GSC Biological and Pharmaceutical Sciences, 2019, 09(02), 102–109

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Essential oil compositions and antioxidant properties of *Cycas revoluta* (Thunb.)

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Publication history: Received on 28 October 2019; revised on 13 November 2019; accepted on 14 November 2019

Article DOI: https://doi.org/10.30574/gscbps.2019.9.2.0203

Abstract

Cycas revoluta is an ornamental plant which has had some of its parts used in treating some forms of cancers, relieving headaches, giddiness and sore throat. Essential oils from both fresh and air-dried *C. revoluta* leaf and stalk samples were obtained by hydro-distillation using a Clevenger apparatus with the constituents identified and quantified by Gas Chromatography – Mass Spectrometry (GC-MS) analysis. The fresh leaf and stalk essential oils had 22 and 45 identified essential oil components respectively while the air-dried parts had 25 leaf essential oil and 18 stalk essential oil components. γ-ketovaleric acid (29.36 %), 2,4,4-Trimethyl-2-pentene (13.27 %), lauric acid (9.84 %) and 7-Nonenamide (58.71 %) constituted the major components of the fresh leaf, fresh stalk, dry leaf and dry stalk essential oils respectively. Free radical scavenging ability of the essential oils was assayed with 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method and the four essential oil samples recorded anti-oxidant activities which were concentration dependent with the dry leaf essential oil exhibiting the highest activity at 1 mg/mL with a 72 % inhibition. These values were comparable with the currently known antioxidant standards.

Keywords: Antioxidant assay; *Cycas revoluta*; DPPH; essential oils; GC-MS; Hydro-distillation

1. Introduction

Cycas revoluta (Thunb) is the only known genus of the family, Cycadaceae. It is a popular ornamental plant known as sago or long sago palm [1, 2]. The species could be found throughout the world although this cold hardy palm is native to the Far East. *Cycas revoluta* (Thunb) has become very popular for indoor decorations and has also been used for outside landscaping for centuries.

The seeds are used in China as an anti-rheumatic, expectorant and a tonic. The shoots are utilised as an astringent and diuretic [1, 2]. The young leaves are edible and the juice of tender leaves is valuable for the treatment of flatulence and vomiting [3]. It has also been used to relief headaches, giddiness and sore throat. *C. revoluta* appears to be the most studied followed by-*C. circinalis* due to their chemical constituents and biological activities. Both have been found to contain toxin, cycasin - an azoxy-glucoside that is carcinogenic [4]. Biflavonoids, lignans, flavan-3-ols, flavone-c-glucosides, nor-isoprenoids and flavanone have been isolated from the chloroform extract of *C. revoluta.* The bioflavonoids exhibited moderate activity against *Subtilis aureus* and methicillin-resistant *Subtilis aureus* [5]. The presence of saponins, tannins and sugars in the chloroform and hydro-alcoholic extracts of *C. revoluta* leaves has also been reported [6].

Estragole was reported to be the primary volatile compound in the male and female cones of *C. revoluta* [7]. Leaves of *C. revoluta* afforded lariciresinol, naringenin and flavonoids which are derivatives of amento-flavone and hinoki-flavone. The anti-microbial, anti-malarial and anti-leshmanial activities of these compounds have also been reported [8]. A tincture of *C. revoluta* leaves has been reported to contain inhibitors of cytochrome P-450 aromatase [9] and thus may

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be efficacious in treating oestrogen-dependent carcinoma. The essential oils from *C. revoluta* have not however been analysed or reported to the best of our knowledge. This paper focuses on identification of the chemical constituents and antioxidant properties of the essential oils from the fresh and dry leaves and stalk of *C. revoluta*.

2. Material and methods

2.1. Plant materials

Fresh leaves and stalks of *C. revoluta* samples were collected from the Botany Department environs of the University of Ibadan in August, 2018. A voucher specimen with voucher number FHI 6013157596 was deposited at the Herbarium of the Forest Research Institute of Nigeria (FRIN), Ibadan where the plant was identified and authenticated.

