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Microwave Exposure Sensitive Response of Antioxidant Properties of *Nelumbo nucifera* rhizome at Various Levels of Extraction and Analysis

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Abstract

Effect of microwave treatment on phytochemical composition and free radical scavenging capacity of *Nelumbo nucifera* rhizome was determined. Paste of fresh rhizome and powder were treated with microwave (MW) radiation for different time periods 0, 4, 8, 12, 16, 20 minutes at medium low intensity. Total phenolic content (TPA), Linoleic acid reduction capacity (LARC), β-carotene bleaching assay (BCBA), DPPH radical scavenging capacity and Superoxide radicals scavenging activity (SORSA) was found to be decreased and total antioxidant activity by Phosphomolybdenum assay (TAOA), reducing power (RP) and iron chelation activity (ICA) is increased in response of increased microwave treatment. TPA content of fresh and dry samples ranged from 4.38±0.13 to 4.67±0.14 and 4.35±0.11 to 4.72±0.14 g/100g extract respectively. TAOA and RP (absorbance at 700 nm) content of fresh and dry samples ranged from 1.91±0.33 to 9.08±0.34 and 2.83±0.22 to 8.58±0.34 g/100 extract and from 0.72±0.04 to 1.29±0.02 and 0.73±0.05 to 1.29±0.02 respectively. ICA of fresh and dry samples ranged from 6.51±0.13 to 8.90±0.17 and 6.31±0.11 to 8.99±0.13 g/100 g extract respectively. BCBA values ranged from 4.69±1.02 to 16.19±1.16 and 2.60±1.08 to 15.56±1.41 %. DPPH and SORSA content of fresh and dry samples has significant values. It is therefore concluded that the microwave treatment has a significant effect on the antioxidant properties of *N.nucifera* rhizome. The data would be useful for the researches concerning with the analysis of effect of microwave treatment on the antioxidant activity of *N. nucifera* rhizome.

Key words: Antioxidant activity, *Nelumbo nucifera*, rhizome, Microwave exposure, Regression analysis

Introduction

Antioxidants are the substances which play a significant role in preventing the oxidative stress caused by endogenous and exogenous free radicals in living and nonliving systems respectively. These substances protect lipids, proteins and nucleic acids from oxidative damage by reducing or scavenging free radicals due to their hydrogen donating ability[1, 2]. Plants are the good source of phytochemical compounds which possess antioxidant properties. The natural antioxidants are more effective and nontoxic as compared to synthetic antioxidants. However, there are several factors which may affect antioxidant activity of these compounds during extraction, isolation, purification and analysis. The processing conditions such as light intensity and temperature during soaking, boiling, steaming and drying are the major factors which have been found to affect antioxidant activities of plant material[3].

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High temperature drying of plant material has been observed to accelerate degradation of antioxidant compounds and decrease their activity[4, 5]. The intensity of direct sunlight and microwave during drying has been also found to decreases total antioxidant and phytochemical contents of plant materials[6, 7, 8, 9, 10, 11]. Lotus (*Nelumbo nucifera*) is a perennial water plant grown as an ornamental plant produce rhizome that is very popular as a vegetable and its parts leaves, stems, seeds, roots and other parts are harmless to eat and many parts of the plant are thought to have multiple medicinal properties like antidiarrhoeal, psychopharmacological, diuretic, antipyretic, antimicrobial hypoglycaemic activity [12, 13]. Fresh rhizome contains abundant starch and minerals including calcium, copper, iron, magnesium, and zinc[14]. Approximately all parts of the plant are used in indigenous system of medicine[15]. *N. nucifera* is reported to possess Antioxidant activity of various parts e.g. leaf, stamens and rhizomes of *N. nucifera* are well established [16]. Among various vegetables, the lotus rhizome exhibited the strongest antioxidant activity that is why it is used as a medicine in

treatment of all the diseases caused by oxidative stress [17]. Exposure to microwave *N. nucifera* during drying, storage, extraction and analysis may affect the antioxidant activity of *N. nucifera* rhizome. A careful literature survey showed that no work has been done on the effect of microwave on antioxidant properties of *N. nucifera*. Therefore, the present study was planned to evaluate the effect of microwave exposure on the antioxidant properties of *N. nucifera* rhizome at different stages of extraction and analysis.

Material and Methods

Rhizomes of *N. nucifera* (6 Kg) were collected from damp areas near the east bank of river Indus in Muzaffargarh, South Punjab, Pakistan were washed with distilled water and their ends were cut with a sharp knife. The lotus rhizomes kept at room temperature for drying until constant weight. This is known as dry sample. For the Dry powder, samples were fully dried and ground in electric grinding machine. It was ready for microwave treatment. For the fresh paste of rhizome, the fresh lotus rhizomes after washing were ground in pestle mortar until it converted into very small pieces. Then it was ready for microwave treatment. This is known as fresh sample.

