Resource-Efficient Technologies 3 (2017) 385–393

Contents lists available at ScienceDirect

Resource-Efficient Technologies

journal homepage: www.elsevier.com/locate/reffit

Research paper

Optimization studies on extraction of phytocomponents from betel leaves

Muruganandam L*, Anantha Krishna, Jashwanth Reddy, G.S. Nirmala

Chemical Engineering Department, School of Civil and Chemical Engineering, VIT University, Vellore, Tamil Nadu 632014, India

ARTICLE INFO

Article history:
Received 12 October 2016
Revised 31 January 2017
Accepted 27 February 2017
Available online 19 April 2017

Keywords:
Betel leaves
Box–Behnken response surface design (BBD)
Phytocomponents
Antioxidants

ABSTRACT

The present study aims at finding out the optimum parameters for the extraction of components from Betel leaves possessing medicinal applications using ethanol solvent by Soxhlet apparatus. The optimum conditions for the extract were calculated based on the extract yield by varying four parameters: material quantity (A: 2–4 g), solvent quantity (B: 250–300 ml), mantle temperature (C: 65–75 °C) and extraction time (D: 1–3 hours) and optimized using a four factor three level Box–Behnken response surface design (BBD) coupled with desirability function methodology. Results showed that temperature and extraction time had significant effect on yield of extract. Optimum conditions for highest yield of extract (10.94%) are as follows: material quantity (2 g), solvent quantity (281.4 ml), temperature (72 °C) and time (3 hours). The extract at the maximum yield condition was analyzed for phytocomponents by FTIR and GC–MS. The results indicated the presence of Hydroxy chavicol (69.46%), 4-Chromanol (24%) and Eugenol (4.86%), which possess wide application including as antioxidant, anti-inflammatory, anti-platelet and antithrombotic, antibacterial and antifungal agents.

© 2017 Tomsk Polytechnic University. Published by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license.
(http://creativecommons.org/licenses/by-nc-nd/4.0/)

1. Introduction

Piper betel, belonging to the *piperaceae* family, is one of the precious medicinal herbs found in central and eastern Malaysia, Southeast Asia. In India, it is commonly known as Paan, which is second to tea and coffee based on daily consumption. In spite of its alienness, the plant is much more popular in India than in any other country in the world since antiquity. This would be evident from the numerous citations laid down in the ancient literature, particularly the Indian scriptures [1,2]. Betel leaves are very nutritive and contain substantial amount of vitamins and minerals [3]. The leaves also contain the enzymes like diastase and catalase besides a significant amount of several essential amino acids including lysine, histidine and arginine [1,2,4–6]. Most of the previous studies on Betel leaf concentrated more on the components present in betel leaf extract [7–12]. There is a large scope in determining an appropriate extraction technique that extracts the phyto-components from betel leaves. Selection of solvent is an important aspect in any extraction; in general, solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate, have been commonly used for the extraction of phenolics from fresh products [13]. Appropriate extraction technique coupled with the optimization of the parameters involved in extraction and using a right optimization technique proves essential in order to capture medicinal components for further processing in pharmaceutical industries.

Scientific research on betel leaf reveals that it possesses many beneficial bioactivities and its extract has a great potential to be used in developing commercial products [14–19]. Though research has been done extensively on the components of betel leaf, its medicinal properties and potential applications, few research studies focused on the maximization of the yield of extract for various parameters.

The present study mainly concentrates on maximizing the yield of the extract by varying the parameters such as quantity of material fed, quantity of solvent, heating temperature in the mantle and extraction time. Further, the optimization of parameters using right optimization tools would reduce number of experimentation focusing on maximum medicinal components of betel leaf for further processing. The prediction of number of experiments to be performed was governed by using an appropriate response surface model as in Design Expert 9.0.4.1 software. Sirisomboon and Kitchaiya [20] reported that the total amount of oil extracted

* Corresponding author. Chemical Engineering Department, School of Civil and Chemical Engineering, VIT University, Vellore, Tamil Nadu 632014, India. Tel.: +9104162202515.
E-mail address: lmn@vit.ac.in (M. L).

https://doi.org/10.1016/j.reffit.2017.02.007
2405-6537/© 2017 Tomsk Polytechnic University. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license.
(http://creativecommons.org/licenses/by-nc-nd/4.0/)

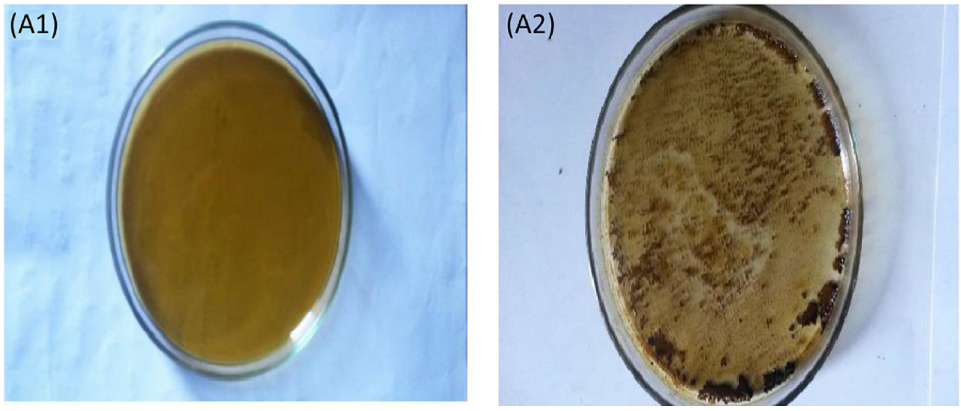

Fig. 1. Final extract before (A1) and after evaporation of solvent (A2).

using Soxhlet from jatrohpa kernels depends mainly on the extraction time and temperature. Ahmad et al. [21] reported the effects of extraction time and solvent type on the extracted oil of Herba leonuri, a medicinal plant. Authentication of plant material should be done before performing extraction. Any foreign matter should be completely eliminated. Selection of right extraction procedure considering the pros and cons of other extraction alternatives is a key step in extraction. Soxhlet extraction has been the most used extraction technique worldwide for a number of decades, surpassing the performance of other extraction alternatives and being used as an efficiency reference for the comparison of its conventional and new counterparts [21]. Being a continuous–discrete technique, it shows some important advantages. Specifically, in Soxhlet extraction, the sample is repeatedly brought into contact with fresh portions of solvent facilitating the displacement of the transfer [22].

