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Optimization of recovery of antioxidants from the coconut shell using Response Surface Methodology

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ABSTRACT

Coconut shell is an important byproduct of *Cocos nucifera* L. which is largely being wasted. The present study is focused on optimizing the extraction process of phenolic compounds and also to evaluate its physico-chemical and antioxidant properties. After studying the effect of different solvents and different extraction conditions, methanol was found to be the most effective solvent and extraction at 90°C was suitable to give high phenolic yield (4045 mg GAE/100 g). For further precision of the ideal conditions, response surface methodology was carried out to optimize the solvent concentration, temperature and extraction time. The results revealed that methanol at 72%, extraction time 167 min and temperature 68°C yielded maximal level of polyphenols from coconut shell. Both extraction temperature and solvent concentration were found to have significant effect on the phenolic yield. Suitable conditions could be applied to recover the antioxidant phenolic compounds from the coconut shell, an agricultural byproduct available at huge quantity in India. The physico-chemical and antioxidant properties of methanolic extract of coconut shell were noticed to be suitable for its application in food and pharmaceutical industries.

Keywords: Coconut shell; Solvent extract; Polyphenols; RSM; Antioxidant

INTRODUCTION

Coconut (*Cocos nucifera* L.) is believed to be originated from Indian-Indonesian region and is known for its versatile uses and hence called the 'Tree of life'. It is spread across the tropical countries, especially in the coastal regions.[1] It belongs to the family Arecaceae and grows up to 30 m in height, has pinnate leaves and produces male and female flowers in the same inflorescence. According to the Coconut Development Board, India is one of the leading producers of coconut with the cultivation area of 12196 hectares. In particular, India is the third largest coconut producing country, after Indonesia and the Philippines.[2] On an average, 10345 nuts per hectare are being produced annually in India.[3] In India, the four states namely Kerala, Tamil Nadu, Karnataka and Andhra Pradesh account for 99% of the coconut production.

Coconut fruit composed of outer fibrous husk, hard shell and edible kernel with high nutritional value. Several products ranging from grated coconut to coconut milk with vital culinary

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uses have been observed, especially in India.[4] Tender coconut water refers to the liquid endosperm which is rich in electrolytes, vitamins, minerals and has excellent medicinal properties.[5, 6] Oil, obtained from the dried coconut kernel, is a principal component in cosmetic and food industries due to its fatty acid profile, vitamin E content, antioxidant, antibacterial and antifungal properties.[7-9]

Coconut husk and shell are the major byproducts with minor uses in various domains. Husk fiber is used as raw material for carpets, car seat stuffing and fertilizer. In the traditional medicine of Eastern Brazil, coconut husk decoction has been used to treat diarrhea and arthritis.[10] Husk fiber extract is used to treat fever, renal inflammation, dermatitis, abscesses, injuries, oral asthma and diabetes.[11] The polyphenols and catechins present in the husk has the potential to capture free radicals and pro-oxidants and confers to its antioxidant,[12,13] anti-mutagenic,[14] antibacterial, antiviral and anti-inflammatory activities.[15]

Coconut shell is the strongest outer covering and exactly located between the husk and kernel.[15] It is composed of mainly lignin and cellulose similar to a hard wood. The oil obtained by heating coconut shell has been used in treating ringworm infection in India.[12] Venkataraman et al.[16] have reported the anti-fungal activity[17] of alcoholic extract of coconut shell. In India, a traditional preparation containing coconut shell and cow's urine is prescribed as a hypocholesterolemic agent. Charcoal is a vital product obtained from coconut shell which has been widely used as domestic and industrial fuel. The shell is also used to produce various handicraft products and is highly regarded throughout the world. Phenolics from coconut shell are similar to the woods which have been used to store and age alcoholic beverages.[18]

Annual production of coconut shell in India is 6.7 million tons[1] and only a small portion is being utilized as fuel. The remaining portion may cause environmental burden as a solid waste, if not resolved properly. Since the nut byproducts such as cashew nut shell liquid, walnut shell, peanut shell, pistachio shell and hazelnut shell are reported as a natural source of antioxidants,[19-21] we intend to investigate the polyphenolic content of coconut shell and also to optimize the extraction process by response surface methodology.

