BBD Driven Optimization of Extraction of Therapeutically Active Xanthanoid Mangiferin from Mangifera indica L. Leaves and its Antioxidant Activity

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ABSTRACT
Background: Mangiferin, a C-glucosyl xanthone present in Mangifera indica leaves exhibits profuse pharmacological activities. Our research highlights the process parameter at which high yield of Mangiferin can be extracted from Mangifera indica leaves in “one run.” Objectives: The study compares the efficacy of different modern and traditional methods for mangiferin extraction. Box-Behnken Design (BBD), was employed for optimizing the process parameters for the Mangiferin extraction from Mangifera indica leaves.

Materials and Methods: Extraction conditions (extraction temperature, drug to solvent ratio and extraction time) were optimized by Response-Surface Methodology (RSM), specifically BBD. Quantification analysis of Mangiferin in different extracts was done using HPLC. Further, the antioxidant potential of M. indica extracts in different solvents were evaluated using DPPH method.

Results: Reflux technique, a hot solvent extraction method, conferred the highest yield of Mangiferin and ethanol was found to be the most efficient extractive solvent. Through the use of BBD, the optimal conditions for mangiferin extraction were established as extraction time 63.653 min, extraction temperature 63.563°C and drug to solvent ratio 1:22.634 g/ml. Under such conditions, Mangiferin was yielded as 90.31 mg/g, which was nearly close to the predicted value of 91.096 mg/g. The ethanolic extract has revealed significant antioxidant potential with a percentage inhibition of 59.76 %.

Conclusion: The reflux technique stood out to be the best amongst all the other thermal and non-thermal modes of extraction used, and ethanol proves to be the most efficient extracting solvent. Additionally, Mangiferin extraction was significantly affected by all three different variables. Our study highlights the use of RSM, a modern-day statistical technique in the extraction field of therapeutically potent phyto compounds, which makes the optimization method cheap and less laborious than the traditional optimization method.

Keywords: Mangiferin, Mangifera indica, Extraction, Optimization, Response surface methodology.

INTRODUCTION

Mangifera indica (Linn.), commonly kenned as Mango, belongs to Anacardiaceae family. It is a prime herb in indigenous and ayurvedic medical systems. As claimed by Ayurveda, diversified medicinal properties are ascribed to various parts of mango tree.[1] Mango possesses anti-inflammatory, anti-diabetic, hypotensive, anti-oxidant, cardiotoxic and anti-viral activities.[2,3] Various effects like anti-tumor, antibacterial, anthelmintic, antifungal, antiparasitic, anti-HIV, antispasmodic, anti-bone resorption, anti-diarrhoeal, antipyretic, antiallergic, hypolipidemic, immunomodulation, antimicrobial, gastroprotective, and hepatoprotective have also been explored.[4-6] Various bio-active constituents have been identified by researchers viz. mangiferone, Mangiferin, quercetin, rutin, myricetin, myricitrin, etc. However, Mangiferin is the main bioactive constituent amongst them.[7]


Owing to its health-endorsing properties, ergo can be availed as a propitious candidate in research and development areas.

*Mangifera indica* is the central hub for the isolation of Mangiferin. However, it can also be extracted from other plants like *Iris unguicularis, Salacia chinensis, Cyclopi genusoides, Anemarrhena asphodeloides* rhizomes, *Bombax ceiba* leaves, as well as from coffee leaves.[25,26]

In these circumstances, where the phytocompounds manifest vast therapeutic potential, optimizing its extraction parameters becomes significant. The maximum amount of the therapeutically active constituents can be extracted from the plants in a single run.

