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Optimization of Ultrasound Assisted Extraction on Total Phenolic Content of Saussurea lappa Root Using Response Surface Methodology and Evaluation of their Antioxidant Properties

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Abstract

Response Surface Methodology (RSM) is a scientific method are useful in optimization of experimental set ups. It requires observing and gathering information about how process and system works. In an experiment, some input x's transform into an output that has one or more observable response variables y. Therefore, useful results and conclusions can be drawn by experiment. In the present paper, Box-Behnken Design (BBD) was applied to the ultrasound assisted reaction using three factors and three variables to optimize the extraction process. The three independent variables during the extraction of phytochemicals from the Saussurea lapparoot were temperature, concentration of solvent a time of sonication and dependent variable is yield, total phenolic content and antioxidant activity. The total phenol contents of the optimized extraction process were studied. The optimized extraction was evaluated for antioxidant studies using DPPH (diphenyl-1-picrylhydrazyl) method. The optimized conditions for the maximum extraction 50% were when temperature was maintained at 45 °C with 60% methanol concentration and sonication time around 50 mins and the 80% of inhibition of DPPH free radical was achieved.

Keywords: *Response Surface Methodology (RSM), Saussurealappa root, Total Phenolic Content, Ultrasound Assisted Extraction, 2-diphenyl-1-picrylhydrazyl*

1.0 Introduction

Response Surface Methodology (RSM) is a mathematical and statistical techniques useful for optimization of a response influenced by several variables resulting to maximize the production of a special substance[1-3]. RSM can be applied to any branch of science which aid to understand the connection associated with the

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dependent and response variables leading to optimize the experimental conditions[4-8] is dependent on several variables such as choice of solvent, temperature, concentration of the solvent, solvent to material ration, time duration of extraction, pH of solvent etc., all needs to be experimentally studied and optimized to increase the high percentage of extraction yield. Hence is time consuming at the same time waste of valuable resource[9-11]. In such case response surface methodology plays a crucial role in optimizing the parameters and reducing the number of sets of experiments, instead narrow downs the number of steps and increases the yield of the end product which is a value addition to the natural product industries[12-13].

$$y = f(x_1, x_2) + \varepsilon \quad (1).$$

variables x_1 and x_2 are independent variables (extraction conditions) and the response variable y (yield of the natural product) depends on x_1 and x_2. The dependent variable y is a function of x_1 x_2 and the experimental error term is denoted as ε (It is an experimental error which has been assumed to distribute normally with zero mean and variance σ^2).

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon \quad (2).$$

If it is a convex in the response surface, then a higher order polynomial has to be used. The approximation of function with two variables is called a second-order model and can be expressed as

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_{22}^2 + \beta_{12} x_1 x_2 + \varepsilon \quad (3).$$

In the present work, *Saussurea lappa* was used for the extraction of phytochemiclas. *S. lappa* is known for its medicinal purpose in various native Indian systems of medicine[14-18]. *S. lappa* is well disturbed all over the world and in India it is found in Himalayan region. *S. lappa* is a perennial, pubescent, robust, erect with stem height of 1-2m[19]. There are many studies that have been carried out on various activities of *S. lappa* by several researchers through several statistical applications. Research findings reported 85.2% of scavenging activity of lipid peroxidase DPPH-85.2%, whereas 58.4% inhibition of nitric acid, 66% inhibition of superoxide of 66% Chlorogenic acid present in 0.027%[20-24].

However, to increase the total phenolic component of the plant source, response surface methodology has been incorporated in several studies to reduce the number of experimental runs. Correlation studies, Response Surface Methodology were carried out and found that total phenolic extraction can be enhanced by optimizing the extraction conditions which enhances the biological activities such as antioxidant, antimicrobial, anticancer activities[25,26].

In the present work, optimization of parameters for ultrasound assisted extraction of *S. lappa* root was carried out using Box-Behnken method using Minitab 9 software. The extract obtained from the optimized process was screened for antioxidant activities.

2.0 Materials and Methods

Methanol (purity 98 %), 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH, purity 98 %), Ascorbic acid (purity 99 %), Folin-Ciocalteu reagent (purity 99 %), were procured from Sigma –Aldrich. Acetic acid, Ethanol (AR grade) were procured from SD-Fine chem Limited. All chemicals were of analytical grade and used without any further purification. Ultrasound batch used for extraction of phytochemicals. UV-visible spectrophotometer (Shimadzu UV-2600) was used to record the total phenolic content and Minitab® 19 software (Minitab Inc., USA) was used to run experimental models.

