Agriculture and Natural Resources 52 (2018) 451–459

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Evaluation of antioxidant and antimicrobial properties of solvent extracts of agro-food by-products (cashew nut shell, coconut shell and groundnut hull)

Anand Prakash,[a] Vellingiri Vadivel,[a,*] Sanaulla Farisa Banu,[b] Paramasivam Nithyanand,[b] Cheepurupalli Lalitha,[c] Pemaiah Brindha[d]

[a] Chemical Biology Lab, School of Chemical and Biotechnology, SASTRA Deemed University, Thirumalaisamudram, Thanjavur, 613401, Tamilnadu, India
[b] Biofilm Biology Lab, School of Chemical and Biotechnology, SASTRA Deemed University, Thirumalaisamudram, Thanjavur, 613401, Tamilnadu, India
[c] Actinomycetes Bioprospecting Lab, School of Chemical and Biotechnology, SASTRA Deemed University, Thirumalaisamudram, Thanjavur, 613401, Tamilnadu, India
[d] Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA Deemed University, Thirumalaisamudram, Thanjavur, 613401, Tamilnadu, India

ARTICLE INFO

Article history:
Received 10 April 2017
Accepted 14 February 2018
Available online 2 November 2018

Keywords:
Cashew nut shell
Coconut shell
Groundnut hull
Polyphenols
Antioxidant
Anti-microbial

ABSTRACT

In India, agro-food by-products such as shell cake of cashew nut (*Anacardium occidentale* L.), shell of coconut (*Cocos nucifera* L.) and hull of groundnut (*Arachis hypogaea* L.) are cheaply available on a vast scale. Even though a small portion of these solid waste materials is being used, a large quantity is not being utilized. Based on literature data, these by-products could be used as a source of valuable phytochemicals. The present study explored a suitable solvent system and extraction conditions for the recovery of polyphenols from three different agro-food by-products. The optimal conditions for the recovery of polyphenols from agro-food by-products were investigated in addition to evaluating their antioxidant and antibacterial properties. Among the three investigated by-products, methanolic extract of cashew nut shell was the most prominent source of antioxidants (3412.28 mg gallic acid equivalent (GAE)/L) compared to coconut shell (1056.32 mg GAE/L) and groundnut hull (426.35 mg GAE/L). The *in vitro* antioxidant assay produced promising radical scavenging activity of shell extract of coconut (concentration at which the response was reduced by half; IC_{50} = 12 μg/mL) compared to cashew nut (IC_{50} = 44 μg/mL) and groundnut hull extract (IC_{50} = 48 μg/mL). The anti-bacterial activity of different solvent extracts revealed that the methanolic extracts from cashew nut and coconut shells were more effective in inhibiting the growth of both Gram-positive and Gram-negative bacteria. The present work revealed the possibility of recovery of useful phytochemical compounds from agro-food byproducts which could be used subsequently as natural food preservatives.

Introduction

Spoilage of food during processing, transportation and storage is a serious problem in food processing industries and chemical oxidation and microbial action are the prime factors for the spoilage of food (Shahidi and Shong, 2010). Oxidation of nutrients (lipids, proteins, and vitamins) as well as microbial decomposition of food products gives rise to the development of off-flavour, loss of nutrients and the formation of potentially toxic compounds and finally make the food unfit for consumption (Shahidi and Shong, 2010). In addition, oxidation of nutrients causes defective nutrition due to the formation of reactive oxygen species and consequently may exhibit deleterious effects on consumers (Esterbauer et al., 1991). Food producers are using synthetic antioxidants such as BHA and BHT to prevent food spoilage and to extend the shelf life of processed foods. Nonetheless, such synthetic compounds have been identified as toxic in the long run and are reported to cause various chronic diseases in humans (Branen, 1975). Hence, there is an urgent need to identify alternative, safe and natural food preservatives. Of late,

* Corresponding author.
E-mail address: vadivel@carism.sastra.edu (V. Vadivel).

https://doi.org/10.1016/j.anres.2018.10.018

consumer preferences for plant-based natural preservatives have resulted in an increased interest towards the use of phytochemicals as antioxidants or antimicrobials in the food system (Vadivel et al., 2013). Agro-food by-products from the food industry could be exploited as a natural source of phytochemicals for food preservation (Vadivel et al., 2014). In this context, agro-food by-products such as the shells of cashew nut or coconut and hulls of groundnut received greater attention as they are locally available on a large scale at a cheap cost in India.

Among the tropical nuts, cashew nut (*Anacardium occidentale* L.) plays a vital role as an edible nut. World production of cashew nut kernels was 3.59 million t from 4 million ha in 2012 (FAOSTAT, 2012). India is the largest producer and exporter of cashew kernels, accounting for almost 50% of world export (Paramashivappa et al., 2001). Cashew nut comprises a hard, outer shell (epicarp), a tightly fitted testa (endocarp) and an edible portion (kernel). While the cashew nut kernel is nutritionally valuable, the shell is considered as a by-product, which may be an environmental problem if not handled properly. The shell constitutes around 50% of the weight of the nut-in-shell, and around 300,000 t of shell is available per year in India (Carr, 2014; Patel et al., 2006). Several studies have been conducted on the polyphenolic compounds and antioxidant activity of cashew nut testa and cashew nut shell liquid. However, there is not much information regarding the phytochemical compounds of the cashew nut shell. A small portion of the shell is used as a substitute for firewood in some places, and a large portion is wasted without any particular use.

Coconut is an edible nut from the coconut palm (*Cocos nucifera* L.), which belongs to the family Arecaceae. India, with cultivation area of about 1.78 million ha is the third largest producer of coconut, after Indonesia and the Philippines (Gunasekaran et al., 2012). Annual coconut production in India is about 7562 million nuts with an average of 5295 nuts/ha (National Multi-Commodity Exchange of India Limited, 2007). Four South Indian states (Kerala, Tamil Nadu, Karnataka and Andhra Pradesh) account for around 90% of the total coconut production in the country (Mandal and Mandal, 2011). On average each coconut tree yields 70–100 nuts per year which in turn will provide about 21–30 kg of coconut shells (Manjula et al., 1985). The coconut shelling process leads to the production of two major by-products, the fibrous husk and the hard shell (Duke, 1992). The annual production of coconut shell is approximately 3.18–4.20 million t (Gunasekaran et al., 2012). Coconut shell is composed mainly of lignin and cellulose with a chemical composition very similar to wood. Because there are few useful applications, a large portion of coconut shell is being wasted and it is one of the main contributors to the nation's pollution problem as a solid waste, representing more than 60% of the domestic waste volume and a serious disposal problem (Gunasekaran et al., 2012).

