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Chemical composition, antibacterial and antioxidant activities of hydrosols from different parts of *Areca catechu* L. and *Cocos nucifera* L.

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ABSTRACT

Hydrosols are promising natural anti-microbial and anti-oxidant agents with potential applications in afro-industries. Hydrosols obtained by hydrodistillation in Clevenger-type apparatus from arecanut (*Areca catechu* L.) and coconut (*Cocos nucifera* L.) were analyzed by GC–MS and evaluated for the antibacterial and antioxidant activities. GC–MS analysis for hydrosols of areca flower (AFH), areca floral axis (AFAH), areca root (ARH), coconut flower (CFH), coconut floral axis (CFAH) and coconut root (CRH) resulted in the detection of 44, 24, 61, 39, 22, and 29 different volatile components, respectively. All tested bacteria were inhibited by all hydrosol samples using paper disc diffusion and serial dilution methods *E. coli* O157:H7 ATCC 33150 and *S. aureus* ATCC 2392 were significantly inhibited by AFH and AFAH, while *E. coli* ATCC 25922 and *C. albicans* ATCC 10231 were the most sensitive bacteria against CFH. Hydrosols from the flowers of arecanut and coconut had the highest total phenolic contents, the best scavenging effect on DPPH and ABTS, and the strongest reducing power than those from floral axes and roots.

1. Introduction

Arecanut is an important commercial plantation crop cultivated mainly in South and Southeast Asia. The economic part of arecanut is called "betel nut" and can be chewed for the purpose of dispersing accumulated fluid in the abdominal cavity and killing worms. Peng et al. (2015) reported that more than 59 compounds have been isolated and identified from arecanut, with pyridine-type alkaloids and condensed tannins as the characteristic constituents. Areca inflorescence including flowers and bracts in the common erect is rich in polyphenols, polysaccharide, alkaloids and other various physiologically active substances and microelements, and is well known as miniature nourishment and longevity food in China (Chen et al., 2012). A lot of literatures had indicated that areca flower extracts were useful nutritional antioxidants, suggesting a possible use in nutraceutical industry (Chen et al., 2012; Cheng et al., 2011; Lin and Li, 2010; Zhang et al., 2009). Arecanut has a shallow root system with more than 70% roots occurring in 60 cm depth and distance from stem of the palm (Bhat and Leela, 1969). The root of arecanut contains higher contents of phenolics and alkaloids than fresh unripe fruit, leaf, spike and vein (Wang et al., 1997). However, methanolic and aqueous extract of seeds contains higher antioxidant activity and total phenolic content than the extract of areca root (Hamsar et al., 2011). Additionally, the antibacterial and antifungal effects both in vitro and vivo conditions of bioactive compounds isolated from arecanut have been documented by Peng et al. (2015).

Coconut is commonly known as "tree of life" since each part of the coconut tree has great utility to humans. Coconut is monecious, and each spadix usually containing several hundred male flowers and 20–30 female flowers (Free et al., 1975). The versatile coconut tree is a source of various chemical compounds, as reported for the composition of coconut sap, coconut inflorescence, and coconut flower extract, etc (Borse et al., 2007; Renjith et al., 2013; Soumya et al., 2014). Coconut flower has been indicated to have antioxidant, antitumor and antimicrobial activities (Kavitha and Beena, 2012), as well as cytoprotective and antihyperglycemic properties (Kabra Mahaveer et al., 2012; Renjith et al., 2013). Coconut has an adventitious root system and the coconut roots have no root hairs and no nutrient-scavenging mycorrhiza. The alcohol and water soluble extracts of coconut root were suggestive of presence of more polar secondary metabolites like glycosides, steroids, triterpenoids, flavonoids, coumarins, phenols and tannins (Saraswathy et al., 2010). Although the antimicrobial activity of coconut female flower extract has been well documented by Raja and Poonkuil (2015), data about the antibacterial property of coconut male flower are lacking.

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Hydrosols are waters obtained during hydro-distillation of plant material, which can be additional products of essential oil isolation or the only product of plant material distillation (Maciag and Kalemba, 2015). The increasing social and economic implications caused by bacteria appeals for a constant striving to produce safer food crops and to develop new anti-bacterial agents. Although increasing use of chemical preservatives can effectively prevent the growth of most bacteria, safety problems related to chemical preservatives are receiving growing attention. Therefore, much effort has been expended in the search for new types of effective and nontoxic antimicrobial activity against some representative spoilage pathogens. Being the by-products coproduced during water or steam distillation of plant material, hydrosols were commonly used in aromatherapy and cosmetic for the therapeutic properties as antibacterial, anti-infectious, antioxidant, anticoagulant, anti-inflammatory, cicatrizing, analgesic, digestive or respiratory (Paolini et al., 2008). The practival applicability of hydrosols has been observed as fumigant in food system in plastic storage containers. Although hydrosols obtained from typical Mediterranean species (Paolini et al., 2008), rose (Kurkcuoglu and Baser, 2003; Ulusoy et al., 2009), lavandula (Moon et al., 2006), *Satureja thymbra* (Chorianopoulos et al., 2008), summer savory (Boyraz and Ozcan, 2006), citrus (Lante and Tinello, 2015), and various spices (Boyraz and Ozcan, 2005; Sagdic, 2003; Sagdic and Ozcan, 2003; Sagdic et al., 2013; Tornuk et al., 2011) were documented, there has been limited information about hydrosols from arecanut and coconut. This report was conducted to extend a developed application of arecanut and coconut as a hydrosol product.

The objectives of this study were to analyze the volatile chemical composition of hydrosols from the flower, floral axis and root of arecanut and coconut, and provide scientific evidence to justify the antibacterial and antioxidant activities of hydrosols from different parts (flower, floral axis and root) of arecanut and coconut. In addition, hydrosols from which part of arecanut and coconut had the highest antibacterial and antioxidant activities were also investigated.

2. Materials and methods

2.1. Plant materials

Different parts of arecanut and coconut such as male flower, floral axis and root were obtained in August 2015 from the Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wenchang, Hainan Province, China. Plant materials were identified by the scientists of botany at Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences.