Extraction procedure: *S. lappa* roots were procured from the Malleswaram market, Bangalore and were identified by the botanist. Roots were ground powdered, dried and sieved at 200 mesh size and used for extraction of phytochemicals.

DPPH inhibition activity: The scavenging effect of Ls-Cs NPs, was done by using the 2, 2-diphenyl-1-picrylhydrazyl radical method as per the standard methods mentioned in literature[27,28].

3.0 Statistical Models

The present study focuses on optimization of total phenolic content from *Saussurea lappa* root which is extracted by the following statistical tools:

- Exploratory analysis
- RSM methodology

3.1 Regression

Regression is a technique used to examine all responses after a series of experimental procedures that involves fitting the response into a polynomial model, such as the second order polynomial model provided in

$$y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \sum_{i=1}^{4} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2 \quad (4).$$

where y stands for the independent responses; $\beta 0$, βi, βii, and βij are, respectively, the intercept, linear, quadratic, and cross product terms' regression coefficients for the process variables. Analysis of variance (ANOVA) must be performed for the reliability test, which assesses the model's fitness using the response's coefficient of R square as well as the F-test for lack of fit[29,30].

3.2 Optimization using Box Behnken Design (BBD)

Another approach in the response surface methodology is called Box-Behnken (BBD), and its goal is to identify the factors that will result in the best possible response or output. Box-Behnken design is regarded as a design without the embedded factorial or fractional factorial point that could be identified as the variable condition located at the midpoint borders of the variables space as well as at the centre, according to the literature[31]. The number of experiments by BBD can be calculated by

$$N = 2k^*(k-1) + C0 \quad (5).$$

where C0 is the number of replicates of the centre point, and k is the factorial number. The central point also makes it possible to calculate the response function at intermediate levels and to estimate the pure error. Following the replication of the centre point, a system performance estimation within the researched range is thus feasible.

Independent variables for extraction of phytochemicals from *S. lappa* roots were concentration of methanol (0, 50 an d 100 %) with temperature (30, 40 and 50 ˚C) and sonication time (40, 60 and 80 mins) whereas dependent variables were yield of phytochemicals, total phenolic content and DPPH free radical scavenging activity.

4.0 Results and Discusssion

Regression Equation in Coded Units

The quadratic regression model for yield of phytochemicals obtained through ultrasound extraction of *S. lappa* are indicated in the equation 6 and Figure 1. Represents the counter and response surface plots of yield (%) Vs methanol concentration, temperature and time of sonication. From the 3D response surface graphs, it is observed tah the yield of the phyto-chemical was achieved

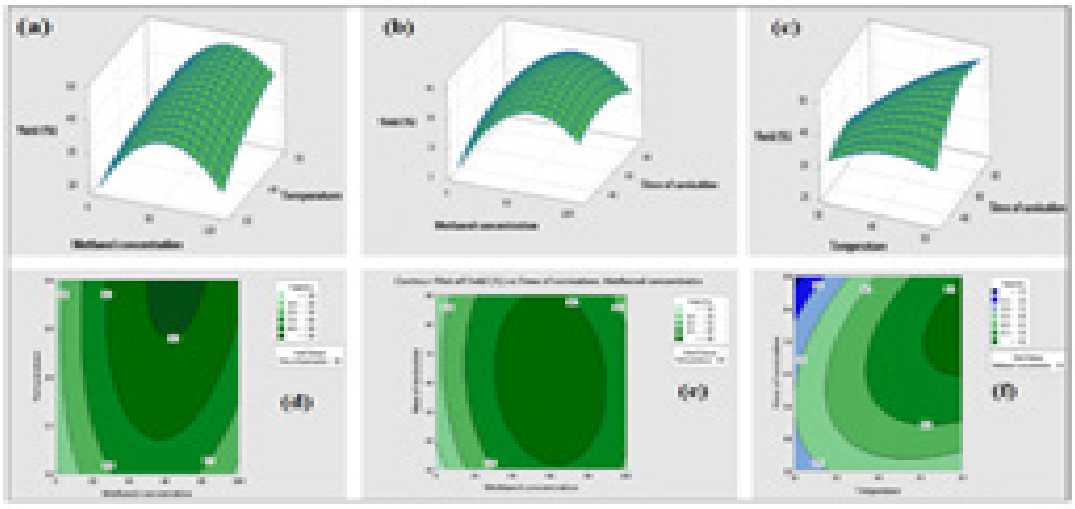


Figure 1. Response surface and Counter plots of yield (%) Vs methanol Concentration, temperature and time of sonication

