HEMOLYTIC PROPERTY AND GC –MS ANALYSIS OF COCOS NUCIFERA FIBER EXTRACTS FROM MARINE COASTAL AREA

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Received: 22 Nov 2013 Revised and Accepted: 10 Apr 2014

ABSTRACT

Objective: The main objective of the present study was to determine the hemolytic property of Cocos nucifera fiber crude extracts.

Methods: The aqueous crude extracts were tested for in vitro hemolytic property and the chemical constituents of the crude fiber extract of Cocos nucifera was determined by GC-MS (Gas chromatography–mass spectrometry).

Results: The crude extract revealed maximum lysis of RBCs with 100% inhibition. Nineteen chemical constituents have been identified and the major chemical constituents were 9-Octadecenoic acid methyl ester (58.86%), Hexadecanoic acid methyl Ester (19.025%), 6-Octadecanoic acid methyl ester (9.14%).

Conclusion: These findings demonstrate that the crude fiber extracts of Cocos nucifera serves as the excellent bioactive potential with beneficial virtues.

Keywords: Bioactive compounds, Fiber crude extract, Cocos nucifera, Hemolytic activity, Natural products.

INTRODUCTION

Natural products are currently the leading source for new biologically active compounds. Plants with medicinal values contain several chemical substances with important therapeutic properties which are used for treating human diseases. The healing power of herbs had been recognized since creation and botanic medicine is one of the oldest practiced by mankind [4]. The use of plant extracts and phytochemicals with known bioactive components can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [5, 7]. The discovery of new active metabolites must be followed by adequate biological testing [9] Therefore, such plants should be investigated to understand their properties, safety and efficiency [2]. Cocos nucifera is an important member of the family Araceaeae (palm family) and it is the only accepted species of the genus Cocos. The plant kingdom comprises many species of plants containing substances with biological activity, which are yet to be explored. The most interesting feature of the fibrous coconut fruit is not only edible but also suitable for multipurpose uses [1]. Cocos nucifera is known for its natural source of bioactive metabolites which perhaps provide novel or lead compounds that may be employed in controlling some infections globally. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. The study was especially selected due to lack of scientific data on its hemolytic property of Cocos nucifera origin. Hence the investigation will ensure the better understanding of Cocos nucifera properties from the marine source.

MATERIALS AND METHODS

Authentication of the plant

Cocos nucifera were collected from the sea shore of Bay of Bengal, Chennai, Tamilnadu, India in the month of May and it was authenticated by National Institute of Herbal Science, Chennai.

Extraction Process:

The coconut fiber was sun dried, milled and sieved manually to obtain fine powdered particles. About 50 g of the powder was dissolved in sterile distilled water (500 mL) for 24 h with shaking. The extract was filtered, lyophilized and stored at 5 °C for further use [3].

Hemolysis by qualitative method:

Hemolytic activity was carried out using (5%) human blood. The blood agar base was sterilized by autoclaving at 121°C at 15 lbs pressure for 20 min. Blood was added prior to pouring and the plates were allowed to solidify. 100μl of fiber crude aqueous extract with the concentration of 1mg/ml was added in the well on blood agar plate and were incubated at 37°C for 24 hours. The plates were then examined for zone of clearance.

In vitro Hemolytic Activity

In vitro hemolytic activity was evaluated for fiber crude extracts on human erythrocytes (RBCs) following the method [6]. The blood was obtained from the peripheral blood of (0 positive) individual, further the blood was centrifuged. 1 ml of 10% RBCs suspension was dispersed in dried, clean glass tubes. Erythrocytes were washed 3-4 times in sterile 0.85% NaCl saline solution later cells were centrifuged at 1500rpm for 5 min and the supernatant was discarded. The pellet was diluted 1.9 (v/v) in sterile 0.85% NaCl saline solution and fiber crude extract with the various concentrations from 5μg/ml - 1mg/ml was added. 5% Triton X was used as positive control. The blanks were prepared in separate tubes without addition of blood. PBS served as negative control. The mixture was incubated at 37 °C for 1 h and centrifuged at 8000 rpm for 10 min. The resulting supernatant was evaluated using spectrophotometer at 540 nm. The doses of substances inducing 50% hemolysis were calculated graphically. The data have been presented as an average of replicates and the percentage of hemolysis was calculated

GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID × 1mL df, composed of 100% Dimethyl poly siloxane, operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 μl was employed (split ratio of 10:1).
injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Interpretation on mass spectrum of GC-MS was done using the database NIST08 and WILEY8. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

RESULTS AND DISCUSSION

The use of plant extracts with medicinal potentials represents a valid alternative for the treatment of different ailments and diseases. Many reports have shown the enormous property of Cocos nucifera ingredients which act synergistically to confer bio activity on a plant as an active material. In the present study the preliminary screening of hemolysis of Cocos nucifera was carried out by agar well diffusion method. The zone of inhibition was found to be 8mm in diameter shown in figure 1.

The in vitro hemolytic activities of the fiber crude extracts of Cocos nucifera were assayed with heparinized human RBCs and found to exhibit potent hemolytic activity. The Cocos nucifera fiber crude extracts were tested with different concentration i.e. 5µg/ml - 1000µg/ml. 100% inhibition was observed at the maximum concentration of 1mg/ml shown in Figure 2 and 3. Data observed is expressed in % of Hb release by comparing with 100% hemolysis.

The chemical constituents of aqueous fiber crude extract of Cocos nucifera was identified by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The aqueous extract showed 19 peaks indicating the presence of nineteen phytochemical constituents which includes several important organic metabolites namely 9-Octadecenoic acid methyl ester(58.86%), (E)- HEXADECANOIC ACID METHYL ESTER (19.02%), 6 Octadecanoic acid methyl ester(9.14%). Interpretation on mass spectrum of GC-MS was done using the database NIST08, WILEY8 (Figure 4, Table 1)

The in vitro hemolysis of Rbcs

Fig. 1: Agar well diffusion assay of the aqueous fiber crude extract of Cocos nucifera

Fig. 2: In vitro hemolysis of Rbcs

Fig. 3: Reflection of hemolytic activity of the Cocos nucifera crude extract at 1000µg/ml on human erythrocytes

Fig. 4: The GC-MS chromatogram of the Cocos nucifera fiber extract
Though the potential of fibre extracts from *cocos nucifera* has been studied for its bioactive potential. [3, 8] This is the first report on the hemolytic property of fibre extracts from *cocos nucifera*. Hence the presence of various bioactive compounds justifies the use of the crude fiber extract for various ailments of diseases. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. However, further studies will need to be undertaken to study its toxicity profile.

**CONCLUSION**

The result from the present study indices the property of hemolysis against the RBCs which indicates the predominant role of cytotoxic effect. Further studies are needed to elucidate the active components and their modes of action as well as their potentials.

**ACKNOWLEDGEMENTS**

We are greatly indebted to Vellore Institute of Technology for the constant encouragement, help and support for extending necessary facilities.

**REFERENCES**

2. Eloff JN Which extract should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethnopharmacology.1998; 60:1-6.

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**Table 1: The GC-MS chromatogram of the *Cocos nucifera* aqueous fiber extract**

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<tr>
<th>Peak#</th>
<th>R. Time</th>
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<tr>
<td>1</td>
<td>14.564</td>
<td>1965067</td>
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<td>2</td>
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<td>3</td>
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<td>5</td>
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Int J Pharm Pharm Sci, Vol 6, Issue 4, 615-620