Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

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

NASIMUNISLAM[1], JEMIMAH NAINE [2],C. SUBATHRA DEVI[2] ,G. JAYARAMAN [2], PANNEERSELVAM. A*[1]

[1]Department of Zoology, Thiruvalluvar University, Serkedu, Vellore, Tamil Nadu, India, [2]School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India. Email: sagoselvam@yahoo.co.in

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 constitutes 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 Aracaceae (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* are 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 nuciferera* origin. Hence the investigation will ensure the better understanding of *Cocos nuciferera* 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 121ºC at 15lbs pressure for 20 min. Blood was added prior to pouring and the plates and was 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 (O positive) individual, further the blood was centrifuged. 1 ml of 10% RBCs suspension was dispensed 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 ×1EM 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 EI 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 bioactivity 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.

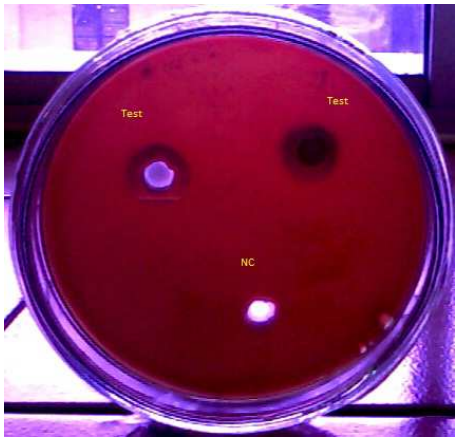


Fig. 1: Agar well diffusion assay of the aqueous fiber crude extract of *Cocos nucifera*

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 - 1000mg/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.

Fig. 2: *In vitro* hemolysis of Rbcs

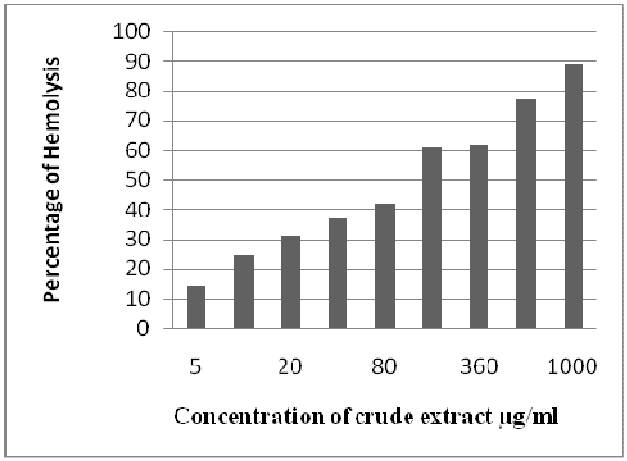


Fig. 3: Reflection of hemolytic activity of the *Cocos nucifera* crude extract at 1000µg/ml on human erythrocytes

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 (19.025%), 6 Octadecanoic acid methyl ester(9.14%). Interpretation on mass spectrum of GC-MS was done using the database NIST08, WILEY8 (Figure 4,5, Table 1)