2.2. Preparation of test bacteria

The following bacterial species were used as test organisms: *E. coli* ATCC 25922, *C. albicans* ATCC 10231, *E. coli* O157:H7 ATCC 33150, and *S. aureus* ATCC 2392. The four bacterial species obtained from stock cultures were grown in nutrient broth and incubated at 37 °C for 18 h. According to Sagdic (2003), broth cultures were prepared overnight in nutrient broth and later adjusted so that the final concentration of each sample following inoculation was approximately 10^6–10^7 cfu/ml.

2.3. Preparation of hydrosols from different parts of arecanut and coconut

Hydrosols from different parts of arecanut and coconut were obtained from distillation of fresh plant materials in water. Fresh materials of the male flower, floral axis and root of arecanut and coconut were washed with distilled water for three times to remove the surface contamination. After that, the floral axes and roots of arecanut and coconut were cut into small pieces (about 1 cm). Hydrosols were produced by the method described by Lante and Tinello (2015) with some modifications. Fresh materials (250 g) were placed into a flask (1 l) with 500 ml of distilled water (1:2 w/v) and hydrodistilled for approximately 30 min with a Clevenger apparatus. Hydrosol of 250 ml was obtained by hydrodistillation of each batch. This procedure was repeated to acquire 2 l of each type of hydrosol at least. Hydrosols obtained were kept in sterile dark colored bottles at 4 °C until use.

2.4. Volatile analysis of the hydrosols

Volatile composition analysis of the hydrosols was run on an Agilent 7890A gas chromatograph system (Agilent, Avondale, USA) coupled to a mass selective detector (Agilent Technologies, Agilent, Avondale, USA) and HP-5MS column (0.2 mm × 50 m, film thickness 0.25 μm) according to the procedure of Sagdic et al. (2013). The compounds adsorbed by the fibers were desorbed from the injection port for 20 min at 50 °C in the splitless mode. The oven temperature was held at 40 °C for 5 min, and then heated to 125 °C at 3 °C/min, and further increased to 230 °C at 5 °C/min, and finally it was held at 230 °C for 5 min. The carrier gas was helium with a flow rate of 1.0 ml/min. Qualitative analysis was based on the comparison of retention times and the computer mass spectra libraries using Wiley GC/MS Library and Nist, Tutore Libraries. Components relative concentrations were calculated based on GC peak areas.

2.5. Antibacterial activities

The antibacterial activities of the hydrosol products were carried out according to the paper disc diffusion method and the serial dilution method (3) using four bacteria (*E. coli* ATCC 25922, *C. albicans* ATCC 10231, *E. coli* O157:H7 ATCC 33150, and *S. aureus* ATCC 2392). The plates and tubes inoculated with the four bacterial were inculated at 37 °C for 18 h. The determinations were performed in triplicate for each sample and the values were averaged. Benzylpenicillin with a concentration of 5 μg/ml was used as a positive control in the paper disc diffusion method.

2.6. Antioxidant properties

2.6.1. Total phenolic contents

The total phenolic content was determined according to the Folic-Ciocalteu colorimetric method, as proposed by Shen et al. (2014) with a slight modification. Sample solution (3 ml) was added to 1 ml of 10-fold diluted Folin-Ciocalteu's reagent. After an interval of 5 min, 1.5 ml of 6% sodium carbonate were added. The reactions were placed in the dark for 2 h, and the absorbance was recorded at 725 nm (Libra S35 UV/Vis-spectrophotometer, Biochrom Ltd., Cambridge, UK). Distilled water was used as a blank and gallic acid was used as the standard. The standard curve was presented in Fig. 1. Samples were assayed in triplicate.

2.6.2. Reducing power assay

Reducing power was measured as described by Shen et al. (2014) with a slight modification. Sample solution of 1 ml was mixed with 2.5 ml of 0.2 M phosphate buffer (Ph 6.6) and 1.5 ml of 1% potassium ferricyanide solution. The mixture was incubated at 50 °C for 20 min, and then 1.5 ml of 10% trichloroacetic acid was added to the mixture to terminate the reaction. Then the solution was centrifuged at 4000 rpm for 10 min. The supernatant (4 ml) was mixed with 0.5 ml of 0.1% ferric chloride solution. The absorbance of hydrosol samples as compared with that of ascorbic acid (5 μg/ml)

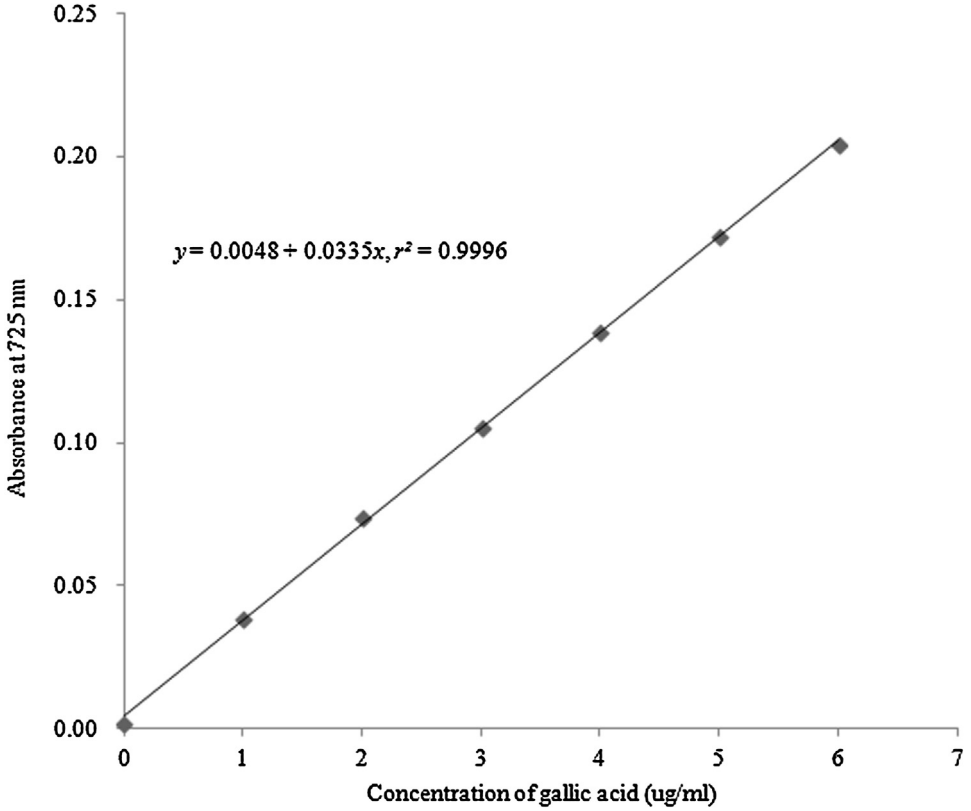

Fig. 1. Standard curve of gallic acid for detecting total phenolic content of hydrosols.

was measured at 700 nm after 10 min. The determinations were carried out in triplicate.