Traditionally, Optimization is carried out using “one-factor-at-a-time” method, wherein one factor is varied at a time. Howbeit, this method is laborious, time-consuming, and requires more amount of solvent. Moreover, it fails to study the interaction of different variables. Therefore, other techniques like Response surface methodology (RSM) have come into play, a statistical, mathematical technique introduced by Box and Wilson in the year 1951 for the purpose of analysing and modeling any process wherein the response of interacting variable is dependent on different variables. RSM can be effectively operated wherein different combinations of the input variables (for example, extraction time, extraction temperature, and pH) are specified, and their effect in the response (quantity of phytocompound) is determined.[26,37]

RSM is time-saving and less laborious and helps in studying the interactive effects of different variables and then overcomes the drawbacks associated with traditional optimization methods.[27] The multivariate technique has been copiously exploited by the researchers for the purpose of optimization of extraction parameters of various phytocompounds like atropine from *Atropa balleldona,[28]* carthamin from *Carthamus tinctorious,[29]* embelin from *Emuica ribes,[30]* flavonoids from *Vitis vinifera,[31]* glycyrrhizinic acid from *Glycyrriza glabra,[32]* karanjin from *Pongamia pinnata,[33]* lupeol from *Ficus racemosa,[34]* quercitrin from *Herba polygoni capitati[35]* and baikalin from *Oroxylum indicum.[36]*

Our current study utilizes RSM to optimize the extraction parameters (drug to solvent ratio, extraction temperature, extraction time) of Mangiferin from *Mangifera indica* leaves, and quantification of the phytoactive constituent is done with the help of HPLC. The Optimization of Mangiferin extraction parameters from *Mangifera indica* leaves via Ultrasound extraction technique was reported by Tang-Bin Zou et al. in 2014.[39] However, our study employs different techniques like soxhlet, maceration, reflux technique, and UAE for the extraction of Mangiferin, which has not been reported by any other researcher yet.

**MATERIALS AND METHODS**

**Collection and authentication of the Plant Material**
The leaves of *Mangifera indica* were acquired from the Herbal Garden, Jamia Hamdard, New Delhi, India. Authentication of the leaves was made by a Taxonomist and for further reference, the voucher specimen was preserved.

**Chemicals**
Standard Mangiferin was acquired from Sigma Aldrich. HPLC grade acetonitrile, water and o-phosphoric acid were purchased from S.D. Fine Chemicals, Mumbai, India. All other chemicals were of analytical grade and obtained from S.D. Fine Chemicals, Mumbai, India.

**Preparation of Plant Material**
The leaves were thoroughly cleansed to eliminate all the cling foreign material and dust particles and washed under running water. Further, they were dried at room temperature, powdered and passed through 40 mesh sieves, and stored in an air-lock container.

**Extraction of Mangiferin**
Different solvents - acetone, ethanol, dimethylformamide (DMF), and dimethyl sulphoxide (DMSO) of varying polarity were employed to extract Mangiferin from *Mangifera indica* using four different modes of extraction viz. reflux, soxhelation, maceration, and ultrasound-assisted extraction.

**Soxhlet Extraction**
Extraction was carried out using a soxhlet apparatus (continuous hot solvent extraction) at 50°C for 1 hr using the solvent-to-drug ratio- 10 ml/g. After extraction, the plant residue was filtered, and a rotary evaporator dried the filtrate under a vacuum.[40]

**Reflux Extraction**
The extraction process was carried out in a reflux apparatus (hot solvent extraction method) using 50 ml solvent at 50°C for 1 hr with solvent-to-drug ratio- 10 ml/g. After extraction, the plant residue was filtered, and a rotary evaporator dried the filtrate under a vacuum.

**Ultrasound-Assisted Extraction**
Extraction was done using ultrasound-assisted extraction (UAE) method for 1 hr using the solvent-to-drug ratio- 10 ml/g at 50°C in a sonicator (TOSCHON, SW7). After extraction, the plant residue was filtered, and a rotary evaporator dried filtrate under a vacuum.

**Extraction by Maceration**
Two grams of the powdered drug were taken in a beaker and soaked in 20 ml of solvent for 72 hr at room temperature.
(solvent-to-drug ratio- 10 ml/g). The menstruum was filtered in a china dish, and the filtrate was allowed to evaporate at room temperature to obtain a brownish-colored sticky mass. The extract was then stored for further analysis.