Variables such as temperature, extraction time, material and solvent quantity on the extraction yield of extract were optimized using a four factor three-level Box–Behnken response surface design coupled with desired function methodology. Box–Behnken design has proven to be an extremely valuable tool, it not only helps in determining the accurate optimum values of experimental parameters but also provides the possibility to evaluate the interaction between variables with a reduced number of experiments [23]. The optimized controlled conditions determined in this study should offer important reference values for any subsequent studies. The solvent used for extraction was ethanol, a polar molecule due to the presence of OH group, which attracts non-polar substances because the ethyl (C_2H_5) in ethanol is non-polar. Thus, it can dissolve both polar and non-polar substances.

During Soxhlet extraction, the system remains at a relatively higher temperature by effect of the heat applied to the distillation flask reaching the extraction cavity to some extent. In addition, no filtration is required after leaching and sample throughput can be increased by performing several simultaneous extractions in parallel, which is facilitated by the low cost of the basic equipment [24]. The sample corresponding to the maximum yield was analyzed for functional groups and the corresponding compounds and their quantity were analyzed by FTIR and GC–MS respectively. FTIR is certainly one of the most important analytical techniques available for identifying the types of chemical bonds (functional groups) present in compounds. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. GC–MS technique was used in this study to identify the components present in the extract. The structures of the components were identified using a mass spectrophotometer.

The objectives of the study include setting up different experimental parameters for Soxhlet extraction and performing selective experiments as per Design of Experiments (DOE) 9.0.4.1 Box–Behnken methodology; finding out optimum conditions for Betel leaf extract based on the effect of various parameters like solvent quantity, leaf mass fed, mantle heating temperature and contact time; and the influence of these parameters on the mass of extract obtained. The FTIR and GCMS analysis was done for the sample at the optimum condition to the components present in the extract corresponding to maximum yield.

2. Experimental methods

2.1. Materials

The plant materials *Piper betle leaves* used for performing extraction was purchased from local market of Vellore, India. The instruments used for analysis of components in extract are as follows: FTIR (Fourier Transform Infrared radiation) Spectrometer – Shimadzu IR affinity-1, and GC–MS (Gas Chromatography and Mass spectroscopy): GC – Perkin Elmer GC Clarus 680 system and MS – Clarus 600 system (GC–MS).

2.2. Extraction parameters

The parameters include material quantity: 2 g, 3 g, 4 g; solvent quantity: 250 ml, 275 ml, 300 ml; mantle temperature: 65 °C, 70 °C, 75 °C and extraction time: 1 hour, 2 hours and 3 hours. The 4 factors (parameters) with 3 levels gave a total of 29 selective experiments (in randomized order), as per Design Expert software 9.0.4.1, Box–Behnken response surface design. The final mass of extract after vaporization of solvent corresponding to each experiment was calculated and the respective percentage yield of extract was governed. Crude extract after the evaporation of the solvent is shown in Fig. 1 .

The extraction yield is a measure of the solvent's efficiency to extract specific components from the original material and it was defined as the amount of extract recovered in mass compared with the initial amount of whole plant. It is presented in percentage (%) and was determined for each technique tested. Further, the extract corresponding to maximum percentage yield was analyzed for components by FTIR and GCMS analysis.

2.3. FTIR analysis

FTIR spectroscopy utilizes ceramic light source with DLATGS detector and an interferometer. The sample was placed between interferometer and detector. Ceramic rods, used to produce infrared light source, produce corresponding interferogram in the detector when they fall on the sample. This interferogram obtained from

Table 1
Experimental results.

Run	Factor 1 A: material Quantity (g)	Factor 2 B: solvent Quantity (ml)	Factor 3 C: Temperature (°C)	Factor 4 D: time (hours)	Mass of extract (g)	Response yield %	Predicted yield %
1	2	275	65	2	0.154	7.70	7.72
2	3	250	75	2	0.252	8.40	8.54
3	4	275	70	1	0.282	7.05	7.35
4	3	275	65	3	0.288	9.63	9.62
5	4	275	65	2	0.324	8.10	8.03
6	3	275	75	3	0.316	10.56	10.53
7	4	275	70	3	0.416	10.40	10.71
8	3	300	65	2	0.228	7.60	7.76
9	3	275	70	2	0.273	9.10	9.02
10	3	300	70	1	0.196	6.56	6.97
11	3	275	75	1	0.231	7.70	7.33
12	2	275	70	1	0.135	6.75	6.75
13	3	300	70	3	0.309	10.30	10.36
14	3	300	75	2	0.265	8.86	8.91
15	3	275	70	2	0.267	8.93	9.02
16	3	275	70	2	0.273	9.10	9.02
17	4	275	75	2	0.372	9.32	9.38
18	3	250	70	1	0.180	6.00	6.01
19	3	275	65	1	0.172	5.76	5.41
20	2	275	70	3	0.216	10.8	10.81
21	4	250	70	2	0.350	8.77	8.62
22	2	300	70	2	0.185	9.25	9.01
23	2	250	70	2	0.162	8.10	8.15
24	3	275	70	2	0.270	9.00	9.02
25	3	250	70	3	0.310	10.36	10.04
26	3	275	70	2	0.270	9.00	9.02
27	3	250	65	2	0.198	6.60	6.86
28	4	300	70	2	0.378	9.47	9.03
29	2	275	75	2	0.181	9.05	9.20

Table 2
Analysis of variance for response surface quadratic model of yield of extract.

Source	Sum of squares	df	Mean square	F value	P value Prob > F
Model	52.667	14	3.761	44.935	<0.0001
A – material quantity	0.181	1	0.181	2.165	0.1633
B – solvent quantity	1.213	1	1.213	14.499	0.0019
C – temperature	6.032	1	6.032	72.057	<0.0001
D – time	41.193	1	41.193	492.040	<0.0001
AB	0.050	1	0.050	0.604	0.4497
AC	0.003	1	0.003	0.046	0.8321
AD	0.12	1	0.12	1.46	0.2464
BC	0.071	1	0.071	0.85	0.3723
BD	0.10	1	0.10	1.20	0.2922
CD	0.25	1	0.25	2.99	0.1060
A^2	0.092	1	0.092	1.10	0.3114
B^2	1.26	1	1.26	15.00	0.0017
C^2	2.06	1	2.06	24.65	0.0002
D^2	0.37	1	0.37	4.47	0.0530
Residual	1.17	14	0.084		
Pure error	0.021	4	5.222E-003		
Cor total	53.84	28		R-Squared	0.9782
Std. dev.	0.29			Adj R-Squared	0.9565
Mean	8.56			Pred R-Squared	0.8762
C.V. %	3.38			Adeq Precision	25.950

the spectroscopy was Fourier transformed and the resultant spectrum was analyzed using chemometric Technique.

