JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

A PRELIMINARY INVESTIGATION OF A MICROWAVE ASSISTED EXTRACTION STRATEGY FOR OPTIMUM EXTRACTION OF PHENOLICS FROM *FICUS RACEMOSA* FRUITS

Anup Kumar Das[1], Subhash C Mandal[2], Sarbani Dey Ray[1], Adriana Kolesarova[3,4], Shubhadeep Roychoudhury[5]**

Address(es):
[1] Department of Pharmaceutical Sciences, Assam University, Silchar 788011, India.
[2] Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India.
[3] AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, 94976 Nitra, Slovakia.
[4] Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, 94976 Nitra, Slovakia.
[5] Department of Life Science and Bioinformatics, Assam University, Silchar 788011, India.

*Corresponding author: sarbanideyray09@gmail.com, shubhadeep1@gmail.com

https://doi.org/10.55251/jmbfs.12457

ARTICLE INFO

Received 27. 2. 2025
Revised 26. 7. 2026
Accepted 27. 7. 2026
Published 1. 8. 2026

Regular article

OPEN ACCESS

ABSTRACT

Polyphenolic compounds provide numerous benefits to cells, not just through their antioxidant properties but also by influencing various signaling pathways. The fruits of *Ficus racemosa* are recognized for containing valuable phenolic compounds such as gallic acid and ellagic acid; however, they have largely been overlooked, primarily being used for animal feed and ceremonial purposes in northeastern India. This study outlines an energy efficient approach to extract a phenolic-rich fraction from this underutilized wild fruit by employing modern green extraction techniques with less carbon foot print. To achieve this, microwave-assisted extraction (MAE), an automated green extraction method, was utilized to efficiently extract the phenolic-rich fraction from *Ficus racemosa* fruits, supported by multivariate statistical optimization tools. The significant parameters with optimal values obtained from optimization study were microwave power (457 Watts), extraction time (12.20 min) and ethanol concentration (61.0% v/v). The total phenolic content (TPC) and IC_{50} values (Antioxidant potential) as obtained from optimization design were 69.43 mg gallic acid equivalent (GAE) / g DM and 211.32 µg /ml respectively under optimal conditions. Validation experiments confirmed reproducibility of the optimized conditions, with 95% confidence intervals of 68.02–69.04 mg GAE/g DW for TPC and 211.26–216.54 µg/ml for IC_{50}, thereby strengthening confidence in the optimization outcome. MAE was found to be approximately 50 % and 120 % more efficient than ultrasound and Soxhlet extraction respectively. MAE was found to be more energy efficient in extracting out phenolics with less electrical consumption and carbon emission into the environment in comparison to other techniques. Thus, MAE showed obvious advantages over conventional extraction techniques in terms of better extraction efficiency, reduced extraction time and environment effect.

Keywords: *Ficus racemosa,* Microwave assisted extraction, Total phenolic content, Optimization

INTRODUCTION

Consuming foods rich in antioxidants on a daily basis can significantly help combat complications arising from oxidative stress in humans. As a result, plants and their components that boast high antioxidant properties should be considered valuable options for regular dietary inclusion. Numerous phytochemical investigations by various researchers have highlighted the antioxidant capabilities of certain Ficus species, attributed to their phenolic content (**Al-Fatimi *et al*., 2007; Manian *et al*., 2008**). In this regard, the fruit of Ficus racemosa stands out as an important yet underappreciated resource from a scientific perspective.

Ficus racemosa L. (syn. *Ficus glomerata* Roxb, Family: Moraceae) is commonly known as Cluster fig in English. Many medicinal utilities of Fig fruits have been reported by several researchers (**Rao *et al.*, 2008; Trivedi *et al*., 1969; Jahan *et al*., 2009**). Fig fruits are known to contain several phenolic compounds such as gallic acid, chlorogenic acid and ellagic acids which are known for their potent antioxidant potential and protection against DNA damage.

Additionally the nutritional analysis of the fruits revealed the presence of 32.7mg of carotenoids per 100g of fruit , 41.9 mg of vitamin C per 100g of fruit along with sugar and protein content of 15.3% and 29.4% respectively (Verma et al., 2010).The experimental work conducted by **Verma *et al*., (2010)** has gathered enough evidences regarding the potential of this fruit so that it can be used as an edible natural antioxidant owing to its capacity to scavenge reactive oxygen species or free radicals. In India *Ficus racemosa* is often cultivated in villages for its edible fruit (**Anonymous, 1952**). Evidences of historical use of the fruits of several Ficus species in several forms ranging from eating raw or applying as poultices on wounds are present (**Lansky & Paavilainen, 2011**). Despite these beneficial properties, fig still remain an underutilized natural resource in India. In order to develop this health promoting fruit for daily consumption, more research is immediately required to facilitate scientific exploitation of this fruit for human consumption.

The use of microwave energy in extracting the several phyto-constituents of plant material have shown remarkable research interest and prospect due to its many advantages over conventional methods (**Song *et al.*, 2011, Mandal & Mandal, 2010**). Research indicates that MAE has been widely employed to extract total phenolics from different medicinal plants (**Beejmohun *et al.*, 2007; Sutivisedsak *et al*., 2009**). To achieve a high analyte yield, it is absolutely necessary to design optimal extraction conditions as well. A single factor analysis technique does not convey anything about the collective influence of all the parameters involved in any operation since here other factors are maintained at a constant level. Additionally, it is a time-consuming operation and requires a large number of experiments to find out the optimum levels (Sun et al., 2011). Such limitations can be overcome by using a multivariate optimization tool namely response surface methodology (RSM) (**Das *et al*.,2012**). Unlike the conventional methods, RSM generates a mathematical model which takes into account all possible interrelationships among the test parameters (i.e. extraction parameters) while reducing the number of experiments needed to be done.

Therefore, in this study we report a detailed and a systematic optimization of MAE of phenolics for the first time from *Ficus racemosa* fruits using RSM in determining the TPC and IC_{50} of fig fruit extract. Furthermore, the extraction potential of microwave extraction was compared with ultrasound and Soxhlet extraction technique with respect to total phenolic content and free radical scavenging potential. So, the aim of or investigation was to develop a cost effective and energy efficient extraction strategy for phenolic extraction from fig fruit using microwave energy. An attempt was made to analyze the environmental impact with respect to carbon foot print while using MAE and a comparison study was performed as well with other extraction techniques.

MATERIALS AND METHODS

Plant Materials

Green unripe fruits of *Ficus racemosa* were collected from Cachar district of Barak Valley region of Assam, India. Shade dried fruits were grounded into powder using a milling machine and the powdered materials which passed through sieves of

different sizes and were kept in sealed polyethylene bags under a controlled environment. The moisture content of the dried fruit powder was measured and was found out to be 9.3 %.

Chemicals

Gallic acid was obtained from SD FINE CHEMICALS LIMITED (Mumbai, India) and DPPH from SIGMA CHEMICAL Co. (St. Louis, MO, USA). Folin-Ciocalteu reagent was obtained from FLUKA (Buchs, Switzerland). Doubled distilled water was used for the preparation of all the solutions and was purified by Millex®GV Durapore Filter unit, 0.45 µm (Millipore, Carrigtwahill, Co. Cork, Ireland) All other chemicals were ultra-pure or analytical grade.

Apparatus and conditions

The preparation process of a phenolic rich fraction from the dried fruits of *Ficus racemosa* by microwave treatment was performed using a modified microwave extraction system provided with different microwave power levels, a time controller, a temperature sensing probe, an exhaust system and an inbuilt magnetic stirrer (CATA R, by Catalyst Systems, Pune, India). Ultrasound assisted extraction (UAE) was performed with a digital sonication bath (PHOENIX Digital Ultrasonic Cleaner PHUC-300). Soxhlation was done with a Soxhlet apparatus (500 ml) using heating mantle of 500W.