2.2. Isolation of essential oils

The fresh plant samples were immediately chopped while the air-dried samples were air-dried for 7 days and pulverised. Both the freshly chopped and air-dried samples were subjected to hydro-distillation for the extraction of essential oils. Essential oil from each plant part was separately isolated for three hours using an all-glass-clevenger-type apparatus according to the British Pharmacopoeia specifications [10]. The oil yields were calculated and thereafter, the oils were stored in vials in the refrigerator at about 4^{o}C prior analysis.

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Perkin Elmer Gas Chromatography (Clarus 580) equipped with MSD mass spectrometer (Clarus SQ8S) instrument with built-in auto sampler was used in identifying the chemical constituents of the volatile oils. Capillary column type was an Elite-5MS (30.0 m × 0.25 mm id× 0.25 µm). Gas Chromatographic (GC) operating conditions were as follows: a sample volume of 1.0 µL was injected in helium carrier gas at a split flow of 20 mL/min; column temperature was programmed from 37-320 ˚C at a rate of 18-25 ˚C/ min and held for 0.5 and 1.85 mins at 18 and 320 ˚C respectively. The injector temperature was 250 ˚C and MS Ion source temperature was 280 ˚C with full scan and solvent delay of 0-2.30 min. MS Scan range was m/z 35-500 in 0.10 sec.

Individual constituents of the oil were identified based on their retention indices and their mass spectral fragmentation patterns. The NIST 11.L database/ChemStation data system was used to acquire the mass spectral data and compared with published data [11]. The GC retention index data were also compared with literature values.

2.4. Antioxidant assay

The method of Bruits [12] was adopted and modified for the investigation of the DPPH scavenging activity of the essential oils. Five concentrations of the volatile oils (1-0.015) mg/mL were measured in test tubes; 0.1 mM methanol solution of DPPH (2 mL) was added to each test tube and vigorously shaken. The mixture was then incubated at room temperature for 30 minutes. The absorbance of the treated essential oil samples at different concentrations and the blank DPPH solution (control) were thereafter measured at 517 nm with the aid of a UV/Visible spectrometer (GS-UV12 spectrometer). Butylated hydroxyl anisole (BHA) and α- tocopherol which are established anti-oxidants were used as standards. The analyses were carried out in triplicates and the average values were obtained [13-16]. The activities of all the analysed samples were calculated as a function of their % inhibition using the equation:

$$\% \text{ Inhibition} \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100 \qquad [1]$$

The results were expressed as mean ± standard deviation of three parallel measurements. Microsoft Office excel, 2007 software was used to plot the graph.

3. Results and discussion

3.1. Properties and composition of *C.revoluta* essential oils

Fresh leaf, fresh stalk and dry leaf essential oils of *C. revoluta* gave leafy odours while the dry stalk essential oil had a woody scent. The fresh leaf essential oil was pale white in colour; the fresh stalk was pale green in colour while the essential oils from the dry samples were colourless. Dry *C. revoluta* leaf-stalk essential oil had the highest yield (0.57 %w/w) while the fresh leaf, fresh leaf-stalk and dry leaf essential oils yielded 0.20, 0.42 and 0.50 %(w/w) respectively.

Individual constituents of the oils were identified by GC-MS based on the comparison of their mass spectral fragmentation patterns using the NIST 11.L database/ChemStation data system and comparison of their retention indices by with published spectra. Twenty two and forty five components were identified in the fresh leaf and stalk essential oils while twenty five and eighteen components were identified in the air-dried leaf and stalk essential oil samples by GC/MS analysis as displayed in Tables 1.