2.6.3. DPPH assay

The determination of the scavenging effect on DPPH• radical by the sample was carried out according to the method of Shen et al. (2014) with a slight modification. Sample of 5 ml was mixed with 1 ml of DPPH• in methanol prepared daily (0.1 mM). After 30 min incubation in darkness, the absorbance at 517 nm was measured against 80% methanol blank. The inhibition percentage of DPPH• radical was calculated according to the formular: DPPH• radical scavenging capacity (%) = $[(A_0–A)/A_0] \times 100$, where A_0 and A are the absorbance values of the control and tested samples, respectively. Ascorbic acid (5 μg/ml) was used for comparison. The determinations were performed in triplicate.

2.6.4. ABTS assay

The ABTS radical scavenging assay was estimated according to the previously reported procedure with a slight modification (Cao et al., 2015). ABTS radical cation solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium persulfate, after incubation at 23 °C in the dark for 16 h. The ABTS radical cation solution was then diluted with PBS (Ph 7.4) to obtain an absorbance of 0.700 ± 0.005 at 734 nm. The ABTS radical cation solution of 2 ml was added to 0.7 ml of the test sample and mixed vigorously. Following kept in the dark at room temperature for 60 min, the absorbance was spectrophotometrically determined at 734 nm. The $ABTS^+$ scavenging activity was calculated using the following formula: ABTS radical scavenging capacity (%) = $[(A_0–A)/A_0] \times 100$, where A_0 is the absorbance of the control, and A is the absorbance of the sample. Ascorbic acid (5 μg/ml) was used for comparison. The determinations were performed in triplicate.

2.7. Statistical analysis

The experimental data were subjected to an analysis of variance (ANOVA) for a completely random design using a statistical system of SPSS 16. 0. Ducan's range tests were used to detect significance of difference at $P < 0.05$.

3. Results and discussion

3.1. Volatile components of the hydrosols

Major volatile components, retention index and total peak areas of the hydrosols obtained by GC–MS analysis were presented in Table 1. GC–MS profile of the tested hydrosols showed the presence of a wide range of compounds, including hydrocarbons, alcohols, ketones, aldehydes, esters, acids and others. In another study, twenty-five volatile and semi-volatile phytoconstituents, such as carbohydrates, alkaloids, phenols, tannins, flavonoids, and phytosterols, were identified in the aqueous alcoholic extract of coconut female flower (Soumya et al., 2014). Qualitative and quantitative differences were observed in the components of different hydrosol samples. Among the 44, 24 and 22 volatile constituents identified by GC–MS analysis of AFH, AFAH, and CFAH, benzyl alcohol was found to be the major compound (14.39%, 20.52% and 21.11%, respectively), followed by 1-heptanol of 13.84% for AFH, and ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate of 17.85% and 17.41%, respectively for AFAH and CFAH. High amount of benzyl alcohol was beneficial because of its allergenic, anesthetic, antiodontalgic, antipruritic and antiseptic activities (Duke, 1992). In addition, Zhang et al. (2014) reported 47 and 24 aromatic components from ground areca male flower and areca floral axis by SPME coupled with GC–MS, respectively. 3,7-Dimethyl-1,6-octadien-3-ol was the main component of ARH

Table 1
GC–MS data for volatile components of hydrosols from different parts of arecanut and coconut.