Extraction procedures

Microwave assisted extraction (MAE)

Initially a screening design using Plackett–Burman design was used and experiments were performed as per the design matrix generated. 10g of dried powdered fruit sample was introduced into a microwave heating flask. The extraction process was subsequently conducted utilizing various parameters, including microwave power levels, extraction time, solvent-to-sample ratios, particle sizes of the fruit powder, ethanol concentrations, and soaking or preleaching time.

Following the extraction process, all extracts underwent centrifugation for 15 minutes at 4°C and 4000 rpm using an R-8C centrifuge (REMI, Mumbai, India). The resulting supernatants were subjected to evaporation under reduced pressure, subsequently reconstituted in HPLC-grade ethanol, filtered through a 0.45 mm membrane filter, and stored for analysis. A same quantity of the dried powdered sample was utilized for each experimental trial. All experiments were performed in triplicate, and the mean values were employed for statistical evaluation. The results were analyzed using a Pareto chart to locate the significant extraction parameters based on their p values ($p = 0.05$). The so obtained parameters were further optimized using a Box–Behnken design to identify and predict regions with maximum TPC and minimum IC_{50}.

In several studies it has been reported that ethanol and aqueous alcoholic solution are more effective in extracting phenolic compounds than water and is in agreement with the results published by other researchers (**Karacabey *et al.*,2010; Zhang *et al*., 2007**). So, in order to establish an eco-friendly extraction process, cheap, reusable and less harmful ethanol was employed as an extracting solvent.

After the completion of each extraction, the extracts were filtered through a Whatman No. 1 filter paper and the filtrates were then concentrated to obtain a semisolid residue with a rotary evaporator under vacuum and was preserved prior to analysis.

Soxhlet extraction

The Soxhlet extraction method was executed utilizing a Soxhlet apparatus and a heating mantle with 10g of dried powdered fruit sample. Ethanol served as the solvent, and the extraction was continued until the complete extraction of the desired constituents was achieved. Upon completion of the extraction, the resultant extract was filtered using Whatman No. 1 filter paper, and the filtrate was subsequently concentrated to yield a semisolid residue via rotary evaporation under vacuum conditions. This residue was then stored for further analysis.

Ultrasound assisted extraction (UAE)

Ultrasound-assisted extraction was carried out using a digital sonication bath. 10 g of sample was mixed with 100 ml of ethanol in a 250 ml beaker that was placed inside the sonication bath and was operated for 30 minutes at 40 Khz. Following the extraction process, the extract was filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated using a rotary vacuum evaporator to produce a semisolid residue. The residue was then stored for further analysis.

Optimization approach

Optimization study was applied in two stages, first to identify the significant extraction parameters using a Plackett–Burman design (a screening design) and later the significant variables that resulted from a Plackett– Burman design was optimized by using a Box–Behnken design (an optimization design). The entire experimental flow is given in **Supplementary Figure 1.**

Plackett–Burman design (PBD): The Plackett–Burman design criterion was applied to identify the significant extraction parameters responsible for phenolic extraction from *F. racemosa* fruits. This design criterion is based on the first-order model:

$$Y = \beta o + \sum \beta i x i \quad \text{(Eq.1)}$$

Here Y is the estimated outcome (TPC, IC_{50}), β_i the regression coefficients, β_0 is the constant (model intercept), i is the parameter number (and can have any value from 1.....k) and x_i is the independent extraction parameter. Initially, the effect of six parameters (microwave power, extraction time, solvent: sample ratio, ethanol concentration, particle size, soaking/preleaching time) on TPC and IC_{50} was tested at two experimental levels (**Table 1**).

Table 1 Initial level of the extraction parameters used in Plackett–Burman design

PBD Extraction factor code	Extraction condition	Low level (-)	High level (+)
A	Microwave power	140 Watts	700 Watts
B	Extraction time	2 min	20 min
C	Solvent: Sample ratio	10:1mL /g	50:1mL/ g
D	Particle size	10 mesh	120 mesh
E	Ethanol concentration	10 % v/v	100%v/v
F	Soaking/Pre-leaching time	5 min	30 min

Box–Behnken design (BBD):

After using PBD, we have used BBD to optimize the significant extraction parameters obtained from screening design (**Table 2**). This methodology allows for an intricate exploration of factor interactions and assists in determining the specific factor level combinations that result in the optimal response.

All the data were analyzed by multiple regressions to fit into the following non-linear software generated quadratic (second order) polynomial equation:

$$Y(TPC / IC50) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^{\,2} + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \quad \text{(Eq.2)}$$

Where Y is yield, *b0, bi, bii*, and *bij* are the regression coefficients for intercept, linear, quadratic and interaction terms respectively, and *Xi*,
and *Xj* are the independent variables. The regression coefficients of individual linear, quadratic and interaction terms were determined according to the analysis of variance (ANOVA).

Analysis of the response surfaces generated from optimization study using polynomial equation and graphical optimization

The regression coefficients were then used to make statistical calculation to generate three-dimensional contour maps from the polynomial equation. The P values of < 0.05 were considered to be statistically significant. All the experiments were carried out in triplicate and the mean value was taken for statistical analysis.

Table 2 Extraction parameters and their levels used in Box- Behnken experimental design

Extraction parameters	Factor levels			
	Coded	-1	0	+1
A: Microwave power (Watts)	x_1	140	420	700
B: Extraction time (min)	x_2	2	11	20
C: Ethanol concentration(%v/v)	x_3	10:1	55:1	100:1

Determination of total phenolic content

The total phenolic content was determined using the method of **Macdonald *et al.* (2001)** with slight modification. A specified amount of sample was taken from the extracts of the experimental runs or standard solution of gallic acid (5, 10, 20,30,40,50 µg/ml) and was added to 25 ml volumetric flask, containing 9 ml of double distilled water. 1 ml of 2 N Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7 % Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 ml) with double distilled water and mixed. The samples were incubated for 90 min at 25°C. The absorbance of the diluted solution was measured at 765 nm versus the blank. The total phenolic content in all the extracts was expressed as mg of gallic acid equivalent (GAE) / g of extract. All estimations were conducted in triplicate and the mean value was used for statistical analysis.

Determination of DPPH free radical scavenging activity

The DPPH assay model was used to evaluate the antioxidant potential of the various fruit extracts obtained through different extraction techniques. The DPPH free radical scavenging activity of the extract was determined according to the method described by **Bhandari *et al.* (2010)** with slight modification. The DPPH free radical scavenging activity was expressed by IC_{50} value which is the concentration of extract required to decrease the absorbance at 517 nm by 50%. Measurements were performed in triplicate and the mean value was used for statistical analysis.

Validation experiment to check the reproducibility of the MAE process

The precision of proposed the MAE method was evaluated by running a validation experiment. 5 gm of dried fruit sample was processed under the optimum extraction conditions as predicted by the software but with slight modifications in order to overcome operational limitations. The values for TPC and IC_{50} obtained from this validation run were compared with the software predicted values and % RSD (relative standard deviation) was calculated to check whether any major deviation is happening or not. This study was done to check the precision level of the proposed MAE process and to ascertain whether the optimization study was reliable or not. A similar approach was adopted by **Talebpour *et al.*, 2009.**

Comparison with other extraction methods

In this study, MAE was compared with Soxhlet and ultrasound assisted extraction method and TPC and IC_{50} were determined and compared. During comparison study sample – solvent ratio was kept constant at 1:10 ratio (**Supplementary Table 4**). The extraction conditions for MAE were microwave power of 457 W, extraction time of 12min 20 sec and 100 ml solvent where ethanol conc is 61%. For UAE, 100 ml of solvent with 61% ethanol conc was used for 30 mins and Soxhlation was done with same solvent conc as above with the extraction time of 3 hr. Measurements were performed in triplicate and the mean value was used for statistical analysis.