Table 1 Compounds obtained from GC-MS analysis of fresh *C. revoluta* leaf and leaf- stalk essential oils

Peak No	Retention Index	Identified Compounds	Composition (%)			
			fCrL	dCrL	fCrS	dCrS
1	690	2-Methyl-3-pentanone	1.44	3.09	-	0.34
2	717	2,4,4-Trimethyl-2-pentene	4.32	-	13.27	-
3	742	3-Methylhept-1-ene	-	-	2.81	-
4	780	2-Hexanol	2.62	-	-	-
5	788	2-Methyl-3-ethylhexane	-	-	0.98	-
6	794	Toluene	4.32	-	5.95	-
7	816	(2E)-3,4,4-Trimethyl-2-hexene	-	-	0.43	-
8	824	Dimethylfulvene	-	5.31	-	-
9	842	1,trans-2-Dimethylcyclohexane	-	-	7.37	-
10	851	3,4-Dimethyl-2-hexanol	-	-	0.43	-
11	868	3,3-Dimethyl-2-hexanone	-	-	2.18	-
12	887	2,7-Dimethyloctane	-	1.89	1.23	-
13	907	m-Xylene	-	3.22	3.00	-
14	912	Butenediol	1.81	-	-	-
15	914	3-Hexyl hydroperoxide	3.42	-	-	-
16	935	1-Chloro-3-methyl-3-pentanol	1.70	-	-	-
17	953	(1E)-1-Chloro-4,4-dimethyl-1-penten-3-one	-	4.92	-	-
18	969	cis-3-Methylcyclohexanol	-	4.49	-	-
19	1006	m-Methylethylbenzene	-	-	1.39	-
20	1011	gamma.-Ketovaleric acid	29.36	-	1.87	-
21	1044	2-hydroxy-2-methyl-4-heptanone	1.45	-	-	-
22	1066	2-Isopropyl-5-methyl-1-hexanol	-	2.56	1.05	-
23	1086	2,9-Dimethyldecane	-	8.77	-	2.14
24	1115	5-Octanol-4-one	1.75	-	-	-
25	1150	4-Hydroxy-3-propyl-2-hexanone	2.96	-	-	-
26	1164	(1S,2R,5R)-(+)-Isomenthol	-	-	3.30	-
27	1185	3,8-Dimethylundecane	-	1.60	-	-
28	1190	Phenethyl alcohol	6.02	-	-	-
29	1249	5-Propyldecane	-	1.42	-	-
30	1320	2,6,11-Trimethyldodecane	4.80	-	-	1.29
31	1322	5-Bromo-2-adamantanone	-	-	0.72	-
32	1333	7-Nonenamide	-	-	-	58.71
33	1365	1-Iodo-2-methylnonane	-	-	1.61	-
34	1389	3-Cyclohexylpropanamide	-	-	-	0.54
35	1402	Lauraldehyde	-	-	1.10	-

36	1471	Allyl caprate	1.59	5.02	-	-
37	1483	2-Methyl-6-propyldodecane	-	-	-	0.99
38	1484	4-epi-cubedol	-	2.52	-	-
39	1490	1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)- Azulene	-	-	1.21	-
40	1519	2,6,10-Trimethyltetradecane	-	3.35	-	-
41	1530	Viridiflorol	-	-	0.79	-
42	1555	3,5-Di-tert-butylphenol	-	-	0.41	-
43	1564	1-Iodo-2-methylundecane	-	1.60	-	-
44	1574	beta.-Humulene	-	-	8.64	2.42
45	1580	alpha.-Cadinol	-	2.80	-	2.00
46	1575	1,2-Dicyclohexylpropane	-	-	0.68	-
47	1612	n-Cetane	-	-	0.49	-
48	1616	Tumerone	-	-	1.25	-
49	1668	4,6-di-tert-Butyl-m-cresol	-	-	-	0.90
50	1701	n-Pentadecanal	-	-	1.94	
51	1753	Crocetane	3.36	-	2.61	0.65
52	1754	Hexahydrofarnesyl acetone	4.58	-	1.52	-
53	1770	2-Ethyl-2-methyl-1-tridecanol	-	-	0.46	0.27
54	1771	(6Z,9Z)-6,9-Pentadecadien-1-ol	-	-	1.93	-
55	1808	Z-9-Hexadecenal	2.29	-	2.35	-
56	1822	Myristic amide	-	-	-	14.12
57	1843	(8Z)-14-Methyl-8-hexadecenal	-	-	5.53	-
58	1846	2-Methyloctadecane	-	-	0.95	-
59	1852	2,6,10,14-Tetramethylheptadecane	2.73	-	-	0.30
60	1680	Methyl myristate	-	-	1.29	-
61	1924	Palmitic acid chloride	-	-	0.40	-
62	1930	Myristyl monoethoxylate	-	4.01	-	-
63	1945	9-Methylnonadecane	-	-	2.23	-
64	1976	(2E)-2-Hexadecenoic acid	-	2.20	-	-
65	1978	[3R-(3.alpha.,4a.beta.,6a.alpha.,10a.beta.,10b.alpha.)]-3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-1H-Naphtho[2,1-b]pyran	-	-	0.46	-
66	2009	Eicosane	-	-	4.33	-
67	2021	Palmitamide	-	-	-	4.38
68	2026	Hexadecyl iodide	-	-	1.42	-
69	2045	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	6.28	1.92	1.26	2.09