Experimental design for preparation of extracts

Microwave treatment To study the effect of microwave exposure on antioxidant properties of plant material at different stages of extraction and analysis lotus rhizome powder (5 g) and fresh paste (150 g) of *N. nucifera* were subjected to microwave treatment at low medium intensity in transparent glass bottles on different duration of treatment (4, 8, 12, 16, 20 min). The microwave treated and non treated dry samples of lotus rhizome flour were stored in sealed bottle at customary laboratory environment during the study period. Fresh lotus rhizome paste was not stored. Treated and non treated fresh paste was immediately used for the extraction with other samples.

Extraction

The powder (5 g) of each sample and paste (150 g) of each sample were extracted in 80% methanol, dried in vacuum desiccators and weighted. The extracts were stored in air tight glass container for further analysis.

Antioxidant Analysis

Total phenolic acids content

The total phenolic acids (TPA) content were determined by following the method described by Taga *et al*[18].

BHT equivalent total antioxidant activity by phosphomolybdenum assay

The total antioxidant activity (TAOA) by Phosphomolybdenum assay was determined by using the method of Prieto *et al*[19]. BHT equivalent total antioxidant activity was measured using the regression equation obtained from the standard curve of BHT (R^2=0.9811).

Reducing power

The reducing power (RP) was determined by following the method developed by Oyaizu[20]. The absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power.

DPPH radical scavenging capacity

The DPPH radical scavenging capacity (DPPH RSC) was assayed by the method of Moreno *et al* [21]. Methanol was taken as blank and DPPH solution, without the plant extracts, was used as positive control. The radical scavenging capacity was calculated as follows:

$$\text{DPPH radical scavenging capacity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Beta carotene bleaching assay

To calculate antioxidant activity of samples by the beta carotene linoleate system the method of yae *et al.*[22] Blank solution (without beta-carotene) was also prepared for background subtraction. The beta-carotene bleaching activity of the extracts was calculated as:

$$AAC\ (\%) = (Aa_{(120)} - Ac_{(120)}) / (Ac_{(0)} - Ac_{(0)}) \times 100$$

Where Aa is the absorbance of sample and Ac is the absorbance of control.

Superoxide radicals scavenging activity (SORSA)

Scavenging ability on superoxide radical was determined by the procedure of Marklund with slight modifications in Yea *et al.*[22] method. The mixture without samples extracts was the control and mixture without pyrogallol was taken as blank. The scavenging activity was calculated by following formula.

Scavenging activity (%) = [1-(Abs of sample-Abs of blank)/Abs of control)] ×100

BHT was used as standard

Iron Chelation assays

The iron chelation capacity of samples was estimated according to the method of puntel[23].

Linoleic acid reduction capacity

The antioxidant activity of extracts was determined according to the ferric thiocyanate method by Osawa and Namiki[24]. BHT solutions were used as standards. The percentage inhibition of lipid per oxidation was calculated by the following equations:

% Inhibition of lipid per oxidation = 100 - [A_s /A_c x 100]

A_c is the absorbance of solution taken as control and A_s is the absorbance of sample solution.

Statistical analysis

The results were expressed as mean values±standard deviation of three parallel replicates. The means were separated at confidence level $p \leq 0.05$ by using analysis of variance (ANOVA) using Tukey's multiple range test were analyzed by regression analysis to investigate the effect of microwave exposure on the antioxidant properties of *N. nucifera* rhizome.

Results and Discussion

Polyphenolic compounds are commonly found in both edible and inedible plants and have been reported to possess multiple biological activities. Phenolic acids and polyphenols are known to act as antioxidants owing to their ability to scavenge free radicals. Antioxidants perform their action by donating electron to reactive oxygen and nitrogen species[25]. The in vitro determination of antioxidant activities of plant materials can be determined as antioxidant content, radical scavenging and reducing abilities[26]. However, each of these methods is sensitive towards direct sunlight and microwave treatment[27, 7]. Microwave drying not only deactivates the enzymes due to increased temperature but also causes photo degradation of phytochemicals[28].

In present study, effect of microwave exposure on antioxidant properties of *N. nucifera* rhizome at different stages of extraction was investigated.

The microwave induced variations in antioxidant parameters of various samples of *N. nucifera* rhizome are presented in Table 1 and Figures 1-4. The regression analysis of experimental data (Table 1) showed an exponential increase in ICA, Phosphomolebdenum assay, RP in response to increase in duration of exposure with high value of coefficient of determination as shown (Table 1) following regression equation was obtained from suggested regression model.

$$ICA\ (g/100g\ extract) = I \circ e^{E_{sc}.DMT}$$

$$TAOA\ (\%) = T_\circ\ e^{E_{SC}.DMT}$$

$$RP = E_{SC} \times DMT + R_\circ$$

where, I_o, T_o and R_o are pre-exponential factors which indicate TEC and OH radical scavenging capacity of antioxidants at negligible time respectively, E_{sc} is light exposure sensitivity coefficient and DMT (0, 2, 4, 6, 8, 10) is the duration of microwave exposure.

