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Extraction of polysaccharides from *Morinda officinalis* by response surface methodology and effect of the polysaccharides on bone-related genes

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ABSTRACT

In this study, the optimization of the main variables involved in the extraction process has been done by response surface methodology (RSM) using, as responses, the extraction yield. The maximum amount of *Morinda officinalis* polysaccharides (7.32%) was obtained when *M. officinalis* were extracted with 4 times volume (v/v) of water above 100 °C for more than 160 min. A second-order polynomial response surface equation was developed indicating the effect of variables on extraction yield. The models were found to agree with the data at the probability level of 99%. The results indicated that the models explained 94% of the variability for *M. officinalis* polysaccharides extraction. Analysis of variance also showed that the regression models for *M. officinalis* polysaccharides extraction were statistically good with a significance level of $P < 0.0001$ and the models had no significant ($P < 0.05$) lack of fit. Pharmacological test showed that *M. officinalis* polysaccharides treatment could stimulate bone-related genes expression.

1. Introduction

In recent decades, polysaccharides isolated from botanical sources (mushrooms, algae, lichens and higher plants) have also attracted a great deal of attention in the biomedical arena because of their broad spectrum of therapeutic properties and relatively low toxicity (Paulsen, 2001; Tzianabos, 2000; Wasser, 2002). While our understanding of the mechanism of action of these substances is still developing, it appears that one of the primary mechanisms involves nonspecific induction of the immune system (Tzianabos, 2000). Indeed, the basic mechanism of the immunostimulatory, anti-tumor, bactericidal and other therapeutic effects of botanical polysaccharides is thought to occur via macrophage stimulation and modulation of the complement system (Chihara, 1992; Wang et al., 1997; Komori & Kishimoto, 1998; Onubogu et al., 2010; Thungrat, Pusoonthornthum, Fish, & Yibchok-anun, 2010; Ananthi et al., 2010).

The dried roots of *Morinda officinalis* How., Bajitian in Chinese, are a famous traditional Chinese medicine and are listed in the Chinese Pharmacopoeia (China Pharmacopoeia Committee, 2000). It has been used to support the entire body treating a wide range of symptoms, including poor digestion, high blood pressure, respiratory problems and immune deficiencies in China since ancient times (China Pharmacopoeia Committee, 2000; Li, Gong, Yang, Zhao, & Luo, 2003; Zhang, Yuan, Yang, Luo, & Zhao, 2002; Zhu, Wang, Zhang, Pei, & Fen, 2008; Li, Nie, Yan, Zhu, & Xie, 2009; Mateos-Aparicio, Mateos-Peinado, Jiménez-Escrig, & Rupérez, 2010; Simelane, Lawal, Djarova, & Opoku, 2010). The principal bioactive constituents of this herb are anthraquinones, which were reported to exhibit anti-HIV, anti-inflammatory, antinociceptive, antimicrobial, antioxidant, antihepatotoxic and antimutagenic (Ali et al., 2000; Choi et al., 2005; Adesokan, Ekanola & Okanlawon, 2010).

The aim of this study was to optimize extraction condition of the polysaccharides from *M. officinalis* by boiling water extraction. In order to obtain basic technological information for the *M. officinalis* polysaccharides, the performance of extraction operation through the approach of response surface was used. The extraction parameters studied were extraction time, extraction temperature, ratio of liquid to sample and extraction number.

2. Materials and methods

2.1. Experimental design

Four independent variables, extraction time (X1), extraction temperature (X2), ratio of liquid to sample (X3) and extraction number (X4), and the dependent response variable extraction yield

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Table 1
Levels of different process variables in coded and un-coded form for *Morinda officinalis* polysaccharides extraction.

Factor	Low	Center	High
X1	−1 (120 min)	0 (140 min)	1 (160 min)
X2	−1 (80 °C)	0 (90 °C)	1 (100 °C)
X3	−1 (3)	0 (4)	1 (5)
X4	−1 (2)	0 (3)	1 (4)

(%) were studied. Each independent variable was studied at three coded levels (−1, 0, +1). The minimum and maximum levels of each independent variable and the experimental design with respect to their coded and uncoded levels were presented in Table 1.

A second-order regression analysis of the data was carried out to get empirical model that defined response in terms of the independent variables. Analysis of variance (ANOVA) was performed in coded level of variables to study the effects of independent variables. The 3D graphs were generated to understand the effect of selected variables individually and in combination to determine their optimum level for maximal production of *M. officinalis* polysaccharides.

The actual and coded levels of the independent variables used in the experimental design and the results obtained were shown in Table 2. The experimental plan was designed and the results statistically analyzed using SAS ver. 8.0 software to build and evaluate models.