EXPERIMENTAL DETAILS

Collection of material

Coconut shells were collected from Kumbakonam, Tamilnadu during August 2016. Shells were dried under shaded condition for 48 h and then pulverized in a mill (Make: Vikrem, 500 rpm) with the particles size of 1 mm and used for further experiments.

Optimization of extraction

The extraction solvent was optimized with hexane, ethyl acetate, methanol, ethanol and water by taking 5 g of the sample in 50 ml of respective solvent and kept for 2 h at room temperature. Then the contents were filtered and analyzed for total phenolic content (TPC). Based on the results, methanol was found to be the effective solvent, and hence it was used to carry out further experiments with different conditions (Heating, Shaking and Soaking). For heating we have used hot plate at 45^{o}C and shaking was done in an orbital shaker at 500 rpm. After analyzing TPC, heating was found to be the most efficient process of extraction and this was further optimized by

Table 1. Factors and levels for response surface methodology, Box–Behnken design matrix (in coded and uncoded level of three variables) and experimental data for the total phenolic content of coconut shell.

Std Order	Run Order	Pt Type	Blocks	Variables			Total phenolic content (mg GAE / 100 g)
				Solvent Conc. (%)	Time (Min)	Temp. (^{o}C)	
1	1	2	1	50	60	60	1793.52 ± 39.88
2	2	2	1	100	60	60	2667.00 ± 52.74
3	3	2	1	50	180	60	2774.72 ± 78.06
4	4	2	1	100	180	60	2863.10 ± 76.37
5	5	2	1	50	120	30	717.44 ± 16.12
6	6	2	1	100	120	30	767.76 ± 17.82
7	7	2	1	50	120	90	2028.80 ±113.14
8	8	2	1	100	120	90	1863.04 ± 58.13
9	9	2	1	75	60	30	654.72 ± 34.82
10	10	2	1	75	180	30	759.36 ± 41.58
11	11	2	1	75	60	90	2276.00 ± 113.14
12	12	2	1	75	180	90	2438.20 ± 50.91
13	13	0	1	75	120	60	2930.40 ± 73.12
14	14	0	1	75	120	60	3096.32 ± 69.58
15	15	0	1	75	120	60	2876.40 ± 42.43

subjecting to varied temperatures (30, 60 & 90˚C), duration of extraction (60, 120 & 180 min) and solvent (Methanol) concentrations (50, 75 & 100%) using response surface methodology (RSM). RSM corresponds to a group of mathematical and statistical techniques used in the development of a functional relationship between a response of interest and a number of control variables linked to it.[22] RSM design was carried out using Minitab software (Version 17, Pennsylvania State University). A total number of 15 randomized experiments were carried out and the complete design of experiments was given in Table 1.

Total Phenolic content

TPC of the extracts was estimated according to the modified method of Singleton et al[23]. Primarily, 0.1 mL of the extract was mixed with 0.5 mL of Follin's Ciocaltue reagent and 2 mL of 4.4% sodium carbonate was added. The reaction mixture was incubated in dark for 30 min and the absorbance was measured at 720 nm. The TPC was determined using standard curve prepared with gallic acid (R^2 = 0.95; y = 0.001 x + 0.021) and results are expressed as gallic acid equivalents (GAE).

Physico-Chemical Properties

The solvent extract yield was determined by taking 10 ml of extract in pre-weighed beaker and heated for 1 h at 105^{o}C in a forced air oven (Memmert, Model: UF-30 plus) and the gross weight was recorded. Based on difference, the extract yield was calculated and expressed on percentage basis. The extract was evaporated using Rotovapor (Buchi, Model: R-300) and the air-dried residue was observed for colour and odour manually. The dry extract was re-dissolved in distilled water at 10 mg / ml ratio and analyzed for pH using a pH meter (Labman Scientific). For solubility test, 100 mg of dry extract was dissolved in 10 ml of distilled water and kept on a magnetic stirrer for 30 min. Then the contents were centrifuged at 3000 rpm for 10 min. After removing the supernatant, the pellet was air-dried, weighed and based on weight difference, the water solubility of the extract was calculated.