Groundnut (*Arachis hypogaea* L.) belongs to the family Leguminosae. World groundnut production is 29.1 million t and it is primarily used for the production of oil. It is also an important source of dietary protein in developing and developed countries (Francisco and Resurreccion, 2012). India is the second largest producer of groundnut (1.5–2 million t annually) next to China (2–2.5 million t annually), followed by Sub-Saharan African countries and Central and South America (Bharthare et al., 2014). Regional estimates show that Tamilnadu (1 million t annually), Gujarat (1–3.5 million t annually), Andhra Pradesh (1–2 million t annually), Karnataka (0.5 million t annually) and Maharashtra (0.5 million t annually) are the major producers of groundnut in India (Bharthare et al., 2014). Groundnut comprises kernels, skin (testa) and hulls (also known as shell). After harvesting, the kernels are separated from hulls in the groundnut processing industry. Groundnut hulls account for approximately 20% of the dry weight of the nut-in-shell. The hull is an extremely low valued by-product of groundnut processing operation that remains under-utilized.

Based on this background information, the present research was carried out to optimize the recovery of phenolic extracts from nut by-products and to investigate their antioxidant and anti-bacterial activities with a view to utilizing them as natural food preservatives.

Materials and methods

Sample collection

Nut by-products (cashew nut shell, coconut shell and groundnut hull) were collected from local food processing industries in Thanjavur, Tamilnadu, India in January 2016. The materials were dried under shaded conditions for 2 d and then fungal contamination and decayed materials were removed. The selected samples were hammered into small pieces and then powdered to a 1 mm particle size using a laboratory mill.

Optimization of phenolic extraction

Different solvent extracts (hexane, ethyl acetate, methanol, ethanol and water) were prepared by taking 10 g of nut by-product in 100 mL of each respective solvent and shaking (500 revolutions per minute, rpm) for 2 h and then the contents were filtered using a Whatman No. 42 filter paper. The extracts were analysed for total phenolic concentration (TPC) and based on the results, methanol was selected as a suitable solvent with a high TPC yield for all the by-products. Then, the methanolic extracts were prepared from the powdered by-products under different conditions (soaking, shaking, mild heating, ultra-sonic assisted, microwave-assisted, acid hydrolysis and alkali hydrolysis) for different periods to optimize the recovery of polyphenols. Solvent extraction used 100 g of sample with 500 mL of methanol in a conical flask. Acid hydrolysis used 2% hydrochloric acid, whereas alkaline hydrolysis was carried out with 2% sodium hydroxide at room temperature. In all experiments, the contents were filtered at regular intervals and analysed for TPC. In the acid and alkali hydrolysates, the pH was adjusted to 7.0 with equal strength of acid or alkali as required and then used for the quantification of TPC.

Total phenolic content

The TPC of prepared extracts was analysed by placing 50 μLwith 50 μL of Folin's reagent and 100 μL of sodium carbonate (4.4%) on a microplate (Vadivel and Brindha, 2015). The contents were incubated for 30 min in the dark and read at 750 nm in the plate reader (Epoch; BioTek, Vermont, USA). The TPC for the samples was calculated using the formula: (absorbance − c/s × dilution factor) where “c” is the constant obtained from the gallic acid curve and “s” is the slope of the gallic acid curve. The formula for calculating TPC was $y = 0.002x + 0.182$ and the value of the correlation coefficient was 0.994. Various physicochemical properties of the extracts (extract yield, pH and solubility) were investigated according to the method described by Joshi and Aeri (2009).

Purification of extracts

The solvent extracts of nut by-products were purified using column chromatography. A glass column (60 cm length × 3 cm diameter) was equipped with a vacuum pump to speed up the elution process and was packed with silica (G-60). The extract was made into slurry using silica and loaded on the stationary phase

Table 1
Effect of different solvents under soaking conditions on the recovery of total phenolic compounds of nut shell by-products.

Organic solvent	Total phenolic content (mg GAE/100 g)		
	Cashew nut shell cake	Groundnut hull	Coconut shell
Hexane	$0.86^{c} \pm 0.04$	$5.93^{c} \pm 0.26$	$2.36^{d} \pm 0.24$
Ethyl acetate	$2.29^{c} \pm 0.15$	$12.71^{c} \pm 0.82$	$7.24^{d} \pm 0.21$
Methanol	$3412.28^{a} \pm 42.17$	$426.35^{a} \pm 64.22$	$1056.32^{a} \pm 71.47$
Ethanol	$1374.11^{b} \pm 21.63$	$308.59^{b} \pm 26.18$	$818.92^{b} \pm 52.80$
Water	$1349.02^{b} \pm 115.52$	$275.30^{b} \pm 24.09$	$696.63^{c} \pm 3.54$

Values are mean ± SD of three separate determinations. Mean values in a column that do not share the same lowercase superscripts are significantly different ($p < 0.05$, confidence interval = 95%).

and separated using various solvents (hexane, chloroform, ethyl acetate, methanol and ethanol). In total, eight fractions were collected from the solvent extract of cashew nut shell (one in hexane, two in chloroform, two in ethyl acetate and three in ethanol). Among these, the sixth fraction eluted with ethanol contained the highest TPC level (247 mg GAE/L) and antioxidant activity (82.59%). Regarding coconut shell extract, four fractions were collected (hexane, chloroform, ethyl acetate and ethanol), among which the maximum phenolic concentration (205.24 mg GAE/L) and antioxidant activity (75.34%) were produced from ethyl acetate fraction. Five fractions were collected in groundnut hull extract (one in hexane, one in chloroform, one in ethyl acetate and two in ethanol). Among these, the fifth fraction eluted with methanol was found to contain the highest TPC level (232.18 mg GAE/L) and antioxidant activity (92.46%). The active fraction in each byproduct with the highest TPC level and antioxidant activity was further analysed using high performance liquid chromatography (HPLC, Agilent Technologies, Infinity 1200, India) using the isocratic mobile phase consisting of methanol, water and acetic acid (20:80:2, volume per volume per volume) in a C-18 column with detection at 280 nm and a the run time of 15 min. Standards such as anacardic acid, catechin, and luteolin were purchased from Sigma Aldrich (St. Louis, Missouri, USA) and used to identify the assigned peaks in the HPLC chromatogram.

The acid and alkali hydrolysates of nut by-products were purified using ion-exchange column chromatography with a commercial type resin (Amberlite XAD-7). The acid and alkali hydrolysate from each by-product was neutralized to pH 7 with equal strength HCl or NaOH and passed through the conditioned resin-packed column followed by washing with distilled water twice and then eluted with absolute ethanol. The total phenolic concentration of hydrolysate and eluents were determined and the recovery percentage was calculated.

