Inhibitory effects of *Lepidium sativum* polysaccharide extracts on TNF- α production in *Escherichia coli*-stimulated mouse

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Abstract

The present study was designed to study the quantitative effects of extraction time, temperature and solvent to sample ratio on the yield of *Lepidium sativum* polysaccharides (LSP) using a Box–Behnken design. The activities of the optimized LSP extract were then tested in an in vivo experimental system of *Escherichia coli* (*E. coli*)-induced endotoxin shock. The optimal polysaccharide extraction conditions were established by the equation of regression and evaluation of the response surface contour plots: extraction time 5.2 h; temperature 95 °C and ratio of water to raw material 31.89 mL/g. Subsequently, an in vivo endotoxin shock was induced in mice with a single *E. coli* i.p. injection. Septic mice showed a substantial raise in the levels of tumor necrosis factor alpha (TNF- α) in plasma, whereas mice treated with LSP after *E. coli* injection showed considerable lower plasma levels of TNF- α ($P < 0.05$). These results suggest that LSP have beneficial effects when administered to mice with endotoxin shock by diminishing the pro-inflammatory response. The systemic activity of LSP indicated that the extract has a significant inhibitory effect against *E. coli*-induced inflammation by reducing the circulating levels of TNF- α . Further studies are warranted to explore the clinical implications of such observations.

Keywords *Lepidium sativum* · Polysaccharides · Box–Behnken design · TNF- α inhibitory property

Introduction

The use of herbal medicines is currently growing exponentially among people. In addition, extensive research is being conducted on herbs to take advantage of their possible medicinal values. Indeed, numerous herbs have been recognized as rich sources of therapeutic agents for the

treatment of various diseases (Saxena et al. 2015; Kirtikar 2005). *Lepidium sativum* (*L. sativum*), also known as garden cress, is a fast-growing vertical herb of the family Cruciferae. It is cultivated throughout the world as a culinary vegetable. *L. sativum* has been shown to be effective against various disorders, including inflammation, hypertension, arthritis, hepatotoxicity, bronchitis, diabetes and cancer (Nadkarni 2005; Sharma and Agarwal 2011).

The use of *L. sativum* seeds has been well-established in ancient India and the Middle East countries for the treatment of various ailments (Juma 2007). The leaves of *L. sativum* have been used for their antibacterial as well as diuretic properties and its roots in the treatment of syphilis (Raval 2016). *L. sativum* seeds are brownish red in color and become oily when soaked in water. They contain different components, including proteins, vitamins, carbohydrates, calcium, fatty acids, phosphorus and trace elements (Rehman et al. 2012; Manohar et al. 2012). They also contain a volatile aromatic oil and are a good source of iron (Manohar et al. 2012; Doke and Guha 2014). Various alkaloids have been isolated for the seeds such as sinapine, sinapic acid and glucotropaeolin. The

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mucilage of *L. sativum*, also known as *L. sativum* polysaccharides (LSP), is a combination of uronic acid and cellulose containing polysaccharides; L-rhamnose, L-arabinose, D-galactose, D-glucose and D-galacturonic acid, which are the products of acid hydrolysis (Doke and Guha 2014). Polysaccharides are recognized as carbohydrate (polymeric) molecules consisting of long chains of monosaccharide units. They play a vital role in the development and growth of living organisms due to their exclusive physical, chemical and biological properties (Samavati and Manoochehrizade 2013). Polysaccharides are heterogeneous and can have distinct properties from their monosaccharide building blocks depending on the structure. Polysaccharides may be amorphous or even insoluble in water. Their range in structure varies from linear to highly branched such as glycogen and starch, chitin and cellulose (Varki et al. 2009). They are also recognized as important constituents for skin care products and therapeutic drugs used in modern medicine (Yang et al. 2008).

