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GC/MS Identification and Biological Evaluation of the Red Sea Soft Coral *Nephthea molle* Extracts.

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ABSTRACT

In-vitro antitumor activity for two non-polar fractions of *Nephthea molle*extract was determined against both human colorectal and prostate carcinoma (HCT116 and PC3 cell lines). Both fractions as well as crude extract exhibited promising cytotoxicity. Antibacterial activity of non-polar fractions of *N. molle* was evaluated also against the pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus iniae* and *Escherichia coli*. Both *N. molle* fractions showed broad anti-bacterial activity towards all pathogenic bacteria. The minimum inhibitory concentration (MIC) of fractions was also determined in ppm. About 35 volatile constituents were identified from N. molle fractions using GC/MS technique.

Keywords: Seseqiterpene; *Nephthea molle*; *In-vitro* antitumor; HCT116; PC3; antibacterial activity

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INTRODUCTION

The Red Sea is considered the world's warmest (up to 35˚C in summer) and most saline marine habitat [1,2]. It is an important sea area, not just as a unique environment, but as one of the most diverse marine ecosystems [3,4].It represents one of the most promising areas as a source of medicinal and nutritional natural products [5-7]. Soft coral is a rich source of bioactive sesquiterpenes which have antibacterial, anti-inflammatory and cytotoxic properties[3,4]. The genus Nephthea (Acyonaceae, subfamily Nephtheidae) is distributed throughout the world mainly in the Indo-Pacific region and is a rich source of sesquiterpenoids and steroids [8].

In this research article,the biological activities as antitumor activity on human colorectal and prostate carcinoma (HCT116 and PC3 cell lines) as well as the anti-microbial activities toward different strain pathogens of different Red sea *Nephthea molle* (Acyonaceae, subfamily Nephtheidae) fractions were evaluated. The tested fractions were characterized using GC/MS analysis in order to identify the major constituents.

MATERIAL AND METHOD

General

GC/MS analyses were performed on the Agilent's 7890B GC. The column used was Agilent 19091s-433:1 (HP-5MS 5%phenyl methyl silox) cross-linked fused silica capillary column (30 mx250 µmx 0.25 µm). The oven temperature was programmed from 90 ^{o}C for 1 min., at isothermal, then heating by 8^{o}C/ min. to 240 ^{o}C and isothermally for 10 min., at 254^{o}C. Injector temperature was 200 ^{o}C and the volume injected was 1 µl. Ionization energy was set at 70eV (National Institute of Oceanography and Fisheries, Egypt).

Chemicals

Hexane, dichloro-methane, ethyl acetate and methanol were obtained from both Sigma Aldrish and Adwic companies

Cell culture for the In-vitro antitumor assay

PC3 human prostate carcinoma, and HCT116 human colorectal carcinoma cell lines were obtained from ATCC and was maintained in RPMI 1640 and McCoy's 5A media, respectively, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin antibiotic. The two cell lines were incubated at 37°C in 5% CO_2 and 95% humidity. Cells were sub-cultured using trypsin versene 0.15% [9].

Strains for anti-microbial assay

Pathogenic bacteria under investigation were *Escherichia coli* (ATCC8739), *Pseudomonas aeruginosa* (ATCC 9027) (as Gram negative strains), *Staphylococcus aureus* (ATCC 6538), *Streptococcus iniae*, a fish pathogen, (as Gram positive strains). All bacterial pathogens were kindly provided by Marine Biotechnology Lab., NIOF, Egypt. Stock bacterial cultures were maintained on nutrient agar slants at 4˚C [10].

Marine organism materials

The soft coral, *Nephthea molle*, was kindly collected and identified by Dr.Elsayedabd-Elaziza researcher at the National Institute of Oceanography and Fisheries (NIOF) in November 2015, from the Red Sea at a depth of 4-5 m at the front of Hurghada marine station of National Institute of Oceanography and Fisheries, Hurghada, Egypt.

Extraction and isolation

The frozen marine organism (wet weight 9 kg) was fragmented to small pieces and extracted several times (4 L × 5)at room temperature with a mixture of CH_2Cl_2/MeOH, 1:1 until complete extraction. After filtration, the extract was concentrated under reduced pressure at 40oC. The residue (40.0 g) was subjected to rapid flash silica gel column eluted with n-hexane, then with (ethyl acetate/hexane, 1:9). Two fractionswere

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collected according to their TLC pattern. Both fractions were evaporated to dryness, where yellow residues were obtained (5g and 8g). Samples from the fraction residues were analyzed by GC/MS technique for identification of their constituents.