Compound	Peak area (%)						
	RI	AFH	AFAH	ARH	CFH	CFAH	CRH
Hydrocarbons							
β-Myrcene	958	–	–	0.18	–	–	–
[[(2-Ethylhexyl)oxy]methyl]-oxirane	1217	–	–	0.12	1.26	3.63	1.30
Tetradecane	1413	–	–		0.74	–	–
Cyclododecane	1439	–	–		–	1.31	
Pentadecane	1512	–	–	0.32	–	–	2.41
Hexadecane	1612	–	–	0.33	–	–	–
Heptadecane	1711	0.31	1.49	–	–	0.93	0.64
(*Z*)-3-Heptadecene	1719	–	–	–	–	–	0.82
1,3,12-Nonadecatriene	1916	–	0.39	–	–	–	–
[4aS-(4aα,4bβ,7α,8aα)]-7-Ethenyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-1,1,4b,7-tetramethyl-phenanthrene	1926	–	–	–	–	1.49	–
Eicosane	2009	–	–	–	–	–	0.21
Heneicosane	2109	0.14	0.85	–	1.35	0.25	–
Total		0.45	2.73	0.95	3.35	7.61	5.38
Alcohols							
3-Methyl-1-butanol	697	–	–	0.17	–	–	–
1-Hexanol	860	1.07	–	0.48	2.14	–	–
(*Z*)-3-Hexen-1-ol	868	2.37	1.01	0.58	1.82	1.05	1.20
1-Heptanol	960	13.84	0.55	3.28	4.43	–	4.58
2-Propyl-1-pentanol	995	–	5.34	–	–	–	–
2-(2-Ethoxyethoxy) ethanol	1012	0.62	–	–	0.48	–	–
Benzyl alcohol	1036	14.39	20.52	1.98	10.19	21.11	7.94
1-Octanol	1059	0.61	0.72	0.94	1.65	–	1.05
(*E*)-2-*O*cten-1-ol	1067	0.22	–	0.13	–	–	–
3,7-Dimethyl-1,5,7-octatrien-3-ol	1072	0.20	–	0.18	–	–	–
3,7-Dimethyl-1,6-octadien-3-ol	1082	7.70	10.54	19.55	13.07	11.52	15.35
α-Terpineol	1143	–	–	1.46	–	–	–
L-α-Terpineol	1143	–	–	–	0.87	–	–
(*R*)-5-Methyl-2-(1-methylethenyl)-4-hexen-1-ol	1146	–	–	–	6.66	–	–
5-Methyl-2-(1-methylethenyl)-4-hexen-1-ol	1146	0.52	4.16	12.94	–	1.43	7.95
1-Nonanol	1159	–	–	1.94	5.24	–	–
(+/−)-(1α,2β,5α)-5-Methyl-2-(1-methylethyl)-cyclohexanol	1164	0.30	1.61	0.37	0.84	1.88	0.78
(*Z*)-3-Nonen-1-ol	1167	–	–	0.26	–	–	–
(*E*)-2-Nonen-1-ol	1167	0.18	–	–	–	–	–
Citronellol	1179	–	–	1.96	–	–	–
α-Methyl-α-[4-methyl-3-pentenyl]oxiranemethanol	1182	5.22	6.18	2.88	4.46	6.09	3.81
1-Methyl-4-(1-methylethylidene)-cyclohexanol	1191	–	–	0.33	–	–	–
7-Methyl-3-methylene-6-octen-1-ol	1210	–	–	0.55	–	–	–
Geraniol	1228	0.26	0.30	2.39	0.62	–	0.69
3-Phenylpropanol	1235	–	–	0.11	–	–	–
3-Phenyl-2-propen-1-ol	1243	0.81	–	0.38	0.30	–	–
4-Cyclooctene-1-methanol	1245	–	–	0.11	–	–	–
6-Ethenyltetrahydro-2,2,6-trimethyl-2H-pyran-3-ol	1255	7.51	6.26	2.52	5.14	8.63	3.80
(3*E*)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-ol	1455	0.33	–	0.13	–	–	–
1-Dodecanol	1457	–	–	0.08	1.68	–	–
Trans-2-dodecen-1-ol	1465	–	–	0.22	–	–	–
1,10-Decanediol	1501	0.32	–	–	–	–	–
Cedrol	1543	0.23	–	–	–	–	–
n-Tridecan-1-ol	1556	0.44	1.68	–	–	–	–

Table 1 (*Continued*)

Compound	Peak area (%)						
	RI	AFH	AFAH	ARH	CFH	CFAH	CRH
(*E*)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	1564	0.38	–	0.35	–	–	–
1-Tetradecanol	1656	–	0.52	–	0.44	–	–
n-Pentadecanol	1755	–	–	0.14	–	–	–
Total		57.75	58.67	55.47	58.38	51.71	46.10
Ketones							
4-Methyl-2-hexanone	789	–	–	0.23	–	–	–
6-Methyl-5-hepten-2-one	938	–	–	0.38	–	–	–
2-Octanone	952	–	–	0.84	–	–	–
Isophorone	1097	1.79	4.20	0.13	1.47	2.07	–
6-Ethenyldihydro-2,2,6-trimethyl-2H-pyran-3(4H)-one	1238	1.81	2.47	3.36	2.91	2.33	2.54
4-Hydroxy-3-methylacetophenone	1363	–	–	0.22	–	–	–
(*E*)-1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	1440	–	–	0.02	–	–	–
6,10,14-Trimethyl-2-pentadecanone	1754	–	–	0.67	–	–	–
Total		3.60	6.67	5.85	4.38	4.40	2.54
Aldehydes							
Heptanal	905	2.74	–	–	–	–	–
Benzaldehyde	982	0.45	2.65	–	–	1.40	0.37
(*E*)-2-Octenal	1013	0.19	–	–	–	–	–
Nonanal	1104	–	–	0.57	7.40	–	–
(*E*)-Cinnamaldehyde	1189	0.32	–	0.07	–	–	–
Decanal	1204	0.56	–	–	–	–	–
2,4-Dimethyl-benzaldehyde	1208	0.62	–	–	–	–	–
2-Undecenal	1311	–	–	–	0.31	–	–
Dodecanal	1402	0.59	1.08	–	0.80	–	–
Pentadecanal	1701	–	–	–	0.34	–	–
Total		5.47	3.73	0.64	8.85	1.40	0.37
Esters							
1-Butanol-3-methyl acetate	820	–	–	0.47	–	–	–
Formic acid hexyl ester	981	–	–	–	–	–	1.86
(*E*)-3-Hexen-1-ol acetate	992	1.00	–	0.72	–	–	–
Benzoic acid methyl ester	1060	0.84	–	–	–	–	–
Acetic acid phenylmethyl ester	1160	8.56	–	5.06	4.63	–	5.36
2-Methoxy-benzoic acid methyl ester	1249	0.77	0.34	–	0.20	0.50	0.18
Propanoic acid phenylmethyl ester	1259	–	–	2.38	0.26	–	0.26
5-Methyl-2-(1-methylethenyl)-4-hexen-1-ol acetate	1270	–	–	3.79	–	–	–
Methyl salicylate	1281	3.28	–	2.24	0.35	1.16	0.49
2-Methyl-propanoic acid phenylmethyl ester	1294	–	–	1.10	–	–	–
2-Methyl-propanoic acid 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	1347	0.32	–	–	–	–	–
Acetic acid cinnamyl ester	1367	0.83	–	–	–	–	–
3-Methyl-butanoic acid phenylmethyl ester	1394	–	–	3.30	–	–	–
2-Methyl-propanoic acid 2-ethyl-3-hydroxyhexyl ester	1415	0.33	0.66	–	0.59	0.63	0.39
Benzyl tiglate	1443	–	–	0.75	–	–	–
Pentanoic acid phenylmethyl ester	1458	–	–	1.25	–	–	–
2-Methyl-propanoic acid 3-phenylpropyl ester	1493	–	–	0.18	–	–	–
Neryl (*S*)-2-methylbutanoate	1586	–	–	0.74	–	–	–
Heptyl caprylate	1680	–	–	0.11	–	–	–

Table 1 (*Continued*)