Environmental impact of the extraction techniques: A carbon foot print analysis

The current research included an analysis of the environmental impact, specifically focusing on the electrical consumption and CO2 emissions resulting from the techniques utilized. Thus, the electrical consumption (EC) was calculated using the following formula (**Drinić *et al.*, 2020**).

$$EC = P \times t$$

Where
EC = electrical consumption (kWh)
P = electrical power (kW) and
t = process time (h).

As per the findings of **Ferhat *et al.* (2006)** to obtain 1 kW h from coal or fuel source, a 800 g of carbon dioxide will be emitted in the environment during the combustion of fossil fuel. Carbon dioxide emission can be denoted by the equation:

$$E_{CO2} = (EC \times 800)/1000$$

Where
E_{CO2} = Carbo dioxide emission (kg)
EC = electric consumption (kWh).

Scanning electron microscopy (SEM) study

In order to understand the effects of extraction techniques, samples from the different extraction methods were used to take scanning electron micrographs. Microscopic structures were examined by using a JEOL JSM-6360 (Tokyo, Japan) scanning electron microscope at an accelerating voltage of 10 kV. Samples were placed on a double-faced adhesive tape and sputtered with platinum before analysis.

Statistical analysis

All experiments were performed in triplicate as independent extraction replicates, and results are expressed as mean ± standard deviation (SD). For optimization studies, predicted values from the Box–Behnken design (BBD) model were compared with experimental means, and 95% confidence intervals were calculated to assess model reliability. The adequacy of the model was determined by evaluating the lack of fit, the coefficient of determination (R^2) and the F-test value obtained from the Analysis of variance (ANOVA).
Statistical differences among extraction methods (MAE, UAE, and Soxhlet) were evaluated using **one-way ANOVA (single factor)** at a significance level of $\alpha = 0.05$. When ANOVA indicated significant differences, **Tukey's Honest Significant Difference (HSD)** test was applied to determine pairwise differences. Analyses were performed using Stat-Ease Design-Expert 8 and GraphPad Prism 9.

RESULTS

Screening of extraction parameters using the Plackett–Burman design criterion

A total of 12 experiments were conducted and all experiments were conducted in triplicate and the mean values were used for statistical analysis (**Table 1**). Among six extraction parameters studied, three parameters (microwave power, extraction time and ethanol concentration) were found to have significant influence on TPC and IC_{50} as evidenced from ANOVA (**Supplementary Table 1**). Pareto plot chart generated by PBD (**Supplementary Figure 2**) showed microwave power (denoted by A), extraction time (denoted by B) and ethanol concentration (denoted by E) are having significant influence on the experimental outcomes. Moreover, the coefficient of determination (R^2) of the PBD model was found to be 0.94, which indicates that the model could explain up to 94% variation of the data. The extraction parameters that were found to be significant from the analysis were taken into consideration for next level optimization design i.e. BBD.

Table 3 Screening with PBD using 12 experimental runs and their corresponding outcomes

Run Order	A	B	C	D	E	F	TPC (Experiment result)	TPC (Software Predicted result)	IC_{50} (Experimentalr esult)	IC_{50} (Software Predicted result)
1	+1	+1	-1	+1	+1	+1	60.10± 0.01	60.13	260.22±0.02	248.53
2	+1	+1	-1	-1	-1	+1	60.88±0.03	58.48	275.11±0.01	291.64
3	-1	-1	-1	+1	-1	+1	34.10±0.01	37.28	490.80±0.02	461.54
4	-1	+1	-1	+1	+1	-1	52.23±0.01	51.28	329.17±0.02	348.35
5	-1	+1	+1	-1	+1	+1	52.71±0.02	54.00	327.14±0.02	317.65
6	+1	-1	+1	+1	+1	-1	57.18±0.01	55.96	293.50±0.02	283.60
7	+1	+1	+1	-1	-1	-1	59.43±0.01	61.69	286.22±0.01	264.45
8	+1	-1	+1	+1	-1	+1	50.16±0.02	49.35	310.11±0.02	334.53
9	+1	-1	-1	-1	+1	-1	55.57±0.01	57.71	300.56±0.01	302.97
10	-1	+1	+1	+1	-1	-1	48.10±0.02	47.87	364.85±0.01	372.09
11	-1	-1	+1	-1	+1	+1	47.90±0.02	46.61	370.43±0.02	379.92
12	-1	-1	-1	-1	-1	-1	44.25±0.02	42.25	450.88±0.03	453.72

Data are expressed as means ± standard deviation (n = 3).

Optimization of the screened extraction parameters to confirm model validity

Following the application of a Plackett-Burman Design (PBD), which is primarily aimed at screening a wide array of parameters to ascertain their significant effects on the response, researchers often turn to the Box-Behnken Design (BBD). The BBD allows for an in-depth exploration of the relationships among the critical factors by analyzing both quadratic effects and interactions within a more limited set of experiments, thereby enhancing the precision of the optimization process. In this case a total of 17 experimental runs were carried out in random order with the most significant extraction parameters obtained from the PBD (**Table 4**).
The predictive equations (Equation 3 and 4) which were obtained by fitting the 17 experimental data to the BBD model represent an empirical relationship between the response (TPC and Antioxidant assay) and extraction parameters is shown below:

$$Y_{TPC} = + 68.68 + 5.73A + 1.67B + 0.66C - 1.18AB - 0.30AC - 0.62BC - 7.92A^2 - 3.86B^2 - 3.86C^2 \quad \text{(Eq.3)}$$

$Y_{DPPH/\ IC50} = +220.28 - 57.60\ A - 20.48\ B - 6.38C + 10.66\ AB - 4.51\ A\ C + 7.68\ B\ C + 90.09A^2 + 13.95\ B^2 + 11.69\ C^2$ (Eq.4)

Where A, B and C represents the microwave power, extraction time and ethanol concentration respectively. In general, exploration and optimization of a fitted response surface may produce poor or misleading results unless the model exhibits a good fit, which checks the model validity (**Liyana-Pathirana and Shahidi, 2005**). In this case from **Supplementary Table 2** it was observed that the *F*-test had a very high model *F*-value (691.54 for TPC and 419.32 for DPPH assay) and a very low *p*-value ($p < 0.0001$), demonstrating that the fitness and validity of the BBD model was highly significant. Moreover, the determination coefficients (R^2) for each model (0.9989 for TPC and 0.9981 for DPPH assay) were close to 1, which represented the satisfactory correlation between actual experimental outcome and software predicted outcome. The *F*-value of 0.15 and *p*-value of 0.9235 for TPC as well as 0.50 and 0.7027 for DPPH assay implied that the lack of fit was insignificant relative to the pure error due to noise. A non-significant lack of fit further confirmed the validity of the model.

ANOVA data in **Supplementary Table 2** indicated that in either of the cases (TPC and DPPH assay) the interactions between microwave power × extraction time (TPC, p=0.0002; DPPH assay, p= 0.0015) and extraction time × ethanol concentration. (TPC, p=0.0085; DPPH assay, p= 0.0085) was found to have significant effect, whereas the interaction between microwave power × ethanol concentration for both TPC and DPPH assay was insignificant (TPC, p=0.1246; DPPH assay, p=0.0710).

All the above findings showed that microwave power was the most significant single factor that influenced the experimental outcome, followed by extraction/irradiation time and ethanol concentration.

