polysaccharide and Mycelium Biomass by Morchella esculenta As 51620. Biochem. Eng. J 39: 66–73.
23. Das S. 2013.Optimization of Ultrasound-assisted Extraction of Total Flavonoids and Antioxidant properties from Trigonella foenum-graecum Seeds with Response Surface Methodology. IJPSR 4(11): 4308-4318.
24. Das S. 2014. Hepatoprotective Activity of Methanol Extract of Fenugreek Seeds on Rats. IJPSR 5(4): 1506-1513.

How to cite this article:
Das S and Mandal SC: Effect of Process Parameters of Microwave Assisted Extraction (Mae) On Natural Product Yield from Onion Peel. Int J Pharm Sci Res 2015; 6(8): 3260-75.doi: 10.13040/IJPSR.0975-8232.6(8).3260-75.