Comparison of Different Extraction Techniques for Extraction of Mangiferin

Quantification of Mangiferin in different extracts of *Mangifera indica* was performed using HPLC Quaternary System (Shimadzu, Japan) with 10 x 4.6 mm and particle size of 5 μm. using a Lichrospher C18 RP column (Merck, Germany) A stock solution of standard Mangiferin and sample solution of different extracts of *Mangifera indica* were prepared in HPLC grade methanol. The dilutions of standard Mangiferin ranged from 20 μg/ml-100 μg /ml were also prepared in HPLC grade methanol. All the solutions were filtered through a 0.2 μm membrane filter (Axiva) before subjecting to the HPLC system. Acetonitrile and 0.1% o-phosphoric acid solution in water (70:30) was used as mobile phase at a 1mL/ minute flow rate in isocratic mode[16] and detection was done at a wavelength of 318 nm. The calibration curve was being made between concentration for standard Mangiferin and peak area. Mangiferin content in different solvents was then estimated from the linear equation of the calibration curve.

Single Factorial Experiments

After establishing the most efficient extraction mode and the best solvent, single factorial experiments were run on three parameters: solvent-to-drug ratio, extraction time and extraction temperature. To study the influence of a particular parameter on the yield of Mangiferin, two parameters were kept constant, and one was varied during the experimental trial. The ranges evaluated for different parameters are shown in Table 1. Mangiferin content in each extract was quantified using HPLC.

### Table 1: Ranges of different parameters assessed in single factorial experiment along with their coded and actual values.

<table>
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<tr>
<th>Independent variables</th>
<th>Lower Range</th>
<th>Higher Range</th>
<th>Coded levels</th>
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<td>-1</td>
<td>1</td>
<td>+1</td>
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<tr>
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<td>1:30</td>
<td>1:10 1:20 1:30</td>
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### Table 2: Experimental design, BBD.

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<th>Extraction temperature $Y_2$</th>
<th>Drug-to-solvent ratio (g/ml) $Y_3$</th>
<th>Response (Z) Mangiferin content (mg/g)</th>
<th>Experimental value, $Z_i$</th>
<th>Predicted Value, $Z_i$</th>
<th>$Z_i-Z_e$</th>
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<td>60</td>
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<td>60</td>
<td>1:30</td>
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</table>
Optimization of extraction parameters of Mangiferin

Box-Behnken Design (BBD), RSM, was used to optimize the parameters of extraction for Mangiferin from Mangifera indica using Design-expert 13.0 software, Stat-Ease, Inc. USA (Version 13). The experimental design comprises five replicates of the center point and twelve factorial experiments. The three chosen factors selected were encrypted as Y₁, Y₂, and Y₃ and were designed into three levels ciphered as -1, 0, +1 for low, intermediate, and high levels respectively, coding of the test variables were done in accordance with the equation mentioned below:

\[ y_i = \frac{(Y_i - Y_0)}{\Delta Y} \]

Where,
Yᵢ represents coded value of an independent factor,
Yᵢ represents actual value of an independent factor,
Y₀ represents actual value of an independent factor at the center point, and
ΔY represents step-change value of an independent factor.

The actual and the coded values of three variable factors are mentioned in Table 1, and the 17 runs of BBD experiments are mentioned in Table 2.

Quantification of Mangiferin in Various Extracts by HPLC

Different extracts for BBD experiments were analyzed using HPLC for the quantification of mangiferin content in Mangifera indica.

Prefatory phytochemical screening

The presence of diverse phytochemicals (glycoside, alkaloids, carbohydrate, flavonoids, resins, phytosterols, steroid, phenolic compounds, triterpenoids, and tannin) in different extracts of M. indica were evaluated by prefatory phytochemical screening.