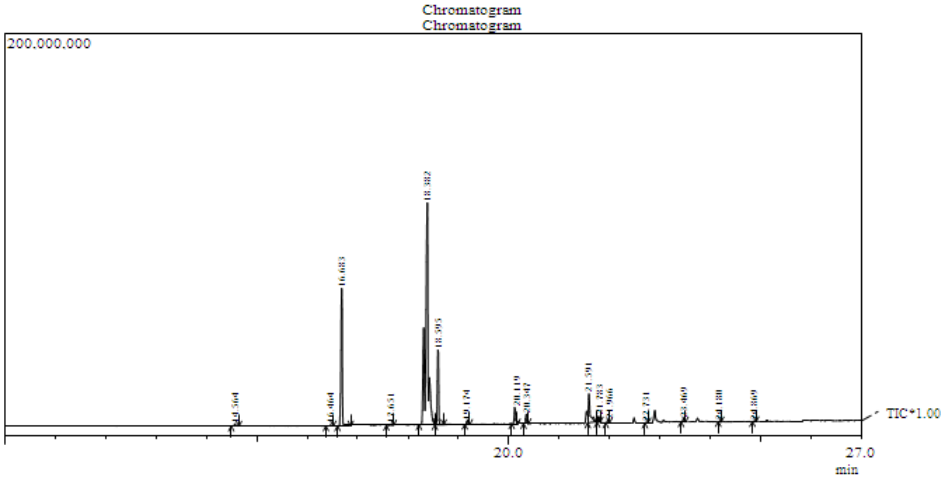
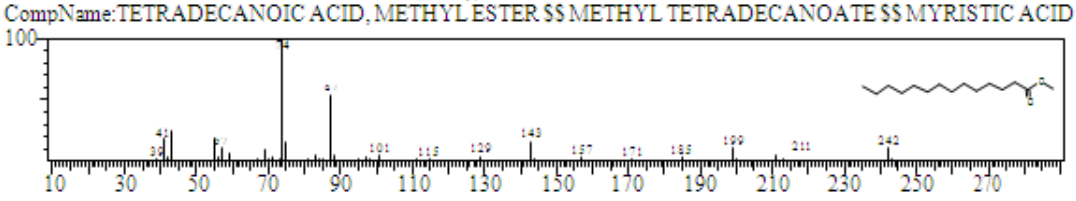
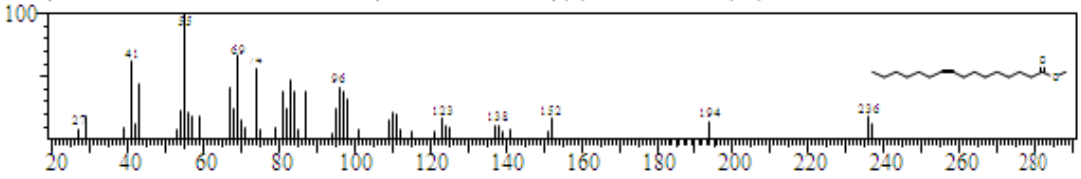


Fig. 4: The GC-MS chromatogram of the *Cocos nucifera* fiber extract

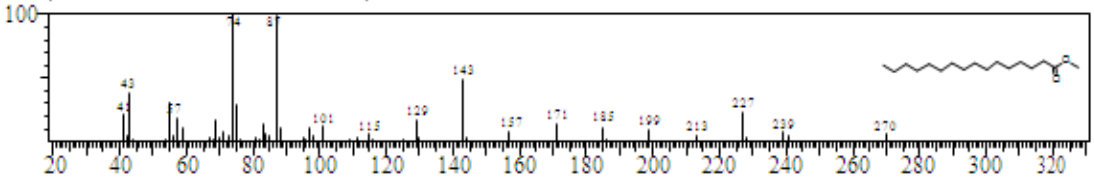
Hit#:1 Entry:164103 Library:WILEY8.LIB
SI:97 Formula:C15H30O2 CAS:124-10-7 MolWeight:242 RetIndex:0
CompName:TETRADECANOIC ACID, METHYL ESTER $$ METHYL TETRADECANOATE $$ MYRISTIC ACID



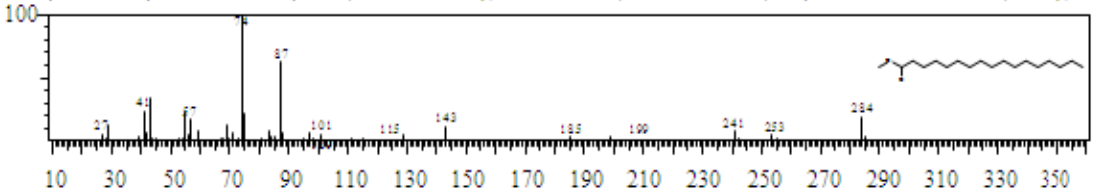
Hit#:1 Entry:199189 Library:WILEY8.LIB
SI:93 Formula:C17H32O2 CAS:1120-25-8 MolWeight:268 RetIndex:0
CompName:9-HEXADECENOIC ACID, METHYL ESTER, (Z)- $$ METHYL (9Z)-9-HEXADECENOATE # $$ AI3-