An ultrasonic extraction process is commonly used to extract polysaccharides or bioactive compounds from herbs as it offers a highly efficient extraction (Yang et al. 2008; Chen et al. 2008; Liu et al. 2010; Ma et al. 2011). The utilization of ultrasonic-assisted extraction (UAE) is beneficial in many ways as it reduces the extraction time and temperature and increases the yield (Entezari et al. 2004). The enhanced extraction yield from UAE is mainly due to the mechanical effects, which enable extensive mass transfer between immiscible phases through superagitation, particularly at low frequency (Vinatoru et al. 1997; Save et al. 1997). Response surface methodology (RSM) is a statistical program used for the development and optimization of multifarious processes (Bezerra et al. 2008; Ahmad et al. 2015). It has numerous advantages over conventional experiments. RSM is a more efficient methodology and provides a vast amount of data with only a small set of experiments needed to be executed to monitor the impact of the independent variables of the response.

Inflammatory shock following the release of lipopolysaccharide (LPS) from Gram negative bacteria is a serious clinical concern. In humans, immune responses to LPS result in the release of many inflammatory cytokines from monocytes and macrophages, in particular tumor necrosis factor alpha (TNF- α), which can cause fever, shock, organ failure and death (Remick et al. 1987; Guha and Mackman 2001). The aim of the present study was to enhance the extraction process (i.e., optimal conditions) of the maximum polysaccharide yield from *L. sativum* using the Box–Behnken design (BBD) and to evaluate the potential beneficial effects of LSP treatment on *E. coli*-induced endotoxin shock using established in vivo models.

Materials and methods

Chemicals and plant materials

Lepidium sativum seeds were purchased from a local herbs shop in Riyadh, Saudi Arabia. A taxonomist identified the seeds of *L. sativum* at the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (no.: 3965-2016) was submitted to the College of Pharmacy Herbarium. The seeds were cleaned and washed with double distilled water, de-shelled, dried and ground to fine powder using a pestle and mortar before extraction. This powder was stored in dark containers away from sunlight and free from moisture until it was used in the experiments. *E. coli* strain ATCC-25992 was obtained from King Khalid University Hospital, Riyadh, Saudi Arabia. All reagents and chemicals used were of analytical grade and were procured from Panrec Chemicals (Barcelona, Spain).

Extraction of polysaccharides

Polysaccharide extraction from *L. sativum* was carried out by UAE according to the scheme described by Ying et al. (2011), Prakash Maran et al. (2013) and Ahmad et al. (2015). An ultrasonic device fitted with a time and temperature controller (Raypa® Instrumentation, Barcelona, Spain) operating at a frequency of 60 Hz, with an input power of 220 V and heating power of 550 W was used for the extraction of polysaccharides from *L. sativum*. About 25 g of powder was mixed with a suitable amount of distilled water for extraction. All tests were conducted according to the BBD shown in Table 1. The percentage of polysaccharide yield (YP %) was calculated by the following equation after the extraction was completed.

$$\text{YP (\%)} = \frac{\text{Mass of crude extract (g)}}{\text{Mass of } L. \text{ sativum powder (g)}} \times 100.$$

Physicochemical properties

The molecular characterizations and chemical compositions of LS seeds were assessed using FT-IR to determine the major sugars/polysaccharides as described by Karazhiyan et al. (2011).

Box–Behnken design (BBD)

BBD, a three-factor design was used for the optimization process with the Design Expert Software (V 8.0.7.1). The design was statistically applied to examine the individual and shared effects of the process variables to allow optimization of the polysaccharide extraction from *L. sativum*.