In vitro antioxidant activity

The solvent of the methanolic extract was evaporated using a rotavapor (Buchi, R-300, Switzerland) and the extract was re-dissolved in water at a ratio of 10 mg/mL. The antioxidant activity of the extracts was analysed for 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging power following the method of Sanchez-Moreno et al. (1998). The extract (100 μL) was added to 3.9 mL of DPPH solution (0.025 g/L), and the reactants were incubated at 25 °C for 30 min. Different concentrations of gallic acid were used as a positive control and ethanol was used instead of extract in the blank. The decrease in absorbance was measured at 515 nm using a spectrophotometer. The radical scavenging activity of tested samples was calculated and expressed on a percentage basis.

Table 2
Effect of different extraction conditions using methanol on the total phenolic content of nut shell byproducts.

Extraction time (min)	Total phenolic content (mg GAE/100 g)		
	Cashew nut shell cake	Groundnut hull	Coconut shell
Soaking at room temperature			
60	$2110.54^{b} \pm 2.14$	$291.75^{b} \pm 7.83$	$815.75^{a} \pm 16.32$
120	$2225.16^{b} \pm 11.42$	$305.50^{c} \pm 9.70$	$852.00^{a} \pm 2.83$
180	$2356.63^{b} \pm 8.34$	$322.25^{c} \pm 2.35$	$884.75^{a} \pm 9.34$
240	$2351.59^{b} \pm 6.48$	$324.68^{c} \pm 5.66$	$902.62^{a} \pm 6.22$
300	$2356.28^{b} \pm 9.62$	$321.54^{b} \pm 3.71$	$916.28^{a} \pm 3.75$
360	$2352.12^{b} \pm 5.84$	$324.39^{b} \pm 6.35$	$919.04^{a} \pm 11.43$
Shaking condition (500 revolutions per minute) at room temperature			
60	$3142.55^{b} \pm 13.26$	$417.50^{c} \pm 4.45$	$1007.56^{a} \pm 5.22$
120	$3272.98^{b} \pm 7.15$	$430.25^{c} \pm 7.83$	$1049.27^{a} \pm 7.83$
180	$3386.43^{b} \pm 14.05$	$451.00^{c} \pm 5.62$	$1081.46^{a} \pm 5.62$
240	$3459.81^{b} \pm 8.28$	$469.73^{b} \pm 9.14$	$1099.75^{a} \pm 9.14$
300	$3460.54^{b} \pm 9.80$	$468.32^{b} \pm 7.02$	$1098.25^{a} \pm 7.17$
360	$3449.52^{b} \pm 12.73$	$465.84^{b} \pm 5.21$	$1095.69^{a} \pm 5.34$
Shaking + mild heating conditions (45 °C)			
60	$3297.15^{c} \pm 12.74$	$487.13^{b} \pm 2.16$	$1117.24^{a} \pm 4.45$
120	$3499.25^{c} \pm 11.02$	$540.47^{b} \pm 7.83$	$1130.83^{a} \pm 5.68$
180	$3583.46^{c} \pm 18.18$	$541.30^{b} \pm 5.62$	$1173.05^{a} \pm 6.35$
240	$3580.54^{c} \pm 15.16$	$569.52^{b} \pm 9.14$	$1189.41^{a} \pm 9.23$
300	$3576.17^{c} \pm 18.38$	$568.18^{b} \pm 7.26$	$1186.65^{a} \pm 6.24$
360	$3582.72^{c} \pm 12.93$	$565.28^{b} \pm 3.52$	$1185.58^{a} \pm 5.49$
Microwave-assisted solvent extraction			
10	$3117.45^{c} \pm 17.22$	$316.24^{b} \pm 5.38$	$1405.18^{a} \pm 2.16$
20	$3232.29^{c} \pm 12.51$	$335.52^{b} \pm 6.14$	$1428.30^{a} \pm 4.15$
30	$3364.42^{c} \pm 18.11$	$369.17^{b} \pm 12.56$	$1437.53^{a} \pm 3.56$
40	$3381.15^{c} \pm 8.14$	$389.45^{b} \pm 11.20$	$1439.16^{a} \pm 6.14$
50	$3422.63^{c} \pm 15.06$	$388.04^{b} \pm 8.58$	$1438.84^{a} \pm 4.46$
60	$3421.15^{c} \pm 12.28$	$384.51^{b} \pm 6.32$	$1435.71^{a} \pm 5.25$

Values are mean ± SD of three separate determinations. Mean values in a row that do not share the same lowercase superscripts are significantly different ($p < 0.05$, confidence interval = 95%).

Table 3
Effect of acid and alkali hydrolysis on the recovery of total phenolic compounds of nut shell byproducts.

Extraction time (min)	Total phenolic content (mg GAE/100 g)		
	Cashew nut shell cake	Groundnut hull	Coconut shell
Acid hydrolysis at room temperature			
60	$1155.19^{a} \pm 12.80$	$117.50^{b} \pm 5.45$	$731.25^{b} \pm 8.84$
120	$1185.72^{a} \pm 10.04$	$130.25^{b} \pm 7.83$	$786.13^{b} \pm 2.12$
180	$1202.56^{b} \pm 2.12$	$171.39^{a} \pm 1.62$	$835.04^{b} \pm 5.56$
240	$1214.47^{b} \pm 6.06$	$179.74^{a} \pm 9.14$	$852.52^{a} \pm 4.90$
300	$1218.35^{a} \pm 3.53$	$174.26^{b} \pm 7.02$	$856.41^{c} \pm 7.48$
360	$1212.58^{a} \pm 14.25$	$178.45^{b} \pm 5.26$	$857.21^{c} \pm 5.20$
Alkali hydrolysis at room temperature			
60	$1820.53^{c} \pm 3.54$	$251.50^{b} \pm 1.09$	$961.25^{a} \pm 5.20$
120	$1894.56^{c} \pm 2.75$	$293.72^{b} \pm 2.06$	$1091.57^{a} \pm 9.45$
180	$1942.85^{c} \pm 3.54$	$334.39^{b} \pm 3.94$	$1146.28^{a} \pm 7.19$
240	$1963.57^{c} \pm 2.12$	$383.58^{b} \pm 5.56$	$1259.61^{a} \pm 4.71$
300	$2013.63^{b} \pm 8.39$	$391.85^{b} \pm 3.24$	$1258.27^{a} \pm 1.67$
360	$2015.28^{b} \pm 4.01$	$396.69^{c} \pm 4.15$	$1251.35^{a} \pm 8.43$

Values are mean ± SD of three separate determinations. Mean values in a row that do not share the same lowercase superscripts are significantly different ($p < 0.05$, confidence interval = 95%).

Table 4
Physico-chemical properties of methanolic extracts of nut byproducts.