Compound	Peak area (%)						
	RI	AFH	AFAH	ARH	CFH	CFAH	CRH
1,1′-Oxybis-octane	1688	–	–	–	–	–	17.10
1,2-Benzenedicarboxylic acid bis(2-methylpropyl) ester	1908	–	–	–	0.88	–	–
Total		15.70	1.72	23.03	8.56	2.29	26.69
Acids							
Octanoic acid	1173	–	–	0.83	0.37	–	–
Nonanoic acid	1272	–	–	–	0.76	–	–
Dodecanoic acid	1570	–	–	–	–	2.21	–
Total		–	–	0.83	1.13	2.21	–
Others							
Benzenepropanenitrile	1238	–	–	0.09	–	–	–
4,5-Dihydro-5,5-dimethyl-4-isopropylidene-1H-pyrazole	1244	–	–	–	–	–	0.92
2-Methoxy-4-vinylphenol	1293	–	–	–	0.67	–	1.13
Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	1529	13.27	17.85	6.41	11.62	17.41	10.33
2,4-Bis(1,1-dimethylethyl)-phenol	1555	1.08	5.33	0.90	3.06	8.58	4.72
Total		13.27	17.85	6.50	12.29	17.41	12.38
Total identified		97.32	96.70	94.17	100.00	95.64	98.18
Number of components		44	24	61	39	22	29

RI: Retention index on HP-5 column.
AFH, AFAH and ARH: hydrosols from flower, floral axis and root of arecanut, respectively; CFH, CFAH and CRH: hydrosols from flower, floral axis and root of coconut, respectively.

(19.55%) and CFH (13.07%), and of AFH, AFAH, CFAH and CRH with a higher percentage (7.7%, 10.54%, 11.52% and 15.35%, respectively). The major components of CRH were 1,1′-oxybis-octane (17.10%), 3,7-dimethyl-1,6-octadien-3-ol (15.35%) and ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate (10.33%). A large part of constituents identified in the hydrosols of floral axis of arecanut and coconut were also present in the flower hydrosols. Meanwhile, Borse et al. (2007) detected the volatile composition from fresh, clarified and fermented coconut sap neera by a simultaneous distillation and solvent extraction method and found ethyl lactate and phenyl ethyl alcohol as two major chemical constituents. It could be expected that compositions of hydrosols from a particular plant could differ depending on several factors such as natural origin, harvesting time, genetic structure and plant part. There were a number of common volatile compounds found in the hydrosols, while ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate was characteristic for all the hydrosols.

All the hydrosol samples were dominated by alcohols, with benzyl alcohol and 3,7-dimethyl-1,6-octadien-3-ol as the main representatives. Similar results were reported in the case of *C. clementina* and *I. graevolens* (Paolini et al., 2008), Lamiaceae spices (Sagdic et al., 2013), rose (Ulusoy et al., 2009), and bay leaf, rosemary, sage and thyme (Tornuk et al., 2011). L-α-Terpineol occurred in small amounts of 0.87% in CFH was reported to be antiasthmatic (Duke, 1992). Citronellol was present only in ARH and was reported to be strong tyrosinase inhibitor (Lante and Tinello, 2015). Trace amount of cedrol (0.23%), which was only detected in AFH, could be used as pesticide and termiticide (Duke, 1992). Hydrocarbons representing 0.45–7.61% of total volatile compounds were identified in the corresponding hydrosols. Minor constituent of β-myrcene (0.18%) detected in ARH, was reported to have cancer-preventive activity (Duke, 1992). Among the esters, 1,1′-oxybis-octane was present in a relatively high amount of 17.10% for CRH. Esters such as methyl salicylate were antioxidant and antiseptic (Ulusoy et al., 2009). Aldehyde like nonanal was detected in a relatively high amount of 7.4% for CFH, while the percentage of ketones from volatile components of the corresponding hydrosols ranged from 2.5 to 6.7%. Moreover, acids such as octanoic acid, nonanoic acid and dodecanoic acid were detected in ARH, CFH and CFAH, but not in AFH, AFAH and CRH. However, Maciag and Kalemba (2015) reported that the presence of fatty acids was unprofitable because of irritating action. 2-Methoxy-4-vinylphenol, which was only detected in CFH and CRH, can induce cell cycle arrest by blocking the hyper-phosphorylation of retinoblastoma protein in benzo[a]pyrene-treated NIH3T3 cells (Jin and Hyung, 2010). Components of β-myrcene, benzyl alcohol, α-terpineol, L-α-terpineol, geraniol, cedrol, dodecanal, methyl salicylate, octanoic acid, nonanoic acid, dodecanoic acid might be responsible for the odour of the hydrosols, as these compounds were reported to be used in perfumery (Duke, 1992).

3.2. Antibacterial activities

Microbial control during processing and storage is a key factor that determines the quality and shelf life of cosmetic and pharmaceutical industry. Knowledge of natural-derived products for inhibiting potential sources and routes of contamination with bacteria can be useful in the development of effective control measures throughout the cosmetic and therapeutic system. Chorianopoulos et al. (2008) assessed the antimicrobial action of essential oil, decoction and hydrosol of *Satureja thymbra* and declared that use of natural antimicrobial agents could provide alternative or supplemented ways for the disinfection of microbial-contaminated industrial surfaces. It is necessary to evaluate the disinfection efficiency of hydrosols from different parts of arecanut and coconut.

Hydrosol samples and benzylpenicillin with a concentration of 5 ug/ml revealed significant differences ($P < 0.05$) in the inhibitory zones (inhibition >6 mm) against all bacteria, while the control had no inhibitory effect on any of the tested bacteria by paper disc diffusion method (Table 2). *S. aureus* ATCC 2392 produced significantly

Table 2
Antibacterial activity of hydrosols from different parts of arecanut and coconut against four bacteria.