2.2. Separation and purification of the Morinda officinalis polysaccharides

The crude polysaccharides from *M. officinalis* were redissolved in ultra-pure water, then applied to a DEAE-Sephadex A-25 column (2.4 × 60 cm) for separation. The column was coupled to an ÄKTA Purifier 100 system (Amersham Pharmacia Biosciences). Detailed experimental conditions were as follows: concentration of crude polysaccharides, 3 mg/ml; injection volume, 4 ml; mobile phase, ultra-pure water; flow rate, 0.5 ml/min. Fractions of 5 ml were collected with a Pharmacia LKB Superfrac fraction collector, and the eluent (polysaccharide and protein elution) was monitored with a Shimadzu RID-10A Refractive Index Detector. After fractionation on a DEAE-Sephadex A-25 anion-exchange column, a fraction was obtained in the water eluate. The fraction eluted with water from crude polysaccharides was further purified on a Sephacryl S-400 column (2.4 × 60 cm). The column was coupled to an ÄKTA Purifier 100 system (Amersham Pharmacia Biosciences) for separation. The injection-loop was 5 ml, and 10–20 mg of the isolated fractions were applied onto the column. Sample elution was carried out using ultra-pure water as the eluent, at a flow rate of 0.5 ml/min. The eluent (polysaccharide and protein elution) was monitored with a Shimadzu RID-10A Refractive Index Detector. Three polysaccharide fractions, named as F-1, F-2 and F-3, were separated (Fig. 1). The fractions, F-1, F-2 and F-3, were dialysed and lyophilised, respectively. The yields of F-1, F-2 and F-3 were about 23.14%, 56.22% and 19.47% from the crude polysaccharides, respectively.

Table 2
Experimental data and the observed response values with different combinations of extraction time (X1), extraction temperature (X2), ratio of liquid to sample (X3) and extraction number (X4) for *Morinda officinalis* polysaccharides extraction.

Run	X1	X2	X3	X4	Y1
1	−1	−1	0	0	5.32
2	−1	1	0	0	5.44
3	1	−1	0	0	6.54
4	1	1	0	0	7.18
5	0	0	−1	−1	6.65
6	0	0	−1	1	6.87
7	0	0	1	−1	6.84
8	0	0	1	1	7.06
9	−1	0	0	−1	6.57
10	−1	0	0	1	6.76
11	1	0	0	−1	6.94
12	1	0	0	1	7.08
13	0	−1	−1	0	6.03
14	0	−1	1	0	6.21
15	0	1	−1	0	6.32
16	0	1	1	0	6.54
17	−1	0	−1	0	6.07
18	−1	0	1	0	5.81
19	1	0	−1	0	6.88
20	1	0	1	0	6.80
21	0	−1	0	−1	6.11
22	0	−1	0	1	6.47
23	0	1	0	−1	6.52
24	0	1	0	1	6.83
25	0	0	0	0	7.04
26	0	0	0	0	7.02
27	0	0	0	0	7.03

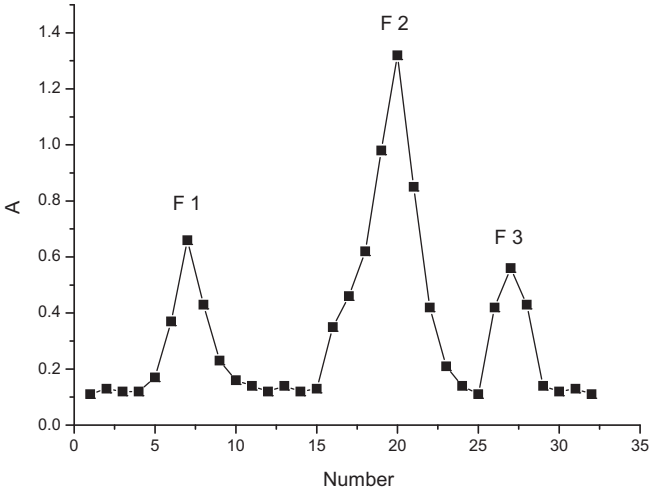

Fig. 1. Isolation and purification of *Morinda officinalis* polysaccharides.

2.3. GC/MS analyses

GC/MS analyses were performed on a Hewlett-Packard 5890 series II gas chromatograph, fitted with an OV1 fused silica capillary column (12 m × 0.30 mm), connected to a Hewlett-Packard 5989X mass spectrometer. In electron impact mode, an ionization potential of 70 eV with an ion source at 200 °C was used. Ammonia was the reagent gas used in the chemical ionization mode (ionization potential 230 eV, ion source 200 °C). The temperature program used was either 100 °C (delay 3 min) to 290 °C at 8 °C min^{-1} (program C) or 200 °C (delay 3 min) and 8 °C min^{-1} to 300 °C (program D).

2.4. IR analysis

FT-IR spectra were recorded as KBr pellets (1/100 mg) in the range 4000–400 cm^{-1} by using a Jasco FT-IR 410 spectrophotometer with a resolution of 4 cm^{-1}. Each spectrum was recorded after 16 scans.

2.5. Animals and treatment

Thirty-two healthy male Wistar rats were obtained from Laboratory Animal Center, our university. At the start of treatment, rats were housed in single cages maintained at 24 °C and 50–60% relative humidity and a 12-h light/dark cycle throughout experiment.

The rats were acclimatized to laboratory conditions for 7 days prior to treatment.

The animals were randomly assigned to four groups, namely control, and three polysaccharides-treated groups, each group containing eight rats. Rats in the control group were given freely tap water and standard chow diet throughout the experiment; rats in the polysaccharides-treated groups accepted the same feeding as that of the control group and orally receive polysaccharides. At the 30th day, animals were decapitated. Tibiaes were collected. These tissue specimen were frozen immediately in liquid nitrogen and stored at −80 °C.