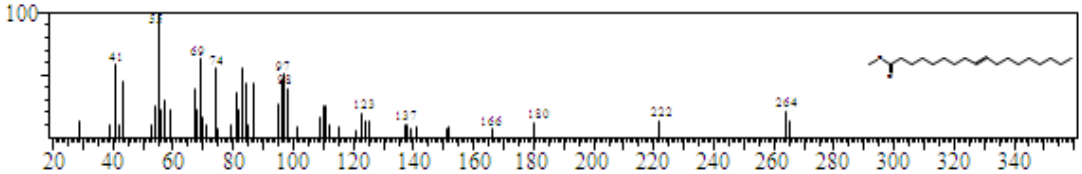
Hit#:1 Entry:201925 Library:WILEY8.LIB
SI:95 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:0
CompName:HEXADECANOIC ACID, METHYL ESTER $$ METHYL HEXADECANOATE $$ PALMITIC ACID M



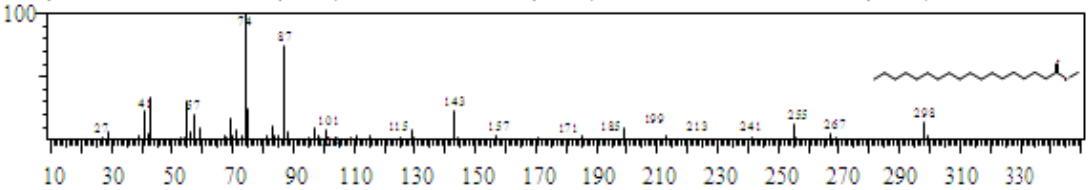
Hit#:2 Entry:23519 Library:NIST08s.LIB
SI:94 Formula:C18H36O2 CAS:1731-92-6 MolWeight:284 RetIndex:1978
CompName:Heptadecanoic acid, methyl ester $$ Margaric acid methyl ester $$ Methyl heptadecanoate $$ Methyl margar



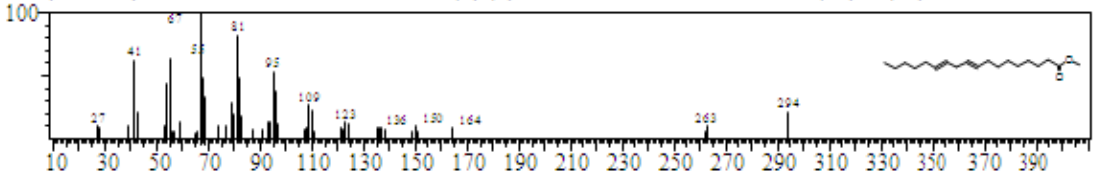
Hit#:1 Entry:24160 Library:NIST08s.LIB
SI:97 Formula:C19H36O2 CAS:1937-62-8 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid, methyl ester, (E)- $$ Elaidic acid, methyl ester $$ Methyl elaidate $$ Methyl trans-9-oc



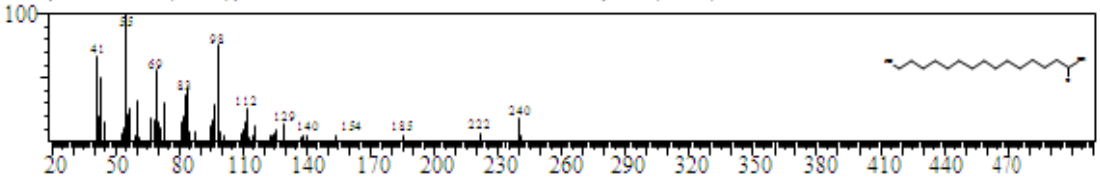
Hit#:1 Entry:110813 Library:NIST08.LIB
SI:97 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077
CompName:Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid, methyl ester $$ Kemes