Hydrosols	Diameter of the zones of inhibition in mm (5 mm disc)			
	E. coli ATCC 25922	*C. albicans* ATCC 10231	*E. coli* O157:H7 ATCC 33150	*S. aureus* ATCC 2392
Control	0	0	0	0
AFH	11.2 ± 0.4^{c}	12.1 ± 0.4^{b}	10.2 ± 0.4^{a}	12.7 ± 0.7^{b}
AFAH	12.6 ± 0.8^{b}	11.6 ± 0.4^{bc}	9.9 ± 0.6^{a}	12.9 ± 0.6^{b}
ARH	9.8 ± 0.8^{d}	10.3 ± 0.6^{d}	8.5 ± 0.7^{b}	11.7 ± 0.4^{cd}
CFH	13.8 ± 0.6^{a}	14.2 ± 0.6^{a}	6.8 ± 0.9^{c}	12.3 ± 0.2^{bc}
CFAH	11.7 ± 0.4^{bc}	14.0 ± 0.6^{a}	6.9 ± 0.7^{c}	11.3 ± 0.3^{d}
CRH	12.5 ± 0.4^{b}	12.5 ± 0.8^{b}	7.4 ± 0.3^{c}	7.2 ± 0.3^{e}
Benzylpenicillin	7.6 ± 0.2^{e}	10.8 ± 0.6^{cd}	9.4 ± 0.2^{ab}	32.9 ± 0.6^{a}

AFH, AFAH and ARH: hydrosols from flower, floral axis and root of arecanut, respectively; CFH, CFAH and CRH: hydrosols from flower, floral axis and root of coconut, respectively.
Mean values with different superscript along the columns were significantly different at $P < 0.05$. Each value is expressed as mean ± standard deviation ($n = 3$).

larger zone of inhibition for benzylpenicillin than all the hydrosols, while *E. coli* ATCC 25922 was slightly inhibited by benzylpenicillin at the concentration of 5 μg/ml. *E. coli* ATCC 25922 (13.8 mm inhibition zone) and *C. albicans* ATCC 10231(14.2 mm inhibition zone) were significantly prevented by CFH, while slightly inhibited for 9.8 and 10.3 mm inhibition zone by ARH, respectively. *S. aureus* ATCC 2392 was extremely inhibited by AFAH (12.9 mm inhibition zone), but slightly prevented by CRH (7.2 mm inhibition zone). All the hydrosol samples except CRH exhibited the lowest antibacterial performance against *E. coli* O157:H7 ATCC 33150. CFH showed the highest efficiency against *E. coli* ATCC 25922 and *C. albicans* ATCC 10231, whereas AFH and AFAH exhibited the highest activity against *E. coli* O157:H7 ATCC 33150 and *S. aureus* ATCC 2392.

The increasing of the hydrosol concentrations showed an increase in the growth of the different microorganisms using serial dilution method (Table 3). All hydrosol samples at concentrations of 10–75 ml/100 ml had bacteriostatic effects against all test bacteria during the 4 days. However, Sagdic (2003) recorded spice hydrosols at 50 and 75 ml/100 ml concentrations had a bactericidal effect and completely inhibited the test bacteria in serial dilution method. At concentration of 75 ml/100 ml, CFH exhibited a growth inhibition level of more than 90 against *E. coli* ATCC 25922 and *C. albicans* ATCC 10231 on the fourth day, while AFAH showed a value of more than 90 against *S. aureus* ATCC 2392. All the hydrosol samples revealed a growth inhibition level of less than 80 on *E. coli* O157:H7 ATCC 33150 even when the concentration was 75 ml/100 ml. AFH exhibited a higher growth inhibition level against *E. coli* O157:H7 ATCC 33150 compared with other hydrosol products.

It can be noticed that *E. coli* O157:H7 ATCC 33150 was more resistant than other bacteria against all the hydrosol treatments. The results indicated that arecanut and coconut hydrosols from the flowers, floral axes and roots possessed antibacterial activity against both gram-negative and gram-positive bacteria. However, all the hydrosol samples failed complete elimination of the test bacteria using paper disc diffusion method (Table 2) and serial dilution method (Table 3). Meanwhile, hydrosols from thyme, oregano, summer savory, black cumin, sage, rosemary, bay leaf, anise, cumin, *Satureja thymbra* were reported to have antibacterial activity (Sagdic, 2003; Sagdic and Ozcan, 2003; Sagdic et al., 2013; Tornuk et al., 2011; Chorianopoulos et al., 2008), whereas rose and lavender hydrosols were found to have no antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa,* and *staphylococcus aureus* by Moon et al. (2006) and Ulusoy et al. (2009). The extracts of coconut female flowers showed antibacterial activity against *Staphylococcos aureus*, and was found to be inactive against *Escherichia coli* and *Bacillus subtilis* (Raja and Poonkuil, 2015).

The high alcohol contents, along with some minor constituents and phenolic contents might have key roles for the antibacterial properties of the hydrosol samples. The antimicrobial properties of alcohols have been known for a long time. Duke (1992) had documented the antibacterial and antiseptic activity of alcohols like geraniol, α-terpineol and citronellol, and aldehydes like benzaldehyde. These components were detected in small amounts in the hydrosol samples (Table 1). Ulusoy et al. (2009) also concluded that the antibacterial activity of the rose essential oil, hydrosol and absolute were mainly due to the phenolic contents. Tornuk et al. (2011) hypothesized that antimicrobial activities of the hydrosols might be originated from the monoterpenic essential oil components and phenlic compounds due to a perturbation of the lipid fraction of bacterial plasma membranes, resulting in alterations of membrane permeability and in leakage of intracellular materials. However, further research is required to determine the mechanisms of antibacterial activity of hydrosols.

3.3. Antioxidant properties

Hydrosols from different parts of arecanut and coconut were evaluated for the antioxidant activities through total phenolic content, reducing power assay, DPPH assay and ABTS assay (Table 4). Significant differences ($P < 0.05$) were observed in the antioxidant activities of hydrosol samples obtained from different parts of arecanut and coconut.