Antioxidant Activity

The dry extract was re-dissolved in methanol at 10 mg / ml ratio and analyzed for antioxidant activity using DPPH free radical scavenging assay[24]. Different dilutions were prepared and 0.1 ml from each concentration was added with 1.9 ml of methanolic solution of DPPH (5 mg/100 ml) and incubated for 30 min in dark. The absorbance was then recorded at 520 nm. Gallic acid was used as reference standard and the free radical scavenging activity was calculated using the formula (Antioxidant activity = Abs control – Abs test / Abs control x 100) and the results were expressed on percentage basis.

Purification of the extract

The solvent extract of coconut shell was purified by using column chromatography. Glass column (60 cm length x 3 cm dia) was equipped with a vacuum pump to speed up the elution process and was packed Silica (G-60). The extract was made into the slurry with silica and loaded on the stationary phase and separated by using various solvents (hexane, chloroform, ethyl acetate, methanol, and ethanol). Total number of four fractions collected was analyzed for total phenolic content and antioxidant activity. The active fraction with highest TPC and antioxidant activity was further analyzed using HPLC (Make:

Table 2. Analysis of variance of the response surface analysis on the recovery of total phenolic compounds from coconut shell

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Regression	9	11076549	11076549	1230728	16.42	0.003
Linear	3	4421135	4868809	1622936	21.65	0.003
Solvent Conc.	1	89553	525974	525974	7.02	0.045
Time	1	260693	203087	203087	2.71	0.161
Temp	1	4070889	4793351	4793351	63.95	0.000
Square	3	6488818	6488818	2162939	28.86	0.001
Conc. x Conc.	1	169251	367454	367454	4.90	0.078
Time x Time	1	2716	60170	60170	0.80	0.411
Temp x Temp	1	6316851	6316851	6316851	84.28	0.000
Interaction	3	166596	166596	55532	0.74	0.572
Conc. x Time	1	154096	154096	154096	2.06	0.211
Conc. x Temp	1	11673	11673	11673	0.16	0.709
Time x Temp	1	828	828	828	0.01	0.920
Residual Error	5	374747	374747	74949		
Lack-of-Fit	3	348477	348477	116159	8.84	0.103
Pure Error	2	26270	26270	13135		
Total	14	11451297				

Model Summary					
S	273.769	PRESS	5634746		
R-Sq	96.73%	R-sq (adj)	90.84%	R-sq (pred)	50.79%

Agilent, Model: Infinity 1200) using isocratic mobile phase consists of methanol, water and acetic acid (20:80:2, v/v/v) in C-18 column and detection at 280 nm with the run time of 15 min. Standard catechin was purchased from Sigma and used to identify the assigned peaks in HPLC chromatogram.

RESULTS AND DISCUSSION

Phenolic compounds are regarded as organic compound which plays a vital role in disease prevention and reported to have chemo-preventive effects. Phenolics are reported to possess various biological activities including, antioxidant, anti-mutagenic, anti-carcinogenic, anti-inflammatory, vasodilatory, anti-microbial, immuno-stimulating, anti-allergic, estrogenic and hypo-cholesterolemic effects[25]. Total phenolic content of coconut shell was analyzed by using different solvents namely Hexane, Ethyl acetate, Methanol, Ethanol and Water. Upon examining the results, it was found that significantly ($p < 0.05$) maximum level of phenolic content 701.1 mg GAE/100g was obtained with methanol, which is followed by ethanol (576 mg GAE / 100 g) (Figure 1-A). Contrarily, hexane had nil effect on the extraction of TPC from coconut shell while ethyl acetate (196.56 mg/100 g) and water (179.58 mg/100 g) appears to be the least effective solvents for the recovery of TPC from coconut shell. Having this as an inference, further experiments were conducted using only methanol. Similarly, methanol was reported to be the effective solvent for the extraction of TPC in byproducts such as cashew nut shell liquid.[26]

Table 3. Estimated Regression Coefficients for the recovery of total phenolic compounds from coconut shell

Term	Coef	SE Coef
Constant	-9163.37	1994.24
Solvent Conc	99.97	37.74
Time	20.85	12.67
Temp	202.62	25.34
Conc. x Conc.	-0.50	0.23
Time x Time	-0.04	0.04
Temp x Temp	-1.45	0.16
Solvent Conc. x Time	-0.13	0.09
Solvent Conc. x Temp	-0.07	0.18
Time x Temp	0.01	0.08