Antioxidant activity using DPPH

Different extracts of M. indica were assessed for antioxidant potential by employing the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method using UV spectrophotometer at 517 nm. Methanol (5ml) and 1mM DPPH (0.5 ml) were added to the different tubes containing aliquots of 20, 40, 60, 80 and 100 μg /ml of extracts and standard. A blank solution consisting of methanol (5ml) and 1mM DPPH (0.5ml) was prepared and further all the tubes were incubated at ambient temperature for 30 min. The antioxidant activity of the different extracts were calculated using the equation:

\[ \% \text{scavenging} = \frac{\text{Absorbance of blank solution} - \text{Absorbance of test sample}}{\text{Absorbance of blank solution}} \times 100 \]

Data are represented as mean of 4 and linear regression analysis was employed to assess the sample size.

RESULTS

Comparison of different extraction techniques for extraction of mangiferin

Four different modes of extraction were used to extract Mangiferin from Mangifera indica employing four different solvents of varying polarity divulged that ethanol stood out to be the most efficient solvent, and reflux technique proved out to be the elite extraction mode for the extraction of Mangiferin (Figure 1). Quantitative analysis of Mangiferin in each extract was done via HPLC (Figure 2 and Figure 3).

Single factorial experiments

Holding to the elite extraction mode and the most efficient solvent for Mangiferin extraction, single factorial runs were performed —the results from single runs assisted in selecting the ranges of different parameters of BBD (Figure 4).

Optimization of Extraction parameters by BBD

Different experimental runs were performed in accordance to the design of BBD. A second-order polynomial equation was obtained through the multiple regression analysis, which defines the relationship between tested variables and response variable (mangiferin content) stated below:

\[ Y = + 90.17 + 3.58 A + 13.59 B + 7.74 C + 2.50 AB - 4.74 AC - 1.94 BC - 11.28 A^2 - 15.78 B^2 - 15.00 C^2 \]

where, Y- Mangiferin content, A- Extraction time (minutes), B- Extraction temperature (°C), C = solvent-to-drug ratio (ml/g)

To determine goodness of the model, analysis of variance (ANOVA) was applied (Table 3). The regression coefficient \( R^2 \) was found to be 0.9999, which apprises the closeness of the data with fitted regression. A difference of < 0.2 between predicted \( R^2 \) and adjusted \( R^2 \) signifies an excellent fit of the model. Meanwhile, a low value of the coefficient of variance (% CV of 0.3128) implies better dependability of the experimental values. Signal to noise ratio called Adequate precision is expected to be more than four was 320.0401, which shows the model’s goodness. The lack of fit test provides data variation around the fitted model. The p-value and F-value for the lack of fit were found to be 0.1052 and 4.05, insinuating it to be non-significant, making it a good model. The p-values for each coefficient were checked for their significance, and all the values were found to be less than 0.1, making them...
significant and thus implying that the model can be utilized to predict the responses.

Figure 5 shows Contour plots and Three-dimensional response surface plots, which aids in understanding the interactions between the responses and variables more clearly. It is noticeable from the 3D graph that mangiferin yield increases as the extraction time are increased from 60 min to 63.653 min and drug to solvent ratio from 1:20 g/ml to 1:22.634 g/ml. However, a further increase in both shows a decrease in mangiferin yield. This implies that both factors are significant for mangiferin extraction. Similarly, the yield of mangiferin increases as extraction temperature increases from 60°C to 63.563°C and drug to solvent ratio from 1:20 g/ml to 1:22.634 g/ml. Further increase of both factors shows the decrease in mangiferin yield. Similarly, Mangiferin yield increases as extraction temperature increases from 60°C to 66.563°C and extraction time from 60 min to 78.146 min.

The point prediction analysis revealed that the optimal conditions for Mangiferin extraction from *Mangifera indica* leaves are: extraction time- 63.653 min, extraction temperature- 66.138°C, and drug to solvent ratio- 1:22.634 g/ml. Also, the maximum mangiferin yield at these optimal conditions was found to be 91.096 mg/g of *Mangifera indica*.

**Model Validation**

To validate the adequacy of the model equation, the optimal extraction conditions for Mangiferin extraction from *Mangifera indica* were modified, and experiments were carried out in triplicate to re-evaluate the run. Moreover, the mangiferin content was found to be 90.31 mg/g using drug to solvent ratio- 1:20 g/ml, extraction temperature- 60°C and extraction time- 60 min.