The regression analysis of the experimental data (Table 1) also showed an exponential decrease in TPA, DPPH, RSC, BCBA, LARC and SROSA in response to an increase in the duration of microwave exposure with high values of coefficient of determination, as shown in Figures 2, 3 and 4.

$$TPA\ (\%) = T_\circ e^{E_{SC}.DMT}$$

$$DPPH\ (\%) = D_\circ e^{E_{sc}.DMT}$$

$$BCBA\ (\%) = Esc \times DMT + Bo$$

$$LARC\ (\%) = E_{SC} \times DMT + L_\circ$$

$$SORSA\ (\%) = Soe\text{Esc.DMT}$$

Where T_o, D_o, B_o, L_o, and S_o is pre exponential factor, Esc is extraction sensitivity coefficient and DMT is the the time of microwave treatment. The pre exponential factor and extraction sensitivity coefficient may provide useful information about the extraction of phenolic acids, the antioxidant activity, reducing power and radical scavenging capacity respectively, at negligible time. Thus, using the values of intercept and slope of the regression equations were written in Table 1.

The predicted values were calculated by putting the values of input variables, pre exponential factors and light exposure sensitivity coefficient in above regression equations and regression models were plotted against the experimental values to test the validity of the suggested model. A good agreement between the experimental and predicted values of ICA, RP, TAOA by Phosphomolybdenum assay, TPA, DPPH RSC, BCBA, LARC and SORSA was observed with high values of co-efficient of determination (R^2 = 0.9504, 0.9845, 0.9844, 0.9791, 0.9948, 0.9872, 0.9862 and 0.9917 respectively). The relatively low degree of scattering and high values of regression coefficient of the observed values about the regression line favors the applicability of the suggested model to study the time dependent effect of microwave exposure on antioxidant extraction.

The present findings showed that methanolic extracts of *N. nucifera* rhizome strongly scavenged the free radicals which indicated that the extract had good potential as a source for natural antioxidant to prevent free radical mediated oxidative damage. Of course, there could be few explanations for the loss of phenolics and antioxidant activity due to microwave treatment that attributes the deactivation of the polyphenol oxidases by absorbing the water molecule. Data in the effects of microwave treatment, antioxidant activity and TPC of fruits and vegetables are conflicting due to several factors like different

drying conditions, type of extraction solvents, and antioxidant assays used. In conclusion, this indicated that the traditional microwave treatment method had an adverse effect in the TPC and TAOA resulting in all the extracts of the dried plant material possessing lower antioxidant properties than fresh plant material. Microwave treatment of plant materials has been reported to significantly decrease phenolic antioxidants by 23-88% [27, 7]. However some studies reported an increase in both TPC and TAA of samples after processing[29, 30]. The present results are in agreement with those reported earlier[27, 7, 9, 10, 11].

In conclusion *N. nucifera rhizome* contains significant amount of antioxidant compounds which makes this plant an important consideration for pharmaceutical applications. The present data suggests that researchers should avoid their samples being exposed to microwave for long duration because it affects the antioxidant properties of samples even in dry or liquid form.

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Conflict of interest

The research project was not funded by any funding agency and collaborated with any other institute. Therefore, there is no conflict of interest regarding this research work.

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Figure Captions
Left to right

Figure 1
A-B: Time dependant variation in iron chelation assay of *N. nuciferia* flour and its extracts exposed to direct microwave
C-D: Agreement between the experimental and predicted values of iron chelation assay of *N.nuciferia* flour and its extracts exposed to direct microwave

A-B: Time dependant variation in TAOA of *N. nuciferia* flour and its extracts exposed to direct microwave
C-D: Agreement between the experimental and predicted values of TAOA of *N. nuciferia* flour and its extracts exposed to direct microwave

A-B: Time dependant variation in reducing power of *N. nuciferia* flour and its extracts exposed to direct microwave
C-D: Agreement between the experimental and predicted values of reducing power of *N. nuciferia* flour and its extracts exposed to direct microwave
Figure 2
A-B: Time dependant variation in TPA of *N.nuciferia* flour and its extracts exposed to direct microwave
C-D: Agreement between the experimental and predicted values of TPA of *N.nuciferia* flour and its extracts exposed to direct microwave
A-B: Time dependant variation in DPPH radical scavenging capacity of *N. nuciferia* flour and its extracts exposed to direct microwave
C-D: Agreement between the experimental and predicted values of radical scavenging capacity of *N. nuciferia* of flour and its extracts exposed to direct microwave
Figure 3
A-B: Time dependant variation in β-carotene bleaching assay (BCBA) of *N. nuciferia* flour and its extracts exposed to direct microwave
C-D: Agreement between the experimental and predicted values of β-carotene bleaching assay (BCBA) of *N.nuciferia* flour and its extracts exposed to direct microwave