In addition to this, we have also investigated the effect of various conventional extraction conditions (heating, shaking and soaking) on the recovery of TPC of coconut shell and the results are given in Figure 1-B. It was observed that, heating for 2 h at 45°C had a significant ($p < 0.05$) effect in enhancing the TPC of the extract (4045.50 mg GAE/100 g), which is followed by shaking at 500 rpm for 2 h (3182.40 mg GAE/100 g). This value is higher when compared to TPC of peanut hull.[27] Thus, heating was found to be the best condition for extracting antioxidants from coconut shell and hence, further RSM experiments were carried out to optimize heating process at three different temperatures (30, 60 & 90°C).

Table 4
Physico-chemical properties of solvent extract of coconut shell.

S. No.	Physico-chemical properties	Solvent extract
1	Colour	Dark Brown
2	Odour	Odourless
3	Extract yield (%)	2.12 ± 0.07
4	pH	5.29 ± 0.02
5	Water solubility (%)	64.50 ± 4.94

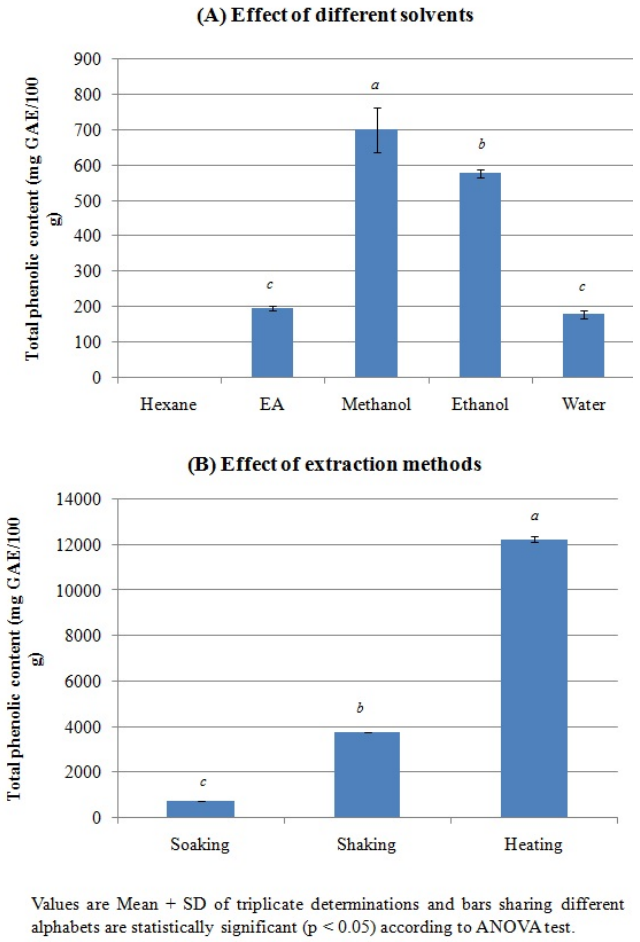

Figure 1. Effect of different solvents (A) and extraction methods (B) on the recovery of total phenolic compounds from coconut shell

To further investigate the ideal extraction temperature, concentration of methanol and extraction time needed to achieve maximum phenolic yield, we adopted RSM. This is the most popular optimization technique consisting of both mathematical and statistical tools[28]. RSM has been employed to optimize the suitable conditions for the extraction of antioxidants from various nut byproducts, such as hazelnut skin and Macadamia nut skin[29, 30]. In the current project, 15 randomized experiments were performed with fixed variables