70	2054	Lauric acid		9.84	-	-
71	2109	Heneicosane	-	-	1.28	-
72	2119	Isobutyl .alpha.-methylacrylate	-	3.93	-	-
73	2127	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	2.24	-	-	-
74	2144	2.alpha.,3.alpha.-epithio-17.alpha.-methyl-5.alpha.-androstan-17.beta.-ol	2.86	-	-	-
75	2190	Verticiol	7.56	-	-	-
76	2192	trans-Geranylgeraniol	-	-	0.44	-
77	2211	Thunbergol	-	-	0.43	-
78	2225	1-Iodooctadecane	-	2.80	-	-
79	2243	2-Methyldocosane	-	3.06	-	-
80	2422	4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate	-	4.06	-	-
81	2542	11-Butyldocosane	-	-	-	0.48
82	2561	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	-	-	-	2.75
83	2641	2-methylhexacosane	-	-	-	0.61
84	2714	4,4-Dimethylcholest-7-en-3-one	-	-	0.78	-
85	2840	2-methyloctacosane	-	-	1.35	-
86	2944	Andrographolid	-	3.83	-	-
87	3493	3,5,6,12-Tetrahydroxyergostan-25-yl acetate	-	-	0.78	-
88	4339	1-Hentetracontanol	-	2.31	-	-
89	Unidentified		4.87	-	0.53	2.52
	Total[7]		**99.53**	**90.52**	**93.82**	**97.5**

Peak number: as eluted on GC column with Elite-5MS (30 m x 0.25 mm id x 0.25 µm). Programmed temperature from 37 to 320 °C at 18- 25 °C/min in a split mode with helium stationary phase

fCrL: fresh *Cycas revoluta* leaves; dCrL: dry *Cycas revoluta* leaves; fCrS: fresh *Cycas revoluta* stalk; dCrS: dry *Cycas revoluta* stalk

The fresh leaf, fresh stalk, dry leaf and dry stalk volatile oils were observed to consist mainly of hydrocarbons (62.43, 54.96, 78.81 and 70.85 % respectively). Of the classes of terpenes possible, monoterpenes, diterpenes, sesquiterpenes, triterpenes and aprocarotenoids were observed. Monoterpenes were present in abundance of 6.02, 2.84 and 2.56 % in the fresh leaf, fresh stalk and dry leaf essential oils respectively while no monoterpene was identified in the dry leaf essential oil. For the fresh leaf, fresh stalk, dry leaf and dry stalk essential oils, an abundance of 4.80, 23.54, 10.64 and 6.61 % were identified as sesquiterpenes while abundance of 22.79, 11.39, 3.83 and 14.77 % were identified as diterpenes respectively. Of all the essential oils, only the fresh *Cycas revoluta* leaf essential oil had triterpenes (2.13 %) present and dry *Cycas revoluta* stalk essential oil had an apocarotenoid (2.75 %) present.