Physico-chemical properties	Nut byproduct extract		
	Cashew nut shell cake	Groundnut hull	Coconut shell
Colour	Blackish-brown	Orange-brown	Reddish-brown
Odour	Astringent	Nutty	Pleasant
pH	6.58 ± 0.13	6.36 ± 0.76	7.12 ± 0.48
Extract yield (%)	3.82 ± 0.25	2.14 ± 0.43	2.95 ± 0.17
Water solubility (%)	65.82 ± 1.08	73.54 ± 0.93	78.43 ± 1.22
Total phenolic content (mg GAE/100 g)	4045.50 ± 63.78	1384.20 ± 50.91	3182.40 ± 25.46

Values are mean ± SD of three separate determinations; GAE = gallic acid equivalents.

Antibacterial activity

The antibacterial activity of the solvent extracts and acid or alkali hydrolysates from nut by-products were evaluated against a panel of both Gram-positive and Gram-negative bacteria consisting of *Staphylococcus aureus* subsp. *aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 12384) and against some pathogens responsible for creating foodborne illness consisting of *Escherichia coli* (ATCC 25922), *Salmonella enterica* subsp. *enterica* (ATCC 13311) and *Shigella flexneri* (ATCC 9199). *E. coli*, *S. enterica*, and *S. flexneri* were maintained in nutrient agar (Himedia, M-001; Mumbai, Maharashtra, India), *S. aureus* was cultured in soybean-casein digest agar (Himedia, M-290; Mumbai, Maharashtra, India), whereas Todd Hewitt agar (Himedia, M-313; Mumbai, Maharashtra, India) was used to grow *S. pyogenes*. Antibacterial activity was assayed using agar well diffusion assay (Nithyanand and Pandian, 2009) wherein the plates were prepared using Mueller Hinton agar (Himedia, M-173; Mumbai, Maharashtra, India) and wells were made with the help of a sterile cork borer. Freshly grown colonies of the above-mentioned pathogens were used to inoculate 25 mL of Muller Hinton broth in a shaking water bath for 4–6 h until reaching a turbidity of 0.5 McFarland (1×10^8 colony forming units (CFU)/mL). Final inocula were adjusted to 5×10^5 CFU/mL, and 100 μL of the final inocula was applied to each agar plate and uniformly spread over the surface using a sterilized cotton swab. Samples of 100 μLof the different solvent extracts (10 mg/mL) were loaded into the wells, the plates were incubated at 37 °C and the zones of inhibition were measured after 24 h. The respective solvents alone loaded in individual wells were used as the control. Determination of the minimum inhibitory concentration (MIC) of the methanolic extracts of nut by-products was carried out using the broth microdilution method in a 96 well microtiter plate (Srinivasan et al., 2010). In brief, 50 μL of different concentrations of extracts were mixed with 50 μL of bacterial cultures (1×10^7 CFU) and 100 μL of their respective broth medium. The

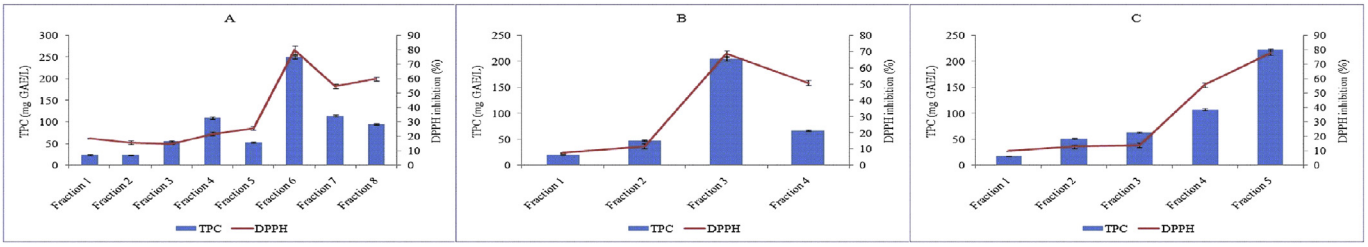

Fig. 1. Total phenolic concentration (TPC) and 2,2-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH) of numbered column fractions of: (A) cashew nut shell; (B) coconut shell; (C) groundnut hull.

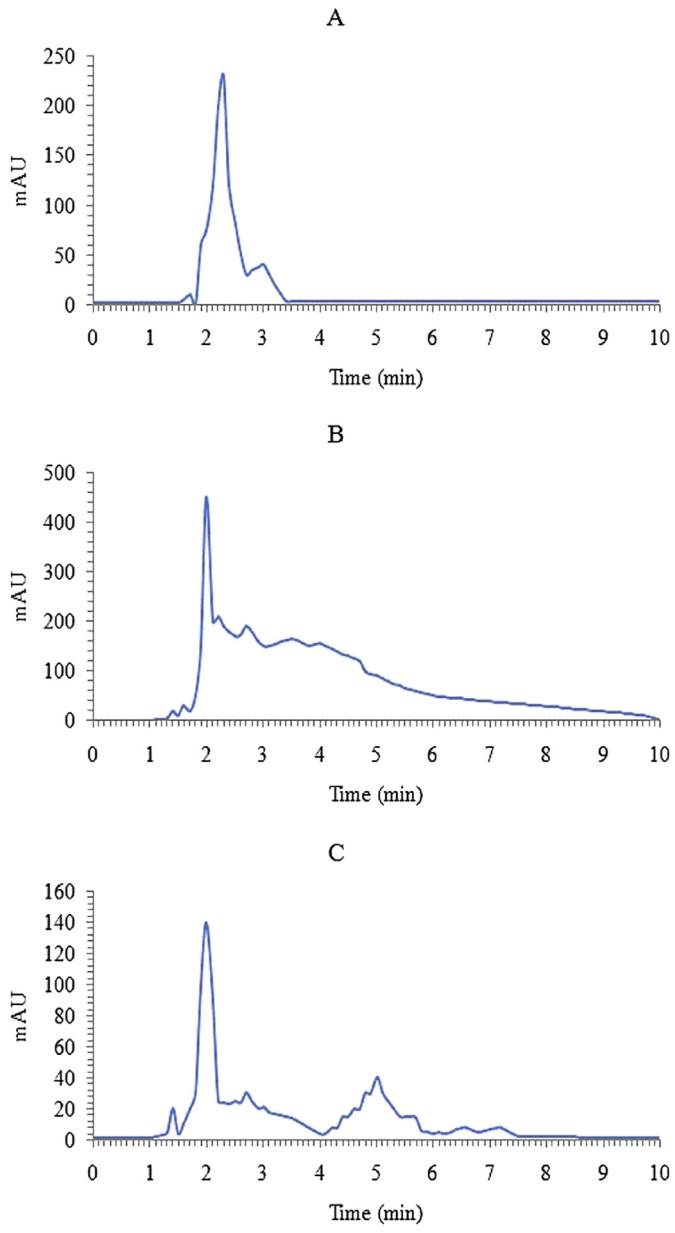

Fig. 2. High performance liquid chromatography chromatogram of active fractions of: (A) cashew nut shell; (B) coconut shell; (C) groundnut hull.

plates were then incubated at 37 °C for 18 h and then 20 μL of MTT (Thiazolyl Blue Tetrazolium Bromide) was added in each well. The lowest concentration at which no growth of colonies was observed was considered as the MIC.