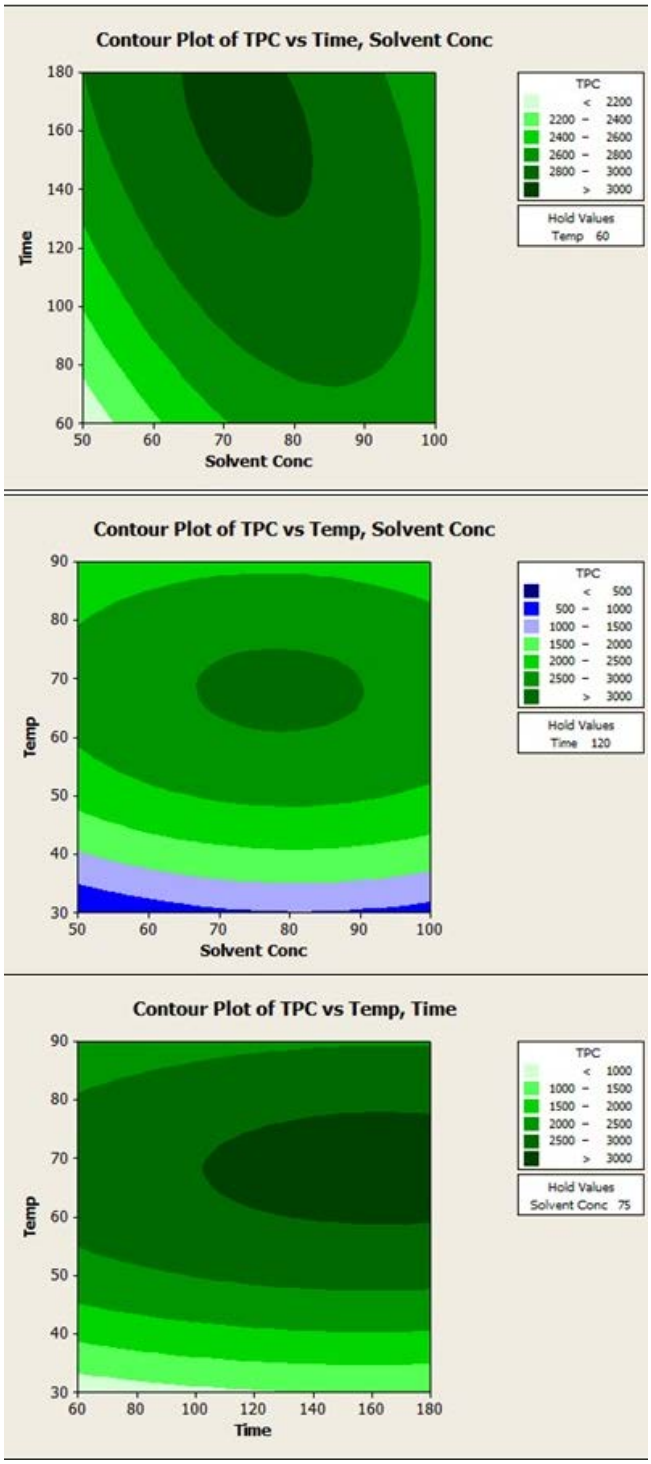

Figure 2. Effect of different variables (solvent concentration, extraction time and temperature) on the TPC yield of coconut shell