Assessment of Cytotoxicity

The acid phosphatase assay method was used to assess the cytotoxicity test according to the method described by [9].

Assessment of antimicrobial activity

The antimicrobial activity was carried out using agar well diffusion method. All tests were performed in duplicates. After incubation, the zone of inhibition was measured and expressed in mm diameter [11].

Determination of the Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined by the broth dilution method.The MIC was taken as the lowest concentration of extracts that did not permit any visible growth for each of bacterial species [12].

RESULTS AND DISCUSSION

Identification of N. molle compounds using GC/MS technique

Table 1displayed the identified compounds from *N. molle*fractions.GC/MS analysis of both fractions revealed that both fractions are rich in sesqueiterpenes.2-methyl-4-methylene-2-(2-methyl-1-propenyl)-1-vinylcycloheptane **(1)**, guaia-1(10),11-dien **(2)**, (−)-alloaromadendrene**(3)**, (+)-valencene**(4)**, (+)-γ-gurjunene**(5)**, guaiene**(6)**, 4,11(13)-eudesmadien-12-ol **(7)**, aromadendrene oxide-(2) **(8)**, ledene oxide-(II) **(9)**, widdrol**(10)**,corymbolone**(11)**, 8,9-epoxyacorenon-B **(12)**, 6-(1-hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one **(13)**, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-2-naphthalenyl acetate **(14)**, sclaral**(15)**, methyl 11,14-eicosadienoate,4,8,13-duvatriene-1,3-diol **(16)**, methyl 4,7,10,13,16-docosapentaenoate,2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde **(17)**, 2-(acetyloxy)-1-[(hexadecyloxy)methyl]ethyl acetate were identified from fraction 1 (Fr1). (-)-Alloaromadendrene**(3)**, 4,11(13)-eudesmadien-12-ol **(7)**, sclaral**(15)**, 4,8,13-duvatriene-1,3-diol **(16)**, 4a-methyl-3,4,4a,5,6,7,8,9-octahydro-2H-benzo[a]cyclohepten-2-one **(18)**, cubedol**(19)**, 4-epi-cubedol**(20)**, calamenene**(21)**, β-vatirenene**(22)**, 6-[1-(hydroxymethyl)vinyl]-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-2-naphthalenol **(23)**, aristolene epoxide **(24)**, 9,10-dehydrofukinone **(25)**, methyl palmitate, 2-heptadecanone, geranyl linallol**(26)**, thunbergol**(27)**, methyl arachidonate were identified from fraction 2 (Fr2).Fraction 1 recorded higher sesqueiterpene hydrocarbons and oxygenated sesqueiterpene.

Table 1: Identified *N. molle* compounds using GC/MS analysis.

Compound name	RT	Mol. For.	Area%		m/z
			Fr1	Fr2	
Sesqueiterpenehydrocarbons					
2-Methyl-4-methylene-2-(2-methyl-1-propenyl)-1-vinylcycloheptane **(1)**	12.52	$C_{15}H_{24}$	0.25		204 (1) $[M^+]$, 189 (6) $[C_{14}H_{21}]^+$, 176 (47) $[C_{13}H_{20}]^+$, 161 (30) $[C_{12}H_{17}]^+$, 147 (17) $[C_{11}H_{15}]^+$, 135 (100) $[C_{10}H_{15}]^+$, 105(43) $[C_8H_9]^+$, 93(36) $[C_7H_9]^+$, 79 (41) $[C_6H_5]^+$.
Guaia-1(10),11-diene **(2)**	12.68	$C_{15}H_{24}$	0.57		204 (6) $[M^+]$, 189 (28) $[C_{14}H_{21}]^+$, 176 (6) $[C_{13}H_{20}]^+$, 161 (32) $[C_{12}H_{17}]^+$, 147 (21) $[C_{11}H_{15}]^+$, 133 (21) $[C_{10}H_{13}]^+$, 107 (100) $[C_8H_{11}]^+$.
(-)-Alloaromadendrene **(3)**	13.34	$C_{15}H_{24}$	4.67	0.75	204.35 (33) $[M^+]$, 189 (46) $[M^+\text{-}CH_3]^+$, 161 (100) $[M^+\text{-}C_3H_7]^+$, 147 (46) $[C_{11}H_{15}]^+$, 105 (100) $[C_8H_9]^+$, 91 (100) $[C_7H_7]^+$.