ϒ-Ketovaleric acid (29.36 %), 2, 4, 4- Trimethyl-2-pentene (13.27 %), 2.9- Dimethylundecane (8.77 %) and 7-Nonenamide (58.71 %) which are all hydrocarbons were respectively identified as the most abundant components in the fresh leaf, fresh stalk, dry leaf and dry stalk essential oils. Verticiol, a diterpene was the most abundant terpene (7.56 %) in the fresh leaf essential oil; beta-Humulene, a sesquiterpene was the most abundant terpene (8.64 %) in the fresh leaf essential oil while Andrographolid (3.83 %) and Capramide (14.12 %) which are diterpenes were the most abundant terpenes in the dry leaf and dry stalk essential oils respectively.

3.2. Antioxidant activity

1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used in measuring the antioxidant activity of the essential oil samples. Free radical scavenging activities of the samples are as shown in Table 3 while the graphical representation of the activity is depicted in Figure 1.

Table 2 Absorbance values for DPPH free radical scavenging activity of essential oils of the fresh leaf, fresh leaf-stalk, dry leaf, and dry leaf-stalk of *Cycas revolute*

Concentration (mg/mL)	Fresh leaf	Dry leaf	Fresh Leaf-stalk	Dry Leaf-stalk	BHA	α – Tocopherol
1.0	0.633± 7.07e-4	0.322 ± 0	0.681 ± 0	0.652 ± 0	0.066 ± 1 × 10^{-3}	0.640 ± 1 × 10^{-3}
0.5	0.670± 7.07e^{-4}	0.405 ± 0	0.685± 7.07e^{-4}	0.660 ± 7.07e^{-4}	0.074 ± 1 × 10^{-3}	0.657 ± 1 × 10^{-3}
0.25	0.682±0	0.441 ± 1.414e^{-3}	0.691±7.07e^{-4}	0.695 ± 7.07e^{-4}	0.063 ± 1.1 × 10^{-3}	0.663 ± 2 × 10^{-3}
0.125	0.691± 7.07e^{-4}	0.449 ± 7.07e^{-4}	0.698±7.07e^{-4}	0.671 ± 0	0.078 ± 1.1 × 10^{-3}	0.672 ± 2 × 10^{-3}
0.0625	0.704± 7.07e^{-4}	0.504±7.07e^{-4}	0.717±7.07e^{-4}	0.719 ± 0	0.072 ± 1 × 10^{-3}	0.681 ± 2 × 10^{-3}
0.03125	0.716± 7.07e^{-4}	0.495±1.414e^{-3}	0.807±7.07e^{-4}	0.721 ± 1.414e^{-3}	0.082 ± 1 × 10^{-3}	0.692 ± 2 × 10^{-3}
0.015625	0.731± 7.07e^{-4}	0.518±7.07e^{-4}	0.928±7.07e^{-4}	0.568 ± 7.07e^{-4}	0.078 ± 1 × 10^{-3}	0.700 ± 2 × 10^{-3}

