World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Cytotoxic, thrombolytic, antioxidant and antimicrobial activities of *Cocos nucifera* linn. endocarp extracts

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Received: 04-01-2015 / Revised: 11-05-2015 / Accepted: 20-05-2015

ABSTRACT

Different partitionates of methanol extract of *Cocos nucifera* Linn. were subjected to screening for cytotoxic, thrombolytic, antioxidant and antimicrobial activities. Cytotoxicity was determined using brine shrimp nauplii in which vincristine sulfate was used as positive control and gave LC_{50} of 0.45±0.08 µg/ml. Among the partitionates, carbon tetrachloride soluble fraction demonstrated the highest cytotoxic activity (LC_{50} value of 0.82±0.19 µg/ml). While assaying for thrombolytic activity, the petroleum ether soluble fraction demonstrated highest thrombolytic activity (37.44±0.33%) compared to the standard streptokinase (66.76±0.03%). In total phenolic content assay, the highest amount of phenolic compounds was found in the crude methanol extract (113.94±2.01 mg of GAE/g of sample). In antioxidant assay, crude methanol extract (IC_{50} value of 4.39±0.69 µg/ml) showed maximum free radical scavenging activity whereas reference standards tert-butyl-1-hydroxytoluene and ascorbic acid gave IC_{50} values of 27.50±0.95 µg/ml and 5.80±1.03 µg/ml, respectively. In antimicrobial screening, the crude methanol extract and its carbon tetrachloride and chloroform soluble fractions exhibited mild zone of inhibition against the test organisms.

Keywords: *Cocos nucifera* Linn., cytotoxic, vincristine sulfate, thrombolytic, streptokinase, antioxidant, ascorbic acid, antimicrobial

INTRODUCTION

The coconut palm (also, cocoanut), *Cocos nucifera* Linn., is a member of the family Arecaceae (palm family) [1]. It is widely distributed in differents parts of the world especially in the regions of India, Srilanka, Bangladesh, Malaysia, Indonesia, Maldives, Middle East, United States and Australia [2]. The term coconut can refer to the entire coconut palm, the seed, or the fruit, which, botanically, is a drupe, not a nut. *C. nucifera* is a large palm, growing up to 30 m (98 ft) tall, with pinnate leaves, 4–6 m (13–20 ft) long, and pinnae 60–90 cm long; old leaves break away cleanly, leaving the trunk smooth. Coconuts are generally classified into two general types: tall and dwarf. Tall selections may attain a height of 24-30 m; dwarf selections also exist. Fruit roughly ovoid, up to 5 cm long and 3 cm wide, composed of a thick, fibrous husk surrounding a somewhat spherical nut with a hard, brittle, hairy shell. The nut is 2-2.5 cm in diameter and 3-4 cm long. Inside the shell is a thin, white, fleshy layer known as the 'meat'. The interior of the nut is hollow but partially filled with a watery liquid called 'coconut milk'. The meat is soft and jellylike when immature but becomes firm with maturity. Coconut milk is abundant in unripe fruit but is gradually absorbed as ripening proceeds. The fruits are green at first, turning brownish as they mature; yellow varieties go from yellow to brown.

Coconut is a very versatile and indispensable fruit for most people under the tropical belt. It is a complete food is rich in calories, vitamins, and minerals. Coconuts may help benign prostatic hyperplasia [3]. In rats, virgin coconut oil reduced total cholesterol, triglycerides, phospholipids, LDL, and VLDL cholesterol levels and increased HDL cholesterol in serum and tissues [4]. The hexane fraction of coconut peel may contain novel anticancer compounds [5]. Young coconut juice has estrogen-like characteristics [6]. It can also serve as an emergency short-term intravenous

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hydration fluid. This is possible because the coconut water has a high level of sugar and other salts that makes it possible to be used in the bloodstream, much like the modern lactated Ringer solution or a dextrose/water solution as an intravenouus solution (IV). Coconut is also commonly used as a traditional remedy in Pakistan to treat bites from rats. The tea from the husk fiber is widely used to treat several inflammatory disorders [7].

MATERIALS AND METHODS

Plant materials*:* The shell of *C. nucifera* Linn. was collected from Dhaka, Bangladesh and the endocarps were extracted.

Extraction and fractionation*:* The collected plant parts were sun dried for several days and then oven dried for 24 hours at 40^{o}C to facilitate grinding. The powdered endocarps (450 gm) were macerated in 1.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number-1. The extracts were concentrated with a rotary evaporator at low temperature (40-45 ^{o}C) and reduced pressure. The concentrated methanol extracts were fractionated by the modified Kupchan partitioning protocol [8] and the resultant partitionates i.e., petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fractions.

Biological Investigations

Cytotoxic activity / Brine shrimp lethality bioassay: This technique was applied for the determination of general toxic properties of the dimethylsulfoxide (DMSO) solutions of plant extractives against *Artemia salina* in a single day in vivo assay. Vincristine sulphate was used as positive control [9].

Thrombolytic activity: The thrombolytic activity of all extractives was evaluated by the method [10] using streptokinase and distilled water as positive control and negative control, respectively.

Antioxidant activity:

a) ***Total phenolic content***: The total phenolic contents of the extractives were determined with Folin-Ciocalteau reagent by the method followed by Harbertson [11]. To 0.50 ml of each sample, 2.5 ml of 1/10 dilution of Folin-Ciocalteau reagent and 2.0 ml of sodium carbonate (7.5%, w/v) in water were added and incubated for 15 minutes at 45 °C. The absorbance of all samples was measured at 765 nm with a visible spectrophotometer. The phenolic contents were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of dry weight of extract.

b) ***DPPH free radical scavenging assay***: Following the method adopted by William [12], the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

Antimicrobial screening: Antimicrobial activity was determined by disc diffusion method [13].