Table 4 Optimization with BBD using 17 experimental runs and their corresponding outcomes

Run Order	Coded			TPC (Experiment result)	TPC (Software Predicted result)	IC_{50} (Experimentalr esult)	IC_{50} (Software Predicted result)
	x_1	x_2	x_3				
1	-1	0	-1	50.20±0.02	50.20	380.10±0.02	397.21
2	-1	0	1	52.24±0.02	52.11	378.15±0.02	377.92
3	1	1	0	63.22±0.02	63.11	258.15±0.01	256.77
4	0	0	0	69.14±0.01	68.68	224.59±0.01	220.28
5	1	0	1	62.98±0.01	62.98	255.00±0.06	253.70
6	1	0	-1	62.13±0.02	62.26	275.00±0.05	275.23
7	0	1	1	62.55±0.01	62.66	224.05±0.01	226.73
8	0	0	0	68.25±0.02	68.68	220.00±0.02	220.28
9	-1	-1	0	48.20±0.03	48.31	411.81±0.01	413.19
10	-1	1	0	53.99±0.06	54.01	353.09±0.01	350.64
11	0	0	0	68.29±0.02	68.68	219.72±0.04	220.28
12	0	0	0	69.10±0.01	68.68	224.32±0.02	220.28
13	0	-1	-1	58.12±0.01	58.01	283.15±0.01	280.47
14	0	1	-1	62.59±0.02	62.58	222.24±0.02	223.39
15	1	-1	0	62.16±0.01	62.14	274.21±0.02	276.66
16		-1	1	60.55±0.02	60.57	253.24±0.01	252.09
17	0	0	0	68.60±0.02	68.68	212.77±0.03	220.28

Data are expressed as means ± standard deviation (n = 3).

Analysis of response surface generated by the BBD model

The polynomial equation 3, graphically represented a three-dimensional response contour plot for TPC. From the three-dimensional response surface shown in **Figure 1 (a, b, c)** the effect of the significant extraction parameters and their mutual interaction on TPC can be seen.

The 3-D response surface plot based on **microwave power** and **extraction time** for TPC is shown in **Figure 1a.** A gradual increase in microwave power coupled with a lesser extraction time has resulted in a gradual increase in the TPC value. Therefore, both these factor and their interactive effects do carry some significance which has been supported by the ANOVA data (**Supplementary Table 2**).

This phenomenon is considered to be caused by a gradual rate of phenolic mass transfer from the ruptured cells with increasing microwave power settings. A similar argument can be postulated for extraction time as well. TPC was observed to be more with microwave power between 400-450 Watts and extraction time between 11-14 min.

Any setting above this range has not improved the TPC due to the fact that an increased temperature environment might have induced an equilibrium in phenolic mass transfer from the ruptured cells. Moreover, degradation of total phenolics at elevated temperature also cannot be ruled out. This suggests that a mid-range microwave power and a short extraction time are effective in extracting phenolic compounds. A similar observation was also reported by **Li *et al.* (2012).**

Figure 1b depicts a 3-D response surface plot for the TPC with varying **microwave power** and **ethanol concentration**. When the ethanol concentration was at 50-70% and the microwave power at 400 – 450 Watts, the TPC value increased. On the other hand, when microwave power and ethanol concentration went higher there was no significant effect on the TPC. This fact is supported by the fact that the ANOVA data also showed that the interactive effect between microwave power and ethanol concentration on TPC was insignificant (p-0.1246) as seen in (**Supplementary Table 2**).

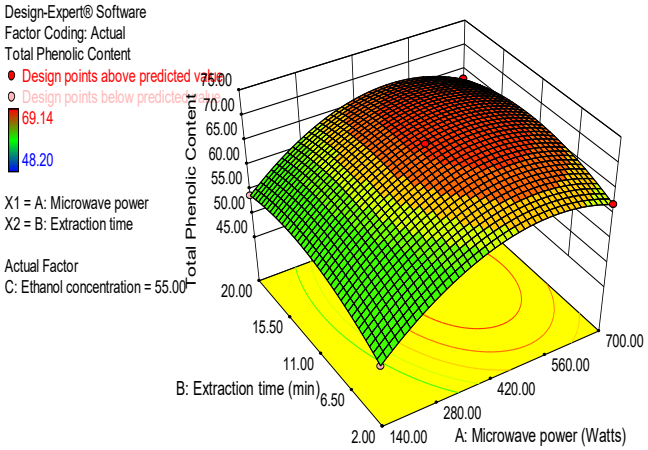
As evident from **Figure 1c**, the TPC value increased initially, but became a plateau afterward when the **ethanol concentration** and **extraction time** increased. It is clear that with an increase in ethanol proportion over 80 % (v/v), the total phenolic content gradually decreased. Solubility of phenolics could be enhanced using an aqueous ethanol over a limited compositional range. In general, it was found that ethanol concentration that ranged from 40 to 80 % had a better efficiency in the extraction of phenolics compared to 100% ethanol concentration. (**Hayouni *et al.*, 2007**). From the industrialization point of view, low ethanol concentration with shorter extraction time would be more appropriate as long extraction period makes the extraction process time consuming, uneconomical and cumbersome.

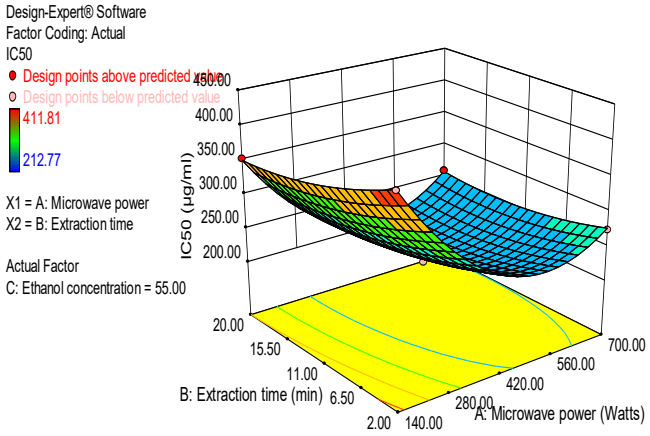
The polynomial equation 4, graphically represented a three-dimensional response contour plot for IC_{50} i.e. antioxidant potential. From the three-dimensional response surface shown in **Figure 2 (a,b,c)**, the effect of the significant extraction parameters and their mutual interaction on IC_{50} can be seen.

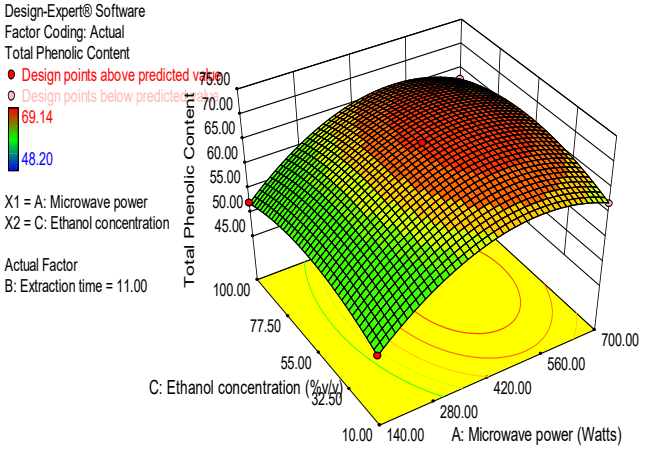
The 3-D response surface plot based on **microwave power** and **extraction time** for IC_{50} is shown in **Figure 2a.** A controlled and gradual increase in microwave power and extraction time has resulted in a decreased IC_{50} value thus signifying a better antioxidant potential. Therefore, both these factor and their interactive effects must be having some significant influence ($p = 0.0015$) on the final outcome and the same has been supported by the ANOVA data (**Supplementary Table 2**).

