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Research Article

Coconut Waste as a Potential Source for Cytotoxic and Antioxidant Compounds

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ABSTRACT

Despite the reported cytotoxic activity of the endocarp of *Cocos nucifera* (Arecaceae), the active principles were never characterized. The total extract (**T**), methylene chloride (**M**) and ethyl acetate (**E**) fractions were investigated for selective cytotoxic activity towards prostate cancer cell line (PC3) compared to normal cells (WI38), T showed remarkable activity (IC_{50}=10.89 ± 2.1 µg/ml), M and E showed comparable moderate activity. All showed significantly higher IC_{50} values towards WI38 with selectivity index (SI) values of >18.3, >11.8 and 6.6, respectively. The phytochemical investigation of **M** has led to isolation of coniferaldehyde (**1**), sinapaldehyde (**2**), *p*-hydroxy benzoic acid (**3**), protocatechuic acid (**4**), vanillic acid (**5**), protocatechuic aldehyde (**6**), balanophonin (**7**), guaiacylglycerol-β-coniferyl aldehyde ether (**8**), *E*-piceatannol (**9**), kompasinol A (**10**) and apigenin (**11**). The cytotoxic and antioxidant potential of the isolates was investigated. Compounds **1**, **4**, **7**-**9** and **11** showed selective cytotoxic activity against PC3. Compounds **6**, **9** and **10** showed remarkable antioxidant activity. . These results added a medicinal value for the coconut endocarp, instead of being as a wasted resource; it could be renewable inexpensive source of potential leads for selective cytotoxic drugs.

Keywords: antioxidant, *Cocos nucifera*, lignan, selective cytotoxicity, stilbene, waste.

INTRODUCTION

Cocos nucifera L. Var. typica (Tall) (Arecaceae) commonly known as coconut, is an important fruit crop in the tropical countries with a total production area of approximately 11.16 Mha. The plant is extensively cultivated for food industry and green coconut water consumption which generates a huge bulk of waste[1]. The discarded parts are the outer epicarp (husk), the mesocarp (husk fibres), and the inner endocarp[2]. Exploring coconut waste as an abundant, renewable inexpensive resource for production of potential active compounds has an economic and environmental appeal. The plant is widely used in food industry, the discarded parts generates tons of waste. For instance in Malaysia, estimated 3950 metric tons are generated form coconut industries[3]. In other terms, the continuous depletion of medicinal plants compels researchers to explore new resources for bioactive leads for drug discovery.

The different parts of coconut fruit are reported to have many bioactivities, including antimicrobial, antineoplastic, analgesic, antinflammtory, antimalarial, antiviral, and antileishmanial activities[1]. For the endocarp, vasorelaxant, antihypertensive[4], antibiofilm[5], cytotoxic, thrombolytic, antioxidant and antimicrobial activities are reported[6].

Despite this multitude of bioactivities, the studies have focused on the effects of the different parts of *C. nucifera* but without demonstrating the specific compounds responsible for these effects. Few phytochemical investigations addressed some polyphenols from the green husk[7] and a lignin from the husk fibres[8] however, no previous studies concerning the chemistry of the endocarp, even though it is reported to be the richest part regarding the phenolic and the flavonoid content[9]. Thus, the objective of this study is to isolate and characterize the active principles of the endocarp.

Prostate cancer is the second most frequently diagnosed cancer in men and the fifth leading cause of cancer death worldwide[10]. Although being effective, the current chemotherapeutic drugs are associated with multiorgan toxicities, debilitating effects in cancer patients, secondary malignancies and they even impact the life of survivors for years after the treatment. This necessitates the development of new pharmaceuticals with scientifically proven selective cytotoxic activity, being harvested from nature affords the potential for chemical diversity, biological selectivity and favourable properties.