Total phenolic contents of hydrosols from different parts of arecanut and coconut varied from 0.17 to 3.88 ug GAE/ml (Table 4), with reference to gallic acid standard curve ($y = 0.0048 + 0.0335x$, $r^2 = 0.9996$) in Fig. 1. The total phenolic content was 19.0% higher in CFH (3.88 μg GAE/ml) than AFH (3.26 ug GAE/ml). CFH had the highest total phenolic content among the samples. The total phenolic contents of AFAH and ARH were 2.79- and 3.71-fold greater than those of CFAH and CRH. In addition, 2-methoxy-4-vinylphenol was only detected in CFH and CRH according to the profile of volatile components, while 2,4-bis(1,1-dimethylethyl)-phenol was contained in all the hydrosols with concentration ranging from 0.90% to 8.58%. These phenols identified in the volatile fractions contributed to the total phenolic compounds content in the hydrosols. Total phenolic contents of 5.2 mg GAE/L for rose hydrosol (Seyhan et al., 2009), 54.5 and 49.0 mg GAE/g for aqueous extracts of arecanut of root and adventitious root, respectively (Hamsar et al., 2011), 406.43 ug GAE/ml for areca flower extracts using 100% distilled water under reflux for 5 h at 70 °C (Lin and Li, 2010) were reported previously. The phenolic compounds may contribute to antioxidant activity, because these compounds have direct antioxidant properties due to the presence of hydroxyl groups, which can act as H_2 donors (Dreosti, 2000).

In reducing power assay, higher absorbance values at 700 nm indicated higher reductive abilities and antioxidant activities. The reducing power capacity of all the hydrosol samples was found to be lower when compared with ascorbic acid at the concentration of 5 ug/ml. CFH revealed a highest reducing power value (0.122) than those of other hydrosol samples, which indicated that CFH repre-

Table 3
Growth inhibition levels of hydrosols from different parts of arecanut and coconut against four bacteria.

Hydrosols	ml/100 ml	*E. coli* ATCC 25922 (days)				*C. albicans* ATCC 10231 (days)				*E. coli* O157:H7 ATCC 33150 (days)				*S. aureus* ATCC 2392 (days)			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
AFH	10	28.6	42.6	42.3	45.8	10.1	24.3	23.9	28.9	20.3	30.8	36.8	43.7	37.8	37.1	43.4	46.1
	25	40.5	46.8	63.5	64.4	11.8	26.1	35.1	45.5	21.1	32.1	39.3	46.0	51.4	55.4	58.6	62.2
	50	59.5	66.0	67.3	74.6	30.2	45.1	45.9	47.9	37.5	48.5	54.9	61.4	62.2	70.7	73.7	75.1
	75	66.7	74.5	75.0	71.2	87.6	85.8	84.2	87.2	60.5	68.9	72.7	78.9	83.8	85.4	86.0	86.2
AFAH	10	38.1	40.4	46.2	54.2	19.5	15.9	18.5	39.7	16.2	18.2	18.6	19.6	16.2	19.3	27.1	37.0
	25	57.1	63.8	67.3	71.2	30.8	33.2	11.2	33.5	23.8	30.4	37.4	41.4	29.7	38.9	49.4	58.3
	50	66.7	72.3	75.0	79.7	53.3	68.1	64.5	64.9	48.6	44.3	48.0	50.8	37.8	45.7	61.7	69.1
	75	78.6	83.0	88.5	89.8	82.2	80.5	81.9	80.6	56.8	65.0	68.9	76.3	86.5	90.0	92.6	92.0
ARH	10	31.0	36.2	34.6	42.4	11.8	11.5	16.6	15.7	17.8	18.9	19.4	22.4	15.3	23.0	30.1	41.4
	25	45.2	51.1	42.3	47.5	14.2	26.5	22.4	21.9	24.3	28.9	34.6	36.7	18.4	28.5	34.0	43.4
	50	50.0	55.3	51.9	57.6	27.8	37.2	36.7	36.0	45.9	42.5	50.0	51.9	40.2	47.2	52.1	61.1
	75	61.9	63.8	65.4	67.8	72.8	68.6	68.7	69.4	64.9	66.4	67.7	71.1	68.6	73.4	77.3	81.4
CFH	10	33.3	46.8	59.6	66.1	18.9	24.8	28.2	31.0	25.3	32.8	37.7	42.0	27.6	28.9	38.3	37.6
	25	42.9	68.1	67.3	72.9	27.8	24.8	35.9	30.6	22.6	33.8	40.2	43.1	35.7	43.9	51.7	50.8
	50	64.3	76.6	71.2	81.4	38.5	64.6	68.0	62.4	38.3	47.2	53.4	58.3	45.9	57.9	59.1	63.5
	75	81.0	87.2	92.3	93.2	75.7	99.1	94.6	95.5	50.6	57.7	65.3	68.2	78.4	81.8	83.4	85.9
CFAH	10	28.6	29.8	32.7	64.4	20.7	20.4	25.1	28.9	18.4	30.2	36.2	41.7	20.7	30.2	37.1	44.6
	25	48.3	53.2	57.7	69.5	31.4	34.5	30.1	42.6	25.3	36.7	41.4	45.7	21.5	33.8	40.8	47.7
	50	54.8	63.8	59.6	80.5	42.6	48.7	75.3	73.1	33.0	43.0	49.7	55.7	40.6	52.1	55.5	60.9
	75	76.2	74.5	71.2	81.4	68.0	93.4	94.6	94.2	47.5	56.1	65.0	68.0	67.8	72.8	75.8	80.3
CRH	10	21.4	27.7	30.8	45.8	22.5	38.5	45.6	43.8	13.4	19.3	27.3	32.0	13.5	17.9	23.1	28.7
	25	28.6	48.9	51.9	61.0	29.6	48.7	51.7	52.5	25.3	33.8	41.4	48.3	16.2	26.8	38.6	46.1
	50	52.4	73.2	73.1	74.6	45.0	69.9	74.9	62.4	36.8	45.9	52.5	61.4	21.6	33.9	47.1	54.4
	75	69.0	80.9	84.6	86.4	76.3	88.5	85.3	86.4	52.5	60.3	68.4	71.0	51.4	63.9	66.9	70.0

AFH, AFAH and ARH: hydrosols from flower, floral axis and root of arecanut, respectively; CFH, CFAH and CRH: hydrosols from flower, floral axis and root of coconut, respectively.

Table 4
Total phenolics, reducing power assay, DPPH assay and ABTS assay of hydrosols from different parts of arecanut and coconut.