Table 1 The Box–Behnken response surface design and corresponding response values

Run	Extraction time (h)	Extraction temp (°C)	L:S ratio (mL/g)	Actual	Predicted	Residual
1	3	70	25	5.56	5.65	−0.089
2	6	70	25	4.02	3.95	0.069
3	3	110	25	3.26	3.33	−0.069
4	6	110	25	4.23	4.14	0.089
5	3	90	10	5.36	5.21	0.148
6	6	90	10	2.13	2.14	−0.010
7	3	90	40	3.26	3.25	0.010
8	6	90	40	5.29	5.44	−0.148
9	4.5	70	10	2.41	2.47	−0.059
10	4.5	110	10	2.95	3.03	−0.079
11	4.5	70	40	4.84	4.76	0.079
12	4.5	110	40	2.13	2.07	0.059
13	4.5	90	25	5.23	5.32	−0.092
14	4.5	90	25	5.37	5.32	0.048
15	4.5	90	25	5.38	5.32	0.058
16	4.5	90	25	5.26	5.32	−0.062
17	4.5	90	25	5.37	5.32	0.048

Three independent variables, extraction temperature A; time B, and liquid–solid ratio C, at three levels were selected for the study and the yield of polysaccharide extracted (Y) was selected as the response. The levels of these tested variables and the outcomes of the full method consisting of 17 set of experimental points carried out in standard order are presented in Table 1. These experimental settings were established after the results of the initial tests. For statistical calculation each variable was coded as −1, 0 and +1 at three levels. The UAE conditions for LSP were used by RSM.

Validation of optimized conditions

The experiment was finally carried out under the optimum values as described by the point prediction tool of the RSM for the concentration of polysaccharides, which should result in maximum yield. The model validity was performed by valuation of the optimal situations as foreseen by the model. These normal values were equated with the values (predicted) of the developed model to define the precision and relevance of the proven model.

Endotoxin shock and treatment

Albino Wistar male rats, obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, were used in this study (clearance no. 2289-EACC, 2016). The animals, weighing approximately 200 ± 20 g, were kept under controlled environmental conditions (23 °C and a 12 h light/dark cycle) and given free access to standard rat pellet food and tap water.

The animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals by the Animal Care Center. The animals were divided into four groups of ten rats each ($n = 10$). Group I served as a vehicle control and was treated with Tween-80 in saline. Group II was injected with *E. coli* at 50 µg/kg i.p., and served as positive control. Animals in groups III and IV received LSP extract at 250 and 500 mg/kg p.o., respectively, for 7 days prior to the induction of systemic infection induced by *E. coli* (50 µg/kg i.p.) The animals were killed under mild ether anesthesia 6 h after *E. coli* challenge and blood samples were collected from the retro-orbital plexus in sterile heparinized tubes and plasma was separated. The resultant plasma samples were stored at −80 °C for TNF-α analysis. The levels of TNF-α were determined using ELISA according to manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Employing BBD, multiple regression analysis and analysis of variance (ANOVA) were used to fit the model. Various statistical analysis parameters were used to evaluate the data such as *P* value, *F* value, determination coefficient (R^2), adjusted determination of coefficient (R^2a), sum of squares (SS), degrees of freedom (*df*), coefficient of variation (CV), mean sum of squares (MSS), absolute average relative deviation (AARD), and coefficient correlation (*R*) to mimic the statistical importance of the established quadratic mathematical model. The calculations and analysis of the experiment were carried out using Microsoft Excel 2007 (Microsoft Inc., Seattle, WA, USA). The data obtained was

used for plotting contour plots and response surfaces. The analysis of variance with Dunnett's post hoc testing was used to determine the differences in TNF- α concentration between groups.