Results and discussion

Optimization of polyphenol recovery

Among the different solvents investigated, methanol registered a significant ($p < 0.05$) effect with the maximum recovery of phenolic compounds from cashew nut shell (3412.28 mg GAE/100 g), coconut shell (1056.32 mg GAE/100 g) and groundnut hull (426.35 mg GAE/100 g) as shown in Table 1. Based on the analysis of the TPC, high polar solvents like methanol solvent, which was followed by ethanol were more efficient in the recovery of polyphenols from all the by-products, whereas the non-polar hexane and the medium polar ethyl acetate were not effective and hence, the polyphenols present in the nut by-products analysed in the current study could be considered to be high polar.

The effect of different conditions on the recovery of phenolic compounds from nut by-products is shown in Table 2. With soaking conditions at room temperature, cashew nut shell produced the significantly ($p < 0.05$) highest level of phenolic yield (2110.54 mg GAE/100 g) at 60 min compared to groundnut hull (291.75 mg GAE/100 g) and coconut shell (815.75 mg GAE/100 g). The phenolic yield of cashew nut shell and groundnut shell increased with extraction time until 180 min whereas for coconut shell, the phenolic yield steadily increased until 240 min and after that no improvement was noticed with extraction time.

The shaking treatment (Table 2) resulted in maximum recovery at 240 min in cashew nut shell (3456.81 mg GAE/100 g), followed by coconut shell (1099.75 mg GAE/100 g) and groundnut hull (469.73 mg GAE/100 g). In all the studied samples, the phenolic yield gradually increased up to 240 min after which there was no further improvement. Compared to the soaking treatment, shaking has released notable amounts of phenolic compounds from nut by-products due to the application of mechanical shaking force.

Shaking and mild heating resulted in a recovery of 3583.46 mg GAE/100 g polyphenols (180 min), 1189.41 mg GAE/100 g polyphenols (120 min) and 540.47 mg GAE/100 g polyphenols (240 min) from cashew nut shell, coconut shell, and groundnut hull, respectively (Table 2). Among the different treatments, shaking and mild heating was an effective treatment for the extraction of maximal levels of phenolic compounds in both cashew nut shell and groundnut hull. Hence, this treatment could be considered for the efficient extraction of phenolic compounds from cashew nut shell and groundnut hull. This treatment was effective, especially in comparison to the separate soaking and shaking treatments because both mechanical shaking and mild heating were applied, which helped to solubilize the free phenols as well as release the bound phenols.

Microwave-assisted extraction produced 3422.63 mg GAE/100 g of phenolics after 50 min from cashew nut shell, 389.45 mg GAE/100 g of phenols after 40 min from groundnut hull and 1437.53 mg GAE/100 g of phenols after 30 min from coconut shell (Table 2). Compared to the other treatments, microwave-assisted extraction

Table 5
Purification of acid and alkali hydrolysates of nut shell byproducts using commercial resin based chromatography.

Sample	TPC of hydrolysate (mg GAE/L)	TPC of eluent (mg GAE/L)	Recovery (%)
Acid hydrolysate	216.28 ± 1.38	158.35 ± 2.50	73.20
Alkali hydrolysate	225.74 ± 1.65	116.67 ± 1.83	51.68
Acid hydrolysate	138.19 ± 0.75	105.68 ± 1.33	76.46
Alkali hydrolysate	238.26 ± 0.48	96.92 ± 3.15	40.67
Acid hydrolysate	165.06 ± 1.44	115.30 ± 1.23	69.85
Alkali hydrolysate	208.82 ± 0.39	83.72 ± 1.69	40.09

Values are mean ± SD of three separate determinations; TPC = total phenolic concentration.

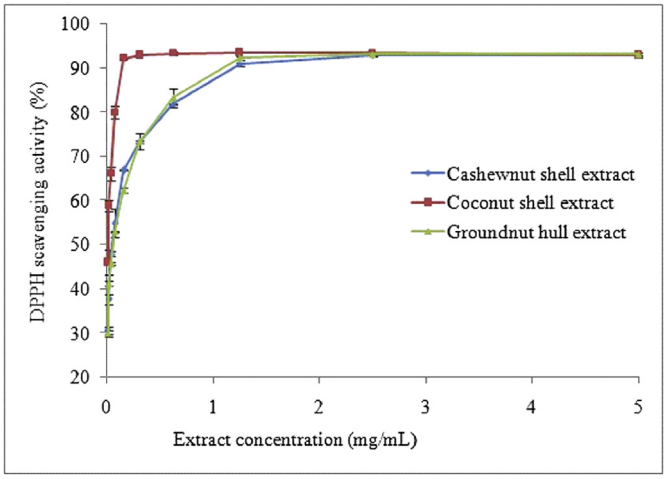

Fig. 3. Antioxidant activity measured using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity for methanolic extract of nut byproducts, where IC_{50} (half maximal inhibitory concentration) of cashew nut shell extract was 44 μg/mL, coconut shell extract was 12 μg/mL and groundnut hull extract was 48 μg/mL.

resulted in the recovery of the maximal level of phenolic compounds from coconut shell. Furthermore, appreciable levels of polyphenols were extracted in all the by-products within a short time compared to the other treatments. Such efficient treatment could be recommended for the recovery of phenolic compounds from coconut shell, and the remarkable level of phenolics noted during this treatment might have been due to the breakage of bonds between phenolic compounds and lignins and their subsequent release into the methanolic solvent because of the microwave irradiation.

The study also focused on the bound polyphenols (which are linked with cell wall materials and lignins through ether and ester bonds) of nut by-products after extraction of free phenols using the solvent (ethanol). Acid and alkali hydrolysis were proven as effective methods to release the bound phenols in lingo-cellulosic materials like nut by-products, and hence these treatments were used and the results are shown in Table 3. Acid hydrolysis produced a significant ($p < 0.05$) release of phenols from residue after solvent extraction of cashew nut shell after 180 min (1202.56 mg GAE/100 g), of coconut shell after 240 min (852.52 mg GAE/100 g) and of groundnut hull after 180 min (171.39 mg GAE/100 g). The release of such notable amounts of phenols from solvent-extracted residue might have been because of the breakage of the ether bonds of phenolic acids and cell wall material of the nut by-products. However, compared to acid hydrolysis, alkali hydrolysis had the significantly ($p < 0.05$) maximum level of polyphenols in cashew nut shell (2013.63 mg GAE/100 g after 300 min) compared to coconut shell (1259.61 mg GAE/100 g after 240 min) and groundnut hull (391.85 mg GAE/100 g after 300 min) as shown in Table 3. The higher levels of phenolic compounds under alkali hydrolysis in all the nut by-products indicated that the bound phenols were linked with cell wall material by ester bonds and their release was caused by the alkaline solution resulting in a higher phenolic content.