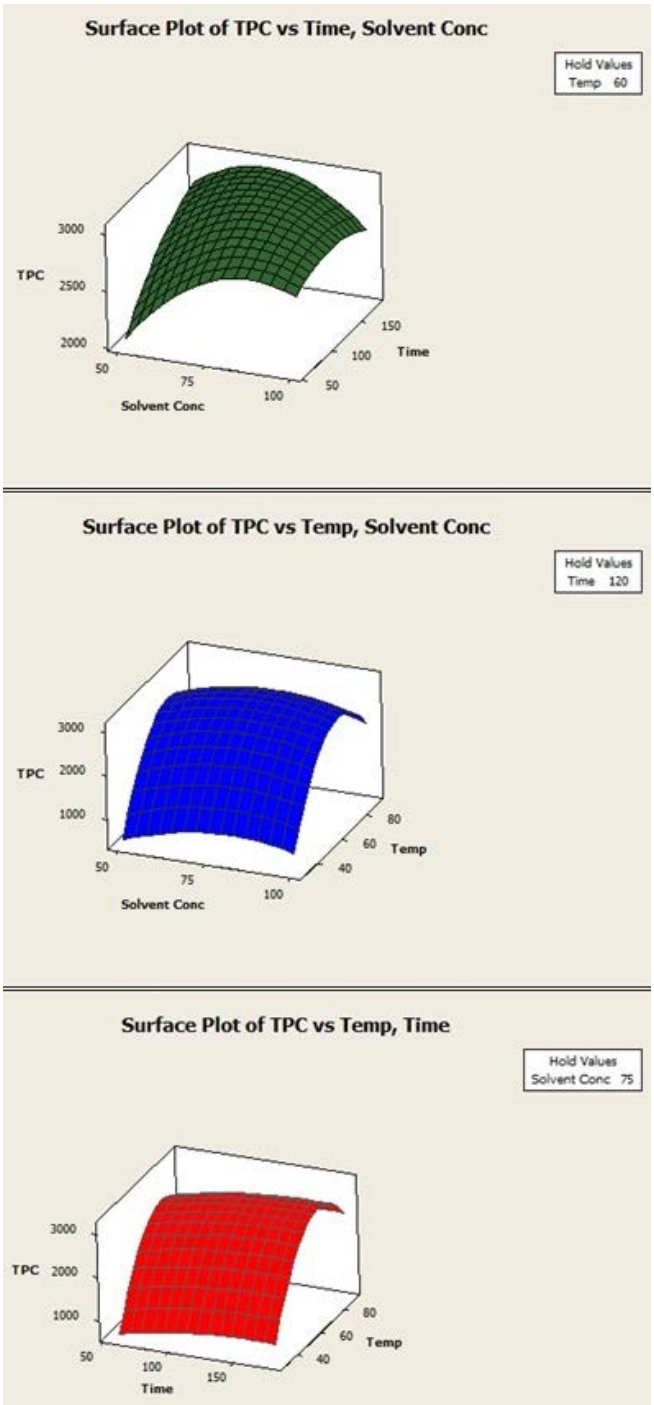

Figure 3. Effect of interaction of variables (solvent concentration, extraction time and temperature) on the yield of coconut shell

and the analysis revealed the presence of TPC (654.72 - 3096.32 mg GAE/100 g coconut shells) and the highest TPC level was recorded in experiment No. 14 with the 75% solvent concentration, 120 min extraction time and 60°C temperature (Table 1). In a previous study, TPC content of 522 – 2137 mg tannic acid equivalents/100 g coconut shell was reported.[18]

Among the three variables, both solvent concentration and temperature were observed to have significant effect on the TPC yield according to the results of ANOVA test (Table 2). In square design, only the interaction of temperature vs. temperature had significant effect. The model summary value of R^2 = 96.73% indicates the adequacy of the design of experiments. The interaction of variables showed the maximum TPC yield could be obtained at extraction time above 100 min and solvent concentration ranges from 65 – 90% at the temperature of 60 – 75°C (Figure 2). In general, both temperature and solvent concentration were noted to play significant role in the extraction of TPC from coconut shell

(Figure 3). The optimal conditions for the recovery of TPC from coconut shell were predicted as the solvent (methanol) concentration of 72%, extraction time of 167 min and temperature of 68°C (Figure 4). By adopting the predicted optimal conditions, a maximum TPC level of 3134 mg GAE/100 g could be obtained from coconut shell. This level appears to be higher than the TPC yield reported in coconut shell by ultrasonic treatment (2244 mg tannic acid equivalents / 100 g sample).[31]

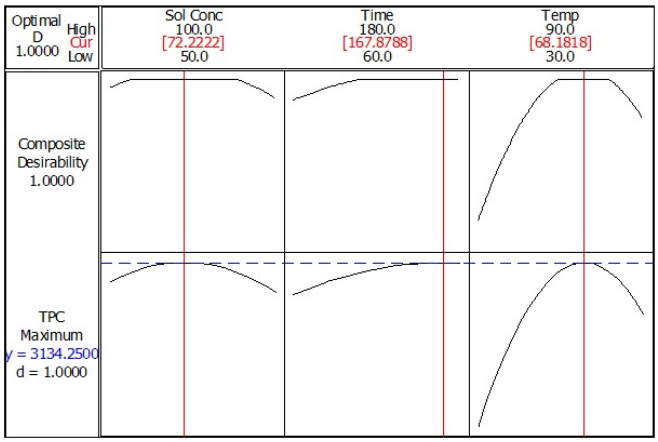

Figure 4. Optimization of extraction conditions (solvent concentration, time and temperature) for the recovery of antioxidants from coconut shell