2.4. GC–MS analysis

GC–MS analysis of the extract was performed using a Perkin Elmer GC Clarus 680 system and gas chromatograph interfaced to a Mass Spectrometer Clarus 600 system (GC–MS) equipped with Elite-1 fused silica capillary column (30 m × 1 µl was Mdf, composed of 100% Dimethyl poly siloxane). For GC–MS detection, an electron ionization energy system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 1 µl was employed (Split ratio of 10:1). Injector temperature was 250 °C. The initial oven temperature was programmed from 60 °C for 2 min, with an increase of 10 °C/min to 300 °C, ending at 6 min. Mass spectra were taken at 70 eV; scan interval, 0.5 seconds and fragments, from 50 to 600 Da. Total GC running time was 32 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was Turbo-

Mass Ver 5.4.2. Compound identification was obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from library data of the corresponding compounds.

3. Results and discussion

The various parameters investigated for maximum percentage of extraction of betel leaf, the mass of extract and the corresponding yield for the 29 experiments are shown in Table 1 . The predicted yield (%) was obtained using the second-order polynomial equation obtained in ANOVA. As analyzed using ANOVA (Table 2), the higher model F-value (44.935) and the associated lower p-values ($p < 0.0001$) demonstrated that the polynomial regression models were suitable to determine optimum conditions for the extraction of the three components. The p-values of the two main variables (temperature and extraction time) were less than 0.0001 indicating that change of temperature and extraction time had significant effects on the yield of extract [25,26], whereas for material and solvent quantity it was more than 0.0001 indicating that these quantities had relatively lesser effect on the yield of extract. High R^2 (0.9782), adj-R^2 (0.967) and pre-R^2 (0.912) values indicate that the variation could be accounted for by the data satisfactorily fitting the model. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable [27]. Since CV is a measure expressing the standard deviation as a percentage of the mean, smaller values of CV give better reproducibility. The coefficient of variation (CV) of less than 10 clearly exhibits a very high degree of precision and good reliability of the conducted experiments [28].

By applying multiple regression analysis on the experimental data, the dependent variable and independent variable are related by the following quadratic equation with interactive parameters:

$$\begin{aligned}\text{yield}\% = {} & -209.2487 + 1.4320 * X_A + 0.5008 * X_B \\ & +3.712 * X_C + 8.5797 * X_D - 4.5 \times 10^{-3} * X_A * X_B \\ & -6.25 \times 10^{-3} * X_A * X_C - 0.175 * X_A * X_D \\ & -1.06 \times 10^{-3} * X_B * X_C - 6.33 \times 10^{-3} * X_B * X_D \\ & -0.05 * X_C * X_D + 0.1193 * {X_A}^2 \\ & -7.04 \times 10^{-4} * {X_B}^2 - 0.0225 * {X_C}^2 - 0.24 * {X_D}^2\end{aligned}$$

where, X_A is material quantity (g); X_B is solvent quantity (ml); X_C is Temperature (°C); and X_D is time (h). After setting up the criteria for optimization, i.e., maximum yield percentage and inde-

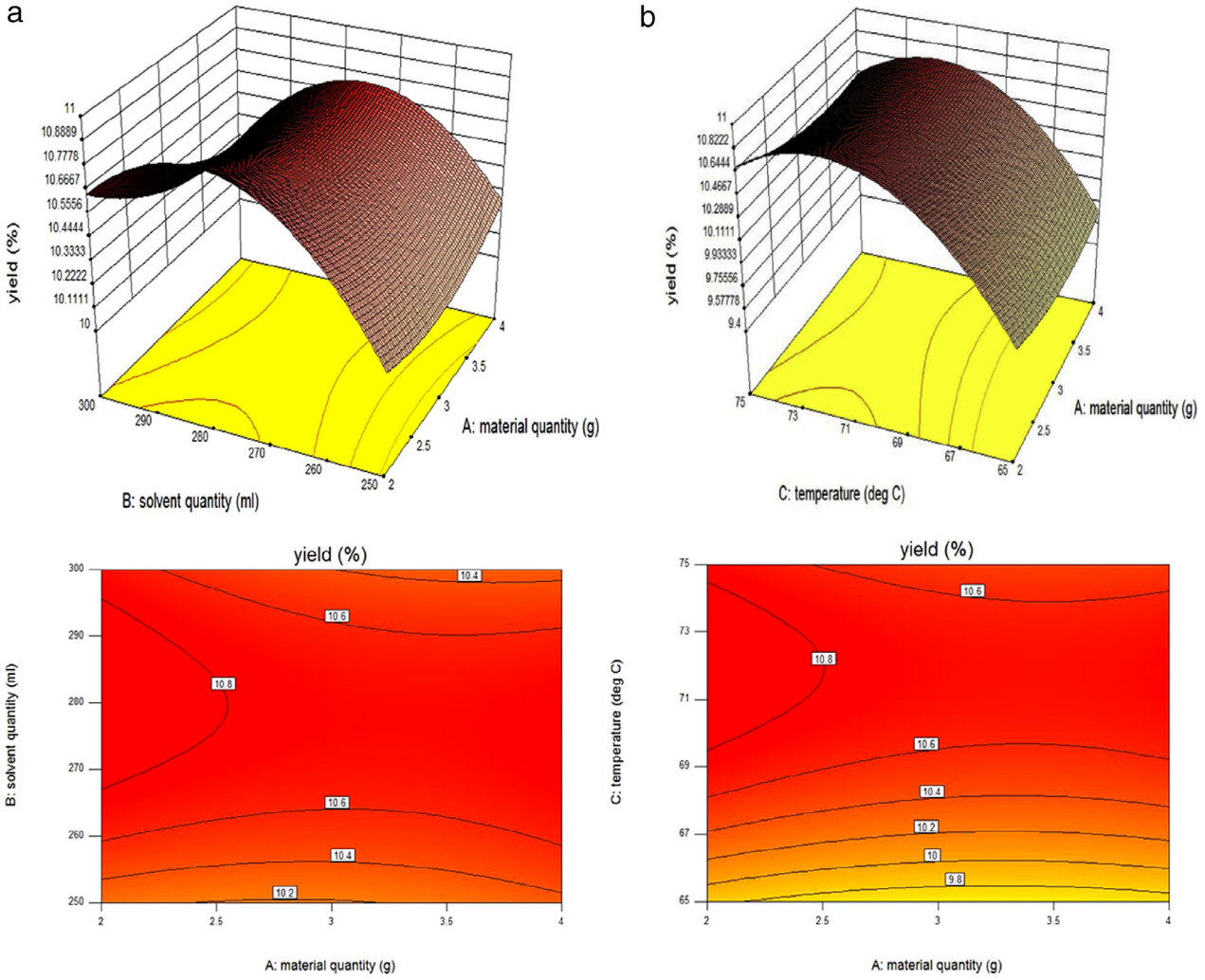

Fig. 2. (a) 3-D response surface and 2-D contour plot for the effect of material and solvent quantity on yield % at constant temperature (71.91 °C) and time (3 hours). (b) 3-D response surface and 2-D contour plot for the effect of material quantity and temperature on yield % at constant solvent quantity (281.434 ml) and time (3 hours). (c) 3-D response surface and 2-D contour plot for the effect of material quantity and time on yield % at constant solvent quantity (281.434 ml) and temperature (71.91 °C). (d) 3-D response surface and 2-D contour plot for the effect of solvent quantity and temperature on yield % at constant material quantity (2 g) and time (3 hours). (e) 3-D response surface and 2-D contour plot for the effect of solvent quantity and time on yield % at constant material quantity (2 g) and temperature (71.91 °C). (f) 3-D response surface and 2-D contour plot for the effect of temperature and time on yield % at constant material quantity (2 g) and solvent quantity (281.434 ml).