2.6. Evaluation of mRNA expression

Total cellular RNA was isolated by RNA isolation kit according to the manufacturer's instructions and treated with DNase. RNA purity was confirmed with the spectrophotometric absorbance ratio at 260/280 and RNA quantity was determined by the absorbance at 260 nm. cDNA was synthesized from 240 ng of total RNA with a two-step reverse transcription-polymerase chain reaction (RT-PCR) kit using a thermal cycler (Gene Amp® PCR system 2400, Perkin–Elmer, Roche, NJ, USA). Specific oligonucleotides sequences were as follows: BMP-2 F: 5′-TCAGCGAGTTTGAGTTGAGG-3′. R: 5′-GATGGCTTCTTCGTGATGG-3′; Cbfal F: 5′-CCAAGAAGGCACAGACAGAA-3′. R: 5′-TGGCTCAGATAAGAGGGGTAAG-3′; Dmp1 F: 5′-CTGTCCTGTGCTCTCCCTGT-3′. R: 5′-TCGTCTTCATCCTCCTCCTT-3′; rRANKL F: 5′-GGCTGGGCCAAGATCTCTAAC-3′. R: 5′-GTGTCCAACCCTTCCCTGTTG-3′; rALP F: 5′-CCTTGAAAAATGCCCTGAAA-3′. R: 5′-CTTGGAGAGAGCCACAAAGG-3′; rOPG F: 5′-CTGTGAAAGCAGTGTGCAACG-3′. R: 5′-AGGTTCTTGCACAGCTTCACC-3′; rPPARγ2 F: 5′-CCCTGGCAAAGCATTTGTAT-3′. R: 5′-ACTGGCACCCTTGAAAAATG-3′; GAPDH F: 5′-CAAGTTCAACGGCACAGTCAA-3′. R: 5′-TGGTGAAGACGCCAGTAGACTC-3′. A dynamic test and cycle determination of PCR protocols were performed to determine the linear range of products and ensure an accurate semiquantitative analysis. There were 30–35 cycles of denaturation at 95 °C for 2 min, annealing at TA for 1 min and primer extension at 72 °C. The reactions were finally extended at 72 °C for 10 min. A blank control (RT-PCR without RNA template) and RT (−) reactions (PCR without reverse transcription) were executed along with all RT-PCR reactions. The PCR products were analyzed by electrophoresis in 1.5% agarose gel and visualized by ethidium bromide staining. Quantitative analysis was performed using the photographs and image-analysis software (Gel Documentation InGenius® L, Bio-Rod Lab, Hercules, CA, USA). The relative amount of BMP-2, Cbfal, Dmp1, rRANKL, rALP, rOPG, rPPARγ2 mRNA expression was calculated as its ratio to GAPDH from the same template.

2.7. Statistical analysis

Results were expressed as mean ± standard error (SE). Differences among groups were determined using one-way and/or two-way analysis of variance (ANOVA), followed by Dunnett's two-tailed *t*-test for comparison with control. A *P* value less than 0.05 was considered statistically significant.

3. Result and discussion

3.1. Optimization of extraction condition of Morinda officinalis polysaccharides

Box–Behnken design was employed for optimization of extraction condition of *M. officinalis* polysaccharides. The analysis of variance for these models was given in Table 3. Predicted response *Y* for the yield of Longan polysaccharides could be expressed by the following second-order polynomial equation in terms of coded values: Eq. (1). According to this model, linear terms of extraction time (X1, $P < 0.01$), extraction temperature (X2, $P < 0.01$), quadratic terms of extraction time (X1 × X1, $P < 0.01$), extraction temperature (X2 × X2, $P < 0.01$), and ratio of liquid to sample (X3 × X3, $P < 0.01$) reached significant. The result suggested that the change of the two factors, extraction time, and extraction temperature had a significant effect on extraction yield of *M. officinalis* polysaccharides. In contrast, the interaction between extraction time and ratio of solvent to sample (X1 × X3), extraction temperature and ratio of solvent to sample (X2 × X3), and ratio of solvent to sample and extraction number (X3 × X4) were not significant.

Table 3
Model coefficients estimated by multiples linear regression (significance of regression coefficients).

Effect	Estimate	Std error	*t* ratio	*P* value
X1	0.45417	0.074993	6.0561	<0.0001
X2	0.17917	0.074993	2.3891	0.0342
X3	0.036667	0.074993	0.48893	0.6337
X4	0.12	0.074993	1.6001	0.1355
X1X1	−0.33458	0.11249	−2.9743	0.0116
X1X2	0.13	0.12989	1.0008	0.3367
X1X3	0.045	0.12989	0.34644	0.7350
X1X4	−0.0125	0.12989	−0.096233	0.9249
X2X2	−0.56958	0.11249	−5.0634	0.0003
X2X3	0.01	0.12989	0.076987	0.9399
X2X4	−0.0125	0.12989	−0.096233	0.9249
X3X3	−0.24833	0.11249	−2.2076	0.0475
X3X4	0	0.12989	0	1.0000
X4X4	0.079167	0.11249	0.70377	0.4950

The models were found to agree with the data at the probability level of 99%. The accuracy of the models was evaluated by coefficient of determination (R^2 and adjusted R^2 values). Adjusted R^2 is a measure of the amount of variation with respect to the mean explained by the model. The results indicated that the models explained 94% of the variability for *M. officinalis* polysaccharides extraction. Analysis of variance (Table 4) also showed that the regression models for *M. officinalis* polysaccharides extraction were statistically good with a significance level of $P < 0.0001$ and the models had no significant ($P < 0.05$) lack of fit.