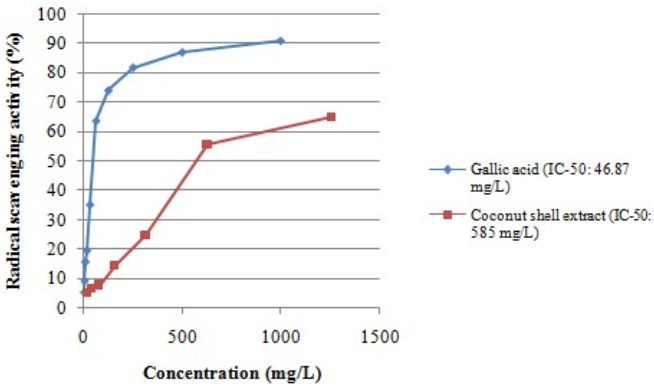

Figure 5. Antioxidant activity of coconut shell extract

The extract characterization revealed that the methanolic extract of coconut shell is dark brown in colour and odourless with a slightly acidic pH (5.29) (Table 4). The extract yield was found to be 2.12% with high water solubility (64.5%). In general, the physico-chemical properties of methanolic extract of coconut is suitable for its further applications in food and pharmaceutical industries. The coconut shell methanolic extract was noticed to possess 65% of free radical scavenging power at a concentration of 1250 mg/L (IC-50: 585 mg/L), which seems to be lower when compared to standard gallic acid (90% antioxidant activity at 1000 mg/L, IC-50: 46.87 mg/L) (Figure 5). The antioxidant power of coconut shell extract is comparable to that of previous report in methanolic extract of hazelnut skin (80% antioxidant activity at a concentration of 1000 mg/L).[32] The antioxidant activity of coconut shell (IC-50: 585 mg/L) was remarkably higher when compared to by-product of *Lavandula latifolia* (IC-50: 5090 mg/L).[33] Antioxidants are known for their remarkable effect in the prevention of cardiovascular diseases, cancer and other neurological disorders.[34,35] Free radicals are generated in our body due to various metabolic processes and results in consequences like lipid peroxidation and ultimately cellular damage. Reactive oxygen species like superoxide and hydroxyl radicals cause oxidative stress, aging and toxicity which can be effectively prevented by antioxidants.[36] Antioxidants prevent the oxidation of free radicals by oxidizing itself.[37] Polyphenols from natural sources like plant have been reported as strong antioxidants. In this prospect, coconut shell extract could be explored as a source of natural antioxidants for industrial applications.

Upon purification of coconut shell extract through column chromatography, four fractions were collected (hexane, chloroform, ethyl acetate, and ethanol) and among which the maximum phenolic concentration (205.24 mg GAE / L) and antioxidant activity (75.34%) was noted in ethyl acetate fraction. This active fraction with highest TPC and antioxidant activity was further analyzed using HPLC and the chromatogram revealed the presence of the major peak with the retention of 2.0 min (Figure 2). By comparing to the retention time of standard, the peak was identified as catechin and our findings are in agreement with the earlier literature indicating the presence of catechin in coconut husk (Esquenazi et al., 2002) through HPLC studies. Identified major phytochemical in the coconut shell extract of the present study could be responsible for the antioxidant property through *in vitro* assays.

CONCLUSIONS

From the results of present study, the coconut shell can be considered as a rich source of phenolic compounds. This study was focused on optimizing the extraction process of antioxidants from coconut shell and it was predicted that extraction with 72% methanol at 68°C for 167 min could be efficient for the maximum recovery of polyphenolic compounds. Having optimized the method to extraction, this agricultural byproduct could be effectively utilized as a natural source of antioxidants in food and pharmaceutical industries. Since the coconut shell powder along with cow's urine is being used as hypo-chloesterolemic drug by traditional healers in Kerala state of India and the polyphenols from coconut shell is hypothesized to attribute such medicinal effect, we seek to do further research on their hypo-chloesterolemic property to prove its bioactivity.

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