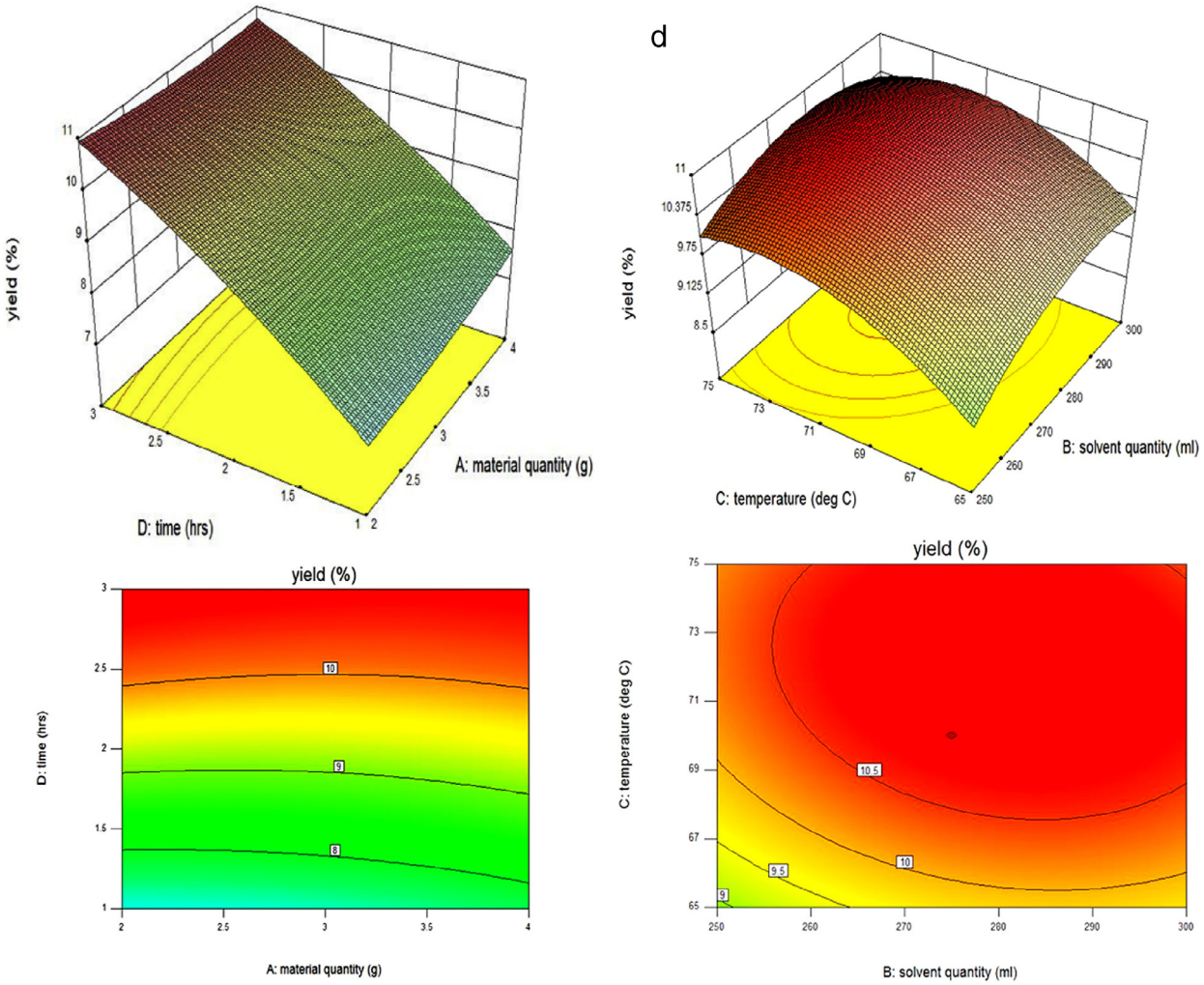

Fig. 2. Continued

pendent variables in range, the Ramp solution showed a desirability of 0.948. The optimum values were as follows, yield percentage: 10.9436, material quantity: 2 g, solvent quantity: 281.434 ml, temperature: 71.91 °C and time: 3 hours. Fig 2 a–f represents the response surface plots (3D) and 2-D contour plots showing the effects of variables (*A*: material quantity (g); B: solvent quantity (ml); C: temperature (°C); D: time (h)) on the response yield percentage of extract.

3.1. Effect of extraction time

The greater the time of contact, the greater is the number of cycles (greater the number of times the fresh solvent comes in contact with the sample in thimble) and hence there is higher possibility of extracting components from the material. Attributing to this fact, the extraction time corresponding to 3 hours as shown in Fig. 3a gave a higher yield to that corresponding to lower extraction time (1 and 2 hours) [21].

3.2. Effect of temperature

It was observed that the percentage yield increases with increase in temperature. A higher temperature favors faster diffusion and better extraction of sensitive substances. The mass transfer coefficient of the extraction process also increases with temperature thus effecting the diffusion [29]. Thus, the yield percentage increased with increase in extraction temperature from 65 °C to 72 °C as given in Fig. 3b. Increasing temperature favored extraction by enhancing both the solubility of solute and diffusion coefficient from a solid matrix to a liquid matrix as mentioned by Luthria [22]. Despite the fact that increasing temperature has the positive effect on the extraction yields, increasing temperatures also affect the stability of phenolic compounds. When extracting phenolic compounds from olive leaves at higher temperature, both effects need to be considered [30]. Temperatures above 72 °C probably caused a decrease in the extraction yield for a known material quantity due to possible degradation of phenolic compounds, caused by hydrolysis, internal redox reactions and polymerization [31].