$$\begin{aligned} Y1 = {} & 7.03 + 0.454167 \times X1 + 0.179167 \times X2 + 0.036667 \times X3 \\ & + 0.12 \times X4 - 0.334583 \times X1 \times X1 + 0.13 \times X1 \times X2 \\ & + 0.045 \times X1 \times X3 - 0.0125 \times X1 \times X4 - 0.569583 \times X2 \\ & \times X2 + 0.01 \times X2 \times X3 - 0.0125 \times X2 \times X4 - 0.248333 \times X3 \\ & \times X3 + 0.079167 \times X4 \times X4 \end{aligned} \quad (1)$$

The 3D response surface and the 2D contour plots of the regression model were used to explain the effects of the independent variables and interactive effects of independent variables on the response. The shape of the corresponding contour plots indicated whether the mutual interactions between the independent variables were significant or not. From the 3D response surface plots and the 2D corresponding contour plots, the optimal values of the independent variables and the corresponding response could

Table 4
Fit statistics for Y1.

	Master model	Predictive model
Mean	6.552963	6.552963
R-square	87.41%	87.41%
Adj. *R*-square	72.73%	72.73%
RMSE	0.259785	0.259785
CV	3.964388	3.964388

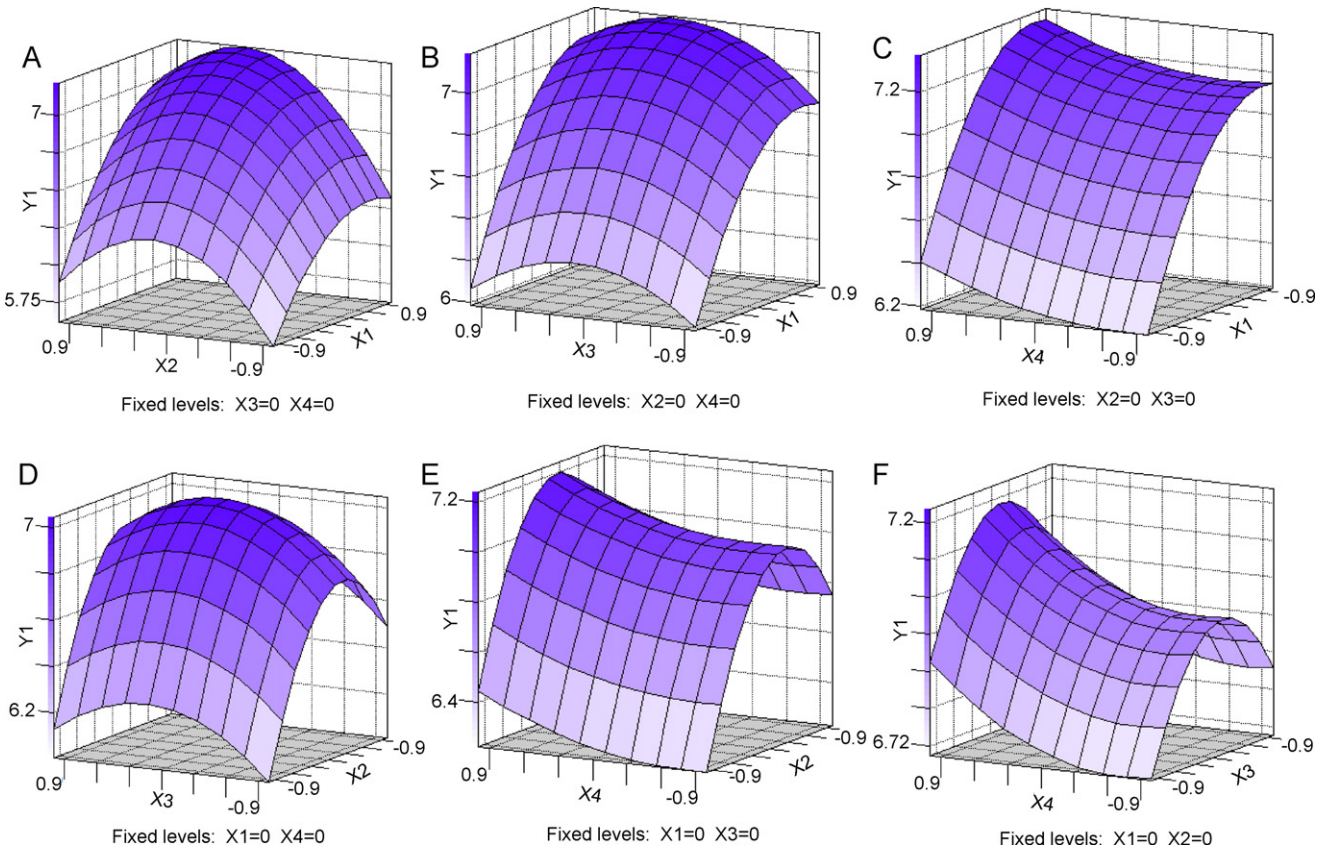

Fig. 2. Three-dimensional mesh plot of *Morinda officinalis* polysaccharides production: (A) the effect of extraction time (X1) and extraction temperature (X2) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (B) the effect of extraction time (X1) and ratio of liquid to sample (X3) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (C) the effect of extraction time (X1) and extraction number (X4) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (D) the effect of extraction temperature (X2) and ratio of liquid to sample (X3) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (E) the effect of extraction temperature (X2) and extraction number (X4) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (F) the effect of ratio of liquid to sample (X3) and extraction number (X4) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level.

be predicted. The interaction between each independent variable pair could be understood. The maximum predicted value was indicated by the surface confined in the smallest ellipse in the contour diagram. The *M. officinalis* polysaccharides production for different concentrations of the variables could be predicted from Fig. 2(A–F) and Fig. 3(A–F), corresponding to the interactive effects of three important variables, respectively. It could be seen from Figs. 2(A–F) and 3(A–F) that the response surface of each variable was almost independent of the concentration of the other. The interactions of three variables were not significant. The plots showed that optimal conditions of *M. officinalis* polysaccharides extraction were 160 min (extraction time), 100 °C (extraction temperature), 4 (ratio of liquid to sample) and 3 (extraction number). Maximum extraction yield under this optimal condition was 7.32%.