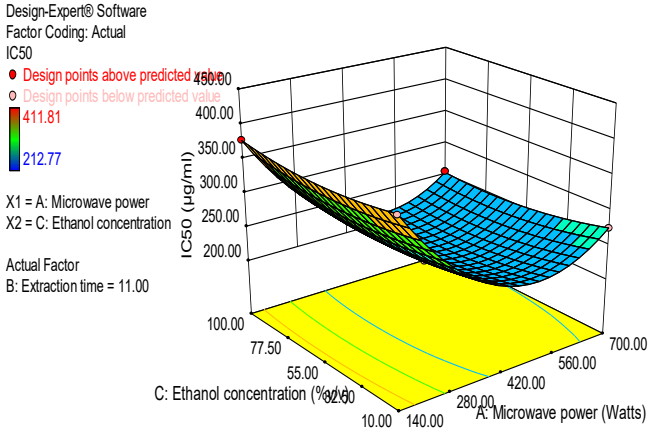
Figure 2b depicts a 3-D response surface plot for the IC_{50} with varying **microwave power** and **ethanol concentration**. It was observed that when the microwave power is in the range of 400 – 450 Watts, the antioxidant potential which is denoted IC_{50} has shown a lower value (downward curve) signifying a better antioxidant potential. With ethanol concentration no significant improvement in antioxidant potential was observed when we varied the ethanol concentration. Moreover, when very high microwave power setting and ethanol concentration was used there was no significant effect on the IC_{50}. High temperature environment might have degraded the phenolic compounds present within the sample. A similar observation was reported by **Hayet *et al.*, 2019, Routray *et al.*, 2012** and **Lovric *et al.* (2017**). This fact is also supported by ANOVA data which showed that the interactive effect between microwave power and ethanol concentration on IC_{50} was insignificant (p=0.0710) as seen in (**Supplementary Table 2**).

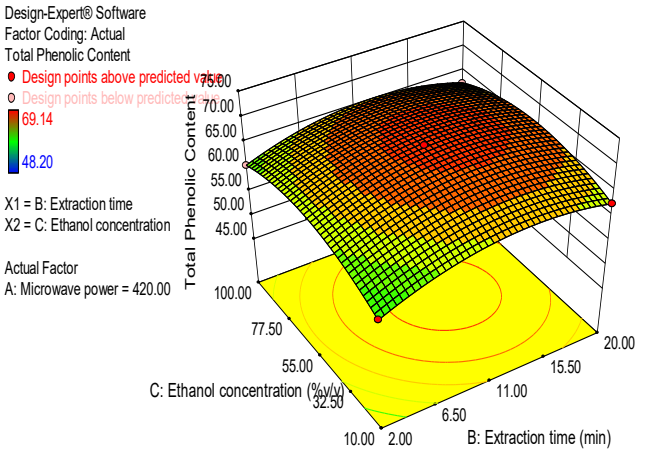
As evident from **Figure 2c**, a low value in IC_{50} (signifying high antioxidant potential) reading was observed with 70% – 100% ethanol concentration along with an extraction time frame of 10 – 15 min. It can be hypothesized that when more water is present with ethanol, then it will extract out water soluble non-phenolic components like carbohydrates which has affected IC_{50} value and has lowered the antioxidant potential. A similar observation was reported by and **Septiana *et al.*, 2022** and **Do *et al.*, 2014.** Thus, these two factors have some impact on the antioxidant potential and is supported by ANOVA result where their interactive effect is shown as significant ($p = 0.0085$).











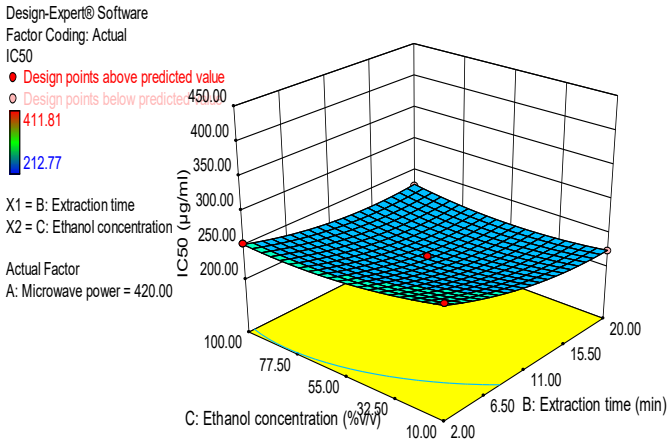


Figure 1 Response surface plots showing the effects of Microwave power vs Extraction time (a) , Microwave power vs Ethanol conc. (b) and extraction time vs ethanol concentration(c) on TPC estimation.

Figure 2 Response surface plots showing the effects of Microwave power vs Extraction time (a)Microwave power vs Ethanol conc. (b) and extraction time vs ethanol concentration(c) on IC50 estimation.

From this graphical optimization step, a region of prime interest that represents the probable zone of maximum TPC and low IC_{50} was obtained including the level of the extraction parameters involved in the process and is shown in **Figure 3a, b, c** as overlay plots. When all the experimental data were analyzed, it predicted that the TPC and IC50 are 69.43 mg GAE/ g of DW and 211.32 µg / ml respectively with a microwave power setting of 457.33 watts, extraction time of 12.20 min and ethanol concentration of 61%.

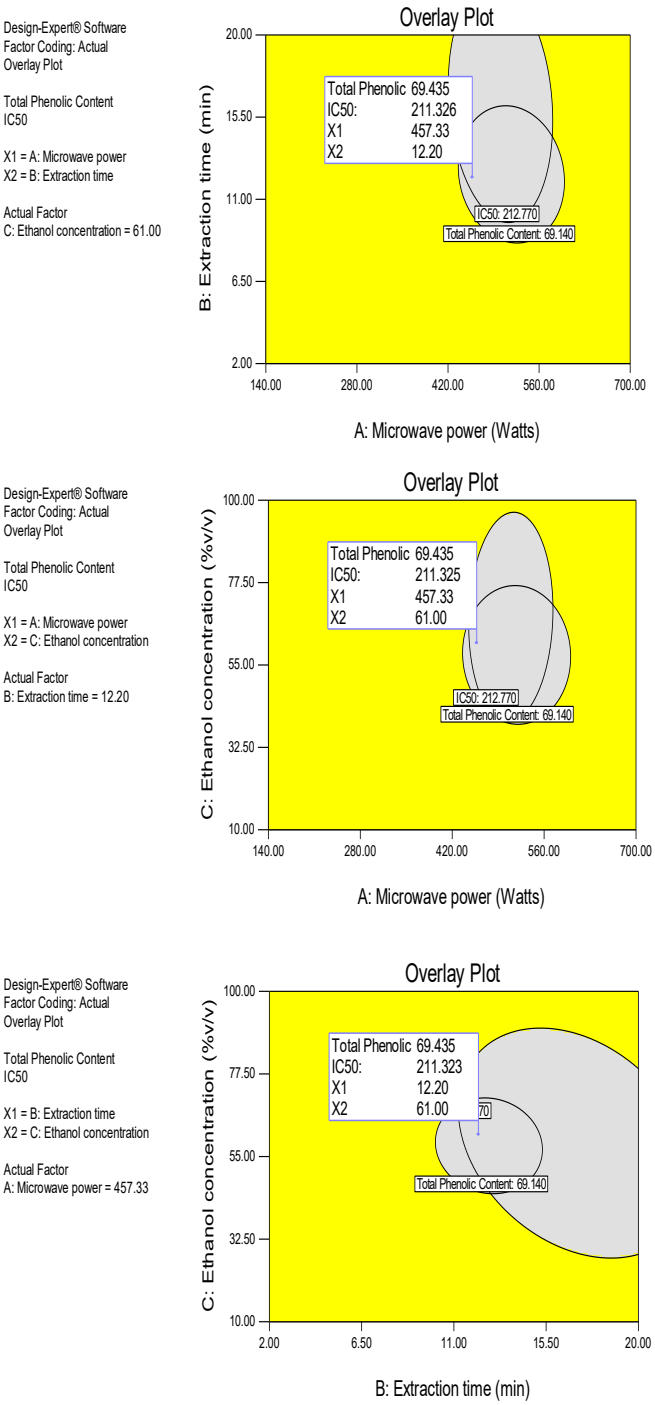


Figure 3 The overlay plots showing region of maximum TPC and optimum IC_{50} value. (a) Microwave power vs Extraction time, (b) Microwave power vs ethanol conc. (c) Extraction time vs ethanol conc. TPC and IC 50 of 69.43 mg GAE/ g of DWand 211.32 µg / ml respectively with microwave power of 457.33, extraction time of 12.20 min and ethanol conc of 61%

Validation of the MAE process

After this we need to ascertain whether the graphical optimization predicted extraction conditions are actually reproducible and repeatable or not in a laboratory set up. So, a validation experiment was carried out by slightly altering the conditions to check the reproducibility of the process. The experimental value for TPC and antioxidant activity as obtained under the modified experimental condition during validation study were found to be 68.53 mg gallic acid equivalent/ g (TPC) and 213.90 µg / ml (IC_{50}/antioxidant assay), respectively which were close to the software predicted values as shown in **Supplementary Table 3**.