BHA: Butylated Hydroxyl anisole

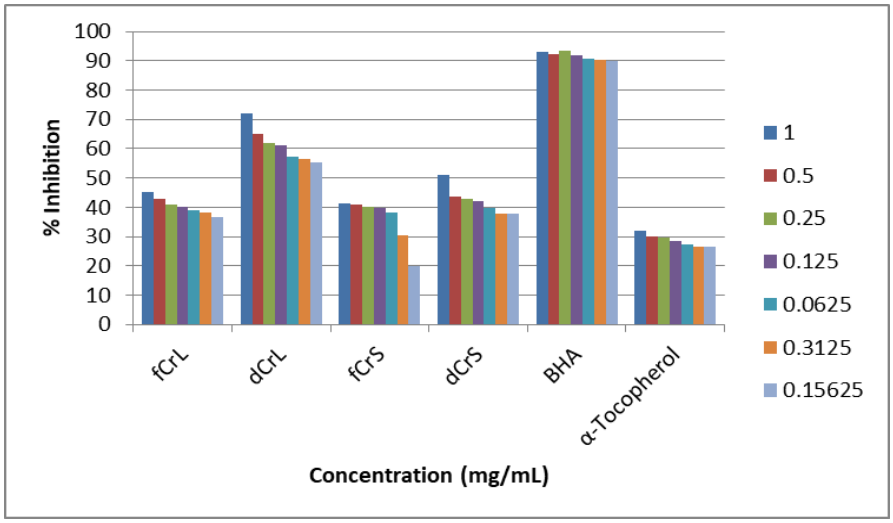

Figure 1 DPPH radical scavenging activity of essential oils and standards at 1.0, 0.5, 0.25, 0.125 mg/mL.
α-tocopherol and BHA (Butylated Hydroxyl Anisole) are the standards; fCrL: fresh *Cycas revoluta* leaves; dCrL: dry *Cycas revoluta* leaves; fCrS: fresh *Cycas revoluta* stalk; dCrS: dry *Cycas revoluta* stalk

Potential antioxidant activities were demonstrated by the essential oils at all concentrations. The potency of these volatile oils was generally concentration dependent with their potency generally reducing as the concentration was reduced including for BHA and α-tocopherol. Dry *C. revoluta* leaf-essential oil had the highest activity at all concentrations used when compared with essential oil samples from other parts with percentage inhibition (% I) values ranging from 72.0 - 55.22 % at 1.0 - 0.015625 mg/mL. The DPPH radical scavenging activity at 0.5 mg/mL showed the fresh *C. revoluta* stalk-essential oil to be the least active (40.88 %). Dry *C. revoluta* leaf-essential oil (61.88 %) and dry *C. revoluta*-stalk-essential oil (42.95 %) were the most active at 0.25 mg/mL with fresh *C. revoluta* leaf essential oil (41.05 %) with fresh *Cycas revoluta* stalk essential oil (40.27 %) being the least active at the same concentration. A similar progression as stated above was observed at concentrations 0.125, 0.0625, 0.03125 and 0.015625mg/mL where the dry *C. revoluta* leaf essential oil had the highest inhibition followed by the dry *C. revoluta* stalk essential oil, the fresh *C. revoluta* leaf essential oil and then the fresh *C. revoluta* stalk essential oil. All the afore-mentioned essential oils had activities higher than α-tocopherol except for fresh *Cycas revoluta* stalk essential oil at 0.15625 mg/mL with an inhibition of 19.77 % but lower activities than butylated hydroxylanisole (BHA) at all concentrations.

4. Conclusion

The essential oils from each plant part differ qualitatively and quantitatively as portrayed by Gas Chromatography-Mass Spectrometer (GC-MS). A total of 22, 45, 25 and 18 components were identified in the fresh leaf, fresh stalk, dry leaf and dry stalk essential oils respectively. 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol was present in fresh leaf, fresh stalk, dry leaf and dry stalk essential oils at concentrations of 6.28, 1.92, 1.26 and 2.09 % respectively. The identified components of the volatile oils could help explain their antioxidant properties. The four volatile oils exhibited good free radical scavenging properties with dry *C. revoluta* leaf-essential oil being the most promising free radical scavenger and at 1 mg/mL with an inhibition of 72.0 %. The antioxidant activities could be a result of synergetic effect of all the identified components. These active samples on subjecting to further tests and analyses could be found to be better and safer agents compared with the synthetic standards currently in use.

Compliance with ethical standards

Acknowledgments

The authors profoundly thank Professor Olusegun O. Ekundayo for supervising this research work as part of the PhD research work of the corresponding author.

The authors thank Dr G.K. Oloyede and Miss Ufuoma Oghenejoboh of Chemistry Department, Faculty of Science, University of Ibadan, for their assistance with the requisite apparatus and antioxidant assay required for this research.

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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How to cite this article

Anifalaje EO and Odeja OO. (2019). Essential oil compositions and antioxidant properties of *Cycas revoluta* (Thunb.). GSC Biological and Pharmaceutical Sciences, 9(2), 102-109.