Industrial Crops and Products 84 (2016) 13–21

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Ultrasound assisted extraction of total phenolics from *Cassia auriculata* leaves and evaluation of its antioxidant activities

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ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form
31 December 2015
Accepted 8 January 2016
Available online 8 February 2016

Keywords:
Antioxidant activity
Cassia auriculata
Optimization
Response surface method
Total phenolics
Ultrasound assisted extraction

ABSTRACT

The present study investigated the optimization of ultrasound assisted total phenolics extraction from *Cassia auriculata* leaves by Response surface methodology (RSM). A three-level four-factor Box–Behnken design (BBD) was used to illuminate the optimal points of ultrasound assisted extraction (UAE) process variables, extraction time (5,10,15 min), pH (5,6,7), solvent concentration (40,50,60%) and ultrasonic power (30,40,50 W) for obtaining maximum total phenolics with better antioxidant activities. *C. auriculata* extracts obtained from UAE in RSM experiments were evaluated in terms of total phenolics content (TPC), FRAP (Ferric reducing antioxidant power) and DPPH (1,1′-diphenyl-2-2′-picrylhydrazyl) radical scavenging activities as responses. Second-order polynomial models for TPC, FRAP and DPPH were build by using the coefficients obtained from regression analysis. ANOVA statistics showed that pH had significant effect on TPC, DPPH and FRAP antioxidant activities. The predicted optimum levels of extraction time 5 min, pH 6.2, solvent concentration 60% and power 50 W showed maximum TPC, FRAP and DPPH activities of 59.68 (mg_{GAE}/g), 96.2 (mM Fe^{2+}/g) and 90.5% respectively. Validation experiments results had good agreement with the predicted responses by RSM. Results of this study implied that UAE was the better method for TPC extraction from *C. auriculata* leaves with good antioxidant activities which can be used as natural antioxidant in pharmaceutical and food industries.

1. Introduction

Several diseases like, cancer, arthritis, Parkinson's disease and heart diseases are caused due to oxidative stress effect by reactive oxygen and nitrogen species (super oxide, hydrogen peroxide and hydroxyl radicals) (Tabaraki and Nateghi, 2011). These are produced by exposure to pollutants and cellular metabolism, leading to oxidation of proteins, low density lipoproteins causing DNA, cell damage and loss of nutritional value of packaged foods (Ghafoor and Choi, 2009). The body's defense mechanism neutralize these ROS by antioxidants mainly a secondary metabolites like phenolic compounds produced by shikimic acid and melonic acid pathway in humans and plants. In elderly individuals the synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG) are given as support in the form of medicines, nutraceuticals and cosmetics which are considered as unsafe. Awareness among researchers has increased to explore novel natural antioxidants (flavonoids, carotenoids, vitamin C and polyphenols) which can reduce the risk of diseases when taken as food supplements.

Plants produce phenolic compounds with varied polarities and physiochemical properties for its survival but have good pharmacological activity in humans. Asian (*Cassia auriculata* L.), a fast growing shrub is widespread in India belongs to family of *Caesalpiniaceae* and can able to survive under adverse conditions (Jaydeokar et al., 2014). It is commonly known as Avaram tree in Tamil and Tanner's cassia in English (Vijayaraj et al., 2013). From older days in India, *C. auriculata* is used as folk and ayurvedic medicine to reduce blood glucose level, to treat Diabeties mellitus (Latha and Pari, 2003). The leaves are alternate, closely placed with 16–24 leaflets; possess a large bright yellow flower and short legume fruit having 12–20 seeds in its cavity (Nanjaraj Urs et al., 2015; Joy et al., 2012). Several phytochemical constituents are reported such

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http://dx.doi.org/10.1016/j.indcrop.2016.01.010
0926-6690/

as flavonoids, polysaccharides, anthracene derivatives, alkaloids and tannins having therapeutic properties toward diabetes, dyslipidemia, conjunctivitis, renal and liver disorders, ulcer, asthma, skin disease and cancer (Juan-Badaturuge et al., 2011). Aqueous acetone leaf extract showed presence of benzocoumarin glycoside, avaraoside I, avaraol I with protective effect on liver disorder. Other constitutents include luteolin, kaempferol, quercetin, myricetin, 3-methoxyluteolin, kaempferol 3-*O*-β-D-glucopyranoside, epigallocatechin and emodin which contains functional groups such as hydroxyl, ester, aromatic ring, and ether functionalities which shows antipyretic, antiulcer, antihelmintic and hepatoprotective activity (Nakamura et al., 2014).