However, there was no significant difference between the experimental and predicted yield, which infer that the response model was adequate and satisfactory for Optimization.

**Prefatory Phytochemical Screening**

Phytochemical tests of *M. indica* extracts in acetone, ethanol, DMF and DMSO showed the presence of flavonoids and phenolic compounds (Table 4). The extractive value of mangiferin in diverse solvents signifies the extent and character of phytobioactive constituents in each solvent (Table 5). The ethanolic extract of *M. indica* unveiled the presence of reducing sugars, phenolic
Adin, et al.: Optimization of Extraction of Mangiferin from Mangifera indica L. Leaves

Pharmacognosy Research, Vol 15, Issue 1, Jan-Mar, 2023

89

compounds, alkaloids, phytosterols, triterpenoids, flavanoids, and glycosides as the chief secondary phytochemicals which may be attributed for its therapeutic potential.

Antioxidant activity

The antioxidant activity of the M. indica extracts in different solvents was assessed using DPPH method. Test samples were assessed based on utilization of DPPH free radicals in test samples to give purple color. The antioxidant activity of the M. indica extracts in different solvents were compared with the standard Vitamin C. The IC₅₀ value was estimated graphically for assessing the antioxidant activity of M. indica extracts in different solvents.

The ethanolic extract of M. indica unveiled maximum DPPH scavenging activity at 100 mg/ml concentration, i.e., 59.76 % compared to M. indica extracts in DMSO, acetone, and DMF, which were reckoned to be 38.63 %, 43.12%, and 46.72%. The Vitamin C unveiled the 88.15 % DPPH scavenging activity.

From Figure 6, it is corroborated that antioxidant activity of extracts augments on increasing the extracts concentration and ethanolic extract revealed the maximum activity in comparison with other extracts, and it can be concluded that the antioxidant activity of M. indica ethanolic extract could be ascribable to presence of xanthones and phenolic compounds.

DISCUSSION

A broad range of extraction methods is available to extract therapeutically active phytoconstituents, with every method exhibiting its pros and cons. In our present work, RSM was being exploited to optimize the extraction process of Mangiferin from Mangifera indica leaves. BBD was employed as it does not use any embedded and factorial design and is more systematic than other designs of RSM. In addition, BBD surmounts experiments at extreme conditions, thereby providing adequate results. The regression model of RSM was found to demonstrate the optimal conditions for Mangiferin extraction significantly. Our study deduces that the reflux technique is a better choice for obtaining Mangiferin than other thermal and non-thermal techniques. Ethanol was found to be the most efficient solvent for the extraction of Mangiferin.

In our present study, different extraction techniques, viz. soxhlation, reflux, maceration, and UAE, were employed to extract therapeutically potent xanthanoid, Mangiferin from Mangifera indica leaves. BBD, a modern-day statistical technique, was being exploited for optimizing the extraction parameters of Mangiferin. This method is superior as it is time-saving, economical, less laborious, and it holds supremacy over other traditional methods, and the interaction between different independent variables can be studied. In our work, BBD serves as a valuable tool for optimizing the extraction parameters of Mangiferin from Mangifera indica leaves. Quantitative analysis of Mangiferin was done using HPLC on a C₁₈ reverse-phase column with U.V. detection at 318 nm. An eluting solution consisting of acetonitrile: 0.1% solution of o-phosphoric acid (30:70 v/v) was used as mobile phase at a 1mL/ minute flow rate in isocratic mode.