A-B: Time dependant variation in Linoleic acid reduction capacity (LARC) of *N. nuciferia* flour and its extracts exposed to direct microwave
C-D: Agreement between the experimental and predicted values of Linoleic acid reduction capacity (LARC) of *N.nuciferia* flour and its extracts exposed to direct microwave

Figure 4
A-B: Time dependant variation in superoxide radical scavenging capacity of *N. nuciferia* flour and its extracts exposed to direct microwave
C-D: Agreement between the experimental and predicted values of radical scavenging capacity of *N. nuciferia* of flour and its extracts exposed to direct microwave

Table Captions

Table 1. Regression analysis of experimental data on antioxidant properties of *N.nuciferia* flour and its extracts exposed to direct Microwave

Table 1. Regression analysis of experimental data on antioxidant properties of *N.nuciferia* flour and its extracts exposed to direct Microwave

Sample	Duration of MW treatment (min)						Regression Coefficient	Regression Equation	P value
	0	4	8	12	16	20			
	Total Phenolic Acid Contents (g/100g extract)								
Fresh rhizome	4.67±0.14^{a}	4.58±0.12^{a}	4.57±0.11^{a}	4.48±0.10^{a}	4.44±0.13^{a}	4.38±0.13^{a}	0.9911	$y=4.6995e^{0.0045x}$	0.070
Dry rhizome	4.72±0.14^{a}	4.58±0.15^{ab}	4.55±0.13^{ab}	4.49±0.12^{ab}	4.44±0.12^{ab}	4.35±0.11^{a}	0.9983	$Y=4.7254e^{-0.0045}$	0.031
	Phosphomolybdenum assay (g/100 extract)								
Fresh rhizome	1.91±0.33^{e}	2.71±0.20^{d}	3.20±0.30^{d}	4.69±0.30^{c}	6.91±0.22^{b}	9.08±0.28^{a}	0.9923	$Y=2.0602e^{-0.0747}$	0.000
Dry rhizome	2.83±0.22^{c}	3.39±0.33^{c}	4.07±0.58^{bc}	5.12±0.52^{b}	7.41±0.73^{a}	8.58±0.34^{a}	0.9844	$Y=2.7052e^{-0.058}$	0.000
	Reducing power (absorbance at 700 nm)								

Fresh rhizome	0.72±0.04^{d}	0.99±0.06^{c}	1.02±0.09^{c}	1.11±0.01^{bc}	1.21±0.01^{ab}	1.29±0.02^{a}	0.9302	Y=0.0276x+0.781	0.000
Dry rhizome	0.73±0.05^{d}	1.01±0.01^{c}	1.15±0.02^{bc}	1.16±0.03^{b}	1.22±0.01^{ab}	1.30±0.02^{a}	0.9608	Y=0.0267x+0.7954	0.000

Iron chelating assay (g/100 g extract)

Fresh rhizome	6.51±0.13^{e}	6.10±0.14^{f}	6.96±0.13^{d}	7.79±0.13^{c}	8.36±0.15^{b}	8.90±0.17^{a}	0.954	$y=6.2581e^{-0.0174x}$
Dry rhizome	6.31±0.11^{d}	6.35±0.11^{d}	6.85±0.11^{c}	7.90±0.10^{b}	8.00±0.11^{b}	8.99±0.13^{a}	0.9458	$y=6.0895e^{-0.0186x}$

Linoleic acid Reduction capacity (%)

Fresh rhizome	19.92±2.79^{a}	17.36±1.74^{ab}	13.98±1.73^{bc}	10.49±1.84^{cd}	8.56±1.38^{d}	5.83±1.05^{d}	0.9302	y=0.0267x+0.795
Dry rhizome	18.53±1.43^{a}	16.20±1.73^{ab}	12.93±2.48^{bc}	10.37±1.36^{cd}	5.82±2.51^{d}	4.66±1.31^{e}	0.9608	y=0.0276x+0.781

β-carotene bleaching assay (%)

Fresh rhizome	16.19±1.16^{a}	15.32±1.46^{ab}	12.71±1.16^{bc}	10.55±1.40^{cd}	7.81±1.34^{de}	4.69±1.02^{e}	0.9803	y= -0.587x+17.083
Dry rhizome	15.56±1.41^{a}	14.16±1.32^{ab}	11.28±1.08^{bc}	8.11±1.63^{c}	5.35±0.74^{c}	2.60±1.08^{d}	0.9917	y= -0.647x+16.251