A relative standard deviation (%RSD) was calculated to gather some idea about uncertainty estimates. For precision study, repeatability of the optimized method is always measured in terms of % RSD (relative standard deviation). The precision of the method was considered satisfactory as the %RSD was found to be less than 2% (**Nhavale *et al.*, 2024**)

The calculated % RSD value of 0.92% for TPC and 0.86% of the antioxidant activity between the experimental and software predicted results showed that the proposed microwave extraction method has a satisfactory precision level and the optimization study was reliable as well.

The optimized condition predicted a TPC of 69.43 mg GAE/g DW and an IC_{50} of 211.32 µg/ml. Validation experiments under modified conditions yielded 68.53 ± 0.21 mg GAE/g DW and 213.90 ± 1.06 µg/ml (n = 3). The 95% confidence intervals were 68.02–69.04 mg GAE/g DW for TPC and 211.26–216.54 µg/ml for IC_{50}, confirming that the optimization outcomes are statistically robust and reproducible.

Comparison of MAE with other extraction techniques

In this study, MAE with its optimized levels of extraction parameters was compared with Soxhlet and ultrasound assisted extraction method and the corresponding TPC and IC_{50} values were reported. From **Supplementary Table 4** it can be seen that the best result was obtained with MAE, which gave higher output value when compared with Soxhlet and ultrasound assisted extraction method. Statistical differences among extraction methods (MAE, UAE, and Soxhlet) were evaluated using one-way ANOVA (single factor) at $\alpha = 0.05$, followed by Tukey's HSD post-hoc test. The analysis confirmed that MAE yielded significantly higher total phenolic content (TPC) and lower IC_{50} values compared to UAE and Soxhlet ($p < 0.05$), thereby validating the superiority of MAE beyond descriptive comparison. MAE was found to be about 50 % and 100 % more efficient than ultrasound and Soxhlet extraction respectively in extracting out total phenolic and antioxidant principles from fig fruits.

Environmental impact of the extraction techniques: A carbon foot print analysis

For industrial viability, energy requirements, and carbon emissions are important parameters to be evaluated. Therefore, energy consumption and carbon emissions values were calculated in the present study. The electrical energy consumption required to perform MAE extraction at 457 W for 12 min was 0.0914 KWh, UAE at levels of 300W for 30 min was 0.15 kWh, while Soxhlet extraction using a heating mantle of 500 W for 180 min was 1.5kWh. Meanwhile the CO_2 emission occurred during MAE, UAE and Soxhlation were 0.112 kg, 0.12 kg and 1.2 kg respectively.

From this analysis it can be concluded that the proposed MAE is more energy efficient in extracting out phenolics with less electrical consumption and carbon emission into the environment.

Scanning electron micrographs (SEM) study

To study the surface morphology and microstructure of the sample after each extraction techniques, the samples were examined by scanning electron microscope. **Supplementary Figure 3a-d** represents the micrographs of the microwave treated sample (a), ultrasound treated sample (b), Soxhlation (c) and untreated sample (d).

SEM micrographs revealed visible pore openings and cell-wall disruption in MAE-treated samples compared to UAE and Soxhlet. These observations are presented qualitatively and are consistent with improved extraction efficiency. As no quantitative image analysis was performed, the mechanistic interpretation remains descriptive, serving as supportive visual evidence rather than definitive proof of structural changes.

In MAE the surface of the sample was greatly destroyed with complete loss in cell wall structure and its arrangements followed by massive widening or opening up of the cellular pores (Supplementary Figure 3 a). Microwave irradiation induces a rapid rise in temperature and an increase in internal pressure, which causes the cellulosic material in the cell wall to undergo dehydration. This process reduces the mechanical integrity of the cell wall, thereby enabling the extracting solvent to more easily infiltrate the cellular pores. (**Mandal *et al.*, 2009**).

The voids which got created after ultrasound treatment in the medium are the cavitation bubbles which upon collapsing near the cell envelope produce a shear force thus improving phenolic transfer from inside of the cell towards outside into the solvent outside. As shown in Supplementary **Figure 3b**, perforations were produced into the internal cellular structures due to localized pressure and thermal stress after ultrasound treatment. Similar findings were also reported for the extraction from caraway seeds (**Chemat *et al.*, 2004**) and rosemary leaves (**Yokozawa *et al.*, 1998**).

The changes observed for Soxhlet extraction (Supplementary Figure 3c) were slight ruptures on the surface of the sample accompanied by a slight widening of the cellular channels in comparison to the untreated sample (Supplementary Figure 3c).

Observations made through scanning electron microscopy reveal that microwave energy can significantly alter the structure of plant cell walls. This alteration facilitates the release of internal contents, as the rapid heating and pressure build-up associated with microwave radiation lead to cell wall rupture, thereby streamlining extraction processes. Similar observations were reported by several

researchers while working with microwave assisted extraction (**Nomanbhay *et al.*, 2017; Ghazanfari *et al.*, 2020**).

DISCUSSION

To maximize the extraction of active ingredients from plants, the choice of extraction method is very important. In recent years, considerable efforts have been made by researchers from diverse backgrounds to optimize the extraction process (**Pan *et al.*, 2003**). At present, MAE, a new extraction technology is widely used to extract the active ingredients from plants (**Jafarifar *et al.*, 2005**). On a macroscopic level, microwave energy penetrates evenly into the extraction mixture, and, microscopically, microwave heating generates an electromagnetic field that can accelerate the diffusion of analytes and increase the efficiency of the extraction (**Chen *et al.*, 2015**).

Apart from the extraction method, the choice of analysis methods is also vital. Here RSM was applied as a statistical tool to analyze the polyphenol extraction performance of the proposed MAE. Response surface methodology is a highly accurate and a good optimization tool which is suitable for optimization of process parameters.

In this paper, the microwave-assisted procedure for the extraction of phenolics from *Ficus racemosa* fruits was optimized using RSM, aiming to maximize TPC extraction while minimizing both the processing time and solvent consumption. The optimized condition yielded high amount of TPC, at a quicker processing time compared to other extraction methods.

Predictive model equations of RSM was used to describe the relationship between the response (i.e. TPC and antioxidant potential) and various extraction parameters like microwave power, extraction time and solvent concentration, This has allowed us to predict the optimal conditions to maximize the desired analyte extraction and eventually had helped in establishing a relationship between the response and extraction parameters as well as how parameters amongst themselves are interacting to improve the outcome of the experiment.

ANOVA result showed that microwave power has emerged as the most significant single factor that influenced the experimental outcome, followed by irradiation time and ethanol concentration. Moreover, in both TPC and IC50 assay the interactions of microwave power × extraction time (TPC, p=0.0002; DPPH assay, p= 0.0015) and extraction time × ethanol concentration. (TPC, p=0.0085; DPPH assay, p= 0.0085) was found to have significant effect, whereas the interaction of microwave power × ethanol concentration for both TPC and DPPH assay was insignificant (TPC, p=0.1246; DPPH assay, p=0.0710).