Among the three by-products investigated in the present study, cashew nut shell was the most promising source of antioxidants, followed by coconut while groundnut hull was a poor source. Maximum recovery of polyphenols from cashew nut shell resulted from the treatment involving shaking and mild heating for 180 min and also following alkali hydrolysis for 300 min. With coconut shell, the maximum recovery of polyphenols occurred with microwave-assisted solvent extraction for 30 min and alkali hydrolysis for 240 min. In groundnut hull, solvent extraction in conjunction with shaking and mild heating for 240 min and alkali hydrolysis for 240 min appeared to be efficient.

Physicochemical properties

The physicochemical properties of nut by-products are presented in Table 4. The colour of cashew nut shell, coconut shell, and groundnut hull were blackish-brown, reddish-brown and orange-brown, respectively. Astringent, nutty and pleasant odours were observed for cashew nut shell, coconut shell, and groundnut hull samples, respectively. The pH was nearly for the aqueous extracts of cashew nut shell (6.58), coconut shell (6.36) and groundnut hull (7.12). The extract yield of cashew nut shell was higher (3.82%) than for coconut shell (2.95%) and groundnut hull (2.14%). The water solubility was 65.82%, 73.54% and 78.43% was recorded for cashew nut shell, coconut shell, and groundnut hull extracts, respectively. The observed physicochemical properties of the extracts of nut by-products indicated they were suitable for further industrial applications, especially as natural food preservatives.

Purification by column chromatography

Solvent extracts of the nut by-products were purified using column chromatography, and the fractions were eluted and based

Table 6
Antibacterial activity of different solvent extracts of nut shell byproducts.

Solvent extract	Zone of inhibition (mm)				
	Escherichia coli (ATCC 25922)	*Salmonella enterica* (ATCC 13311)	*Shigella flexneri* (ATCC 9199)	*Staphylococcus aureus* (ATCC 25923)	*Streptococcus pyogenes* (ATCC 12384)
Hexane	0	0	11	13	10
Ethyl acetate	13	12	13	11	13
Ethanol	17	16	16	14	16
Methanol	16	16	17	17	17
Water	0	0	0	0	0
Hexane	0	0	0	0	0
Ethyl acetate	10	10	0	18	10
Ethanol	11	12	13	27	13
Methanol	15	15	16	19	16
Water	0	0	0	0	0
Hexane	0	0	0	0	0
Ethyl acetate	10	13	12	12	11
Ethanol	14	14	12	17	20
Methanol	12	14	14	16	15
Water	0	0	0	0	0

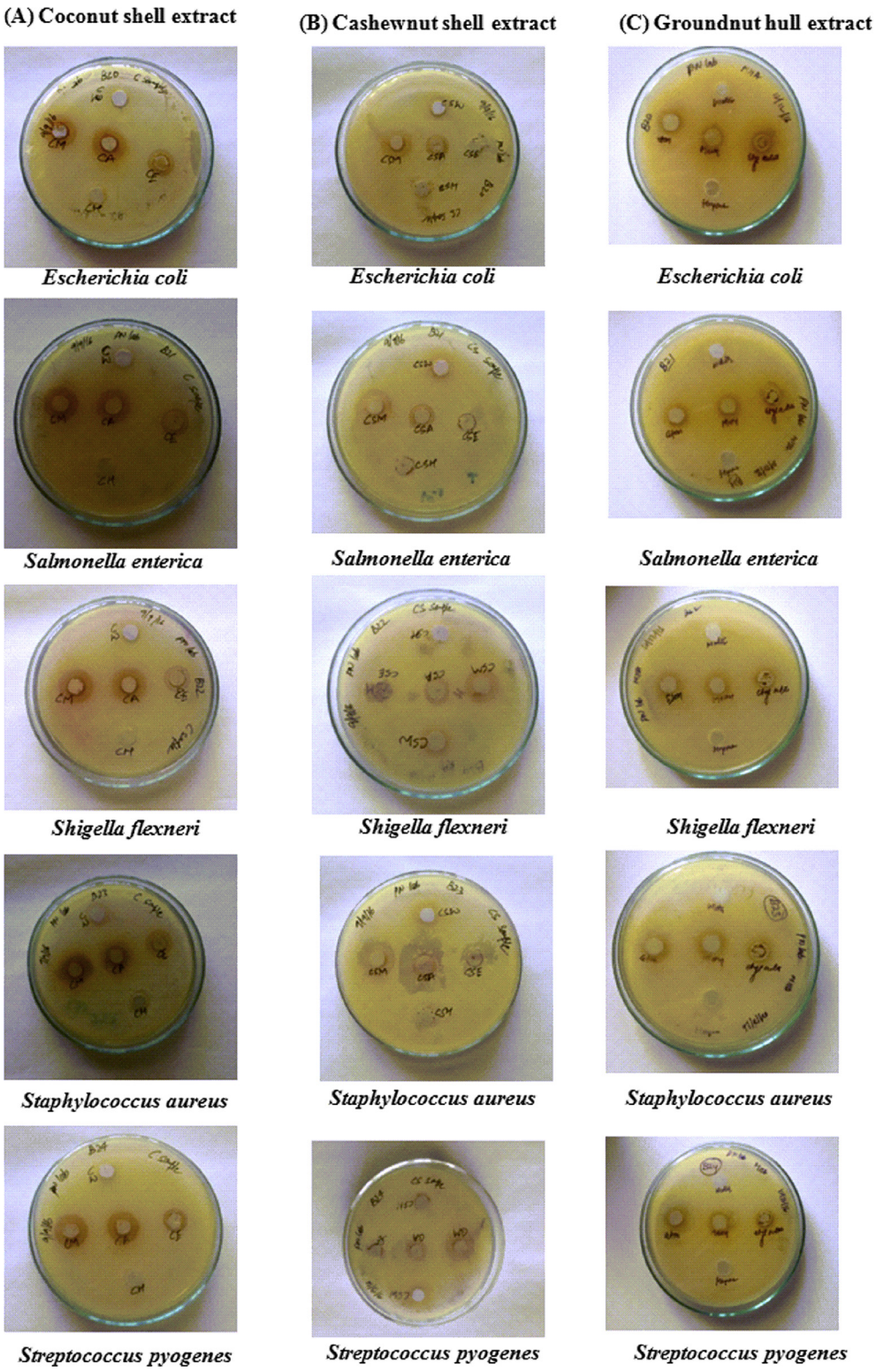

Fig. 4. Antibacterial activity against selected food-borne pathogens of extract of: (column A) cashew nut shell; (column B) coconut shell; (column C) groundnut hull.

on the total phenolic concentration and antioxidant activity, the active fractions were selected (Fig. 1).