	Total phenolics (ug GAE/ml)	RP	DPPH (%)	ABTS (%)
AFH	3.26 ± 0.03^{b}	0.093 ± 0.004^{c}	81.83 ± 0.26^{a}	58.60 ± 6.02^{a}
AFAH	1.48 ± 0.02^{c}	0.051 ± 0.001^{d}	68.02 ± 0.78^{d}	24.28 ± 3.86^{b}
ARH	0.63 ± 0.03^{d}	0.042 ± 0.003^{e}	47.00 ± 0.69^{e}	13.80 ± 4.75^{c}
CFH	3.88 ± 0.05^{a}	0.122 ± 0.001^{b}	71.32 ± 0.69^{c}	63.57 ± 6.00^{a}
CFAH	0.53 ± 0.02^{e}	0.027 ± 0.005^{f}	36.79 ± 1.30^{f}	13.05 ± 3.28^{c}
CRH	0.17 ± 0.02^{f}	0.018 ± 0.002^{g}	25.98 ± 1.30^{g}	4.75 ± 2.16^{d}
Ascorbic acid	NA	0.185 ± 0.006^{a}	73.27 ± 0.01^{b}	60.78 ± 0.02^{a}

AFH, AFAH and ARH: hydrosols from flower, floral axis and root of arecanut, respectively; CFH, CFAH and CRH: hydrosols from flower, floral axis and root of coconut, respectively; NA: not applicable.
Mean values with different superscript along the columns were significantly different at $P < 0.05$. Each value is expressed as mean ± standard deviation ($n = 3$).

sented a best inhibition in the reduction of Fe^{3+} than the others. However, CRH displayed a lowest reducing power of 0.018. It has been reported that the reducing properties may be associated with the high amounts of polyphenolics (Lee et al., 2007) and reductones (Shimada et al., 1992). CFH may have the highest amounts of polyphenolics and reductones, which could exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Areca inflorescence extracts using boiled water exhibited higher reducing power value and phenolic content, followed by those using ambient water and ethanol (Chen et al., 2012).

All hydrosols exhibited DPPH inhibition activity ranging from 25.98% to 81.83%, relative to ascorbic acid with 73.27% inhibition. The poor radical scavenging avtivity by CRH in comparison to other tested hydrosols may be due to its low phenolic content supporting the earlier view that phenolic compounds play major roles in antioxidant activity. Scavenging effect on the DPPH radical decreased in the following order: AFH > Ascorbic acid > CFH > AFAH > ARH > CFAH > CRH. Hydrosols from flowers of arecanut and coconut exhibited the top two highest DPPH inhibition activities. The present study also suggested that hydrosol from areca flower revealed better DPPH scavenging activity than coconut flower. This might be due to the presence of strong biologically active components such as phenols and polyphenol, etc., which show remarkable activity against free radicals (Siddhuraju and Becker, 2001). The antioxidant activity of areca inflorescence extracts (Cheng et al., 2011), coconut inflorescence extracts (Renjith et al., 2013), and areca root extracts (Hamsar et al., 2011) has been defined using similar DPPH scavenging activity methods.

ABTS assay is based on the measurement of the ability of antioxidants towards scavenging the stable ABTS radical. AFH and CFH exhibited significantly higher ABTS scavenging activity than other hydrosol samples. CRH was observed to possess weak free radical scavenging activity against ABTS radical with a scavenging value of 4.75%. The ABTS scavenging activity was moderately inhibited by AFAH, ARH and CFAH ranged from 13.05 to 24.28%. CFH had a scavenging value of 63.57% against ABTS radical, which was significantly higher than ascorbic acid at the concentration of 5 μg/ml. Similar with the total phenolic content assay, the radical scavenging potentiality measured by ABTS assay presented the following order: CFH > Ascorbic acid > AFH > AFAH > ARH > CFAH > CRH. This result supported the report declared by Cao et al. (2015) that polyphenols appeared to fast react with ABTS radical.

Today, antioxidative properties of the plants have become a great interest due to possible uses as natural additives to replace synthetic ones. Both radicals ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) used in this study are nitrogen centered and scavenging of these radical demonstrates the ability to transfer one electron to each of these radicals (Panat et al., 2016). The results showed that all the hydrosols from

arecanut and coconut could effectively scavenge DPPH and ABTS radicals and exhibited potent antioxidant activity. It is interesting that CFH exhibited the highest total phenolic content, reducing power value and ABTS scavenging activity, while AFH showed the strongest DPPH scavenging activity. This difference performed might be attributed to the different mechanisms involved or some masked bioactive compounds. The main mechanism leading to the observed behavior should be the cross-reactions among the radical species generated by the reaction between the highly reactive peroxyl radicals and the antioxidants (Rossetto et al., 2002). Chandra and Uchimaru (2002) also indicated that the radical scavenging/antioxidant potentials measured by ABTS and FRAP assays do not correlate well because differences in reactive chemistry of the analyte may favour one or the other assay.

4. Conclusion

Hydrosols from different parts of arecanut and coconut revealed varying antibacterial and antioxidant activities, which could be attributed to the presence of hydrocarbons, alcohols, ketones, aldehydes, esters, acids and others. These chemical compounds of different classes were responsible for the distinctive aroma of the hydrosols. Among the hydrosol products, CFH was the most efficient as both antioxidant and antibacterial agent against *E. coli* ATCC 25922 and *C. albicans* ATCC 10231. CRH showed the lowest antioxidant properties and not enough antimicrobial performance to inhibit *E. coli* O157:H7 ATCC 33150 and *S. aureus* ATCC 2392. In view of the potential as inhibitory of bacterial growth and antioxidant, hydrosols from flowers of arecanut and coconut may be recommended for plant based preservatives for enhancement of food shelf life by inhibiting bacteria growth and controlling free radical scavenging and oxidation of unsaturated lipids. However, further research is needed to establish these hydrosol products as biofilm sanitizers in real complex food-processing ecosystems. Meanwhile, the safety profile of the hydrosols should be recorded on animal system, although arecanut and coconut from which the hydrosols have been isolated are well known health beneficial plants (Peng et al., 2015; Soumya et al., 2014). The importance of this work lies in the extension of knowledge on the antioxidant and antimicrobial action of arecanut and coconut hydrosols. The result opened the opportunity for the use of hydrosols from arecanut and coconut as raw antibacterial and antioxidant ingredient in medicine, therapeutic system and aromatherapy industries.

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