MATERIAL AND METHODS

General

Thin-layer chromatography was performed on silica gel F254 plates (Merck, Germany) using vanillin–sulfuric acid spray reagent. The solvents used were of reagent grade (El-Nasr Co., Abu Zaabal – Kalyoubia, Cairo, Egypt). For column chromatography, Silica gel G60-230 (Merck, Germany), Sephadex LH-20 (Sigma-Aldrich, Missouri, USA) and reversed phase silica gel (Rp-C18, Bakerbond octadecyl C18, 40 µm) (Phillipsburg, NJ, USA).

NMR Spectra

Jeol 500 MHz ™ spectrometer, δ in ppm relative to Me_4Si as internal standard. For cytotoxicity assay, human cancer

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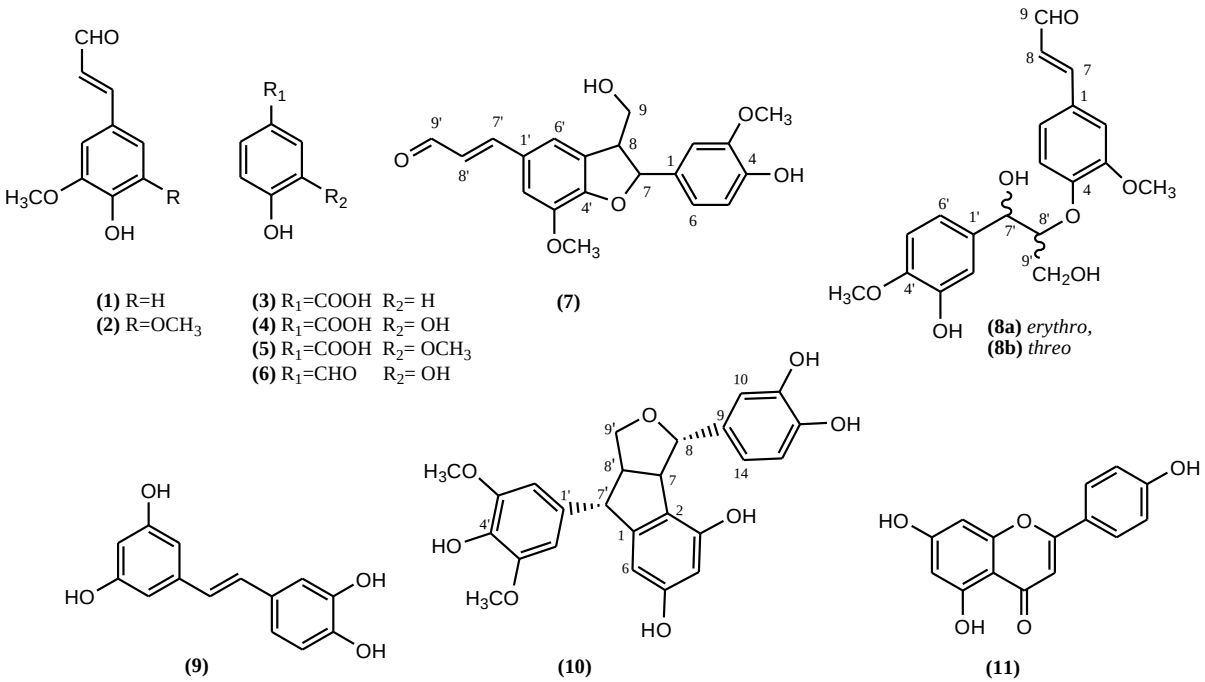

Figure 1: Structures of compounds 1-11.

Table 1: Cytotoxic activity of extracts and compounds of *Cocos nucifera*..

Treatment	IC_{50} [µg/ml for extract], [µM for compounds]		Selectivity Index
	PC3	WI38	
T	10.89 ± 2.1	>200	> 18.3
M	16.86 ± 2.04	>200	> 11.8
E	21.05 ± 2.12	140.83 ± 7.8	6.6
1	23.54± 1.78	>200	> 8.4
2	68.12 ± 2.36	150.66 ± 9.86	2.2
3	>200	>200	in[b]
4	17.09 ± 2.48	>200	> 11.7
5	166.06 ± 9.01	>200	in[b]
6	114.56 ± 6.25	>200	in[b]
7	14.98 ± 1.23	172.13 ± 10.89	11.4
8	10.42 ± 1.94	129. 16 ± 2.14	12.3
9	29.39 ± 1.64	183.7 ± 5.63	6.3
10	>200	>200	in[b]
11	32.88 ± 0.84	140.53 ± 8.18	4.2
Doxorubicin	0.23 ± 0.04	1.69 ± 0.17	7.3
5-FU	14.42 ± 1.42	67.97 ± 4.13	4.7