The HPLC chromatogram of purified fractions revealed the presence of the major peaks with retention of 2.4 min in the case of cashew nut shell, 2.0 min in coconut shell and 1.9 min in groundnut hull (Fig. 2). These peaks were compared with the retention times of standards and identified as anacardic acid, catechin, and luteolin in the extracts of cashew nut shell,

Table 7
Minimum inhibitory concentration of methanolic extracts of nut shell byproducts against selected bacterial species.

Extract	Microorganisms				
	Escherichia coli (ATCC 25922)	*Salmonella enterica* (ATCC 13311)	*Shigella flexneri* (ATCC 9199)	*Staphylococcus aureus* (ATCC 25923)	*Streptococcus pyogenes* (ATCC 12384)
Coconut shell	>10 mg/mL	>10 mg/mL	>10 mg/mL	>10 mg/mL	>10 mg/mL
Cashewnut shell	2.5 mg/mL	2.5 mg/mL	5 mg/mL	5 mg/mL	5 mg/mL
Groundnut hull	>10 mg/mL	10 mg/mL	10 mg/mL	5 mg/mL	>10 mg/mL

coconut shell and groundnut hull, respectively. These results were in agreement with the earlier literature in which HPLC studies reported the presence of anacardic acid, cardanol and cardol in cashew nut shell (Philip et al., 2008), catechin and epicatechin in coconut husk (Esquenazi et al., 2002) and resveratrol and luteolin in groundnut hull (Dean et al., 2008). The major phytochemicals identified in each by-product in the present study could be responsible for the antioxidant and antimicrobial properties of the extracts noticed through *in vitro* assays.

Purification of hydrolysates

The acid and alkali hydrolysates of nut by-products were purified using Amberlite XAD-7 resin to remove impurities like sugars, vitamins, amino acids and acid and alkali chemical residues. Then, the phenolic compounds were eluted with absolute ethanol, and the TPC was estimated using spectrophotometry and the results are given in Table 5. Resin treatment resulted in better recovery of phenolic compounds from acid hydrolysates of cashew nut shell (73.20%), coconut shell (76.46%) and groundnut hull (69.85%). Eluents from acid hydrolysates of cashew nut shell (158.35 mg GAE/L), coconut shell (105.68 mg GAE/L) and groundnut hull (115.30 mg GAE/L) produced the maximum recovery of polyphenols compared to alkali hydrolysates. Eluents recovery from alkali hydrolysates were 51.68% (116.67 mg GAE/L) in cashew nut shell, 40.67% (96.92 mg GAE/L) in coconut shell and 40.09% (83.72 mg GAE/L) in groundnut shell. Similarly, the use of Amberlite XAD-7 resin has been reported to purify polyphenols from wheat straw (Lopes et al., 2016) and *Inga edulis* leaf extract (Silva et al., 2007). Hence, the studied resin type could be more suitable for the purification of polyphenols from acid hydrolysis than alkali hydrolysis.

In vitro antioxidant activity

Since methanol showed produced better recovery of polyphenols from the investigated by-products (Table 1), this solvent was used to prepare the antioxidant extract. Among the studied samples, coconut shell extract recorded higher antioxidant power based on 50 mg/mL (IC_{50}) of 12 μg/mL compared to cashew nut shell (IC_{50} = 44 μg/mL) and groundnut hull extract (IC_{50} = 48 μg/mL) as shown in Fig. 3. Since all the investigated solvent extracts of nut by-products had strong antioxidant power through *in vitro* assay (DPPH radical scavenging activity), the extracts could be expected to provide antioxidant activity and prevent oxidation-mediated spoilage in food systems. Since oxidation of food components (especially unsaturated lipids) is the major cause of food spoilage (Huisin't Veld, 1996), incorporation of the investigated extracts of nut by-products in high-fat foods could inhibit lipid peroxidation and therefore act as natural food preservatives, which will be investigated in future studies.

Antibacterial activity

The antibacterial activity of different solvent extracts of selected nut by-products against important pathogens was studied and the results are shown in Table 6 and Fig. 4. Based on the zone of inhibition, the ethanolic extract of coconut shell was effective in controlling the growth of *E. coli* (17 mm), *S. enterica* (16 mm) and *S. flexneri* (16 mm) whereas the methanolic extract of coconut shell had a maximum level of zone of inhibition against *S. aureus* (17 mm) and *S. pyogenes* (17 mm). Methanolic extract of cashew nut shell effectively inhibited the growth of bacteria such as *E. coli* (15 mm), *S. enterica* (15 mm), *S. flexneri* (16 mm) and *S. pyogenes* (16 mm) while ethanol extract was active against *S. aureus* (27 mm) only. With groundnut hull, the ethanolic extract inhibited *E. coli* (14 mm), *S. enterica* (14 mm), *S. aureus* (17 mm) and *S. pyogenes* (20 mm) and the methanolic extract effectively inhibited the growth of *S. flexneri* (14 mm) alone (Table 6). The control wells (with solvent alone) did not produce any zone of inhibition, indicating that the solvents on their own did not inhibit the growth of bacteria.

Since alcoholic extracts of all three by-products produced noticeable zones of inhibition against the bacterial species, different concentrations of methanolic extract of nut by-products were investigated to determine the MIC. Among the studied by-products, cashew nut shell extract was the most effective antibacterial agent as it inhibited the pathogens at a very low concentration with an MIC value of 2.5 mg/mL against *E. coli*, *S. enterica*, and *S. flexneri* (Table 7). Maximum bactericidal efficacy of groundnut shell extract was observed against *S. aureus* with an MIC value of 5 mg/mL. Coconut shell extract was the least effective antimicrobial agent as it did not produce antibacterial activity against any of the tested bacterial strains even at a high concentration of 10 mg/mL. The current results were in accordance with Martin et al. (2012) who evaluated the antimicrobial potential of seven different agro-industrial wastes and reported the MIC values ranged from 0.78 mg/mL to 25 mg/mL. As the alcoholic extracts of the selected nut by-products inhibited the pathogens that are involved in foodborne illness, it is possible that these agro by-products could be used as natural antimicrobial agents to prevent the growth of food-spoiling bacteria. However, the effectiveness of nut by-product extracts to restrict microbial growth in various food systems and their food preservation capacity against food-spoiling microbes should be studied in model food systems.