3D response surface models generated by RSM showed that the amount of TPC extracted from the sample was on the lower side initially when the microwave power was at lower levels (140 W – 350 W) and took a plateau phase towards the end where higher level of microwave power (more than 550 W) was applied. This is due to the fact that lower temperature environment can't break open the cells to release the phenolic compounds (both bound and unbound phenolics) and on the contrary a higher temperature environment might have caused the degradation of phenolic compounds, resulting in a lower yield of extracted phenolics which ultimately will lower the antioxidant potential (indicated as higher IC_{50} value).

Similar observations were seen with extraction time also. A shorter extraction time means less contact time with the sample and solvent which ultimately will affect the TPC yield and shall always show a higher IC_{50} value (means less antioxidant potential). So, a moderate heating coupled with an optimum extraction time is required for better phenolic extraction with an improved antioxidant potential. These observations are in agreement with the findings of other researchers also (**Jeong *et al.*, 2004; Xu *et al.*, 2007 and Hayat *et al.*,2010**).

The three significant extraction factors along with their optimum level as obtained from RSM were microwave power (457 W), extraction time (12.20 min) and ethanol concentration (61%) with TPC and IC_{50} of 69.43 mg gallic acid equivalent/ g and 211.32 µg / ml respectively.

Later on, a validation experiment was done to check the reproducibility of the proposed MAE process and the predicted optimized value for the extraction parameters. The outcome of the validation experiment was satisfactory as there was no major deviation from the RSM predicted result and was measured in term of %RSD which was found to be less than 2%. Henceforth it can be proposed that the MAE method has a good precision and the optimization study was reliable as well.

To evaluate the performances of MAE, it was compared to Soxhlet and ultrasound assisted extraction method for the estimation of TPC and IC_{50}. MAE was found to be almost 50% and 100 % more efficient than ultrasound and Soxhlet extraction respectively in extracting out total phenolic principles from fig fruits. With respect to extraction time, MAE was the fastest extraction method with only 12.20 min of extraction time. Thus, MAE was found to be more effective when compared to Soxhlet and ultrasound assisted extraction. Another advantage of MAE resides mainly due to its applicability to a wide range of sample types (solid, semi-solid, and liquid matrices) because the selectivity can be easily manipulated by altering solvent polarities. It has been reported that MAE is well suited for the extraction of phenolics at relatively high temperatures (110–150 °C) which is a critical feature to handle antioxidants without degradation (**Grigonis *et al.*, 2005**). In a study, it emerged that MAE allowed higher polyphenols recoveries compared to conventional techniques without altering the antioxidant potential of the extracts (**Spigno and De Faveri, 2009**).

This study has a practical application in preparing a phenolic rich fraction from *Ficus racemosa* fruits using microwave energy, which can be a firsthand information for nutraceutical industry and phytochemical research. Analyzing energy consumption is vital for any technology that aspires to reach industrial scale. Henceforth the total energy consumption figures were calculated by examining the electricity usage of each technology involved and corresponding carbon emission was also analyzed.

The environmental comparison in this study was limited to electrical consumption and estimated CO_2 emissions only. While these parameters indicate that MAE requires less energy and generates lower emissions under the tested laboratory conditions, a comprehensive life-cycle assessment including solvent use, waste generation, and scalability would be necessary to fully establish its environmental sustainability. Therefore, MAE can be considered a relatively energy-efficient technique within the scope of this preliminary analysis.

This study, while offering valuable insights into microwave-assisted extraction of phenolics from *Ficus racemosa* fruits, has certain boundaries that should be noted. The analysis focused on total phenolic content and antioxidant activity, without extending to compound-level profiling of individual phenolics. Biological validation beyond in-vitro antioxidant assays was not included, which leaves scope for future exploration of physiological relevance. Similarly, SEM observations were qualitative in nature, providing supportive visual evidence but not quantitative image analysis. Finally, the environmental assessment was limited to electrical consumption and estimated CO_2 emissions, serving as a preliminary comparison of extraction methods. Future investigations incorporating chromatographic characterization, biological assays, quantitative imaging, and comprehensive life-cycle environmental analysis would further strengthen the applicability and sustainability of this approach.

CONCLUSIONS

he current research focused on optimizing a MAE to for obtaining a phenolic rich fraction from *F. racemosa* fruits. The results collectively affirm that MAE is a viable energy efficient methodology for obtaining phenolics with considerable antioxidant potential. A second-order regression model for predicting the TPC at varying ethanol concentration, Microwave power, and extraction time was developed, with a high R^2 value of 0.99. The diagnostic 3 D response surface plots for the generated model demonstrated good agreement between the predicted and experimentally obtained TPC, proving the validity of the predictive model. Analysis of the 3D response plots derived from the model revealed the presence of significant interactions among the three parameters. The optimized conditions for the MAE parameters were successfully established whereby microwave power of 457 Watts, irradiation/extraction time of 12.20 min and ethanol concentration of 61.0% v/v is giving a TPC and IC_{50} values of 69.43 mg GAE / g DW and 211.32 µg /ml respectively.

Upon comparison with other extraction techniques, MAE was found to more effective than other techniques with less energy consumption and carbon emission. The electrical energy consumption required to perform MAE extraction at 457 W for 12 min was 0.0914 KWh, UAE at levels of 300W for 30 min was 0.15 kWh, while Soxhlet extraction using a heating mantle of 500 W for 180 min was 1.5kWh. Meanwhile the CO_2 emission occurred during MAE, UAE and Soxhlation were 0.112 kg, 0.12 kg and 1.2 kg respectively. Thus, MAE can be a safe alternative to extract out phenolics from various plant sources. Therefore, within the scope of this preliminary analysis, MAE can therefore be considered a comparatively energy-efficient and promising option for valorizing this underutilized fruit."

Overall, this work provides preliminary but promising evidence that microwave-assisted extraction is an energy-efficient strategy for enhancing phenolic yield from *Ficus racemosa* fruits, while recognizing that further compound-level profiling, biological validation, and comprehensive environmental assessment are needed to fully establish its broader applicability.

Acknowledgements: We are thankful to the Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India; Assam University, Silchar, India for providing the adequate facilities and basic equipment to carry out the work

REFERENCES

Anonymous. The Wealth of India. Council of Scientific and Industrial Research, New Delhi, India, 1952, pp.35-36.

Al-Fatimi, M., Wurster, M., Schröder, G., & Lindequist, U. (2007). Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethnopharmacology*, 111(3), 657–666. https://doi.org/10.1016/j.jep.2007.01.018

Beejmohun, V., Fliniaux, O., Grand, É., Lamblin, F., Bensaddek, L., & Christen, P.(2007). Microwave-assisted extraction of the main phenolic compounds in flaxseed. *Phytochemical Analysis,* 18(4), 275-282. https://doi.org/10.1002/pca.973

Bhandari, P., Kumar, N., Singh, B., & Ahuja, P.S. (2010) Online HPLC-DPPH method for antioxidant activity of Picrorhiza kurroa Royle ex Benth. and

characterization of kutkoside by Ultra-Performance LC-electrospray ionization quadrupole time-of-flight mass spectrometry. *Indian Journal of Experimental Biology,* 48,323-328.