DPPH radical scavenging activity (%)

Fresh rhizome	71.86±2.74[a]	71.10±2.66[a]	68.99±1.71[a]	69.36±1.51[a]	68.39±2.08[a]	67.86±2.21[a]	0.9983	$y=71.835e^{-0.0029x}$
Dry rhizome	69.73±2.43[a]	69.83±2.16[a]	69.28±2.42[a]	68.52±2.33[a]	67.98±2.47[a]	67.49±2.28[a]	0.9468	$y=70.062e^{-0.0018}$
	Superoxide radical scavenging activity (%)							
Fresh rhizome	96.06±2.51[a]	92.67±2.31[a]	90.38±2.60[ab]	85.83±2.51[bc]	83.86±2.11[c]	80.65±1.99[c]	0.9929	$y=96.138e^{-0.0088}$
Dry rhizome	95.69±2.84[a]	94.86±2.59[ab]	93.99±2.98[ab]	87.51±2.72[bc]	85.32±2.82[c]	82.99±2.35[c]	0.9311	$y=97.282e^{-0.0079}$

DMT* = Duration of microwave exposure

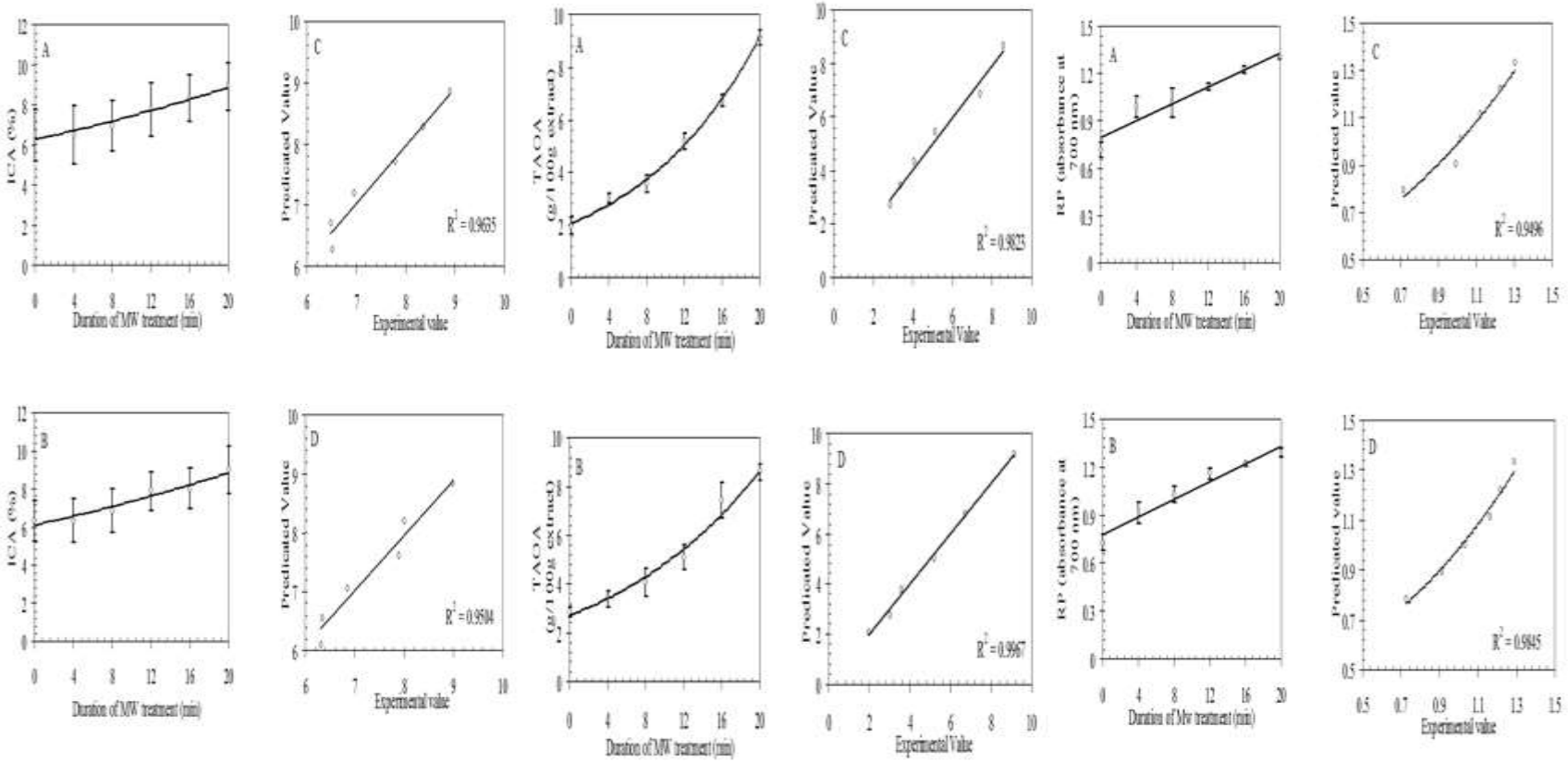
A
ICA (%)
Duration of MW treatment (min)
C
Predicated Value
Experimental value
$R^2 = 0.9655$
A
TAOA (g/100g extract)
Duration of MW treatment (min)
C
Predicated Value
Experimental Value
$R^2 = 0.9825$
A
RP (absorbance at 700 nm)
Duration of MW treatment (min)
C
Predicted value
Experimental Value
$R^2 = 0.9496$
B
ICA (%)
Duration of MW treatment (min)
D
Predicated Value
Experimental value
$R^2 = 0.9504$
B
TAOA (g/100g extract)
Duration of MW treatment (min)
D
Predicated Value
Experimental Value
$R^2 = 0.9967$
B
RP (absorbance at 700 nm)
Duration of MW treatment (min)
D
Predicted value
Experimental value
$R^2 = 0.9845$