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(+)-Valencene **(4)**	14.02	$C_{15}H_{24}$	1.19		204.35 (100) $[M^+]$, 189 (63) $[M^+-CH_3]^+$, 161 (100) $[M^+-C_3H_7]^+$, 147 (83) $[C_{11}H_{15}]^+$, 105 (100) $[C_8H_9]^+$, 91 (100) $[C_7H_7]^+$.
Calamenene **(21)**	14.31	$C_{15}H_{22}$		0.39	202 (17) $[M^+]^+$, 159 (100) $[C_{12}H_{15}]^+$, 144 (20) $[C_{11}H_{12}]^+$, 129 (47) $[C_{10}H_9]^+$, 116 (17) $[C_9H_8]^+$, 105(15) $[C_8H_9]^+$, 91(12) $[C_7H_7]^+$.
(+)-γ-Gurjunene **(5)**	14.40	$C_{15}H_{24}$	1.82		204.35 (33) $[M^+]$, 189 (66) $[M^+-CH_3]^+$, 161 (100) $[M^+-C_3H_7]^+$, 147 (66) $[C_{11}H_{15}]^+$, 105 (100) $[C_8H_9]^+$, 91 (100) $[C_7H_7]^+$.
Guaiene **(6)**	14.64	$C_{12}H_{18}O$	0.74		178 (84) $[M]^+$, 163 (34) $[C_{11}H_{15}O]^+$, 150 (42) $[C_{10}H_{14}O]^+$, 135 (100) $[C_{10}H_{15}]^+$, 121 (94) $[C_9H_{13}]^+$, 107 (81) $[C_8H_{11}]^+$, 93 (73) $[C_7H_9]^+$.
β-Vatirenene **(22)**	15.37	$C_{15}H_{22}$		1.47	202 (92) $[M^+]^+$, 187 (100) $[C_{14}H_{19}]^+$, 173 (17) $[C_{13}H_{17}]^+$, 159 (30) $[C_{12}H_{15}]^+$, 145 (40) $[C_{11}H_{13}]^+$, 131 (47) $[C_{10}H_{11}]^+$, 105(47) $[C_8H_9]^+$, 91(57) $[C_7H_7]^+$.
Total			9.24	2.61	
Oxygenated sesqueiterpene					
Cubedol **(19)**	13.87	$C_{15}H_{26}O$		0.96	222 (1) $[M^+]^+$, 207 (32) $[C_{14}H_{23}O]^+$, 205 (16) $[C_{15}H_{25}]^+$, 179 (7) $[C_{12}H_{19}O]^+$, 161 (100) $[C_{12}H_{17}]^+$, 147 (8) $[C_{11}H_{15}]^+$, 133(15) $[C_{10}H_{13}]^+$, 119(37) $[C_9H_{11}]^+$, 105(52) $[C_8H_9]^+$.
4-epi-cubedol **(20)**	14.25	$C_{15}H_{26}O$		0.75	222 (3) $[M^+]^+$, 207 (32) $[C_{14}H_{23}O]^+$, 205 (32) $[C_{15}H_{25}]^+$, 189 (10) $[C_{14}H_{21}]^+$, 179 (7) $[C_{12}H_{14}O]^+$, 161 (100) $[C_{12}H_{17}]^+$, 147 (10) $[C_{11}H_{15}]^+$, 133(17) $[C_{10}H_{13}]^+$.