Statistical Analysis: Three replicates of each sample were used for each assay to facilitate statistical analysis and the values are reported as mean ± SD.

RESULTS AND DISCUSSIONS

The crude methanol extracts of endocarps of *C. nucifera* Linn. as well as different Kupchan partitionates derived from these extracts were subjected to assays for cytotoxic, thrombolytic, antioxidant and antimicrobial activities by following standard protocols.

In brine shrimp lethality bioassay, the LC_{50} values of the test fractions were found within the range of 0.82±0.19 to 6.63±1.91 µg/ml where the maximum and minimum cytotoxicity were revealed by the carbon tetrachloride soluble fraction (LC_{50} = 0.82±0.19µg/ml) and the methanol fraction (LC_{50} = 6.63±1.91 µg/ml), respectively whereas the standard Vincristine sulphate showed an LC_{50} value of 0.45±0.08 µg/ml. (Table-1)

In thrombolytic activity assay, addition of 100 µl streptokinase as positive control (30,000 I.U.) to the clots and subsequent incubation for 90 minutes at 37 °C, showed 66.76±0.03% lysis of clot. On the other hand, distilled water treated as negative control exhibited a negligible percentage of lysis of clot (6.67±0.02%). In this study, moderate thrombolysis was observed and petroleum ether soluble fraction exhibited highest thrombolytic activity (37.44±0.33%). (Table-2)

The total phenolic contents of the extractives were found between 80.36±2.33 to 113.94±2.01 mg of GAE/g of sample. The highest total phenolic content was demonstrated by the crude methanol extract (113.94±2.01 mg of GAE/g of sample. (Table-3). In the free radical scavenging (DPPH) assay, the IC_{50} values of the test fractions ranged from 4.39±0.69 µg/ml to 16.82±2.30 µg/ml where the highest free radical scavenging activity was demonstrated by the crude methanol extract (IC_{50} = 4.39±0.69 µg/ml) compared to the standard butylatd hydroxytoluene (IC_{50} = 27.50±0.95 µg/ml) and ascorbic acid (IC_{50} = 5.80±1.03 µg/ml). (Table-

3). In antimicrobial activity assay, the crude methanol extract and its carbon tetrachloride and chloroform soluble fractions exhibited mild zone of inhibition against the test organisms. The highest 13 mm zone of inhibition was exhibited against *Salmonella* PARATYPHI by the carbon tetrachloride soluble fraction. This fraction also showed 12 mm zone of inhibition against *Bacillus cereus* and 10 mm zone of inhibition against *Escherichia coli*. (Table-4).

CONCLUSION

From the above results it may be concluded that the endocarp extract of *C. nucifera* Linn. has good cytotoxic and antioxidant activities and also mild to moderate thrombolytic and antimicrobial activities. Therefore, further work especially bioassay-guided fractionation is warranted in order to isolate and characterize the active constituents responsible for the specific biological property.

Table-1: LC_{50} values of the test samples of *C. nucifera* Linn.

Test samples	LC_{50} (µg/ml)
VS	0.45±0.08
ME	6.63±1.91
PESF	3.83±0.50
CSF	2.77±0.90
CTCSF	0.82±0.19
AQSF	5.13±1.20

VS= Vincristine sulfate; ME= Crude methanol extract; PESF= Petroleum ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction

Table-2: Thrombolytic activity (in terms of % of clot lysis) of *C. nucifera* Linn.

Fractions	% of clot lysis
SK	66.76±0.03
ME	30.16±0.20
PESF	37.44±0.33
CTCSF	10.39±1.30
AQSF	29.20±1.50
Blank	6.67±0.02

SK =Streptokinase; ME= Crude methanol extract; PESF= Petroleum ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction

Table-3: Total phenolic content determination and IC_{50} values of the standard and partitionates of *C. nucifera* Linn.

Plant	Sample code	Total phenolic content (mg of GAE / gm of extractives	IC_{50} (µg/ml)
Cocos nucifera L.	ME	113.94±2.01	4.39±0.69
	PESF	89.29±1.28	16.82±2.30
	CTCSF	97.90±0.05	6.28±0.90
	CSF	---	8.65±1.50
	AQSF	80.36±2.33	5.40±2.00
BHT (tert-butyl-1-hydroxytoluene) (standard)			27.50±0.95
ASA (Ascorbic acid) (standard)			5.80±1.03

ME= Crude methanol extract; PESF= Petroleum ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction

Table-4: Antimicrobial activity of test samples of *C. nucifera* Linn.

Test microorganisms	Diameter of zone of inhibition (mm)					
	ME	**PESF**	**CTCSF**	**CSF**	**AQSF**	**STD**
Gram positive bacteria						
Bacillus cereus	7	-	12	10	-	34
Bacillus megaterium	-	-	7	6	-	34
Bacillus subtilis	6	-	5	10	-	38
Sarcina lutea	5	-	8	8	-	34
Staphylococcus aureus	-	-	9	7	-	38
Gram negative bacteria						
Escherichia coli	5	-	10	9	-	34
Salmonella PARATYPHI	9	-	13	5	-	35
Salmonella TYPHI	-	-	7	8	-	38
Shigella boydii	6	-	7	7	-	32
Shigella dysenteriae	-	-	5	11	-	34
Vibrio mimicus	-	-	7	7	-	36
Vibrio parahemolyticus	-	-	8	9	-	34

ME= Crude methanol extract; PESF= Petroleum ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction; STD= Standard (Ciprofloxacin)

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