Figure.1

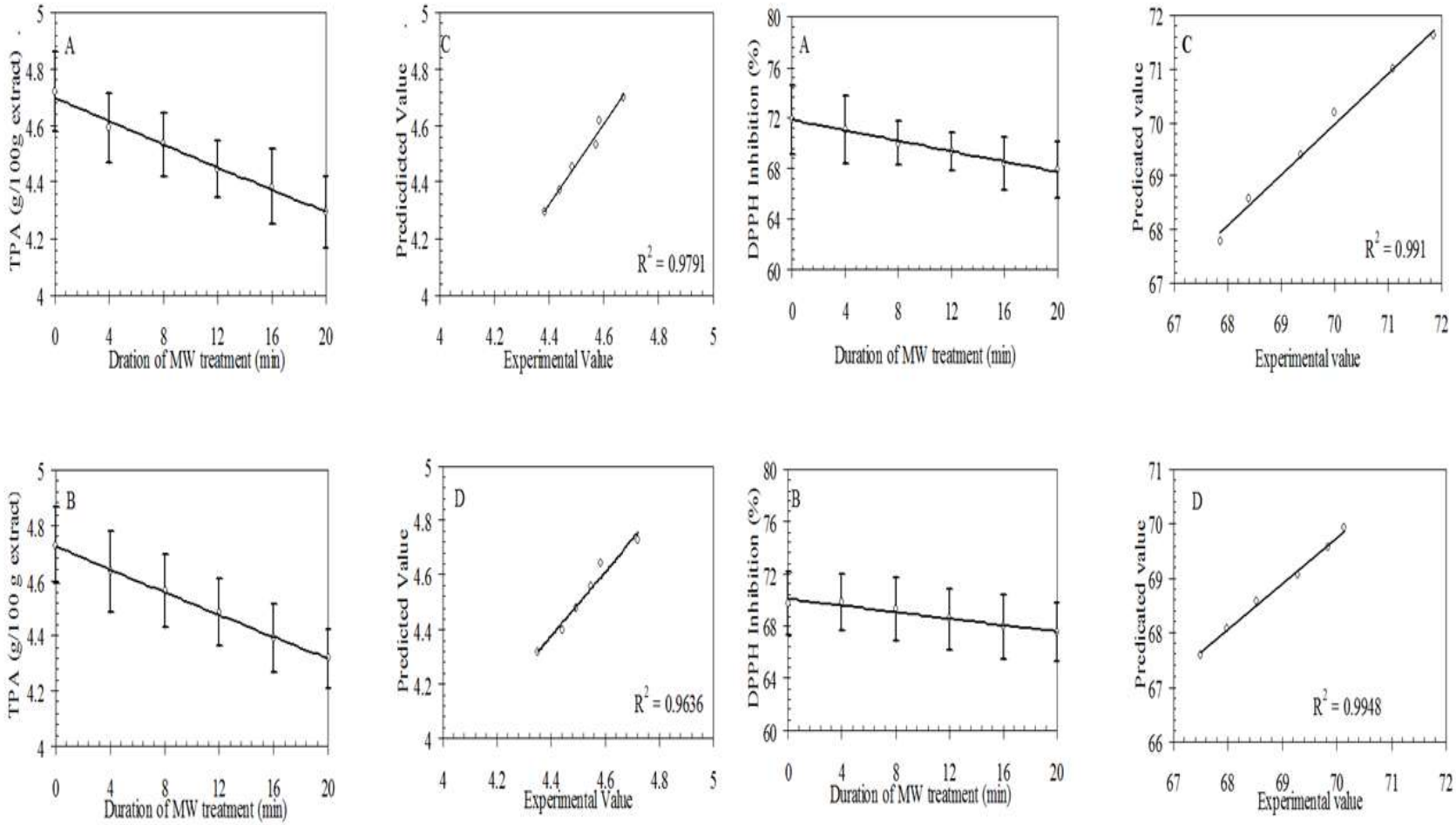
A
TPA (g/100g extract)
Dration of MW treatment (min)
C
Predicted Value
Experimental Value
R^2 = 0.9791
A
DPPH Inhibition (%)
Duration of MW treatment (min)
C
Predicated value
Experimental value
R^2 = 0.991
B
TPA (g/100 g extract)
Duration of MW treatment (min)
D
Predicted Value
Experimental Value
R^2 = 0.9636
B
DPPH Inhibition (%)
Duration of MW treatment (min)
D
Predicated value
Experimental value
R^2 = 0.9948