3.2. Monosaccharide compositions of Morinda officinalis polysaccharides

M. officinalis polysaccharides was hydrolysed by trifluoroacetic acid into individual monosaccharides that were further trimethylsilylated for gas chromatography analysis. In comparison with the retention time of standards, the monosaccharide composition was identified. F-1 was composed of glucose and fructose in a molar ratio of 0.5:1.4. F-2 was composed of glucose, galactose and xylose in a molar ratio of 1.3:0.9:0.6. F-3 was composed of arabinose, glucose, galactose and xylose in a molar ratio of 0.2:0.8:1.6:1.1.

3.3. Chemical structure of Morinda officinalis polysaccharides

The IR microscopy spectrum of *M. officinalis* polysaccharides is shown in Fig. 4. There are several absorptions at 1708, 1642, 1419, 1384, 1258, 1062, 931 and 821 cm^{-1} that are associated with the polysaccharide moiety. A characteristic peak was present at 1419 cm^{-1} corresponding to the C–O stretch and CH or OH bending. The bands at 1384 and 1258 cm^{-1} represent C–H, O–H or $C–H_2$ bending. An extremely broad band due to hydrogen-bonded hydroxyl groups (O–H) appearing at 3408 cm^{-1} is attributed to the complex vibrational stretches associated with free and inter- and intramolecular bound hydroxyl groups. The range of C–H stretching vibrations (2926–2339 cm^{-1}) represents $–CH_2–$ as wells as $–CH_3$ vibrational bands. The peak at 871 per cm in the FT-IR spectra of *M. officinalis* polysaccharides indicated the presence of β-type glycosidic linkages in the polysaccharide.

3.4. Effect of Morinda officinalis polysaccharides on bone-related gene expression in rat

BMP-2 like other bone morphogenetic proteins, plays an important role in the development of bone and cartilage (Chen, Zhao, & Mundy, 2004). It is involved in the hedgehog pathway, TGF beta signaling pathway, and in cytokine-cytokine receptor interaction. It is involved also in cardiac cell differentiation and epithelial to mesenchymal transition. Cbfal (Core-binding factor al) also known as Pebp2aA (polyomavirus enhancer binding protein) or AML3 (acute myeloid leukemia), is one of the transcription factors that