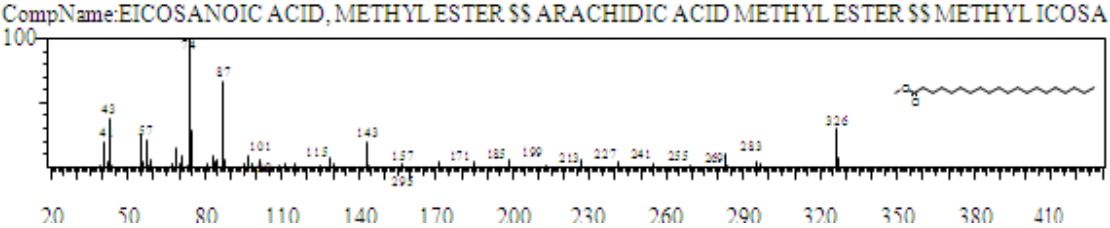
Hit#:1 Entry:232838 Library:WILEY8.LIB
SI:94 Formula:C19H34O2 CAS:112-63-0 MolWeight:294 RetIndex:0
CompName:9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER $$ METHYL (9Z,12Z)-9,12-OCTADECADI



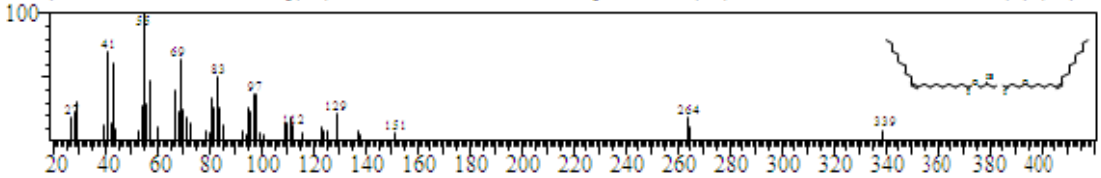
Hit#:1 Entry:82066 Library:NIST08.LIB
SI:81 Formula:C15H30O3 CAS:4617-33-8 MolWeight:258 RetIndex:2111
CompName:15-Hydroxypentadecanoic acid $$ Pentadecanoic acid, 15-hydroxy- $$



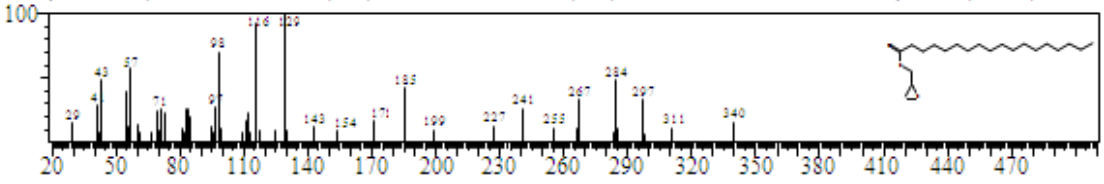
Hit#:1 Entry:270736 Library:WILEY8.LIB
SI:98 Formula:C21H42O2 CAS:1120-28-1 MolWeight:326 RetIndex:0
CompName:EICOSANOIC ACID, METHYL ESTER $$ ARACHIDIC ACID METHYL ESTER $$ METHYL ICOSA



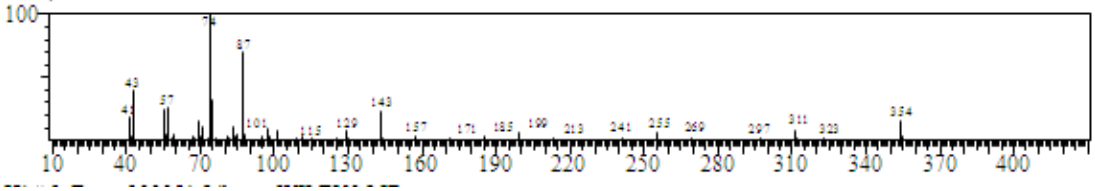
Hit#:1 Entry:391363 Library:WILEY8.LIB
SI:87 Formula:C39H72O5 CAS:2465-32-9 MolWeight:620 RetIndex:0
CompName:2-HYDROXY-3-[(9E)-9-OCTADECENOYLOXY]PROPYL (9E)-9-OCTADECENOATE # $$ (Z,Z)-1,3-D