Chemat, S., Lagha, A., AitAmar, H., Bartels, P.V., & Chemat, F.(2004). Comparison of conventional and ultrasound-assisted extraction of carvone and limonene from caraway seeds. *Flavour and Fragrance Journal,*19(3),188–195. https://doi.org/10.1002/ffj.1339

Das, A.K., Mandal, V., Mandal, S.C.(2012).Design of Experiment (DOE) approach for the process optimization of microwave assisted extraction of Lupeol from *Ficus racemosa* leaves using Response surface methodology. *Phytochemical Analysis*, 24(3):230-47. https://doi.org/10.1002/pca.2403

Do, Q.D., Artik Angkawijaya, A.E., Tran-Nguyen, P.L, Huynh, L.H., Soetaredjo, F.E., Ismadji, S., and Ju. Y. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica. Journal of Food and Drug Analysis*. 22, pp.296-302 https://doi.org/10.1016/j.jfda.2013.11.001

Ferhat, M.A., Chemat, F., Meklati, B.Y., Smadja, J.(2006) . An improved microwave clevenger apparatus for distillation of essential oils from orange peel. J. Chromatogr. A 1112, 121–126 https://doi.org/10.1016/j.chroma.2005.12.030

Ghazanfari, N., Mortazavi, S.,Tabatabaei Yazdi, F.,Mohammadi, M.(2020). Microwave-assisted hydrodistillation extraction of essential oil from coriander seeds and evaluation of their composition, antioxidant and antimicrobial activity. *Heliyon*,6(9), e04893 https://doi.org/10.1016/j.heliyon.2020.e04893

Grigonis, D., Venskutonis, P.R., Silvik, B., Sandahl, M., Eskilsson, C.S. (2005). Comparison of different extraction techniques for isolation of antioxidants from sweet grass (Hierochloe odorata). *The Journal of Supercritical Fluids* 33(3),223–233https://doi.org/10.1016/j.supflu.2004.08.006

Hayat, K & Abbas, S. (2019). Effect of microwave and conventional oven heating on phenolic constituents, fatty acids, minerals and antioxidant potential of fennel seed. Industrial Crops and Products 140, 111610, https://doi.org/10.1016/j.indcrop.2019.111610

Hayouni, E. A., Abedrabba, M., Bouix, M., & Hamdi, M. (2007). The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chemistry*, 105(3), 1126−1134. https://doi.org/10.1016/j.foodchem.2007.02.010

Jahan, I.A., Nahar ,N., Mosihuzzaman, M., Begum, R., Ali, L., Azad Khan, A.K., Makhmur,T., & Iqbal Choudhary, M.(2009). Hypoglycaemic and antioxidant activities of *Ficus racemosa* L. Fruits. *Natural Product Research*, 23(4), 399–408. https://doi.org/10.1080/14786410802230757

Karacabey, E., & Mazza, G. (2010). Optimisation of antioxidant activity of grape cane extracts using response surface methodology. *Food Chemistry,* 119(1), 343–348 https://doi.org/10.1016/j.foodchem.2009.06.029

Lansky, E.P., & Paavilainen, H.M. (2011). Figs : the genus Ficus. (Traditional herbal medicines for modern times ; v. 9). Florida (USA): CRC Press, Taylor & Francis Group, (Chapter 3). https://doi.org/10.1201/9781420089677

Li,Hongyan., Deng, Zeyuan., Wu, Tao., Liu ,Ronghua., Loewen ,Steven., & Tsao, Rong.(2012) Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. *Food Chemistry*, 130(4),928–936. https://doi.org/10.1016/j.foodchem.2011.08.019

Liyana-Pathirana, C., & Shahidi, F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry*, 93(1), 47−56. https://doi.org/10.1016/j.foodchem.2004.08.050

Lovric, V., Predrag, P., Danijela B.K., Marijana, J. and Verica D. (2017). Effect of Microwaveassisted extraction on the phenolic compounds and antioxidant capacity of blackthorn flowers. *Food Technol. Biotechnol*. 55(2), 243–250 doi: 10.17113/ftb.55.02.17.4687

Manian, R., Anusuya, N., Siddhuraju, P., & Manian, S. (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chemistry*, 107(3), 1000–1007. https://doi.org/10.1016/j.foodchem.2007.09.008

MacDonald, S., Prenzler, P.D., Autolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, 73(1), 73- 84. https://doi.org/10.1016/S0308-8146(00)00288-0

Nhavale G, Godge R, Patel A, Barde K. (2024). Quality by Design Approach for the Stability Indicating Method Development and Validation of Selpercatinib Drug Formulation by using RP-HPLC. *International Journal of Pharmaceutical Quality Assurance*.15(2):580-587

Nomanbhay, S.; Salman, B.; Hussain, R.; Ong, M.Y.(2017). Microwave pyrolysis of lignocellulosic biomass—A contribution to power Africa. Energy Sustain. Soc.7, 23. 10.1186/s13705-017-0126-z

Rao, Ch.V., Verma, A.R., Vijayakumar, M., & Rastogi, S.(2008). Gastroprotective effect of standardized extract of *Ficus glomerata* L fruit on experimental gastric ulcers in rats. *Journal of Ethnopharmacoogy,* 115(2), 323–326. https://doi.org/10.1016/j.jep.2007.09.019

Routray W., Orsat V. (2012). Microwave-Assisted Extraction of Flavonoids: A Review. *Food Bioprocess Technology* 5:409–424. https://doi.org/10.1007/s11947-011-0573-z

Septiana, A.T., Wuryatmo, E. 2022. Effect of ethanol concentration and extraction time with microwave assisted extraction on antioxidant activity of temulawak-extract *(Curcuma xanthorrhiza.roxb)*. J. Functional Food & Nutraceutical, 3(2), pp.63-69 https://doi.org/10.33555/jffn.v3i2.86

Song, J., Li, D., & Liu,C. (2011) Optimized microwave-assisted extraction of total phenolics (TP) from Ipomoea batatas leaves and its antioxidant activity. *Innovative Food Science & Emerging Technologies,* 12(3), 282-287. https://doi.org/10.1016/j.ifset.2011.03.001

Spigno, G., De Faveri, M. (2009). Microwave-assisted extraction of tea phenols: a phenomenological study. *Journal of Food Engineering* 93(2), 210–217. https://doi.org/10.1016/j.jfoodeng.2009.01.006

Sutivisedsak, N., Cheng, H. N., Willett, J. L., Lesch, W. C., Tangsrud, R. R., & Biswas, A. (2009). Microwave-assisted extraction of phenolics from bean (*Phaseolus vulgaris* L.). *Food Research International*, 9(2), 143-149. https://doi.org/10.1016/j.foodres.2009.09.014

Sun, Y., Xu, W., Zhang,W., & Qiuhui. (2011) Optimizing the extraction of phenolic antioxidants from kudingcha made frrom Ilex kudingcha C.J. Tseng by using response surface methodology. *Separation and Purification Technology*, 783(3), 311-320 . https://doi.org/10.1016/j.seppur.2011.01.038

Talebpour Z, Ghassempour A, Abbaci M, Aboul-Enein HY. Optimization of Microwave-Assisted Extraction for the Determination of Glycyrrhizin in Menthazin Herbal Drug by Experimental Design Methodology. Chromatographia. 2009;70(1):191-197. 10.1365/s10337-009-1146-4

Trivedi, C.P., Shinde, S., & Sharma, R.C., 1969. Preliminary phytochemical and pharmacological studies on *Ficus racemosa* L. extract (Gular). *Indian Journal of Medical Research,* 57(6), 1070–1074.

Verma, A.R., Vijayakumar, M., Rao, C.V., & Mathela, C.S. (2010). *In vitro* and *in vivo* antioxidant properties and DNA damage protective activity of green fruit of *Ficus glomerata* L. *Food Chemical Toxicology*, 48(2), 704-709. https://10.1016/j.fct.2009.11.052

Yokozawa, T., Chen, C.P., Dong, E., Tanaka, T., Nonaka, G.I, & Nishioka, I. (1998). Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2- picrylhydrazyl radical. *Biochemical Pharmacology,* 56(2) , 213–222. https://doi.org/10.1016/S0006-2952(98)00128-2

Zhang, Z.S., Li, D., Wang, L.J., Ozkan, N., Chen, X.D., & Mao, Z.H.(2007).Optimization of ethanol-water extraction of lignans from flaxseed. *Seperation and Purification Technology*, *57(1), 17–24.* https://doi.org/10.1016/j.seppur.2007.03.006