Results and discussion

The objective of optimizing the extraction process was to enhance the yield of polysaccharide extracts from *L. sativum*. The molecular characterizations and chemical compositions of LSP by FT-IR determines the major sugars of LSP as arabinose, galacturonic acid, mannose, fructose, glucose, glucuronic acid, rhamnose and galactose and uronic acid which imparts a polyelectrolyte nature to the *L. sativum* seed extract (Karazhiyan et al. 2011). The results for the three tested parameters, the maximum predicted and the experimental yield of polysaccharide are presented in Table 1. The experiment was designed according to the BBD containing 17 runs and 5 central points by RSM to optimize these tested factors or parameters. The collaborative and specific effects of these factors were studied in the process of extraction. The outcome of these parameters was quantified in terms of coded factors of yield of polysaccharide. Responses obtained from the investigational tests were analyzed and fitted to non-linear quadratic models for the high polysaccharide yield percentage. A regression (R) analysis (Table 2) was performed to fit mathematical models into the experimental data intended at an optimal value for the responses tested. The tests were executed in a standard order to escape methodical errors. The tested values of the yield of *L. sativum* in ultrasonic extraction were analyzed by regressions (multiple) to fit the second-order polynomial equation. The values of R coefficients were calculated and are related by

the following second-order polynomial equation for coded factors:

$$\begin{aligned} \text{Polysaccharide yield} = & +5.32 - 0.22 \times A - 0.53 \times B + 0.33 \\ & \times C + 0.63 \times A \times B + 1.32 \times A \times C \\ & - 0.81 \times B \times C - 0.064 \times A^2 - 0.99 \\ & \times B^2 - 1.25 \times C^2. \end{aligned}$$

The model validation represents the phenomenon quite well and the response variation was appropriately correlated to the factor variations (Table 2). The investigation and optimization overall of a fitted response surface might yield ambiguous outcomes, which makes the study of the model competence or adequacy indispensable (Liyana-Pathirana and Shahidi 2005). The mean square error to the pure error achieved from the duplicates at the design center as F -ratio (Table 3). Amount of DF in the model depends on the significance of F value and is presented in the P value column (95%, confidence level). Hence, the responses with a P value lower than 0.05 are significant (Morelli and Prado 2012). Importance of every factor and the interaction strength amongst variables is checked by the P value (Karacabey and Mazza 2010).

The higher significance represents the better degree of correlation between values (observed/predicted) (Ravikumar et al. 2006). As shown in Table 3, the model significant response with high R^2 and adj- R^2 values different from 0.999 to 0.994, respectively, and coefficient of variation (CV) was 2.94. The signal-to-noise ($S:N$) ratio was measured by adequate precision measure of the model. A ratio above four is required; in the current model the value was found to be 37.47 (Table 3), indicating a very good $S:N$ ratio. Therefore, this model (quadratic) is appropriate for this experimental set-up. As in Table 3, it is clearly shown that coefficients,

Table 2 Analysis of variance and regression coefficients of the calculated surface quadratic model of polysaccharide yield

Source	Sum of squares	df	Mean square	F value	P value
Model	26.11	9	2.90	187.17	<0.0001
A—extraction time	0.39	1	0.39	25.26	0.0015
B—extraction temperature	2.27	1	2.27	146.34	<0.0001
C—liquid solid ratio	0.89	1	0.89	57.49	0.0001
AB	1.58	1	1.58	101.61	<0.0001
AC	6.92	1	6.92	446.23	<0.0001
BC	2.64	1	2.64	170.36	<0.0001
A ²	0.02	1	0.02	1.10	0.3301
B ²	4.14	1	4.14	266.77	<0.0001
C ²	6.56	1	6.56	423.41	<0.0001
Residual	0.11	7	0.02		
Lack of fit	0.09	3	0.03	5.80	0.0613
Pure error	0.02	4	0.01		
Cor. total	26.22	16			

Table 3 Regression coefficients of the calculated surface quadratic model of polysaccharide yield

SD	0.12	R^2	0.999
Mean	4.24	Adj R^2	0.994
C.V. %	2.94	Pred R^2	0.94
Press	1.44	Adeq precision	37.47

viz., linear (A , B , C), quadratic (A^2 , B^2 , C^2) and cross-product (AB , AC , BC) were statistically significant ($P < 0.05$). All of the above results indicate an excellent competence of the quadratic model for the tested data.