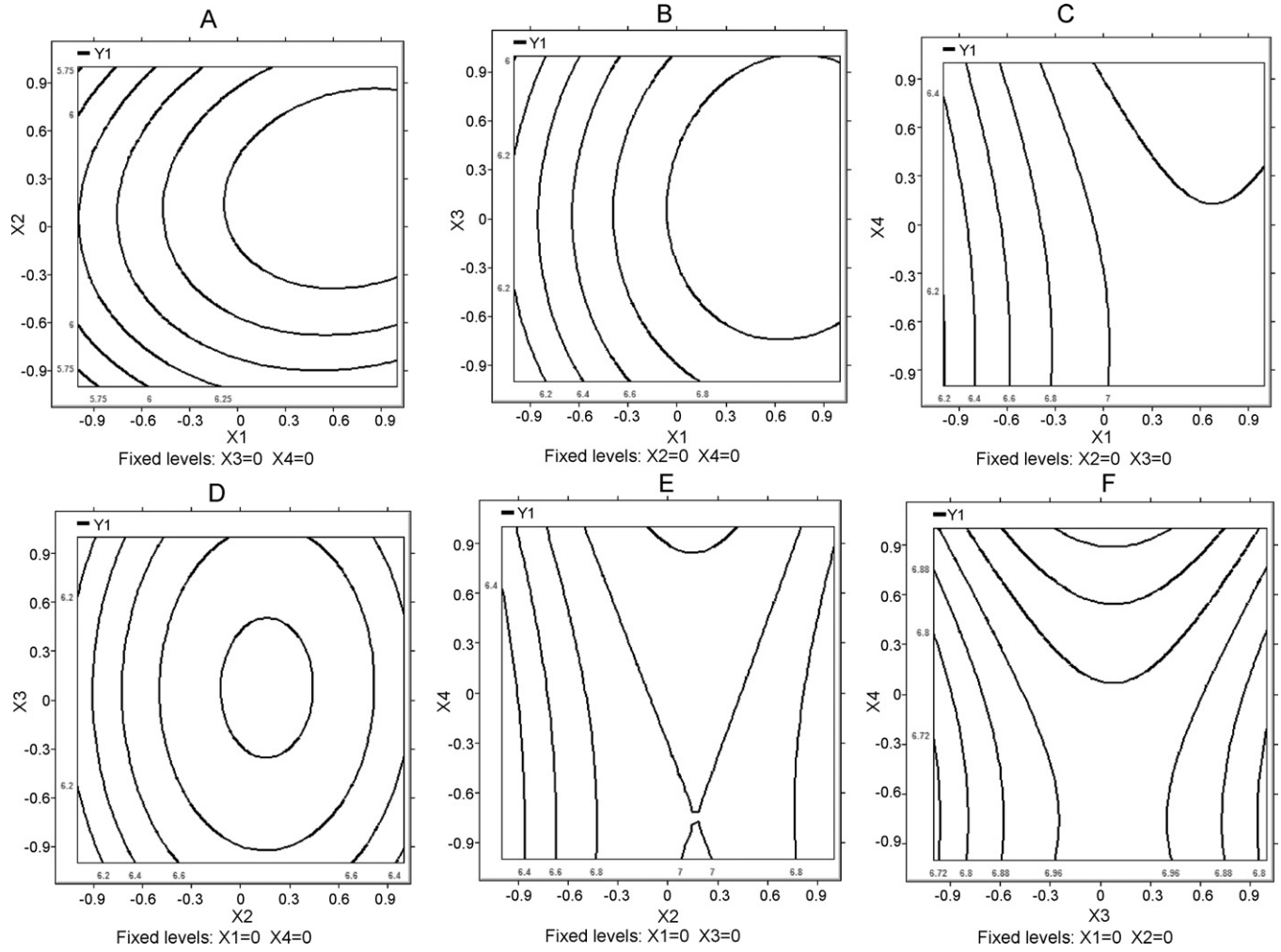

Fig. 3. Two-dimensional contour plot of *Morinda officinalis* polysaccharides production: (A) the effect of extraction time (X1) and extraction temperature (X2) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (B) the effect of extraction time (X1) and ratio of liquid to sample (X3) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (C) the effect of extraction time (X1) and extraction number (X4) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (D) the effect of extraction temperature (X2) and ratio of liquid to sample (X3) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (E) the effect of extraction temperature (X2) and extraction number (X4) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level; (F) the effect of ratio of liquid to sample (X3) and extraction number (X4) on *Morinda officinalis* polysaccharides production (Y) with other components set at center level.

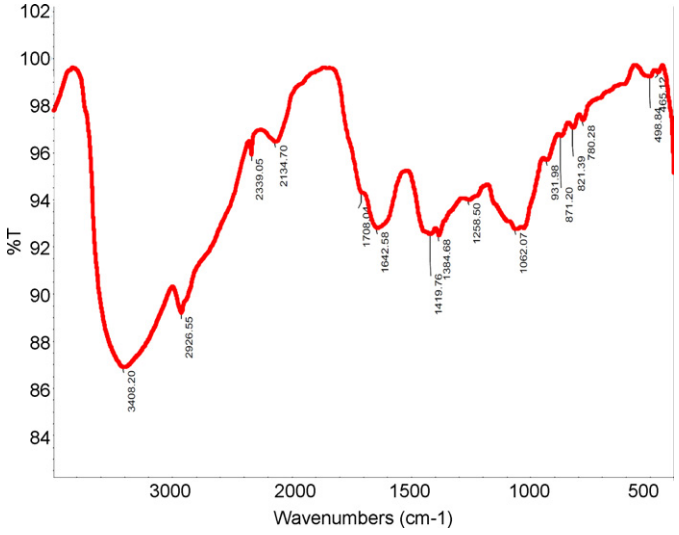

Fig. 4. The FT-IR spectra of *Morinda officinalis* polysaccharides.

belongs to the runt-domain gene family (Komori & Kishimoto, 1998; Levanon et al., 1994; Ogawa et al., 1993). Cbfal/Pebp2aA controls the expression of many genes expressed in osteoblasts (Ducy, Zhang, Geoffroy, Ridall, & Karsenty, 1997) and Cbfal/Pebp 2a A-deficiency in mice resulted in a complete lack of bone formation (Komori et al., 1997; Otto et al., 1997). DMP1 is a major regulator of mineralization and that it may play a role in the transition of osteoblasts/preosteocytes to osteocytes perhaps through maintenance of structure of the lacunae and canaliculi. DMP1 may also be an important regulator of osteocyte-mediated responses to mechanical loading perhaps through its role as a regulator of mineralization (Rios et al., 2005). Receptor activator of nuclear factor kappa-B ligand (RANKL), also known as tumor necrosis factor ligand superfamily member 11 (TNFSF11), TNF-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL), and osteoclast differentiation factor (ODF), is a protein that in humans is encoded by the TNFSF11 gene (Anderson et al., 1997; Wong et al., 1997). RANKL is important in bone metabolism. This natural and necessary surface-bound molecule (also known as CD254) found on osteoblasts serves to activate osteoclasts, which are the cells involved in bone resorption. ALP is a well established osteoblast phenotypic marker, the temporal expression of this gene indi-

Table 5
Effect of *Morinda officinalis* polysaccharides on bone-related genes expression in rat.