3.3. Effect of solvent quantity

An increase of solvent to solid ratio increases the concentration gradient and thus the rate of diffusion of the compounds from the solid to the solvent results in an increased extraction yield. In addition, the chance of bio-active components coming into contact with extracting solvent expanded with increased amount of extraction solvent, leading to higher leaching-out rates [32]. This fact can be attributed to the increase in yield percentage with solvent quantity from 250 ml to 281.43 ml as shown in Fig. 3c; further increase in the solvent quantity above 281.43 ml saturation occurred and not much pronounced effect was observed up to 300 ml.

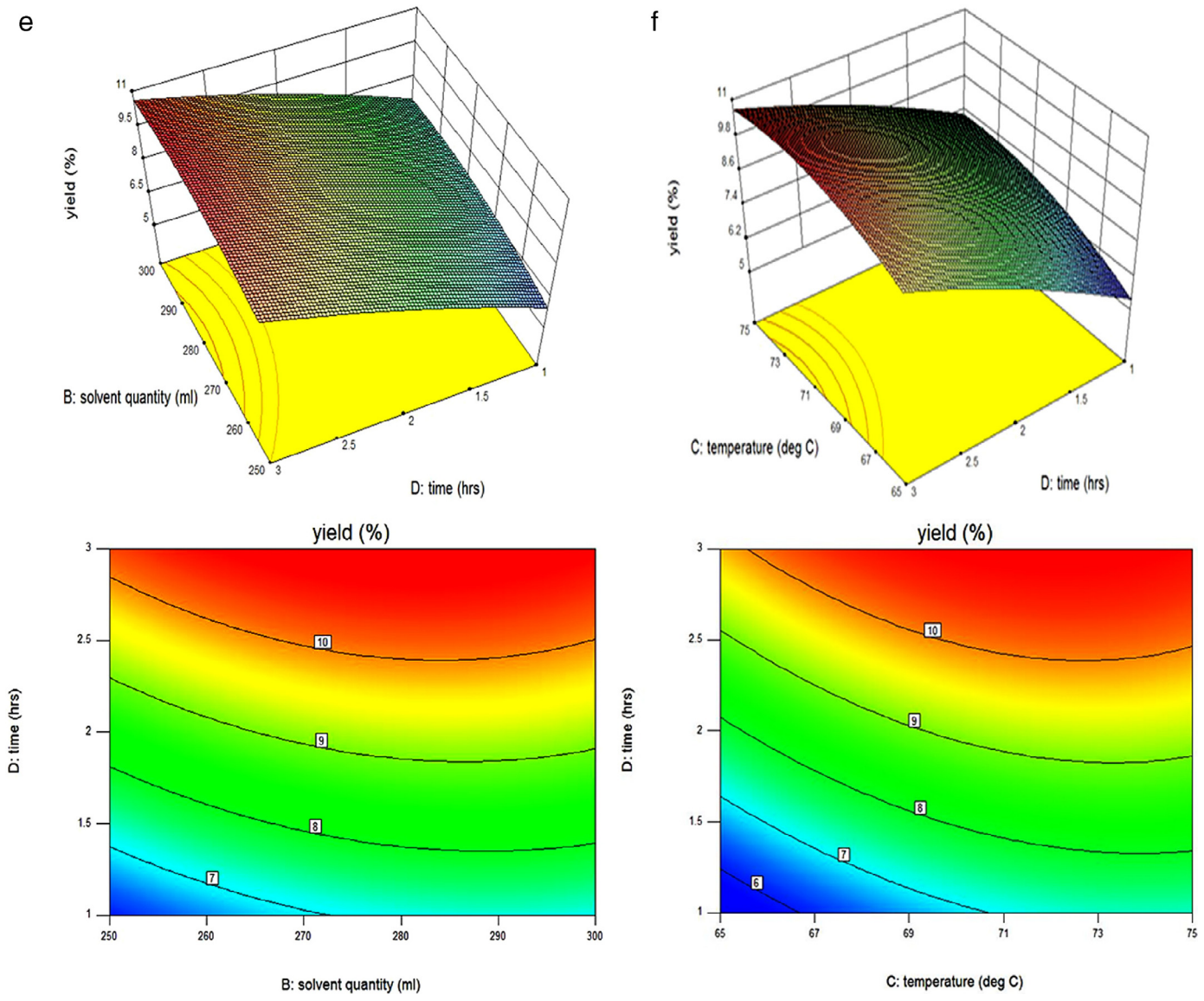

Fig. 2. Continued

Table 3
FTIR analysis of sample corresponding to maximum yield.

Wave number, cm^{-1}	Bond	Functional group
3311.78 (m)	N–H, O–H	Amines, Monomeric – Alcohols, Phenols
2927.94 (w)	O–H	Carboxylic acids
1707.00 (m)	N–H	Amines
1367.53 (v)	CH	Alkanes
1045.42 (s)	C–O	Alcohols, Ethers, Carboxylic acids, Esters
879.54 (m)	C–H	Alkenes

3.4. Effect of material quantity

Upon increasing the material quantity, the mass of extract increases, but there was no significant effect on yield of extract obtained. The effect of material quantity on yield of extract is shown in Fig. 3d. It was observed that the yield of extract corresponding to 2 g with 281.4 ml solvent was highest. The yield percentage decreased later and then slightly increased and became constant. Much pronounced effect of material quantity is expected on yield percentage of extract upon increasing the quantity to 10–15 g.

3.5. FTIR spectral data analysis

The FTIR analysis of ethanolic extract of betel leaves corresponding to maximum yield as shown in Fig. 4 confirmed the presence of amines, monomeric – alcohols, phenols; carboxylic acids; alkanes; ethers, esters and alkenes, which show major peaks at 3311.78; 2927.94; 1707.00; 1367.53; 1045.42; and 879.54 (cm^{-1}) respectively (Table 3) based on the source of absorption intensity (relative transmittance i.e., weak, w, medium, m or strong, s).