4,11(13)-Eudesmadien-12-ol **(7)**	15.26	$C_{15}H_{24}$	2.55	1.62	176 (51) $[C_{13}H_{20}]^+$, 147 (36) $[C_{11}H_{15}]^+$, 133 (81) $[C_{10}H_{13}]^+$, 105 (73) $[C_8H_9]^+$, 91 (100) $[C_7H_7]^+$.
Aromadendrene oxide-(2) **(8)**	15.75	$C_{15}H_{24}O$	1.23		220 (3) $[M]^+$, 205 (10) $[C_{14}H_{21}O]$, 177 (50) $[C_{13}H_{21}]^+$, 161 (26) $[C_{12}H_{17}]^+$, 147 (43) $[C_{11}H_{15}]^+$, 133 (86) $[C_{10}H_{13}]^+$, 105(82) $[C_8H_9]^+$.
Ledene oxide-(II) **(9)**	16.02	$C_{15}H_{24}O$	0.99		202 (100) $[C_{15}H_{22}]^+$, 187 (100) $[C_{12}H_{19}]^+$, 159 (43) $[C_{12}H_{15}]^+$, 145(60) $[C_{11}H_{13}]^+$, 131(56) $[C_{10}H_{11}]^+$.
6-[1-(Hydroxymethyl) vinyl]-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-2-naphthalenol **(23)**	16.25	$C_{15}H_{24}O_2$		1.31	218 (65) $[M-H_2O]^+$, 203 (30) $[C_{15}H_{23}]^+$, 159 (25) $[C_{12}H_{15}]^+$, 145(100) $[C_{11}H_{13}]^+$, 105(25) $[C_8H_9]^+$, 91(35) $[C_7H_7]^+$.
Aristolene epoxide **(24)**	16.33			2.05	220 (5) $[M^+]^+$, 187 (10) $[C_{14}H_{19}]^+$, 161 (7) $[C_{12}H_{17}]^+$, 149 (15) $[C_{11}H_{17}]^+$, 123 (100) $[C_9H_{15}]^+$, 81(50) $[C_6H_9]^+$.
Widdrol **(10)**	17.46	$C_{15}H_{26}O$	3.93		193 (100) $[M^+-C_2H_5]^+$, 189 (21) $[C_{14}H_{21}]^+$, 179 (66) $[C_{13}H_{23}]^+$, 151 (100) $[C_{11}H_{19}]^+$, 138 (53) $[C_9H_{15}O]^+$.
9,10-Dehydrofukinone **(25)**	17.86	$C_{15}H_{22}O$		2.29	218 (98) $[M]^+$, 203 (86) $[M^+-CH_3]^+$, 147 (100) $[C_{10}H_{11}O]^+$, 133 (32) $[C_9H_9O]^+$, 119 (35) $[C_8H_8O]^+$, 105 (42) $[C_7H_5O]^+$, 91 (57) $[C_6H_3O]^+$.
Corymbolone **(11)**	18.40	$C_{15}H_{24}O_2$	1.84		218 (19) $[M^+-H_2O]^+$, 203 (21) $[C_{14}H_{19}O]^+$, 179 (100) $[C_{12}H_{19}O]^+$, 133(47) $[C_{10}H_{13}]^+$, 91 (80) $[C_7H_7]^+$.
8,9-Epoxyacorenon-B	18.68	$C_{15}H_{24}O_2$	1.31		236 (5) $[M^+]$, 179 (100) $[C_{12}H_{21}O]^+$, 151