Figure.2

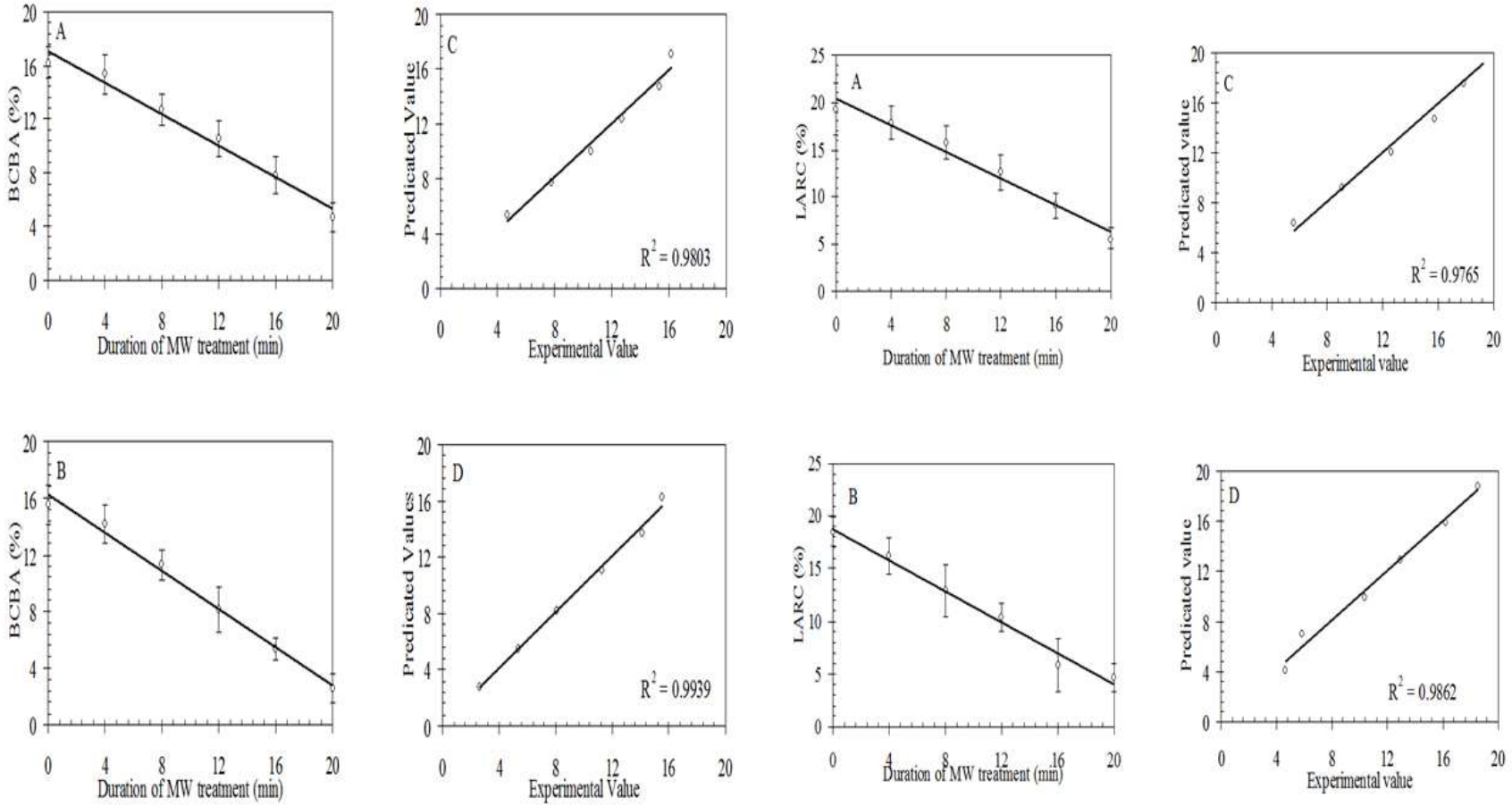
A
BCBA (%)
Duration of MW treatment (min)
C
Predicated Value
Experimental Value
R^2 = 0.9803
A
LARC (%)
Duration of MW treatment (min)
C
Predicated value
Experimental value
R^2 = 0.9765
B
BCBA (%)
Duration of MW treatment (min)
D
Predicated Values
Experimental Value
R^2 = 0.9939
B
LARC (%)
Duration of MW treatment (min)
D
Predicated value
Experimental value
R^2 = 0.9862