3.6. GC–MS interpretation

Interpretation on Mass-Spectrum GC–MS was carried out with reference to the database of National Institute of Standards and Technology (NIST). The spectrum of the unknown components was compared with spectrum of known components stored in NIST library. The GC–MS analysis of betel extract revealed the presence of five phytocomponents as in Fig. 5 . The presence of the phytocomponents was confirmed based on the peak area, retention time and molecular formula. The phytocomponents include hydroxychavicol (69.464%), 4-chromanol (24%), eugenol (4.86%), 1-phenylpropene-3,3-diol diacetate (0.923) and 4-allyl-1,2-diacetoxybenzene (0.765)

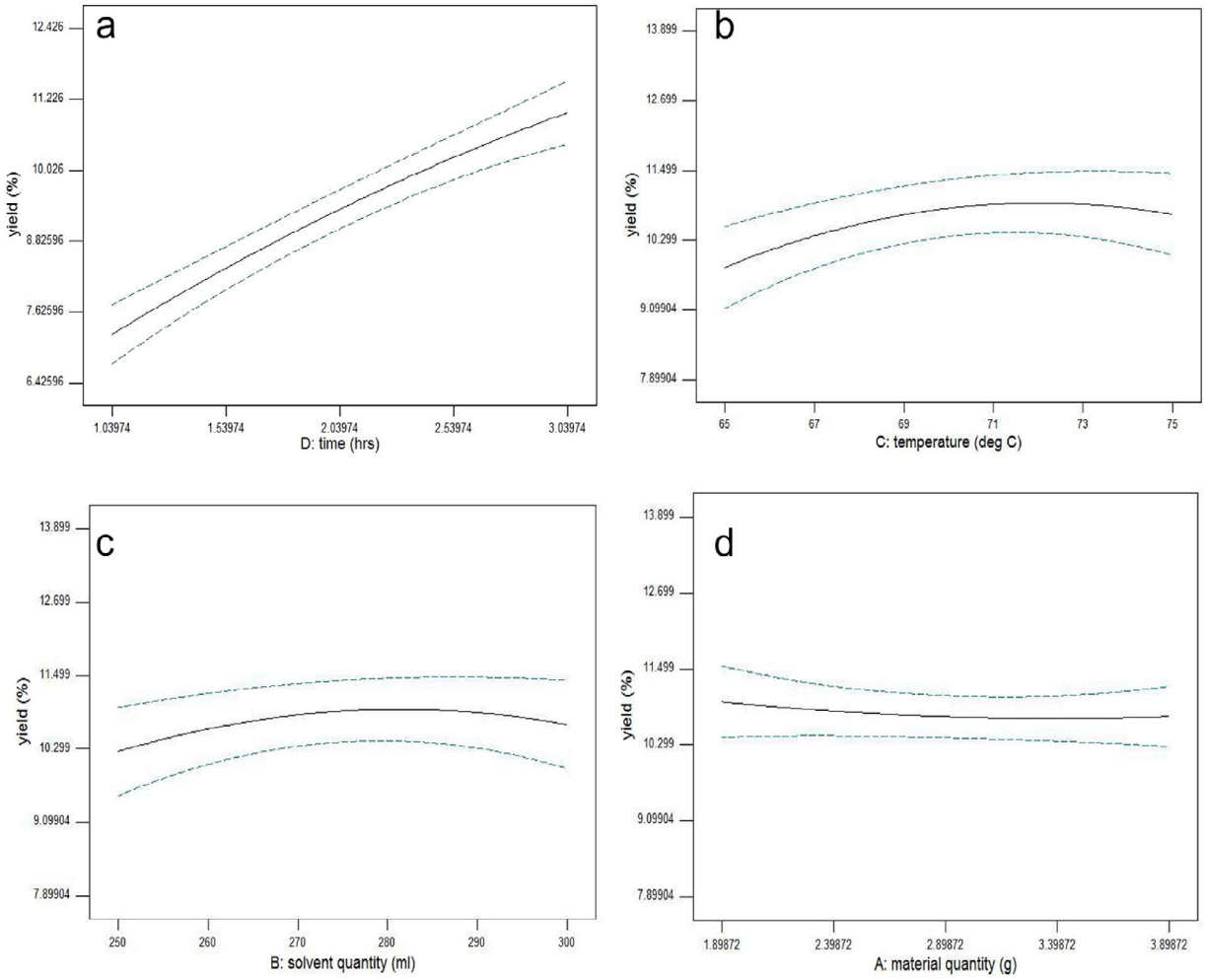

Fig. 3. (a) Effect of yield % with time. (b) Effect of yield % with temperature. (c) Effect of yield % with solvent quantity. (d) Effect of yield % with material quantity.

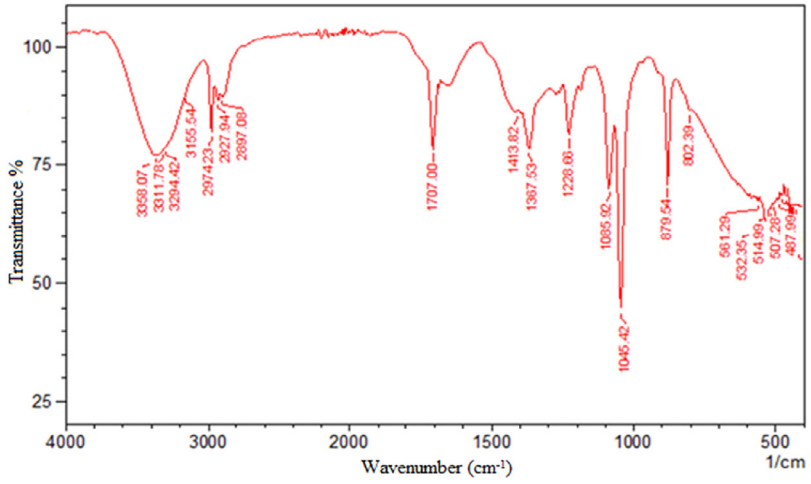

Fig. 4. FTIR image of the extract corresponding to maximum yield.

Table 4
GC–MS analysis of sample corresponding to maximum yield.

S. No	RT	Scan	Height $\times 10^9$ µV	Area $\times 10^9$ µVs	Area%	Norm%	Compound name	Formula	MW
1	11.492	1738	0.443	0.025	0.923	1.33	1-Phenylpropene-3,3-diol diacetate	$C_{13}H_{14}O_4$	234
2	13.068	2053	2.926	0.135	4.857	6.99	Eugenol	$C_{10}H_{12}O_6$	164
3	13.098	2059	5.601	0.670	23.992	34.54	4-Chromanol	$C_9H_{10}O_2$	150
4	13.583	2156	0.384	0.0213	0.765	1.10	4-Allyl-1,2-diacetoxybenzene	$C_{13}H_{14}O_4$	234
5	14.423	2324	14.535	1.940	69.464	100.00	Hydroxy chavicol (1-allyl-3, 4-dihydroxybenzene)	$C_9H_{10}O_2$	150