(12)					(100) $[C_9H_{11}O_2]^+$, 138 (69) $[C_{10}H_{18}]^+$, 123 (90) $[C_9H_{15}]^+$.
6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one **(13)**	18.82	$C_{15}H_{22}O_2$	1.22		236 (5) $[M^+]$, 179 (100) $[C_{12}H_{21}O]^+$,151(100) $[C_9H_{11}O_2]^+$, 138(69) $[C_{10}H_{18}]^+$, 123(90) $[C_9H_{15}]^+$.
3-Hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-2-naphthalenyl acetate **(14)**	19.76	$C_{17}H_{26}O_3$	1.46		216(43) $[C_{15}H_{20}O]^+$, 177 (43) $[C_{12}H_{19}O]^+$, 179 (100) $[C_{12}H_{19}O]^+$, 133 (47) $[C_{10}H_{13}]^+$, 91 (80) $[C_7H_7]^+$.
Total			14.5	8.98	
Total Sesqueiterpene			23.8	11.6	
Oxygenated Diterpene					
Geranyl linalool **(26)**	22.59	$C_{20}H_{34}O$		1.32	121(21) $[C_9H_{13}]^+$, 107(62) $[C_8H_{11}]^+$, 93(100) $[C_7H_9]^+$, 81(65) $[C_6H_9]^+$, 79(85) $[C_5H_9]^+$.
4,8,13-Duvatriene-1,3-Diol **(16)**	23.89	$C_{20}H_{34}O_2$	0.85	1.12	289 (4) $[C_{30}H_{33}O]^+$, 274 (4) $[C_{19}H_{30}O]^+$, 246 (8) $[C_{17}H_{26}O]^+$, 136 (71) $[C_{10}H_{16}]^+$, 107(100) $[C_8H_{11}]^+$, 92(91) $[C_7H_8]^+$.
Thunbergol **(27)**	25.80	$C_{20}H_{34}O$		1.12	290 (3) $[M]^+$, 258 (13) $[C_{20}H_{33}]^+$, 229 (13) $[C_{17}H_{27}]^+$, 161 (28) $[C_9H_{13}]^+$, 121 (78) $[C_9H_{13}]^+$, 107 (78) $[C_8H_{11}]^+$, 93 (100) $[C_7H_9]^+$.
TotalDiterpene			0.85	3.57	
Acetogenines					
Methyl palmitate	21.06	$C_{17}H_{34}O_2$		3.04	270 (21) $[M]^+$, 199 (21) $[C_{12}H_{23}O_2]^+$, 143 (81) $[C_8H_{15}O_2]^+$, 129 (35) $[C_7H_{13}O_2]^+$, 87 (100) $[C_4H_7O_2]^+$.
2-Heptadecanone	22.48	$C_{17}H_{34}O$		0.92	254 (5) $[M]^+$, 225(3) $[C_{15}H_{29}O]^+$, 127 (10) $[C_8H_{15}O]^+$, 99 (7) $[C_6H_{11}O]^+$, 85(40) $[C_5H_9O]^+$, 71(100) $[C_4H_7O]^+$, 57 (100) $[C_3H_5O]^+$.
Methyl (11E,14E)-11,14-eicosadienoate	22.54	$C_{21}H_{38}O_2$	1.64		109 (20) $[C_8H_{13}]^+$, 96 (26) $[C_7H_{12}]^+$, 95 (24) $[C_7H_{11}]^+$ 85 (40) $[C_6H_{13}]^+$, 71 (99) $[C_5H_{11}]^+$, 57 (100) $[C_4H_9]^+$.
2-cis-9-Octadecenyloxyethanol	22.95	$C_{20}H_{40}O_2$	1.77		137 (15) $[C_{10}H_{17}]^+$, 109 (36) $[C_8H_{13}]^+$, 96 (71) $[C_7H_{12}]^+$, 82 (100) $[C_6H_{10}]^+$, 55(78) $[C_4H_7]^+$.
cis-13-Eicosenoic acid	24.07	$C_{20}H_{38}O_2$	1.72	0.84	171 (6) $[C_{10}H_{19}O]^+$, 111 (56) $[C_8H_{15}]^+$, 97 (100) $[C_7H_{13}]^+$, 83 (100) $[C_6H_{11}]^+$, 69 (86) $[C_5H_9]^+$.
Methyl 4,7,10,13,16-docosapentaenoate	27.24	$C_{23}H_{36}O_2$	0.42		177 (8) $[C_{13}H_{21}]^+$, 134 (26) $[C_{10}H_{14}]^+$, 93(100) $[C_7H_9]^+$, 79 (100) $[C_6H_7]^+$.
Methyl arachidonate	27.25	$C_{21}H_{34}O_2$		2.85	318 (1) $[M]^+$, 150 (21) $[C_{11}H_{18}]^+$, 105 (56) $[C_8H_9]^+$, 91 (95) $[C_7H_7]^+$, 79 (100) $[C_6H_7]^+$.
2-(Acetyloxy)-1-[(hexadecyloxy)methyl] ethyl acetate	33.8	$C_{23}H_{44}O_5$	0.55		183 (2) $[C_{13}H_{27}]^+$, 131(7) $[C_6H_{10}O_3]^+$, 117(100) $[C_5H_8O_3]^+$, 103(50) $[C_4H_7O_3]^+$, 71(47) $[C_5H_{11}]^+$, 57(65) $[C_4H_9]^+$.
Total Acetogenines Derivatives			6.09	7.64	
Miscellaneous					
4a-Methyl-3,4,4a,5,6,7,8,9-octahydro-2H-benzo[a]cyclohepten-2-one **(18)**	13.14	$C_{12}H_{18}O$		1.87	178 (65) $[M^+]^+$, 163 (42) $[M^+- CH_3]^+$, 136 (100) $[C_9H_{12}O]^+$, 121 (100) $[C_8H_9O]^+$, 107(77) $[C_7H_7O]^+$, 93(97) $[C_6H_5O]^+$.
Sclaral	20.21	$C_{16}H_{28}O_2$	1.79	1.31	252 (1) $[M^+]$, 237 (92) $[M^+-CH_3]^+$, 191 (17)