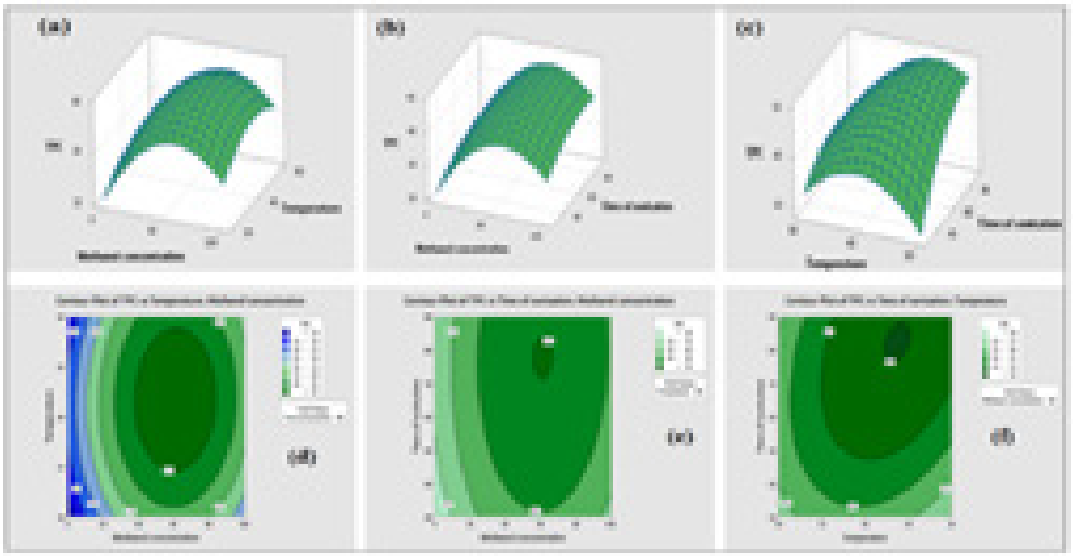


Figure 2. Response surface and Counter plots of TPC (mg) Vs time of sonication, methanol concentration and temperature.

maximum (50 %) when the sonication bath temperature was maintained at 45 ˚C and sonication time up to 70 mins and 60% concentration of methanol was preferred solvent. The R2 value was at 96.87% and Lack-of-Fit was for p Value at 0.092 which was significant for the selected set of experiments. It was observed that time of sonication is significant factor along with the concentration of methanol to achieve the maximum yield of yield.

$$Y1 = -50.9 + 0.755x1 + 2.34x2 + 0.337x3 - 0.0398x22\ 0.00807x32 \quad (6).$$

Total phenolic content was carried out using Folin-Ciocalteu reagent. The regression equation for total