Figure.3

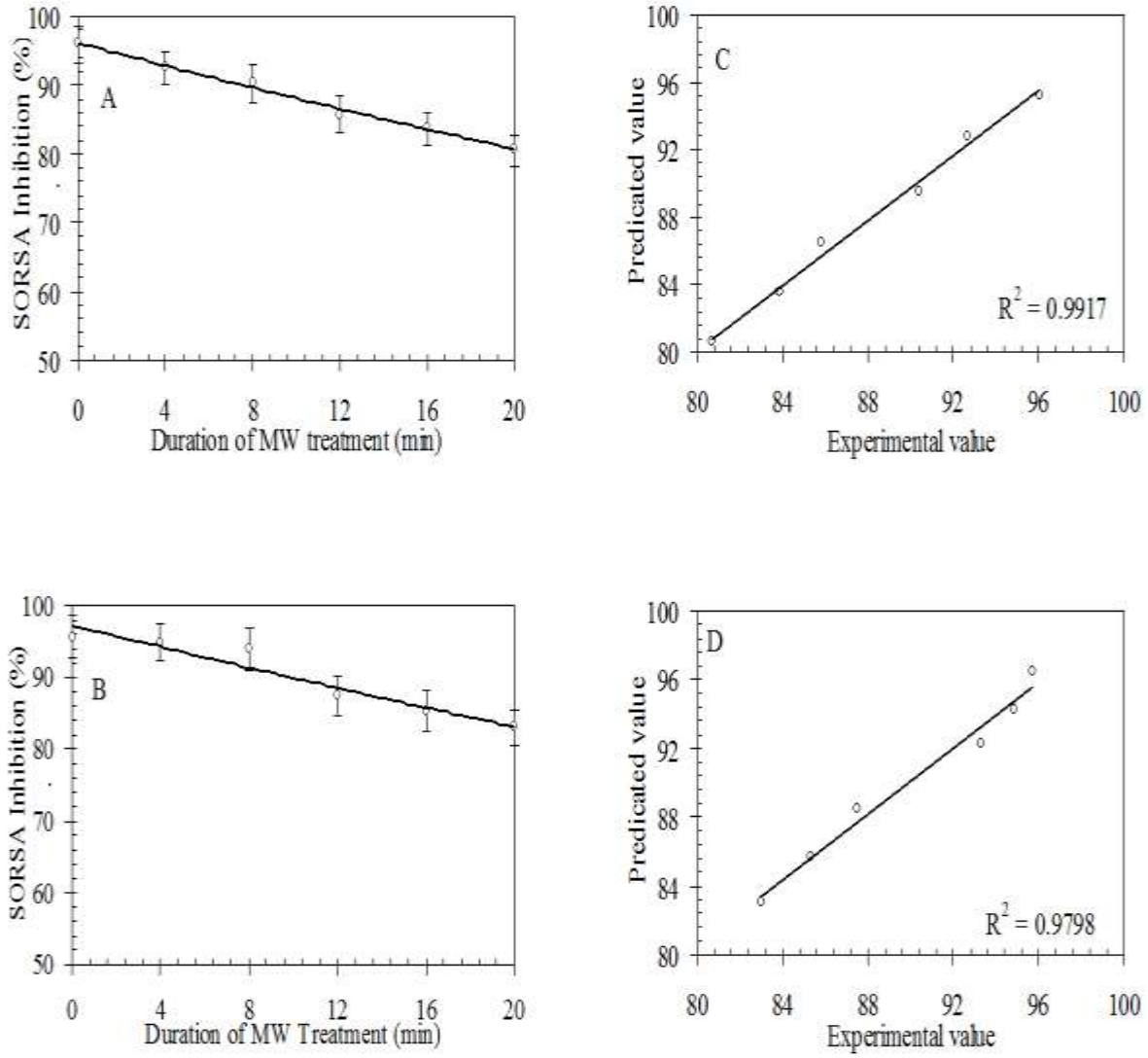

Figure.4

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