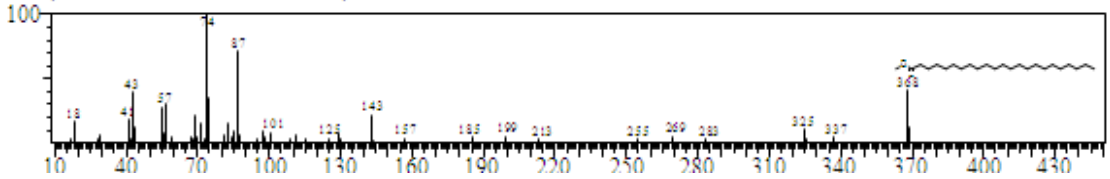
Hit#:1 Entry:139255 Library:NIST08.LIB
SI:85 Formula:C21H40O3 CAS:7460-84-6 MolWeight:340 RetIndex:2366
CompName:Glycidol stearate $$ Glycidyl octadecanoate $$ Glycidyl stearate $$ Octadecanoic acid, oxiranylmethyl ester



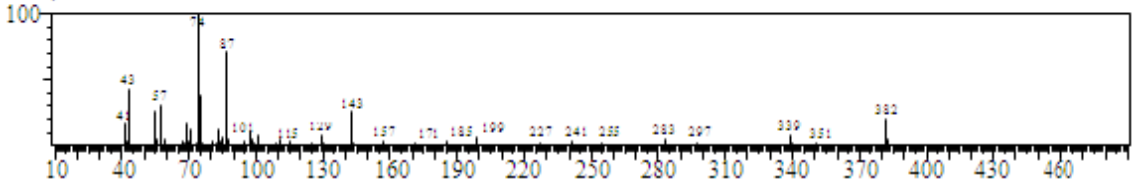
Hit#:1 Entry:52 Library:FA_ME.lib
SI:96 Formula: CAS:0-00-0 MolWeight:0 RetIndex:0
CompName:



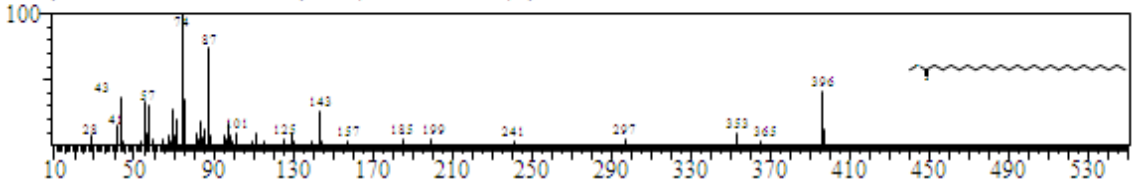
Hit#:1 Entry:311119 Library:WILEY8.LIB
SI:91 Formula:C24H48O2 CAS:2433-97-8 MolWeight:368 RetIndex:0
CompName:TRICOSANOIC ACID, METHYL ESTER $$ METHYL TRICOSANOATE $$ EINECS 219-420-2



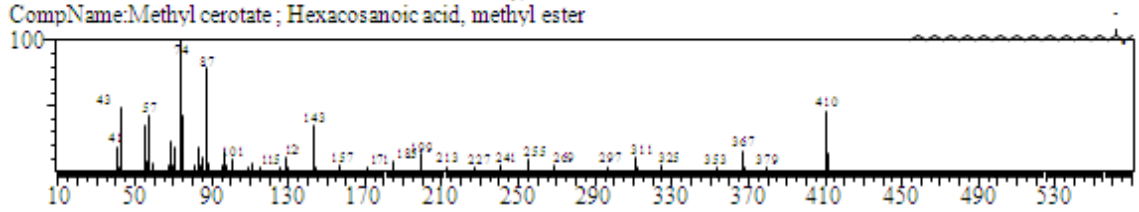
Hit#:1 Entry:56 Library:FA_ME.lib
SI:95 Formula: CAS:0-00-0 MolWeight:0 RetIndex:0
CompName:



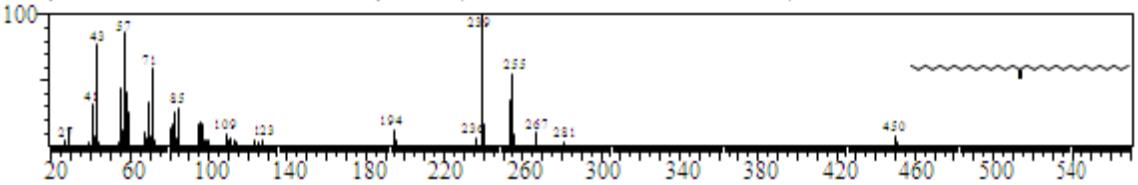
Hit#:1 Entry:27239 Library:NIST08s.LIB
SI:82 Formula:C26H52O2 CAS:55373-89-2 MolWeight:396 RetIndex:2773
CompName:Pentacosanoic acid, methyl ester $$ Methyl pentacosanoate # $$



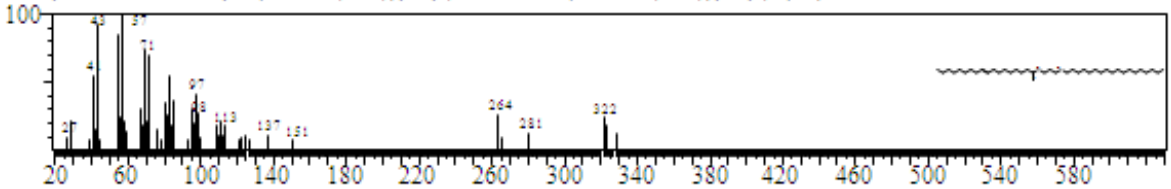
Hit#:1 Entry:45 Library:FA_ME.lib
SI:88 Formula:C27H54O2 CAS:5802-82-4 MolWeight:410 RetIndex:2931
CompName:Methyl cerotate ; Hexacosanoic acid, methyl ester



Hit#:1 Entry:179004 Library:NIST08.LIB
SI:85 Formula:C31H62O CAS:502-73-8 MolWeight:450 RetIndex:3239
CompName:16-Hentriacontanone $$ Dipentadecyl ketone $$ Palmitone $$ Pentadecyl ketone $$



Hit#:1 Entry:188813 Library:NIST08.LIB
SI:77 Formula:C39H76O3 CAS:17367-41-8 MolWeight:592 RetIndex:4149
CompName:Oleic acid, 3-(octadecyloxy)propyl ester $$ 3-(Octadecyloxy)propyl (9E)-9-octadecenoate # $$



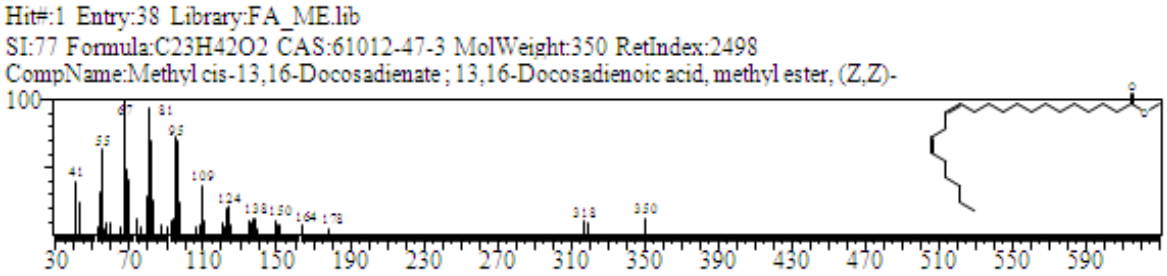


Fig. 5: Molecular structure and corresponding GC-MS peaks of major bioactive metabolites from crude fiber extract of *Cocos nucifera* 9-Octadecenoic acid methyl ester, (E)-, HEXADECANOIC ACID METHYL ESTER (19.025%), 6 Octadecanoic acid methyl ester(9.14%)