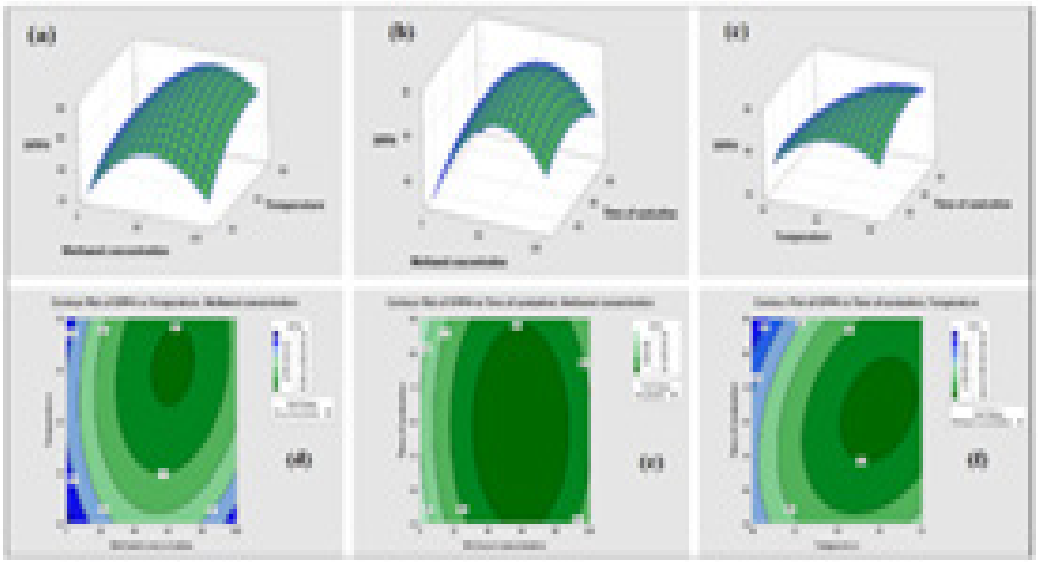


Figure 3. Response surface and Counter plots of DPPH (%) Vs methanol concentration and temperature.

phenolic content present in the root of *S. lappa* were shown using equation 7. The surface response plots indicate that maximum total phenolic content (50%) was achieved at 60 % concentration of methanol maintained at 45 ˚C and extraction time duration between 75-80 mins. The R2 value of the TPC run were 96.97%. To achieve high percentage of TPC, methanol concentration as single factor plays an important role and in two-way interaction the concentration of methanol and temperature are significant factors. 0.079 p value was obtained for the designed experimental runs.

$$Y2 = -101.7 + 0.821x1 + 5.51x2 + 0.164x3 + 0.00050x1x3 + 0.01397x2x30.771x22\ 0.00485x32 \quad (7).$$

Further, to study the antioxidant activity of the phytochemicals present in the *S. lappa* root extract was carried out by DPPH method. The regression equation is indicated I the equation 8. The surface response plots indicate that maximum inhibition of DPPH free radical was achieved by the phytochemicals present in the *S. lappa* root extract when the concentration of methanol was 60 % and temperature of the ultrasound bath maintained at 45 ˚C which were significant in single and square factor. In the two-way interaction, both factor temperature and methanol concentration were significant with 0.097 value. The p value was at 0.070. The R2 was at 96.97%.

$$Y3 = 135.30.773x1 + 7.97x2 + 0.335x3\ 0.1323x2x\ 30.00042x2x10.01078x220.00956x32 \quad (8).$$

Variable interactions vary for each of the above equations. Every model does not need to have the same interaction because the analysis of variance allows for the elimination of non-significant interactions, which improves the precision of response prediction. The accuracy of the response that took into account discrepancies between the predicted and experimental values must then be tested for dependability on all models[32]. The experimental values are produced as average values from an experiment carried out in at least triplicates with assigned values of independent variables, whereas the projected values can be determined using the derived model. In Figure 3, the summary values are displayed.

R and R2 values of the model were reported to be 0.9987 and 0.9975, respectively, from a statistical point of view, indicating a satisfactory agreement between the experimented and projected results assessed using the model.