The three-dimensional representation of the response surface model illustrates the correlation between the dependent and independent variables of polysaccharide yield (Fig. 1). Extraction time is an important variable that might affect the yield efficacy and the fluid selectivity. The effect of extraction time on polysaccharide yield is shown in Fig. 1a. Different time intervals (3, 4.5 and 6 h) were used to carry

out the extraction process along with other extraction variables and conditions such as an ultrasonic temperature range of 70–110 °C, and ratios of water to raw material in the range of 10–40 mL/g. The polysaccharide yield touched a maximum of 5.42% at 95 °C when the time of extraction was increased from 3 to 5.2 h. The polysaccharide extraction yield did not increase on extending the extraction time beyond 5.2 h. This indicated that 5.2 h of extraction was adequate to harvest the optimum polysaccharide production. A lengthier extraction time also grants a positive effect and favors the extraction of polysaccharides (Hou and Wei 2008; Wang et al. 2009; RenJie 2008).

The effect of different ultrasonic temperatures on the yield of polysaccharides from *L. sativum* was also examined. Figure 1b displays the effects of extraction temperature on the polysaccharide yield while keeping other extraction conditions constant—time of extraction 4.5 h and water-to-raw material ratio in the range 10–40 mL/g. Increase in the temperature of extraction (70–110 °C) significantly increases the yield of polysaccharides and the maximum yield (5.42%)

Fig. 1 Response surface 3D plots: **a** the effect of extraction temperature and extraction time; **b** liquid–solid ratio and extraction time; **c** liquid–solid ratio and extraction temperature of polysaccharide yield

was observed when the temperature of extraction reached 95 °C. It is well-known that temperature affects the cavitation verge at which auditory cavitation occurs and also marks in the growth of cavitation nuclei. A higher polysaccharide yield occurred with a gradual increase in temperature at the initial period; however, acoustic cavitation in UAE yielded hydroxyl radicals, leading to chemical decomposition (Koda et al. 2003; Li et al. 2004).

Changes in the water-to-raw-material ratio can also considerably affect the yield of polysaccharides. The manner in which the yield was affected by the ratio is shown in Fig. 1c. The ratio varied between 10 and 40 mL/g while other extraction parameters were kept constant. It was detected that the polysaccharide extraction yield of *L. sativum* escalates with an increase in the fraction of water to raw material and reached a maximum value of about 5.42% when the ratio of water to raw material was 31.89 mL/g, but the yield no longer increased as the ratio was increased further. The diffusion of polysaccharides occurred more rapidly when a higher relation of water to raw material was chosen, which implies an additional concentration difference among the compounds and water. However, a higher water-to-raw-material ratio extended the diffusion space towards the interior tissues of the solid compounds. Consequently, the polysaccharide yield improved slowly as the water-to-raw-material ratio was increased from 10 to 40 mL/g (Govender et al. 2005). In the present work, an optimum water-to-raw-material ratio of 28.64 mL/g was adopted.

The point prediction tool of the software was used to validate and calculate the maximum polysaccharide yield; three experimental variables were used to test the suitability to validate the model adequacy equation. Point prediction tool was used to find out the optimum values and were found to be 5.2 h extraction time, 95 °C extraction temperature, and 31.89 mL/g water-to-raw-material ratio, which resulted in a maximum polysaccharide yield of 5.42%. These optimized values of the tested parameters were validated under the test conditions ($n=3$) and coincided with the predicted value of $5.44 \pm 0.05\%$, which also suggested that the regression model was precise and acceptable for the extraction of LSP. An average of $5.14 \pm 0.03\%$ polysaccharide was obtained in an optimized medium with 94.83%. It demonstrates that the ultrasonic circulating extraction process is operational and feasible to increase the yield.