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

Peak#	R. Time	Area	Area%	Name
1	14.564	1965067	0.28	TETRADECANOIC ACID, METHYL ESTER
2	16.464	1156759	0.16	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-
3	16.683	135790103	19.02	HEXADECANOIC ACID, METHYL ESTER
4	17.651	1125288	0.16	METHYL HEPTADECANOATE
5	18.382	420139249	58.86	9-Octadecenoic acid, methyl ester, (E)-
6	18.595	65212039	9.14	6 Octadecanoic acid, methyl ester
7	19.174	3498576	0.49	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER
8	20.119	18034790	2.53	15-Hydroxypentadecanoic acid
9	20.347	7952653	1.11	EICOSANOIC ACID, METHYL ESTER
10	21.591	36070205	5.05	2-HYDROXY-3-[(9E)-9-OCTADECENOYLOXY]PROPYL (9E)-9-OCTADECENOATE
11	21.783	4553565	0.64	Glycidol stearate
12	21.966	2429707	0.34	DOCOSANOIC ACID, METHYL ESTER
13	22.731	613517	0.09	TRICOSANOIC ACID, METHYL ESTER
14	23.469	3154426	0.44	Tetracosanoic acid, methyl ester
15	24.180	378864	0.05	Pentacosanoic acid, methyl ester
16	24.869	625832	0.09	Methyl cerotate ; Hexacosanoic acid, methyl ester
17	27.317	854775	0.12	16-Hentriacontanone
18	29.065	4492047	0.63	Oleic acid, 3-(octadecyloxy)propyl ester
19	31.488	5726783	0.80	Methyl cis-13,16-Docosadienoate ; 13,16-Docosadienoic acid, methyl ester, (Z,Z)-

Though the potential of fibre extracts from *cocos nucifera* has been studied for it bioactive potential. [3, 8] This is the first report on 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 predominate 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

1. Angieo F, Berenzi A,Benetti A, Rossi E, Del-SOrdo, Sidoni A, Stefani M, Dessy E Expression of p16, p53 and Ki-67 proteins in the progression of epithelial dysplasia of the oral cavity. Anticancer Research. 2008; 28: 2535-9.
2. Ellof JN Which extract should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethnopharmacology.1998; 60:1-6.
3. Esquenazi d, Wigg MD, Miranda M M, Rodrigues HM, Tostes JB, Rozental S, Da silva AJ, Alviano CS Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Research in Microbiology .2002; 153: 647–652.
4. Hugo WB, Russell AD Pharmaceutical Microbiology; 6th ed. Blackwell Science Publishers, Oxford, United Kingdom, 2003; 91-129.
5. Kubo L, Muroi H, Himejima M Structure-antibacterial activity relationships of anacardic acids. Journal of Agricultural and Food Chemistry.1993; 41: 1016-1019.
6. Malagoli A full-length protocol to test hemolytic activity of palytoxin on human erythrocytes D. Invertebrate Survival Journal. 2007; 4: 92-94.
7. Shapoval EES, Silveira SM, Miranda ML, Alice CB and Henriques AT Evaluation of some pharmacological activities of Eugenia uniflora. Journal of Ethnopharmacology. 1994; 44:136-142.
8. Srinivas K, Vijayasrinivas S, Kiran HR, Prasad PM, Rao MEB Antibacterial Activity of *Cocos Nucifera* Linn. Indian Journal of Pharmaceutical Sciences. 2003; 65: 417-418.
9. Jemimah Naine S, Mohana srinivasan V, Subathra Devi C Novel anticancer compounds from marine actinomycetes. Journal of Pharmacy Research. 2011; 4 (4): 1285-1287.