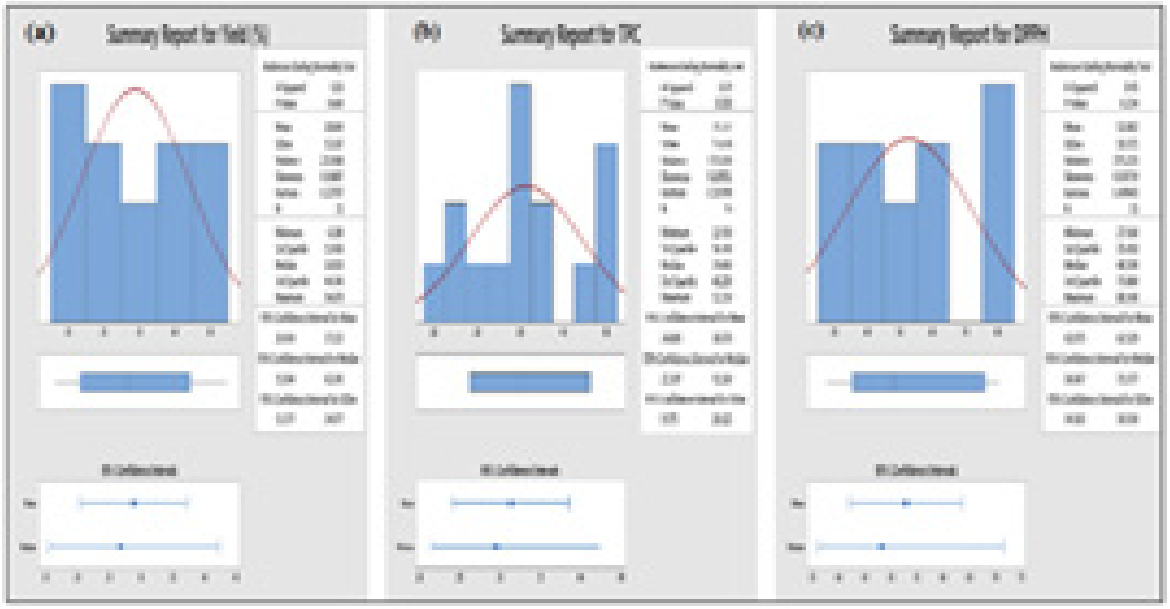


Figure 4. Summary of phytochemical extraction in percentage yield, total phenolic extract and percentage of antioxidant.

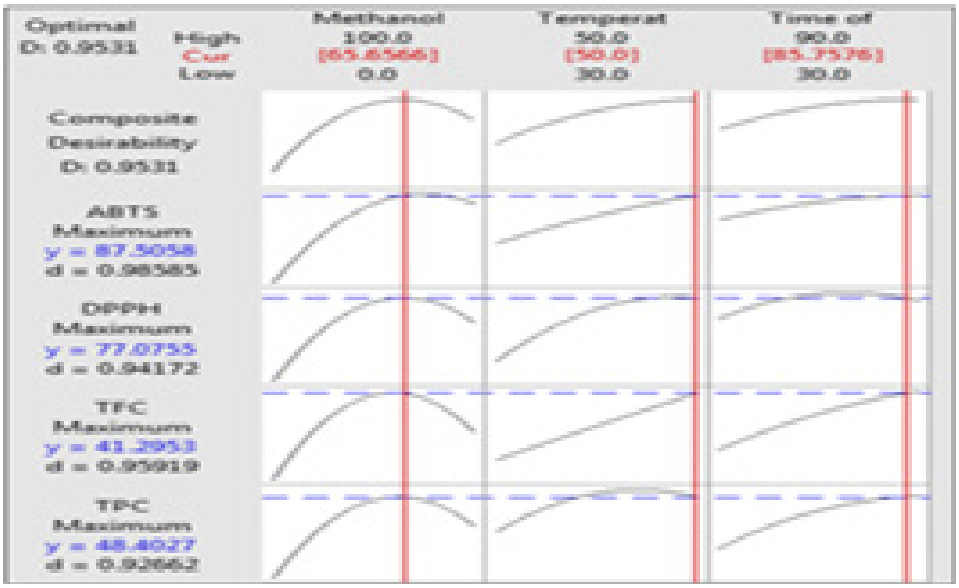


Figure 5. Desirability chart for optimization of phytochemicals extracted from *S. lappa* root extract and its antioxidant activity.

5.0 Conclusions

Box Bekhen model suggested for the three responses (yield, TPC and DPPH) as a function of three independent variables such as concentration of solvent, sonication time and temperature by ultra-sound assisted extraction of *S. lappa* root extract, fitted the data well with an R^2 value of 99.58%. The predicted total phenolic content values are well fitted with experimental observations. The optimized condition for achieving highest phytochemical yields were at 60% methanol concentration with sonication time 60 mins and ultrasound bath temperature at 45˚C. *0 % of DPPH free radical scavenging was achieved with phytochemicals extracted from 60% methanol concentration with ultrasound temperature at 45˚C and time of extraction up to 60 mins. The concentration of methanol and temperature were the two significant parameters that enhanced the high yield of phytochemicals. Through RSM, the antioxidant property carried out by DPPH method could also fit the data well. Based on the RSM model results, further comparative studies can be carried out using various statistical models on numerous factors and best result can be reported. Thus, RSM ensures to minimize the experimental runs improvising the extraction process enhancing higher yields of phytochemical's that can be of pharmacological importance.