[a] The selectivity index is the ratio of the IC_{50} values of the treatments on WI38 cells to those on PC3 cell lines. Results are expressed as Mean ± SD of three replicates. [b]Compounds considered inactive.

cell line from prostate (PC3), normal lung fibroblast (WI38) originated from the American Type Culture collections (ATCC) (Manassas, USA) and were obtained from VACSERA (Cairo, Egypt), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), RPMI-1640 nutrient medium, fetal bovine serum (FBS) and 5-fluorouracil were obtained from Sigma-Aldrich (Missouri, USA), Adricin® (Doxorubcin HCl) from EIMC United Pharmaceuticals (Cairo, Egypt). For ABTS assay, Azino-bis-(3-ethyl benzthiazoline-6-sulfonic acid) (1 mg/ml) was purchased from Fluka (Buchs, Switzerland); Manganese dioxide (25 mg/ml)from Sigma–Aldrich (Steinheim, Germany), Ascorbic acid from Memphis Pharmaceutical Co (Cairo, Egypt) - Phosphate buffer (pH 7, 0.1 M) from BDH Chemicals (London, UK), Elisa microplate reader (Bio-Tek, Winooski, VT, USA).

Plant Material

The plant material consists of the sanded endocarp of *Cocos nucifera* L.Var. typica (Tall). It was purchased from PT. Murvien Global, Jakarta, Indonesia. It was collected from North Sulawesi, North Minahasa in July 2015. Taxonomy was managed by by Indonesian Palmae Crops

Table 2: $ABTS^{\bullet+}$ scavenging activity of isolated compounds.

Compound	% $ABTS^{+\bullet}$ scavenging at 100 μM	IC_{50} [μM]
1	27.21 ± 1.09	>100 μM
2	30.72 ± 0.93	>100 μM
3	37.5 ± 0.24	>100 μM
4	30.8 ± 1.33	>100 μM
5	9.93 ± 0.79	>100 μM
6	56.5 ± 1.17	88.92 ± 3.82
7	46.33 ± 0.15	>100 μM
8	18.01 ± 1.68	>100 μM
9	65.44 ± 1.4	47.7 ± 1.37
10	69.56 ± 1.29	55.55 ± 0.65
11	28.66 ± 0.92	>100 μM
Ascorbic acid	60 ± 0.73	82.06 ± 1.55

[a] Results are expressed as Mean ± SD of three replicates

Research Institute. It was authenticated based on its characters by Prof. Ibrahim Mashaly, at Ecology and Botany Department, Faculty of Science, Mansoura University. An authentic specimen was deposited at Pharmacognosy Department, Faculty of Pharmacy, Mansoura University.

Extraction and Isolation

Powdered plant material (8.5 kg) was exhausted by methanol (7 x 10 L). The extract was evaporated under reduced pressure to yield 419 g. It was then partitioned successively with pet. ether, CH_2Cl_2, EtOAC and n-BuOH. The detailed procedure for isolation of the compounds (1-11) from the CH_2Cl_2 extract is found in supplementary material (Figure 1).

Cytotoxicity and antioxidant assays

The cell viability and the antioxidant activities were measured using MTT assay[11] and ABTS assay[12], respectively. Triplicate repeats were performed; the data was represented as (Mean ± S.D.)