Administration of *E. coli* to rats produced an acute systemic inflammation manifested by a significant increase in TNF- α levels compared to the control group ($P<0.05$). Soon after the *E. coli* administration, lethargy, crouching gait and piloerection were observed in septic rats, but those treated with LSP showed insignificant or none of the above symptoms. TNF- α , the prototype of pro-inflammatory cytokines, was evaluated 6 h after the *E. coli* administration in the serum of in the presence or absence of LSP treatment. Pretreatment

with LSP extract at 250 and 500 mg/kg doses ameliorated the systemic inflammation as indicated by a reduction in TNF- α in a dose-dependent manner (Fig. 2; $P<0.05$).

During homeostatic conditions, cytokines incorporate favorable and protective effects in both innate immunity and hematopoiesis and play a vital role in organogenesis (Gerard and Rollins 2001; Murakami et al. 2004). TNF- α is a well-known cytokine possessing various biological properties involving inflammation, proliferation and differentiation (Aggarwal 2003; Locksley et al. 2001). It also plays an important role in septic shock and cancer (Locksley et al. 2001). In response to TNF- α , endothelial cells promote inflammation by displaying multiple combinations of adhesion molecules for leukocytes in a distinct anatomical, temporal and spatial pattern (Aggarwal 2003). High levels of TNF- α were observed in patients with sepsis as well when bacterial endotoxin administered to human subjects (Gerard and Rollins 2001). Numerous in vitro and in vivo studies suggest that high levels of TNF- α generation lead to aggravation of inflammatory and pro-oxidative responses that are essential in the pathogenesis of many diseases, including

Fig. 2 Plasma TNF- α levels in *E. coli*-induced inflammation measured after bacterial challenge in rats treated with LSP 250 and 500 mg/kg ($n=10$ rats per group; data are given as mean $\pm$ SEM). * ($P<0.05$) In comparison with normal control and # ($P<0.05$) in comparison with *E. coli*

rheumatoid arthritis, atherosclerosis, inflammatory bowel disease and various pulmonary conditions (Bone 1991). Interestingly, inhibition or suppression of TNF- α has proven to be an effective therapy for patients with various inflammatory diseases including rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis and inflammatory bowel disease (Locksley et al. 2001; Aggarwal 2003; Bone 1991). Therefore, the administration of LSP or its equivalent could be of a potential value and may represent a natural method to suppress TNF- α during inflammatory disorders.

Conclusion

RSM was used to regulate the optimum processing conditions resulting in the maximum extraction yield of polysaccharides from *L. sativum* seeds. Under these conditions, the experimental yield of polysaccharides was $5.14 \pm 0.03\%$, which was close to the predicted yield value of $5.42 \pm 0.05\%$. The systemic activity of LSP indicated that the extract has a significant inhibitory effect against *E. coli*-induced inflammation by reducing the circulating levels of TNF- α . Further studies are warranted to explore the clinical implications of such observations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