6.0 References

1. Khuri AI, Mukhopadhyay S. Response surface methodology. Wiley Interdiscip Rev Comput Stat. 2010;2(2):128-49. https://doi.org/10.1002/wics.73
2. Hill WJ, Hunter WG. A review of response surface methodology: a literature survey. Technometrics. 1966; 8(4):571-90. https://doi.org/10.2307/1266632
3. Hadiyat MA, Sopha BM, Wibowo BS. Response surface methodology using observational data: a systematic literature review. Appl Sci. 2022; 12(20):10663. https://doi.org/10.3390/app122010663
4. Khuri AI, Cornell JA. Response surfaces: designs and analyses: revised and expanded. Boca Raton: CRC Press; 2018. https://doi.org/10.1201/9780203740774
5. Teslić N, Bojanić N, Rakić D, Takači A, Zeković Z, Fišteš A, *et al.* Defatted wheat germ as source of polyphenols-Optimization of microwave-assisted extraction by RSM and ANN approach. Chem Eng Process Process Intensif. 2019; 143:107634. https://doi.org/10.1016/j.cep.2019.107634
6. Aydar AY. Utilization of response surface methodology in optimization of extraction of plant materials. In: Statistical approaches with emphasis on design of experiments applied to chemical processes. Boca Raton: CRC Press; 2018. p. 157-69. https://doi.org/10.5772/intechopen.73690
7. Fakayode OA, Abobi KE. Optimization of oil and pectin extraction from orange (*Citrus sinensis*) peels: a response

surface approach. J Anal Sci Technol. 2018; 9(1):1-16. https://doi.org/10.1186/s40543-018-0151-3
8. Jalili F, Jafari SM, Emam-Djomeh Z, Malekjani N, Farzaneh V. Optimization of ultrasound-assisted extraction of oil from canola seeds with the use of response surface methodology. Food Anal Methods. 2018; 11(2):598-612. https://doi.org/10.1007/s12161-017-1030-z
9. Zhang H, Li H, Zhang Z, Hou T. Optimization of ultrasound-assisted extraction of polysaccharides from perilla seed meal by response surface methodology: characterization and in vitro antioxidant activities. J Food Sci. 2021; 86(2):306-18. https://doi.org/10.1111/1750-3841.15597
10. Kashyap P, Riar CS, Jindal N. Optimization of ultrasound-assisted extraction of polyphenols from Meghalayan cherry fruit (*Prunus nepalensis*) using Response Surface Methodology (RSM) and Artificial Neural Network (ANN) approach. J Food Meas Charact. 2021; 15(1):119-33. https://doi.org/10.1007/s11694-020-00611-0
11. Hossain SMZ, Taher S, Khan A, Sultana N, Irfan MF, Haq B, *et al*. Experimental study and modeling approach of response surface methodology coupled with crow search algorithm for optimizing the extraction conditions of papaya seed waste oil. Arab J Sci Eng. 2020; 45(9):7371-83. https://doi.org/10.1007/s13369-020-04551-1
12. Jamaludin R, Kim DS, Md Salleh L, Lim SB. Optimization of high hydrostatic pressure extraction of bioactive compounds from noni fruits. J Food Meas Charact. 2020; 14(5):2810-8. https://doi.org/10.1007/s11694-020-00526-w
13. Esmaeelian M, Jahani M, Feizy J, Einafshar S. Effects of ultrasound-assisted and direct solvent extraction methods on the antioxidant and antibacterial properties of Saffron (*Crocus sativus* L.) corm extract. Food Anal Methods. 2021; 14(1):74-87. https://doi.org/10.1007/s12161-020-01855-8
14. Hassan R, Masoodi MH. *Saussurea lappa*: a comprehensive review on its pharmacological activity and phytochemistry. Curr Tradit Med. 2020; 6(1):13-23. https://doi.org/10.2174/2215083805666190626144909
15. Abd Eldaim MA, Tousson E, El Sayed IET, Awd WM. Ameliorative effects of *Saussurea lappa* root aqueous extract against Ethephon-induced reproductive toxicity in male rats. Environ Toxicol. 2019; 34(2):150-9. https://doi.org/10.1002/tox.22669
16. Kolahalam LA, Prasad KRS, Krishna PM, Supraja N. *Saussurea lappa* plant rhizome extract-based zinc oxide nanoparticles: synthesis, characterization and its antibacterial, antifungal activities and cytotoxic studies against Chinese Hamster Ovary (CHO) cell lines. Heliyon. 2021; 7(6):e07265. https://doi.org/10.1016/j.heliyon.2021.e07265
17. Wu T, Yan XJ, Yang TR, Wang YF, He JY, Feng Y, *et al*. Structure-based molecular networking for the discovery of anti-HBV compounds from *Saussurea lappa* (Decne.) CB Clarke. Molecules. 2022; 27(6):2023. https://doi.org/10.3390/molecules27062023
18. Kumar J, Pundir M. Phytochemistry and pharmacology of Saussurea genus (*Saussurea lappa*, *Saussurea costus*, *Saussurea obvallata*, *Saussurea involucrata*). Mater Today Proc. 2021. https://doi.org/10.1016/j.matpr.2021.11.145
19. Rao VG, Nandakishore P, Reddy KS, Sankaran S. Solid waste disposal practices: implications. Recent Adv Environ Sci. 2003; 211.
20. Habotta OA, Ateya A, Saleh RM, El-Ashry ES. Thiamethoxam-induced oxidative stress, lipid peroxidation, and disturbance of steroidogenic genes in male rats: palliative role of *Saussurea lappa* and *Silybum marianum*. Environ Toxicol. 2021; 36(10):2051-61. https://doi.org/10.1002/tox.23322
21. Ansari S, Hasan K, Bhat S. Anticancer, antioxidant, and hepatoprotective activity of *Saussurea lappa*, CB Clarke (qust) on human hepatoma cell line. J Cancer Res Ther. 2021; 17(2):499. https://doi.org/10.4103/jcrt.JCRT_571_19
22. Ansari S, Potbhare MS, Barik R, Alhoot MA. Study of antiphlogistic effect of *Saussurea lappa* roots' ethanol extract in comparison to paracetamol effect. Malays J Microsc. 2021; 17(1).
23. Singh R, Chahal KK. Phytochemical analysis and in vitro antioxidant capacity of different solvent extracts of *Saussurea lappa* L. roots. J Pharma Phytochem. 2018; 7(3):427-32.
24. Rajha HN, El Khoury G, El Darra N, Raafat K, Debs E, Maroun RG, et al. Biological activities of *Saussurea lappa* antioxidants recovered by solid-liquid, ultrasound and Ired-Irrad®. Curr Bioact Compd. 2021; 17(1):85-97. https://doi.org/10.2174/1573407216666200227094059
25. Andres AI, Petron MJ, Lopez AM, Timon ML. Optimization of extraction conditions to improve phenolic content and in vitro antioxidant activity in craft brewers' spent grain using Response Surface Methodology (RSM). Foods. 2020; 9(10):1398. https://doi.org/10.3390/foods9101398
26. Yang QQ, Gan RY, Zhang D, Ge YY, Cheng LZ, Corke H. Optimization of kidney bean antioxidants using RSM & ANN and characterization of antioxidant profile by