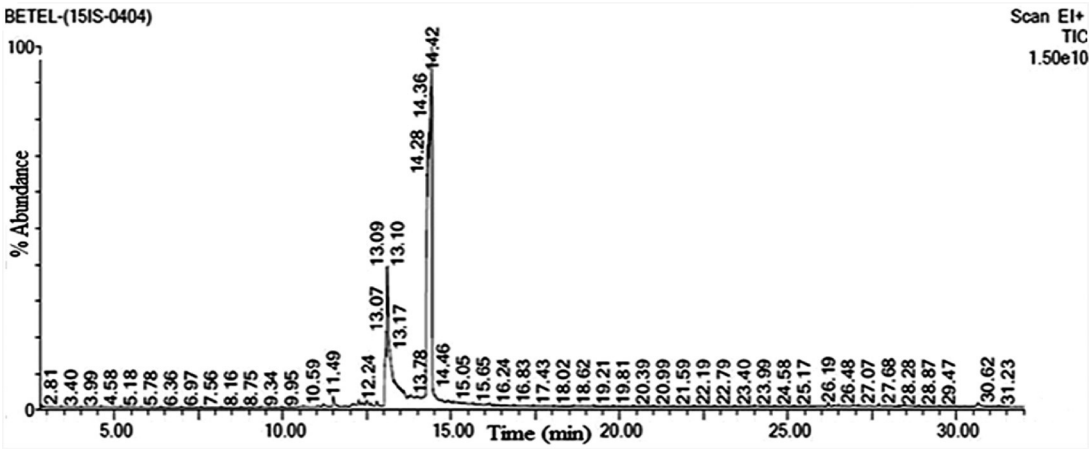

Fig. 5. GC–MS image of the extract corresponding to maximum yield.

listed in Table 4 . Hydroxychavicol is one of the major phenolic compounds which were reported to possess antinitrosation, anti-mutagenic, and anticarcinogenic effects; besides this, it has an enormous potency to act as an antioxidant, antiinflammatory, anti-platelet and antithrombotic agent without impairing hemostatic function [33]. 4-Chromanol has antioxidant activity [34,35]. Eugenol found in the present study has antibacterial and antifungal activities [36]. 4-Allyl-1,2-Diacetoxybenzene, Chavicol and Eugenol are components of Betel extract responsible for anti-fungal medicated activity in shampoo against *pityrosporum ovale*, yeast that causes dandruff [25,37].

4. Conclusions

The variable parameters (independent variables) considered in Soxhlet extraction for performing extraction of Betel leaves at their respective range levels were successfully optimized by Box–Behnken response surface methodology and the maximum yield was calculated by numerical optimization using Design Expert 9.0.4.1 software. The optimum parameters for yield (10.94%) of extract correspond to material quantity (2 g), solvent quantity (281.434 ml), temperature (71.91 °C) and time (3 hours). The model graphs suggest that the mantle temperature and extraction time had a significant effect on yield of extract ($p < 0.001$) whereas for the mass and solvent quantity ($p > 0.001$) suggested relatively lesser effect on yield of extract. The increase of percentage yield (response) with extraction time was almost linear from 1 to 3 hours. The response with change in mantle temperature and solvent quantity showed an increase up to 72 °C and 281.43 ml respectively, and later decreased. Not much pronounced change in response was observed upon changing material quantity. The FTIR analysis of ethanolic extract of betel leaves corresponding to the maximum percentage yield confirmed the presence of amines, monomeric-alcohols, phenols; carboxylic acids; alkanes; alcohols, ethers, carboxylic acids, esters and alkenes, which showed major peaks at 3311.78; 2927.94; 1707.00; 1367.53; 1045.42; and 879.54 (cm^{-1}). The GC–MS analysis of extract corresponding to maximum yield of extract confirms hydroxy chavicol (69.46%), 4-chromanol (24%) and eugenol (4.86%), which possess wide application including as antioxidant, anti-inflammatory, anti-platelet and antithrombotic, antibacterial and antifungal agents.

References

[1] D. Pradhan, K.A. Suri, D.K. Pradhan, P. Biswasroy, Golden heart of the nature: *Piper betle* L, J. Pharmacog. Phytochem 1 (6) (2013) 147–167.
[2] V.P.B. Rekha, M. Kollipara, B.R.S.S. Srinivasa Gupta, Y. Bharath, K.K. Pulicherla, A review on *Piper betle* L.: nature's promising medicinal reservoir, Am. J. Ethnomed 1 (5) (2014) 276–289.
[3] F. Fazal, P.P. Mane, M.P. Rai, K.R. Thilakchand, H.P. Bhat, P.S. Kamble, et al., The phytochemistry, traditional uses and pharmacology of Piper Betel linn (Betel Leaf): a pan-asiatic medicinal plant, Chin. J. Integr. Med (2014), doi:10.1007/s11655-013-1334-1.
[4] D.C. Mohana, S. Thippeswamy, K. Manjunath, R.U. Abhishek, Antioxidant properties of some selected Indian medicinal plants: their correlation with total phenolic contents, Int. J. Green Pharm 7 (2) (2013) 117, doi:10.4103/0973-8258.116388.
[5] S.G. Jaiswal, M. Patel, D.K. Saxena, S.N. Naik, Antioxidant properties of Piper betel (L) leaf extracts from six different geographical domain of India, J. Bioresour. Eng. Technol 2 (2) (2014) 12–20.
[6] L.W. Foo, E. Salleh, S.N.H. Mamat, Extraction and qualitative analysis of *Piper betle* leaves for antimicrobial activities, Extraction 2 (2015) 1–8.
[7] M.K. Dalai, S. Bhadra, A. Bandyopadhyay, P.K. Mukherjee, Evaluation of anticholinesterase activity of the standardized extract of Piper betel L. leaf, Orient. Pharm. Exp. Med 14 (1) (2013) 31–35, doi:10.1007/s13596-013-0141-3.
[8] R. Paranjpe, S.R. Gundala, N. Lakshminarayana, A. Sagwal, G. Asif, A. Pandey, et al., Piper betel leaf extract: anticancer benefits and bio-guided fractionation to identify active principles for prostate cancer management, Carcinogenesis 34 (7) (2013) 1558–1566.
[9] L. Nouri, A.M. Nafchi, Antibacterial, mechanical, and barrier properties of sago starch film incorporated with betel leaves extract, Int. J. Biol. Macromol 66 (2014) 254–259.
[10] L. Nouri, A.M. Nafchi, A.A. Karim, Phytochemical, antioxidant, antibacterial, and α-amylase inhibitory properties of different extracts from betel leaves, Ind. Crop. Prod 62 (2014) 47–52.
[11] B. Jayalakshmi, K.A. Raveesha, M. Murali, K.N. Amruthesh, Phytochemical, antibacterial and antioxidant studies on leaf extracts of *Piper betle* L., Int. J .Pharm. Pharm. Sci 7 (11) (2015) 23–29.
[12] D. Rintu, M. Shinjini, M. Kaustab, P. Pramathadhip, P.S. Umesh, E.R. Banerjee, Anti-oxidant and anti-inflammatory activities of different varieties of piper leaf extracts (*Piper betle* L.), J. Nutr. Food Sci 5 (2015) 415.
[13] N. Durling, O. Catchpole, J. Grey, R. Webby, K. Mitchell, L. Foo, et al., Extraction of phenolics and essential oil from dried sage (Salvia officinalis) using ethanol–water mixtures, Food Chem 101 (4) (2007) 1417–1424, doi:10.1016/j.foodchem.2006.03.050.
[14] A.A. Catherine, H. Deepika, P.S. Negi, Antibacterial activity of eugenol and peppermint oil in model food systems, J. Essent. Oil Res 24 (5) (2012) 481–486, doi:10.1080/10412905.2012.703513.
[15] M.M. Hoque, S. Rattila, M.A. Shishir, M.L. Bari, Y. Inatsu, S. Kawamoto, Antibacterial activity of ethanol extract of betel leaf (*Piper betle* L.) against some food borne pathogens, Banglad. J. Microbiol 28 (2) (2012) 58–63.
[16] A.A. Khan, S.P. Bhatnagar, B.N. Sinha, U.R. Lal, Pharmacognostic specifications of eight cultivars of *Piper betle* from eastern region of India, Pharmacogn. J. 5 (4) (2013) 176–183.
[17] K.N. Akter, P. Karmakar, A. Das, S.N. Anonna, S.A. Shoma, M.M. Sattar, Evaluation of antibacterial and anthelmintic activities with total phenolic contents of Piper betel leaves, Avicenna J. Phytomed 4 (5) (2014) 320–329.
[18] W.Y. Wendy Voon, N.A. Ghali, Y. Rukayadi, M. Hussin, A. Shobirin, Application of betel leaves (*Piper betle* L.) extract for preservation of homemade chili bo, Int. Food Res. J. 21 (6) (2014) 2399–2403.
[19] M. Anugrahwati, T. Purwaningsih, Rustina, J.A. Manggalarini, N.B. Alnavis, D.N. Wulandari, et al., Extraction of ethanolic extract of red betel leaves and its cytotoxicity test on HeLa cells, Procedia Eng 148 (2016) 1402–1407, doi:10.1016/j.proeng.2016.06.569.
[20] P. Sirisomboon, P. Kitchaiya, Physical properties of Jatropha curcas L. kernels after heat treatments, Biosyst. Eng 102 (2) (2009) 244–250, doi:10.1016/j.biosystemseng.2008.11.003.
[21] A. Ahmad, A.F.M. Alkarkhi, S. Hena, B.M. Siddique, K.W. Dur, Optimization of Soxhlet extraction of herba leonuri using factorial design of experiment, Int. J. Chem 2 (1) (2010), doi:10.5539/ijc.v2n1p198.