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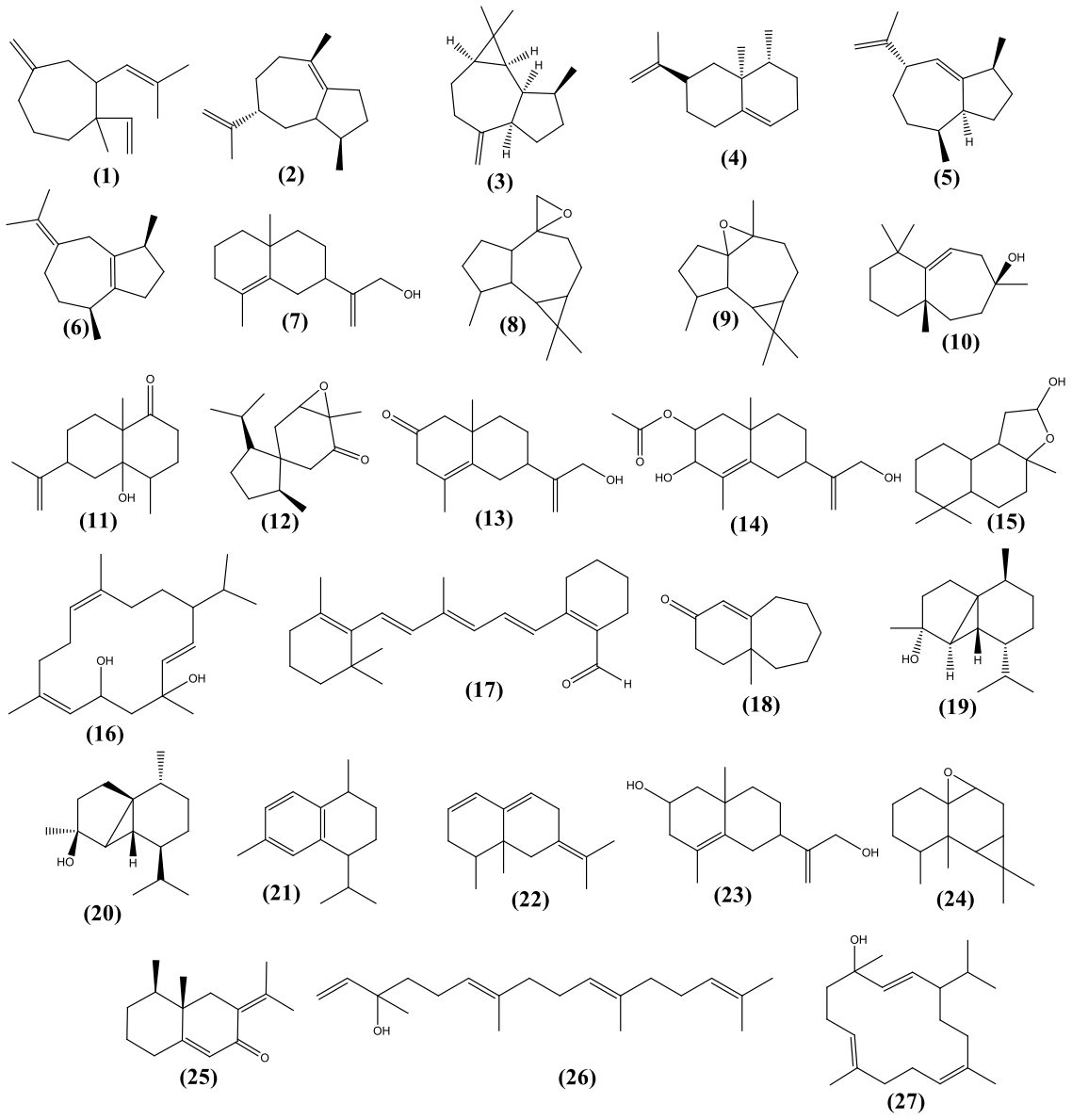
(15)					$[C_{14}H_{23}]^+$, 164 (37) $[C_{12}H_{20}]^+$, 109 (100) $[C_8H_{13}]^+$.
2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde **(17)**	28.52	$C_{23}H_{32}O$	0.47		324 (1) $[M^+]$, 177 (11) $[C_{13}H_{21}]^+$, 149 (69) $[C_{11}H_{17}]^+$,135 (100) $[C_9H_{11}O]^+$, 121 (55) $[C_9H_{13}]^+$, 107 (79) $[C_8H_{11}]^+$.
Total			2.27	3.18	