UPLC-QTOF-MS. LWT. 2019; 114:108321. https://doi.org/10.1016/j.lwt.2019.108321

27. Urmila GH, Rao BG, Satyanarayana T. Phytochemical and in vitro antioxidant activity of methanolic extract of *Lactuca scariola* & *Celosia argentea* leaves. J Drug Deliv Ther. 2013; 3(4):114-7. https://doi.org/10.22270/jddt.v3i4.584
28. Alzoreky N, Nakahara K. Antioxidant activity of some edible Yemeni plants evaluated by ferrylmyoglobin/ABTS assay. Food Sci Technol Res. 2001; 7(2):141-4. https://doi.org/10.3136/fstr.7.141
29. Krishna Kumar G, Velmurugan C, Kannan T. Using the RSM method of improving process parameters of welding AISI 316 and nickel 201 using CO_2 laser. Matéria (Rio J.). 2022; 27(3). https://doi.org/10.1590/1517-7076-RMAT-2022-0129 https://doi.org/10.1590/1517-7076-rmat-2022-0129
30. Radhwan H, Shayfull Z, Farizuan MR, Effendi MSM, Irfan AR. Optimization parameter effects on the quality surface finish of the three-dimensional printing (3D-printing) Fused Deposition Modeling (FDM) using RSM. AIP Conf Proc. 2019; 2129(1):020155. https://doi.org/10.1063/1.5118163
31. Maran JP, Manikandan S, Thirugnanasambandham K, Nivetha CV, Dinesh R. Box-Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide. Carbohydr Polym. 2013; 92(1):604-11. https://doi.org/10.1016/j.carbpol.2012.09.020
32. Said KAM, Amin MAM. Overview on the Response Surface Methodology (RSM) in extraction processes. J Appl Sci Process Eng. 2015; 2(1):8-17. https://doi.org/10.33736/jaspe.161.2015