Gene	Sequences	Expression
BMP-2	F: 5′-TCAGCGAGTTTGAGTTGAGG-3′ R: 5′-GATGGCTTCTTCGTGATGG-3′	Up
Cbfal	F: 5′-CCAAGAAGGCACAGACAGAA-3′ R: 5′-TGGCTCAGATAAGAGGGGTAAG-3′	Up
Dmp1	F: 5′-CTGTCCTGTGCTCTCCCTGT-3′ R: 5′-TCGTCTTCATCCTCCTCCTT-3′	Up
rRANKL	F: 5′-GGCTGGGCCAAGATCTCTAAC-3′ R: 5′-GTGTCCAACCCTTCCCTGTTG-3′	Up
rALP	F: 5′-CCTTGAAAAATGCCCTGAAA-3′ R: 5′-CTTGGAGAGAGCCACAAAGG-3′	Up
rOPG	F: 5′-CTGTGAAAGCAGTGTGCAACG-3′ R: 5′-AGGTTCTTGCACAGCTTCACC-3′	Up
rPPARγ2	F: 5′-CCCTGGCAAAGCATTTGTAT-3′ R: 5′-ACTGGCACCCTTGAAAAATG-3′	Up
GAPDH	F: 5′-CAAGTTCAACGGCACAGTCAA-3′ R: 5′-TGGTGAAGACGCCAGTAGACTC-3′	Up

cates the transient differentiation of MSCs into osteoblasts under mechanical strain (Qi, Zou, Han, Zhou, & Hu, 2009). Osteoporosis (OPG) and osteoclast differentiation factor (ODF) can adjust the differentiation and the bone absorption of the osteoclast. In the bone microenvironment, the ratio of ODF mRNA/OPG mRNA decides the bone metabolic direction (anabolism or catabolism) (Chen, Chen, & Guo, 2007). Overexpression of peroxisome proliferator-activated receptor γ2 (PPARγ2), a member of the nuclear receptor transcription factor family, induces adipogenesis over osteoblastogenesis in pluripotent cells. Interestingly, if PPARγ2 is expressed in osteoblasts it can suppress the mature osteoblast phenotype and induce genes associated with an adipocyte-like phenotype, such as fatty acid binding protein 4 (FABP4/aP2), fatty acid synthase (FAS) and lipoprotein lipase (LPL) (Botolin et al., 2005).

Compared to normal control rats, administration of Lentinus edodes polysaccharides increased bone-related genes (BMP-2, Cbfal, Dmp1, rRANKL, rALP, rOPG, rPPARγ2 mRNA) expression in the polysaccharides-treated rats (Table 5). Among them, BMP-2, Cbfal, rRANKL, rALP and rPPARγ2 mRNA expression levels were markedly increased with *M. officinalis* polysaccharides treatment.

4. Conclusion

RSM was used to determine the optimum process parameters that gave a high extraction yield. ANOVA showed that the effects of all variables (i.e. extraction time, extraction temperature, ratio of liquid to sample and extraction number) were significant and quadratic models were obtained for predicting the responses. The optimal conditions were: extraction time 160 min, extraction temperature 100 °C, ratio of liquid to sample 4 and extraction number 3. Pharmacological test showed that *M. officinalis* polysaccharides treatment could stimulate bone-related genes expression.

References

Adesokan, I. A., Ekanola, Y. A., & Okanlawon, B. M. (2010). Influence of cultural conditions on hydrogen peroxide production by lactic acid bacteria isolated from some Nigerian traditional fermented foods. *African Journal of Microbiology Research.*, *4*(19), 1991–1996.

Ali, A. M., Ismail, N. H., Mackeen, M. M., Yazan, L. S., Mohamed, S. M., Ho, A. S. H., et al. (2000). Antiviral, cytototoxic and antimicrobial activities of anthraquinones isolated from the roots of Morinda elliptica. *Pharmaceutical Biology*, *38*, 298–301.

Ananthi, S., Raghavendran, H. R. B., Sunil, A. G., Gayathri, V., Ramakrishnan, G., & Vasanthi, H. R. (2010). In vitro antioxidant and in vivo anti-inflammatory potential of crude polysaccharide from Turbinaria ornata (Marine Brown Alga). *Food and Chemical Toxicology*, *48*, 187–192.

Anderson, D. M., Maraskovsky, E., Billingsley, W. L., Dougall, W. C., Tometsko, M. E., Roux, E. R., et al. (1997). A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature*, *390*(6656), 175–179.

Botolin, S., Faugere, M.-C., Malluche, H., Orth, M., Meyer, R., & McCabe, L. R. (2005). Increased bone adiposity and PPARγ2 expression in type I diabetic mice. *Endocrinology*, 0, en.2004-1677v1.

Chen, D., Chen, A. M., & Guo, F. J. (2007). Expression of OPGmRNA and ODFmRNA in the patients of hip fracture due to osteoporosis. *Journal of Nanjing Medical University*, *21*, 321–323.

Chen, D., Zhao, M., & Mundy, G. R. (2004). Bone morphogenetic proteins. *Growth Factors*, *22*(4), 233–241.

Chihara, G. (1992). Recent progress in immunopharmacology and therapeutic effects of polysaccharides. *Developments in Biological Standardization*, *77*, 191–197.

China Pharmacopoeia Committee, Pharmacopoeia of the People's Republic China, the first division of 2005 edition, China Chemical Industry Press, Beijing, 2000, pp. 55–56.

Choi, J., Lee, K. T., Choi, M. Y., Nam, J. H., Jung, H. J., Park, S. K., et al. (2005). Antinociceptive anti-inflammatory effect of monotropein isolated from the root of Morinda officinalis. *Biological & Pharmaceutical Bulletin*, *28*, 1915–1918.