[22] D.L. Luthria, Influence of experimental conditions on the extraction of phenolic compounds from parsley (Petroselinum crispum) flakes using a pressurized liquid extractor, Food Chem 107 (2) (2008) 745–752, doi:10.1016/j.foodchem.2007.08.074.
[23] P. Maran, S. Venkatachalam, R. Sridhar, P.I. Victor, Development of model for mechanical properties of tapioca starch based edible films, Ind. Crop. Prod 42 (2013) 159–168, doi:10.1016/j.indcrop.2012.05.011.
[24] M.D. Luque de Castro, F. Priego-Capote, Soxhlet extraction: past and present panacea, J. Chromatogr. A 1217 (16) (2010) 2383–2389, doi:10.1016/j.chroma.2009.11.027.
[25] I. Pratiwi, Application of *Piper betle* Linn Liquid Extraction as Antifungal Medicated Shampoo against *Pityrosporum ovale*, Swiss German University, Indonesia, 2009.
[26] M. Ganesapillai, M. Mathew, A. Singh, P. Simha, Influence of microwave and ultrasound pretreatment on solvent extraction of bio-components from walnut (Julgans regia L.) shells, Period. Polytech. Chem. Eng 60 (1) (2016) 40–48.
[27] R.F. Gunst, R.H. Myers, D.C. Montgomery, Response surface methodology: process and product optimization using designed experiments, Technometrics 38 (3) (1996) 285, doi:10.2307/1270613.
[28] R.L. Mason, R.F. Gunst, J.L. Hess, Statistical Design and Analysis of Experiments (with Applications to Engineering and Science), Wiley, New York, 1989. ISBN 0-471-85364-X.
[29] S. Sayyar, Z.Z. Abidin, R. Yunus, A. Muhammad, Extraction of oil from jatropha seeds – optimization and kinetics, Am. J. Appl. Sci 6 (7) (2009) 1390–1395, doi:10.3844/ajassp.2009.1390.1395.
[30] L.G. Wood, P.G. Gibson, M.L. Garg, A review of the methodology for assessing in vivo antioxidant capacity, J. Sci. Food Agric 86 (13) (2006) 2057–2066, doi:10.1002/jsfa.2604.
[31] R.M. Alonso-Salces, E. Korta, A. Barranco, L.A. Berrueta, B. Gallo, F. Vicente, Pressurized liquid extraction for the determination of polyphenols in apple, J. Chromatogr. A 933 (1) (2001) 37–43.
[32] S. Zhang, H. Bi, C. Liu, Extraction of bio-active components from Rhodiola sachalinensis under ultrahigh hydrostatic pressure, Sep. Purif. Technol 57 (2) (2007) 277–282, doi:10.1016/j.seppur.2007.04.022.
[33] K. Murata, K. Nakao, N. Hirata, K. Namba, T. Nomi, Y. Kitamura, et al., Hydroxychavicol: a potent xanthine oxidase inhibitor obtained from the leaves of betel, *Piper betle*, J. Nat. Med 63 (3) (2009) 355–359.
[34] W. Gregor, G. Grabner, C. Adelwöhrer, T. Rosenau, L. Gille, Antioxidant properties of natural and synthetic chromanol derivatives: study by fast kinetics and electron spin resonance spectroscopy, J. Org. Chem 70 (9) (2005) 3472–3483, doi:10.1021/jo047927s.
[35] P. Kawsud, J. Puripattanavong, R. Teanpaisan, Screening for anticandidal and antibiofilm activity of some herbs in Thailand, Trop. J. Pharm. Res 13 (9) (2014) 1495–1501.
[36] K. Venkadeswaran, P.A. Thomas, P. Geraldine, An experimental evaluation of the anti-atherogenic potential of the plant, *Piper betle*, and its active constitutent, eugenol, in rats fed an atherogenic diet, Biomed. Pharmacother 80 (2016) 276–288.
[37] C. Choopayak, K. Woranoot, P. Naree, A. Kongbangkerd, K. Wongkrajang, R. Buaruaeng, Phytotoxic effects of *Piper betle* L. extracts on germination of Chloris barbata Sw. and Eclipta prostrata L. weeds, NU. Int. J. Sci 12 (1) (2016) 11–24.