Tang-Bin Zou et al. in 2014 reported the optimization of Mangiferin extraction from Mangifera indica leaves by Ultrasound-assisted extraction using RSM. However, our study employs the extraction of Mangiferin from M. indica using different techniques like reflux, soxhlet, UAE, and maceration technique which has not been reported in the literature yet. Further, the antioxidant activity of M. indica extracts in different solvents were evaluated using DPPH method. The DPPH
Figure 5: 3D response surface graphs and Contour plots of (a) A and B, (b) A and C and (c) B and C.
Adin, et al.: Optimization of Extraction of Mangiferin from *Mangifera indica* L. Leaves

**Table 3:** ANOVA for response surface quadratic model.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sum of squares</th>
<th>Degree of Freedom (DF)</th>
<th>Mean square</th>
<th>f-value</th>
<th>p-value</th>
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<td>Lack of fit</td>
<td>0.2553</td>
<td>3</td>
<td>0.0851</td>
<td>4.05</td>
<td>0.1052</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Pure error</td>
<td>0.0841</td>
<td>4</td>
<td>0.0210</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor total</td>
<td>5010.59</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4:** Prefatory phytochemical screening of the extracts of *Mangifera indica* leaves.

<table>
<thead>
<tr>
<th>Plant extract + test reagent</th>
<th>Ethanol</th>
<th>Acetone</th>
<th>DMSO</th>
<th>DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+: Present, - : Absent

**Table 5:** Mangiferin content determined by the HPLC method in the samples

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sample</th>
<th>Mangiferin (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E1</td>
<td>90.17 mg</td>
</tr>
<tr>
<td>2</td>
<td>E2</td>
<td>60.23 mg</td>
</tr>
<tr>
<td>3</td>
<td>E3</td>
<td>88.83 mg</td>
</tr>
<tr>
<td>4</td>
<td>E4</td>
<td>85.67 mg</td>
</tr>
</tbody>
</table>

**Figure 6:** Dose-dependent scavenging of DPPH radicals by the different extracts of *Mangifera indica* compared with standard drug Ascorbic acid. Each value represent mean ± SD (n = 3).
Mangifer, a xanthoid present in *M. indica* possesses multifaceted pharmacological properties. Our research compares the efficacy of different modern and conventional methods for naringin extraction and demonstrates the process parameter at which high yield of mangiferin can be extracted from *M. indica* leaves in "one run". In the present study, Box- Behnken Design, was availed for optimizing the process parameters for the extraction of mangiferin from *M. indica* leaves. Extraction conditions (extraction time, solvent-to-drug ratio and extraction temperature) were optimized by BBD. Quantification analysis of mangiferin in different extracts was done using HPLC. The experimental results revealed that Reflux extraction method stood out to be the best amongst all the other thermal and non-thermal modes of extraction used, and ethanol was proved to be the most efficient extracting solvent. Through the use of BBD, the optimal conditions for naringin extraction were established as extraction time- 63.653 min, extraction temperature- 63.563°C and drug to solvent ratio- 1:22.634 g/ml. Under such conditions, Mangiferin was yielded as 90.31 mg/g, which was nearly close to the predicted value of 91.096 mg/g. Furthermore, mangiferin extraction was significantly affected by all three different variables. The present work highlights the use of BBD, a multivariate statistical technique in the extraction field of therapeutically potent phytoconstituents, which makes the optimization method less laborious and time-saving than the traditional optimization method. The DPPH scavenging activity also reveals that ethanolic extract exhibit highest antioxidant potential and further characterization of this extract can be lucrative for other researchers to discern new therapeutic entities.

Our research will be fruitful for the pharmaceutical industries and the upcoming researchers who wish to extract Mangiferin in a maximum amount from *Mangifera indica* leaves.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**ABBREVIATIONS**

3D: Three-dimensional; °C: Degree Celsius; ANOVA: Analysis of variance; BBD: Box–Behnken Design; G: Gram; HPLC: High performance liquid chromatography; RSM: Response surface methodology; UAE: Ultrasound-assisted extraction.

**SUMMARY**

Mangiferin, a xanthoid present in *M. indica* possesses highest antioxidant potential and further characterization of this extract can be lucrative for other researchers to discern new therapeutic entities.

**REFERENCES**


Adin, et al.: Optimization of Extraction of Mangiferin from Mangifera indica L. Leaves