In-vitro antitumor assay on human colorectal and prostate carcinoma (HCT116 and PC3 cell lines cytotoxicity)

As is shown in figure 1*, N. molle* fractions exhibit a promising cytotoxic activity against both human colorectal and prostate carcinoma (HCT116 and PC3 cell lines) ranging from moderate to highercompare to cisplatin 15µg/ml as reference cytotoxic compound. For prostate carcinoma cell lines *N. molle* fraction 1 showed the highest cytotoxicity percent (67%) which is a little lower than Cisplatin (78.4%) , followed by *N. molle*fraction 2which showed lower cytotoxicity percent (54.60 %) while crude extract showed the lowest cytotoxicity percent (40.94%). For human colorectal cell line (HCT116), *N. molle* fraction 1 showed the highest cytotoxicity percent (90.46%) which is higher than Cisplatincytotoxicity percent (85%), followed by *N.*

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*molle*fraction 2 which showed lower cytotoxicity percent (60.12%) while crude extract showed the lowest cytotoxicity percent (41.59%).

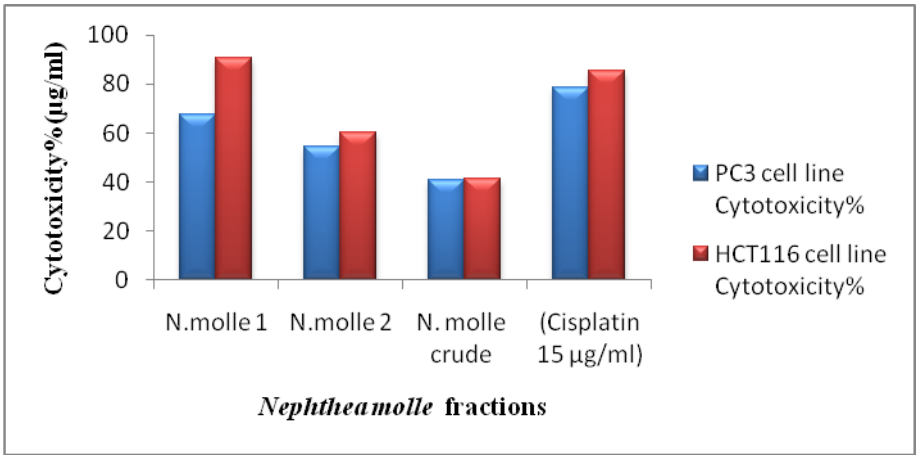

Figure 1: Cytotoxicity of non-polar fractions of N. molle on HCT116 and PC3 cell lines

Antimicrobial activity

As shown in Figure 2, the antimicrobial activity for *Nephthea molle* fractions fluctuated from the highest activity towards *S. iniae* and *S. aureus* to the moderate antibacterial activity for *P. aeruginosas* and finally the lowest one towards *E. coli.* Fraction 2 showed broad anti-bacterial activity toward all pathogenic bacteria. For *S. iniae* both fractions 1 and 2 showed the highest antimicrobial activity (20 mm) followed by crude extract (17 mm). Fraction1 showed the highest inhibition zone (22 mm) for *S. aureus* followed by fraction 2 (17 mm), whereas crude extract showed the lowest antimicrobial activity values of 14 mm. While fraction 1 showed moderate antimicrobial activity toward *P. aeruginosa* (about 19 mm), both crude and fraction 2 showed the lowest antimicrobial activity values of 14 mm and 16 mm. Fraction 2 showed antimicrobial activity of (11 mm) toward *E. coli* which was very low. The rest of the tested fractions showed no antimicrobial activity.