Various extraction methods have been reported for extraction of plant derived phenolic compounds. Conventional extraction procedure involves refluxing of heat treated aqueous extract and soxhlet method, which gives less yield as a result of degradation by oxidation, hydrolysis, increased temperature and long duration of extraction. To overcome the drawbacks obtained by conventional methods of extraction, a suitable method for extraction can be discovered and designed named as 'Green extraction process' which is faster, occupies less working space, increase in heat and mass transfer, consumes less energy, can process with limited solvent and raw materials, flexible to extract with alternative solvents, utilizes a non-dedicated equipment, can yield more product with less unit operations, non toxic and cannot denaturate the product produced. Solvent free extraction or Green extraction tools can be spotlighted on following operations such as pressing, microwave, ultrasonication, supercritical fluid extraction and instant controlled pressure drop process (DIC) (Chemat et al., 2015, 2012; Rombaut et al., 2014). Hence selection of extraction method with less effect on phenolic compounds is crucial. Ultrasound assisted extraction (UAE) is being used in the extraction of nutraceuticals, antibiotics, polyphenols, flavanols and polysaccharides (Azmir et al., 2013; Lianfu and Zelong, 2008; Chen et al., 2007; Liao et al., 2015). UAE breaks plant cell wall by shear force using cavitation produced by compression and expansion of ultrasonic waves (20–100 kHz) causing effective mixing and rate of diffusion. Improved diffusion, mass transfer, maximum breakdown of plant cells, sonocapillary effect and solvent penetration are the properties of ultrasonication extraction process. Moreover the ultrasound is advantageous in both the swelling indices and extractive value, which is quantified in any type of extraction to validate the quality of extractant (Mason et al., 2011). Many investigations proved that ultrasonic assisted extractions carry several advantages and widely accepted for extraction process. But ultrasound treatment results in degradation of antioxidant quality after sonication (Pingret et al., 2013). However, optimizing the experimental parameters such as using mixture of solvents, time, power, ultrasonic frequency etc. can reduce the degree of degradation (Eh and Teoh, 2012) and hence the optimization studies not only enhance the quantity of the product but also the quality. To commercialize UAE in large scale, factors such as wave distribution frequency, power, type of solvent, pressure, temperature and time need to be studied in lab scale (Yingngam et al., 2014; Wu et al., 2014).

Optimization by one factor at a time, an empirical approach which is time consuming and it lacks to explain the interaction effects of the factors studied. Response surface methodology (RSM) is a statistical method to analyze and predict the optimal levels of the selected variables within design space and to determine the interaction effects among the variables with less experiments and time (Myers and Montgomery, 1995). This technique is widely used to optimize process variables in biochemical and other industries (Wu et al., 2015; Sharmila et al., 2013). The objective of the present study was to optimize the UAE process variables to obtain maximum total phenolics with high antioxidant capacities. Box–Behnken design (BBD) of RSM was employed to optimize and study the interaction effects of the process variables; extraction

Table 1
Box-Behnken design matrix for TPC extraction from *C. auriculata* by UAE method and the antioxidant activity assay responses.

Run order	Extraction variables				Responses					
	A (min)	B (−)	C (%)	D (W)	TPC (mg_{GAE}/g)		FRAP (mM Fe^{2+}/g)		DPPH (%)	
					Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	5	6	50	40	49.5	43.69	79.00	86.68	90.23	91.84
2	15	6	50	40	62.6	60.46	43.00	49.83	86.10	86.49
3	5	8	50	40	49.6	47.89	45.83	45.13	83.10	83.09
4	15	8	50	40	37.9	39.86	35.17	33.63	85.40	84.17
5	10	7	40	30	53.4	52.68	44.52	51.23	88.19	87.03
6	10	7	60	30	58.7	61.94	80.86	77.85	86.88	87.50
7	10	7	40	50	64.3	57.21	48.72	57.87	83.72	83.49
8	10	7	60	50	55.2	52.08	76.52	75.94	84.37	85.91
9	5	7	50	30	45	43.98	71.90	71.28	88.37	89.17
10	15	7	50	30	59	55.89	42.28	56.97	88.74	88.90
11	5	7	50	50	44.1	48.86	91.62	83.51	88.93	88.47
12	15	7	50	50	43	45.68	42.28	49.47	85.56	84.46
13	10	6	40	40	62	60.94	69.76	58.97	84.84	85.94
14	10	8	40	40	48.2	47.69	24.66	27.16	82.12	81.88
15	10	6	60	40	55.8	57.96	74.31	78.38	88.93	88.86
16	10	8	60	40	52.1	54.81	35.07	52.44	83.26	81.85
17	5	7	40	40	38.6	44.08	52.17	55.61	90.79	90.12
18	15	7	40	40	54.5	58.40	45.17	34.16	81.95	83.15
19	5	7	60	40	57.8	56.10	82.38	80.68	88.00	86.73
20	15	7	60	40	53.8	50.52	69.93	53.78	88.84	89.43
21	10	6	50	30	49.7	52.83	82.48	76.06	89.02	87.34
22	10	8	50	30	54.7	53.18	59.90	48.54	84.00	85.27
23	10	6	50	50	55	58.72	81.14	79.78	89.58	88.24
24	10	8	50	50	42.9	41.97	55.83	49.54	77.63	79.24
25	10	7	50	40	43.3	45.24	68.55	70.86	86.51	87.20
26	10	7	50	40	43	45.24	72.93	70.86	86.23	87.20
27	10	7	50	40	48.2	45.24	79.07	70.86	88.65	87.20
28	10	7	50	40	44.8	45.24	65.69	70.86	86.05	87.20
29	10	7	50	40	46.9	45.24	68.07	70.86	88.56	87.20