- Aggarwal BB (2003) Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* 3(9):745–756. <https://doi.org/10.1038/nri1184>
- Ahmad A, Alkharfy KM, Wani TA, Raish M (2015) Application of Box–Behnken design for ultrasonic-assisted extraction of polysaccharides from *Paeonia emodi*. *Int J Biol Macromol* 72:990–997. <https://doi.org/10.1016/j.ijbiomac.2014.10.011>
- Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escalera LA (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* 76(5):965–977. <https://doi.org/10.1016/j.talanta.2008.05.019>
- Bone RC (1991) The pathogenesis of sepsis. *Ann Intern Med* 115(6):457–469
- Chen L, Li B, Li D, Gan J, Jiang W, Kitamura Y (2008) Ultrasound-assisted hydrolysis and acidogenesis of solid organic wastes in a rotational drum fermentation system. *Bioresour Technol* 99(17):8337–8343. <https://doi.org/10.1016/j.biortech.2008.02.043>
- Doke S, Guha M (2014) Garden cress (*Lepidium sativum* L.) seed—an important medicinal source: a review. *J Nat Prod Plant Resour* 4(1):69–80
- Entezari MH, Hagh Nazary S, Haddad Khodaparast MH (2004) The direct effect of ultrasound on the extraction of date syrup and its micro-organisms. *Ultrason Sonochem* 11(6):379–384. <https://doi.org/10.1016/j.ultsonch.2003.10.005>
- Gerard C, Rollins BJ (2001) Chemokines and disease. *Nat Immunol* 2(2):108–115. <https://doi.org/10.1038/84209>
- Govender S, Pillay V, Chetty D, Essack S, Dangor CM, Govender T (2005) Optimization and characterization of bioadhesive controlled release tetracycline micro spheres. *Int J Pharm* 306:24–40
- Guha M, Mackman N (2001) LPS induction of gene expression in human monocytes. *Cell Signal* 13(2):85–94
- Hou XJ, Wei C (2008) Optimization of extraction process of crude polysaccharides from wild edible BaChu mushroom by response surface methodology. *Carbohydr Polym* 72(1):67–74. <https://doi.org/10.1016/j.carbpol.2007.07.034>
- Juma A (2007) The effects of *Lepidium sativum* seeds on fracture-induced healing in rabbits. *MedGenMed Medscape Gen Med* 9(2):23
- Karacabey E, Mazza G (2010) Optimisation of antioxidant activity of grape cane extracts using response surface methodology. *Food Chem* 119(1):343–348. <https://doi.org/10.1016/j.foodchem.2009.06.029>
- Karazhiyan H, Razavi SMA, Phillips GO, Fang YP, Al-Assaf S, Nishinari K (2011) Physicochemical aspects of hydrocolloid extract from the seeds of *Lepidium sativum*. *Int J Food Sci Technol* 46(5):1066–1072. <https://doi.org/10.1111/j.1365-2621.2011.02583.x>
- Kirtikar KR (2005) Indian medicinal plant, vol 1. International Book Distributors, Dehradun
- Koda S, Kimura T, Kondo T, Mitome H (2003) A standard method to calibrate sonochemical efficiency of an individual reaction system. *Ultrason Sonochem* 10(3):149–156. [https://doi.org/10.1016/S1350-4177\(03\)00084-1](https://doi.org/10.1016/S1350-4177(03)00084-1)
- Li HZ, Pordesimo L, Weiss J (2004) High intensity ultrasound-assisted extraction of oil from soybeans. *Food Res Int* 37(7):731–738. <https://doi.org/10.1016/j.foodres.2004.02.016>
- Liu J, Li JW, Tang JA (2010) Ultrasonically assisted extraction of total carbohydrates from *Stevia rebaudiana* Bertoni and identification of extracts. *Food Bioprod Process* 88(C2-3):215–221. <https://doi.org/10.1016/j.fbp.2009.12.005>
- Liyana-Pathirana C, Shahidi F (2005) Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chem* 93(1):47–56. <https://doi.org/10.1016/j.foodchem.2004.08.050>
- Locksley RM, Killeen N, Lenardo MJ (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 104(4):487–501
- Ma CH, Liu TT, Yang L, Zu YG, Wang SY, Zhang RR (2011) Study on ionic liquid-based ultrasonic-assisted extraction of biphenyl cyclooctene lignans from the fruit of *Schisandra chinensis* Baill. *Anal Chim Acta* 689(1):110–116. <https://doi.org/10.1016/j.aca.2011.01.012>
- Manohar DVG, Nagesh S, Jain V, Shivaprasad HN (2012) Ethnopharmacology of *Lepidium sativum* Linn (Brassicaceae): a review. *Int J Phytother Res* 2(1):1–7
- Morelli LL, Prado MA (2012) Extraction optimization for antioxidant phenolic compounds in red grape jam using ultrasound with a response surface methodology. *Ultrason Sonochem* 19(6):1144–1149. <https://doi.org/10.1016/j.ultsonch.2012.03.009>
- Murakami T, Cardones AR, Hwang ST (2004) Chemokine receptors and melanoma metastasis. *J Dermatol Sci* 36(2):71–78. <https://doi.org/10.1016/j.jdermsci.2004.03.002>
- Nadkarni KM. (2005) Indian plant and drug with their medicinal properties and uses. Srishti Book Distributors, Delhi, pp 216–217
- Prakash Maran J, Manikandan S, Thirugnanasambandham K, Vigna Nivetha C, Dinesh R (2013) Box–Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk