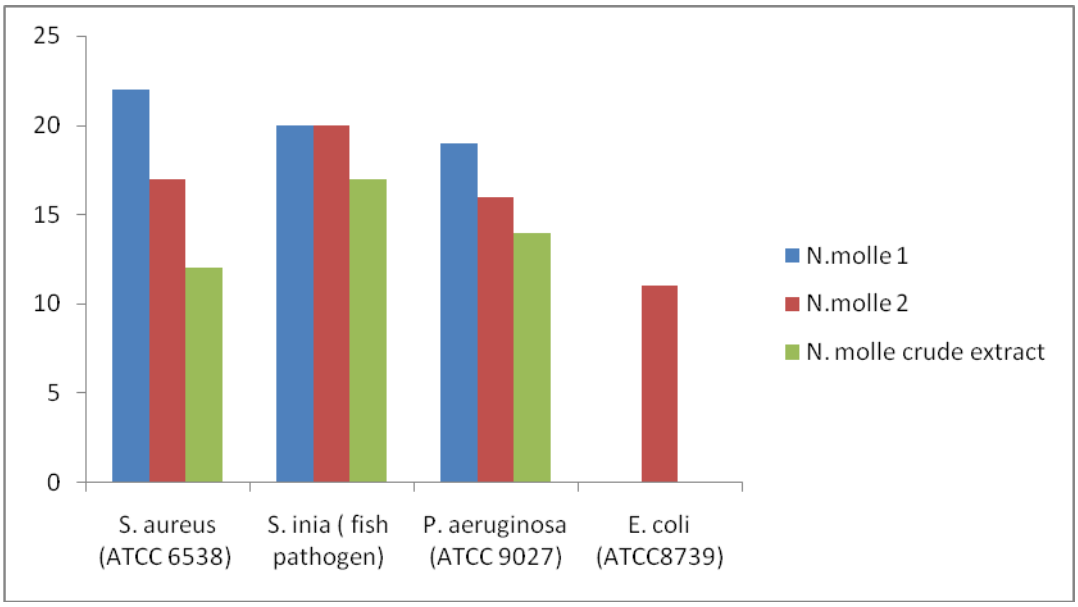

Figure 2: Antimicrobial activity of non-polar fractions of *N. molle* as well as crude extract

Minimum inhibitory concentration for the highly active extracts

The minimum inhibitory concentration of promising fractions that recorded the highest antimicrobial

activity was carried out. Fraction 1 showed MIC against *S. aureus*, *S. iniae* and *P. aeruginosa*recording 62.5µg/ml, 62.5µg/ml and 1000 µg/ml respectively, where MIC of fraction 2 against *S. aureus* and *S. iniae*was (2000 µg/ml). On the other hand, crude extract recorded MIC value (125 µg/ml) against *S. iniae*.

From the results in table1, *N. molle* fraction 1showed a significant relative percentage of Sesquiterpene (23.8%) more than *N. molle* fraction 2 (11.6%) indicating that their percentage may responsible for their highly antimicrobial against Gram positive strains as well as , their highly significant cytotoxicity againstPC3 human prostate carcinoma, and HCT116 human colorectal carcinoma. This finding is highly in agreement with those reported by Zhang *et al.* [13] who attributed that Sesquiterpene lactone is responsible for the anti-cancer function of Sesquiterpene obtained from both in vitro cell culture and in vivo animal models.

CONCLUSION

Investigation of Red Sea soft coral (*Nephthea molle*) led to identification of 35 compound using GC/MS techniques; the majority of them were seseqiterpenes. Non-polar fractions of *N. molle* exhibited promising cytotoxicity against both human colorectal and prostate carcinoma (HCT116 and PC3 cell lines). In addition, promising antibacterial activity towards *P. aeruginosa*, *S. iniae* and *S. aureus* was proven. The minimum inhibitory concentration (MIC) of these fractions were also assessed.

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