Agro-food by-products are considered as solid wastes causing environmental pollution. By adopting suitable methods, valuable products could be developed from the nut by-products and utilized for the betterment of human society. The present work has reported in detail on the optimal extraction conditions and a suitable solvent for the recovery of polyphenols from agro-food by-products such as cashew nut shell, coconut shell, and groundnut hull. Among the investigated by-products, cashew nut shell was a naturally rich source of antioxidants, followed by coconut shell. New attempts were made in the study to recover both the free and bound

polyphenols through acid and alkali hydrolysis and their purification processes. *In vitro* antioxidant and antibacterial experiments revealed the promising effects of methanolic extract of cashew nut and coconut shells. Since the extracts have better antioxidant and antibacterial activities under *in vitro* conditions, they could be explored as natural food preservatives. Accordingly, the current results should be of great use in isolating natural antioxidants and antibacterial compounds from nut by-products in food preservation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The second author received financial support from SERB, New Delhi, India (Start-up Grant No. YSS/2014/000332) and authors are grateful to R. Sethuraman, Vice-Chancellor of SASTRA Deemed University, Thanjavur, Tamilnadu, India for his encouragement to conduct this research project.

References

Bharthare, P., Shrivastava, P., Singh, P., Ttiwari, A., 2014. Peanut shell as renewable energy source and their utility in production of ethanol. Int. J. Adv. Res. 2, 1–12.
Branen, A.L., 1975. Toxicology and biochemistry of butylated hydroxy anisole and butylated hydroxy toluene. J. Am. Oil Chem. Soc. 52, 59–63.
Carr, M.K.V., 2014. The water relations and irrigation requirements of cashew (*Anacardium occidentale* L.): a review. Exp. Agric. 50, 24–39.
Dean, L.L., Davis, J.P., Shofran, B.G., Sanders, T.H., 2008. Phenolic profiles and antioxidant activity of extracts from peanut plant parts. Open Nat. Prod. J. 1, 1–6.
Duke, J.A., 1992. Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants. CRC Press, Boca Raton, FL, US.
Esquenazi, D., Wigg, M.D., Miranda, M.M.F.S., Rodrigues, H.M., Tostes, J.B.F., Rozental, S., Da Silva, A.J.R., Alviano, C.S., 2002. Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Res. Microbiol. 153, 647–652.
Esterbauer, H., Schaur, R.F., Zollner, H., 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 11, 81–128.
FAOSTAT, 2012. Statistical Data Published by Food and Agricultural Organization, Rome, Italy. http://faostat.fao.org/site/567/default.aspx#ancor. (Accessed 25 April 2017).
Francisco, M.L.L., Resurreccion, A.V.A., 2012. Antioxidant capacity and sensory profiles of peanut skin infusions. LWT Food Sci. Technol. 47, 189–198.
Gunasekaran, K., Annadurai, R., Kumar, P.S., 2012. Long term study on compressive and bond strength of coconut shell aggregate concrete. Constr. Build. Mat. 28, 208–215.
Huisin't Veld, J.H., 1996. Microbial and biochemical spoilage of foods: an overview. Int. J. Food Microbiol. 33, 1–18.
Joshi, S., Aeri, V., 2009. Practical Pharmacognosy, first ed. Frank Bros & Co. (Publishers) Ltd., New Delhi, India.
Lopes, A.M.C., Brenner, M., Fale, P., Roseiro, L.B., Bogel-Łukasik, R., 2016. Extraction and purification of phenolic compounds from lignocellulosic biomass assisted by ionic liquid, polymeric resins, and supercritical CO_2. Sust. Chem. Eng. 4, 3357–3367.
Mandal, M.D., Mandal, S., 2011. Coconut (*Cocos nucifera* L.: Arecaceae): in health promotion and disease prevention. Asian Pac. J. Trop. Med. 4, 241–247.
Manjula, S., Sudha, J.D., Bera, S.C., Pillai, C.K.S., 1985. Polymeric resin from renewable resources: studies on polymerization of the phenolic component of coconut shell tar. J. Appl. Polym. Sci. 30, 1767–1771.
Martin, J.G.P., Porto, E., Correa, C.B., Alencar, S.M.D., Gloria, E.M.D., Cabral, I.S.R., Aquino, L.M.D., 2012. Antimicrobial potential and chemical composition of agro-industrial wastes. J. Nat. Prod. 5, 27–36.
Nithyanand, P., Pandian, S.K., 2009. Phylogenetic characterization of culturable bacterial diversity associated with the mucus and tissue of the coral *Acropora digitifera* from the Gulf of Mannar. FEMS Microbiol. Ecol. 69, 384–394.
National Multi-Commodity Exchange of India Limited, 2007. Report on Copra. Ernakulam, Kerala, India.
Paramashivappa, R., Kumar, P.P., Vithayathil, P.J., SrinivasaRao, A., 2001. Novel method for isolation of major phenolic constituents from cashew (*Anacardium occidentale* L.) nut shell liquid. J. Agric. Food Chem. 49, 2548–2551.
Patel, R.N., Bandyopadhyay, S., Ganesh, A., 2006. Extraction of cashew (*Anacardium occidentale*) nut shell liquid using supercritical carbon dioxide. Bioresour. Technol. 97, 847–853.
Philip, J.Y.N., Francisco, J.D.C., Dey, E.S., Buchweishaija, J., Mkayula, L.L., Ye, L., 2008. Isolation of anacardic acid from natural cashew nut shell liquid (CNSL) using supercritical carbon dioxide. J. Agric. Food Chem. 56, 9350–9354.
Sanchez-Moreno, C., Larrauri, J.A., Saura-Calixto, F.A., 1998. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric. 76, 270–276.
Shahidi, F., Shong, Y., 2010. Lipid oxidation and improving the oxidative stability. Chem. Soc. Rev. 39, 4067–4079.
Silva, E.M., Pompeu, D.R., Larondelle, Y., Rogez, H., 2007. Optimisation of the adsorption of polyphenols from *Inga edulis* leaves on macroporous resins using an experimental design methodology. Sep. Pur. Technol. 53, 274–280.
Srinivasan, S., Beema Shafreen, R.M., Nithyanand, P., Manisankar, P., Pandian, S.K., 2010. Synthesis and *in vitro* antimicrobial evaluation of novel fluoroquinolone derivatives. Eur. J. Med. Chem. 45, 6101–6105.
Vadivel, V., Amandola, D., Spigno, G., 2013. Evaluation of antioxidant activity of extracts from four different agro-food by-products. Biochem. Pharmacol. 2, 95.
Vadivel, V., Amandola, D., Spigno, G., 2014. Screening of four different agro-food by-products for the recovery of antioxidants and cellulose. Chem. Eng. Trans. 37, 757–762.
Vadivel, V., Brindha, P., 2015. Antioxidant property of solvent extract and acid/alkali hydrolysates from rice hulls. Food Biosci. 11, 85–91.