- polysaccharide. Carbohydr Polym 92(1):604–611. <https://doi.org/10.1016/j.carbpol.2012.09.020>
- Raval N (2016) A comprehensive review of *Lepidium sativum* Linn, a traditional medicinal plant. World J Pharm Pharmaceutical Sci 5(5):593–1601
- Ravikumar K, Ramalingam S, Krishnan S, Balu K (2006) Application of response surface methodology to optimize the process variables for Reactive Red and Acid Brown dye removal using a novel adsorbent. Dyes Pigments 70(1):18–26. <https://doi.org/10.1016/j.dyepig.2005.02.004>
- Rehman NU, Khan AU, Alkharfy KM, Gilani AH (2012) Pharmacological basis for the medicinal use of *Lepidium sativum* in airways disorders. Evid Based Complement Altern Med eCAM 2012:596524. <https://doi.org/10.1155/2012/596524>
- Remick DG, Kunkel RG, Larrick JW, Kunkel SL (1987) Acute in vivo effects of human recombinant tumor necrosis factor. Lab Invest J Tech Methods Pathol 56(6):583–590
- RenJie L (2008) Orthogonal test design for optimization of the extraction of polysaccharides from *Phascolosoma esulenta* and evaluation of its immunity activity. Carbohydr Polym 73(4):558–563. <https://doi.org/10.1016/j.carbpol.2007.12.026>
- Samavati V, Manoochehrizade A (2013) Polysaccharide extraction from *Malva sylvestris* and its anti-oxidant activity. Int J Biol Macromol 60:427–436. <https://doi.org/10.1016/j.ijbiomac.2013.04.050>
- Save SS, Pandit AB, Joshi JB (1997) Use of hydrodynamic cavitation for large scale microbial cell disruption. Food Bioprod Process 75(C1):41–49. <https://doi.org/10.1205/096030897531351>
- Saxena PK, Gupta DK, Sharma RD, Gupta Ritu SKK (2015) Prospects of phytochemical activity of *Lepidium sativum*: a review. Int J Pharm Bio Sci 5(2):145–151
- Sharma S, Agarwal N (2011) Nourishing and healing powers of garden cress (*Lepidium sativum* Linn); a review. Ind J Nat Prod Resour 2(3):292–297
- Varki A, Cummings RD, Esko JD, Freeze H, Stanley P, Bertozzi CR, Hart GW, Etzler ME (2009) Essentials of glycobiology. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY)
- Vinatoru M, Toma M, Radu O, Filip PI, Lazurca D, Mason TJ (1997) The use of ultrasound for the extraction of bioactive principles from plant materials. Ultrason Sonochem 4(2):135–139
- Wang YJ, Cheng Z, Mao JW, Fan MG, Wu XQ (2009) Optimization of ultrasonic-assisted extraction process of Poria cocos polysaccharides by response surface methodology. Carbohydr Polym 77(4):713–717. <https://doi.org/10.1016/j.carbpol.2009.02.011>
- Yang B, Jiang YM, Zhao MM, Shi J, Wang LZ (2008) Effects of ultrasonic extraction on the physical and chemical properties of polysaccharides from longan fruit pericarp. Polym Degrad Stab 93(1):268–272. <https://doi.org/10.1016/j.polymdegradstab.2007.09.007>
- Ying Z, Han XX, Li JR (2011) Ultrasound-assisted extraction of polysaccharides from mulberry leaves. Food Chem 127(3):1273–1279. <https://doi.org/10.1016/j.foodchem.2011.01.083>