Ducy, P., Zhang, R., Geoffroy, V., Ridall, A. L., & Karsenty, G. (1997). Osf2/Cbfal: a transcriptional activator of osteoblast differentiation. *Cell*, *89*, 747–754.

Komori, T., & Kishimoto, T. (1998). Cbfal in bone development. *Current Opinion in Genetics & Development*, *8*, 494–499.

Komori, T., Yagi, H., Nomura, S., Yamaguchi, A., Sasaki, K., Deguchi, K., et al. (1997). Targeted disruption of Cbfal results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell*, *89*, 755–764.

Levanon, D., Negreanu, V., Bernstein, Y., Bar-Am, I., Avivi, L., & Groner, Y. (1994). AML1, AML2, and AML3, the human members of the runt domain genefamily: cDNA structure, expression, and chromosomal localization. *Genomics*, *23*, 425–432.

Li, Y. F, Gong, Z. H., Yang, M., Zhao, Y. M., & Luo, Z. P. (2003). Inhibition of the oligosaccharides extracted from Morinda officinalis, a Chinese traditional herbal medicine, on the corticosterone induced apoptosis in PC12 cells. *Life Science*, *72*, 933–942.

Li, W.-J., Nie, S.-P., Yan, Y., Zhu, S.-B., & Xie, M.-Y. (2009). The protective effect of Ganoderma atrum polysaccharide against anoxia/reoxygenation injury in neonatal rat cardiomyocytes. *Life Sciences*, *85*, 634–641.

Mateos-Aparicio, I., Mateos-Peinado, C., Jiménez-Escrig, A., & Rupérez, P. (2010). Multifunctional antioxidant activity of polysaccharide fractions from the soybean byproduct okara. *Carbohydrate Polymers*, *82*, 245–250.

Ogawa, E., Maruyama, M., Kagoshima, H., Inuzuka, M., Lu, J., Satake, M., et al. (1993). PEBP2/PEA2 represents a family of transcription factors homologous to the products of the Drosophila runt gene and the human AML1 gene. *The Proceedings of the National Academy of Sciences USA*, *90*, 6859–6863.

Otto, F., Thornell, A. P., Crompton, T., Otto, F., Thornell, A. P., Crompton, T., et al. (1997). Cbfal, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell*, *89*, 765–771.

Onubogu, C. C., Kunle-Ope, C. N., Onyejepu, N., Nwokoye, N. N., Raheem, T. Y., Igbasi, U. T., Tochukwu, N. E., Omoloye, R. M., Ejezie, C. O., Musa, A. Z., Odunukwe, N. N., Onwujekwe, D. I., & Idigbe, E. O. (2010). Prevalence of tuberculosis and human immunodeficiency virus (TB/HIV) co-infections amongst patients with bronchopulmonary disorders in Lagos. *African Journal of Microbiology Research*, *4*(18), 1904–1908.

Paulsen, B. S. (2001). Plant polysaccharides with immunostimulatory activities. *Current Organic Chemistry*, *5*, 939–950.

Qi, M.-C., Zou, S.-J., Han, L.-C., Zhou, H.-X., & Hu, J. (2009). Expression of bone-related genes in bone marrow MSCs after cyclic mechanical strain: Implications for distraction osteogenesis. *International Journal of Oral Science*, *1*, 143–150.

Rios, H. F., Ye, L., Dusevich, V., Eick, D., Bonewald, L. F., & Feng, J. Q. (2005). DMP1 is essential for osteocyte formation and function. *Journal of Musculoskeletal and Neuronal Interactions*, *5*(4), 325–327.

Simelane, M. B. C., Lawal, O. A., Djarova, T. G., & Opoku, A. R. (2010). In vitro antioxidant and cytotoxic activity of Gunnera perpensa L. (Gunneraceae) from South Africa. *Journal of Medicinal Plants Research*, *4*(21), 2181–2188.

Thungrat, K., Pusoonthornthum, P., Fish, K., & Yibchok-anun, S. (2010). Treatment of canine diabetes mellitus using Momordica charantia capsule and a restricted-fat high-fiber diet. *Journal of Medicinal Plants Research*, *4*(21), 2243–2251.

Tzianabos, A. O. (2000). Polysaccharide immunomodulators as therapeutic agents: Structural aspects and biological function. *Clinical Microbiology Reviews*, *13*, 523–533.

Wang, S. Y., Hsu, M. L., Hsu, H. C., Tzeng, C. H., Lee, S. S., Shiao, M. S., et al. (1997). The antitumor effect of Ganoderma lucidum is mediated by cytokines released from activated macrophages and T lymphocytes. *International Journal of Cancer*, *70*, 699–705.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, *60*, 258–274.

Wong, B. R., Rho, J., Arron, J., Robinson, E., Orlinick, J., Chao, M., et al. (1997). TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. *The Journal of Biological Chemistry*, *272*(40), 25190–25194.

Zhang, Z. Q., Yuan, L., Yang, M., Luo, Z. P., & Zhao, Y. M. (2002). The effect of Morinda officinalis How, a Chinese traditional medicinal plant, on the DRL 72-s schedule in rats and the forced swimming test in mice. *Pharmacology Biochemistry and Behavior*, *72*, 39–43.

Zhu, M. Y., Wang, C. J., Zhang, H. S., Pei, X. W., & Fen, J. M. (2008). Protective effect of polysaccharides from morinda officinalis on bone loss in ovariectomized rats. *International Journal of Biological Macromolecules*, *43*, 276–278.