RESULTS AND DISCUSSION

Structure elucidation

Eleven known compounds were isolated from **M** (Figure 1)**.** Their structures were elucidated by comparing their spectral data with those reported in the literature as coniferaldehyde (**1**)[13], sinapaldehyde (**2**)[14], *p*-hydroxy benzoic acid (**3**), protocatechuic acid (**4**), vanillic acid (**5**)[15], protocatechuic aldehyde (**6**)[16], balanophonin (**7**)[17], diastereomeric mixture of (+)-erythro- and (-)-threo-guaiacylglycerol-β-coniferyl aldehyde ether (ratio, 3:2) (**8**)[18], *E*-piceatannol (**9**)[19], kompasinol A (**10**)[20], apigenin (**11**)[21] (Figures S2–S18 in Supplementary Material). To the best of our knowledge, these compounds are isolated for the first time from *C. nucifera*.

Cytotoxic activity

The cytotoxic activity of **T**, **M** and **E** against PC3 and WI38 was investigated (Table 1). **T** showed moderate cytotoxic activity against PC3 with IC_{50} value of 10.98 ± 2.1 μg/ml. Both **M** and **E** showed comparable moderate cytotoxic activity, IC_{50} values of 16.86 ± 2.04 and 21.05 ± 2.12 μg/ml, respectively. Their IC_{50} values were significantly lower than those obtained for WI38, >200, >200 and 140.83 ± 7.8 μg/ml, respectively. Their SI values were >18.3, >11.8 and 6.6, respectively. According to American National Center Institute, extracts with IC_{50} values lower than 30 μg/ml against experimental tumor cell lines are promising anticancer agents for drug development and SI greater than or equal to 2 are promising[22]. In this sense, **T**, **M** and **E** of *C. nucifera* are considered very interesting source for potential selective cytotoxic leads with high safety margin.

Since the SI value of **M** was approximately two folding of that of **E**, SI>11.8 and SI=6.6, respectively, this prompted us to further investigate the cytotoxic constituents of **M**. The lignans **8** and **7** showed the highest cytotoxic activity against PC3; IC_{50} values of 10.42 ± 1.94 and 14.98 ± 1.23 μM, respectively. The cytostatic and apoptotic properties of lignans are previously reported, also their ability to reduce the risk of estrogen dependent cancers including prostate cancer is described[23,24]. Compound **4** was next in activity, IC_{50} value of 17.09 ± 2.48 μM. A previous investigation for this compound reported its apoptotic effects against prostate cancer[25]. The phenolic acid **1**, stilbene **9** and the flavonoid **11** were less active, IC_{50} values of 23.54 ± 1.78, 29.39 ± 1.64 and 32.88 ± 0.84 μM, respectively. These findings are in accordance with those previously reported for piceatannol[26] and apigenin[27]. All these compounds showed significantly higher IC_{50} values towards WI38, SI values of 12.3, 11.4, >11.7, > 8.4, 6.3 and 4.2, respectively. This indicates that these compounds possess cytotoxic abilities with being more than four times, at least, cytotoxic towards prostate cancer cell line than to normal cells. The SI values of compounds, **1**, **4**, **7** and **8** were higher than that of the chemotherapeutic agents used as positive control. These results added a medicinal value for the coconut endocarp, instead of being as a wasted resource; it could be renewable inexpensive source of potential leads for selective cytotoxic drugs.

Antioxidant activity

The antioxidant activity of the compounds was also investigated (Table 2). As compared to Ascorbic acid, compound **6** showed nearly comparable $ABTS^{\bullet+}$ radical scavenging activity; IC_{50} values of 82.06 ± 1.55 and 88.92 ± 3.82 μM, respectively; meanwhile compounds **9** and **10** showed higher activity; IC_{50} values of 55.55 ± 0.65 and 47.7 ± 1.37, respectively. Phenolics are able to act as antioxidants by several mechanisms including donating their hydrogen atom to break the cycle of free radical regeneration[28]. The enhanced activity of compounds 6, 9, 10 could be related to their catechol structure, which confers high stability to the produced phenoxyl radicals via hydrogen bonding or by expanded electron delocalization[29]. Among the cytotoxic compounds, only compound **9** showed promising antioxidant potential. As reported, the cytotoxic activity of phenolic derivatives is directly related as much to their antioxidant potential as to their lipophilicity which favors their better incorporation into cells[30].

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