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Original Research Article

Chemical Compositions and Anticancer Activity of Yemeni Plant *Flemingia grahamiana* Wight & Arn. and *Myrtus communis* L.

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ABSTRACT

Cancer remains one of the world's leading causes of death so, there is considerable scientific interest in the continuing discovery of new anticancer products from natural sources. In the present study chemical composition of methanol extracts of *F. grahamiana* and *M. communis* was assessed by Gas chromatography-mass spectrometry (GC-MS). In the GC-MS analysis of methanol extracts, 21 phytochemical compounds were identified in *F. grahamiana*, and 20 compounds in *M. communis*. The major constituents in *F. grahamiana* were Cyclohexasiloxane, dodecamethyl- (21.24%), Benzoic acid, 2-(dimethylamino) ethyl ester (11.79%), and Cycloheptasiloxane, tetradecamethyl (11.06%), and in *M. communis* were Phenol, 3,4-dimethoxy-(24.41%), 3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo [4.1.0]hept-1-yl)- (17.44%), and 2-Propanamine,N-(1-methylethyl)-N-nitroso-(8.92%). Further, the anticancer activity of methanol and aqueous extracts of these plants was estimated against MCF-7 cell lines by the MTT method *In vitro*. Extracts showed a potent anticancer effect in MCF-7 cells. The highest anticancer activity was recorded of aqueous and methanol extracts of *M. communis* with IC_{50} 20.49 ± 0.29 and 22.10 ± 0.22 µg/ml, respectively. But extracts *F. grahamiana* showed moderate anticancer activity and need concentrations of more than 25 µg/ml to inhibit the growth of 50 % of cells. Our study suggests a promising potential for these plants, as anticancer. However, further studies including pharmacological evaluation, and *in vivo* anticancer activity are required.

Keywords: GC-MS analysis, Anticancer, *Flemingia grahamiana*, *Myrtus communis.*

Introduction

Cancer is a very serious health concern as it is growing in prevalence daily, no satisfactory medical therapy option available until now.[1,2] Despite many studies on cancer worldwide, the majority of drugs used in the treating of cancer are extremely hurtful to both cancer cells and host normal cells. Side effects such as gastrointestinal ulceration, alopecia (hair loss), immune suppression, fertility impairment, and blood dyscrasias are unavoidable in cancer patients on chemotherapy.[3]

Nowadays, scientists focus on discovering new anticancer drugs from natural sources where more than 60 % of commonly used anticancer compounds are extracted from these sources.[4-6] Chemical compounds deriving from plants have been used to remedy human diseases since the dawn of ancient medicine. Through the past 30 years, the natural products have gained growing attention for their potential as novel preventative and therapeutic factors for cancer.[4,7]

In Yemen, traditionally people depend on medicinal plants to treat many diseases example microbial infection, inflammation, etc.[8] Pharmacological and chemical properties of some traditionally used medicinal plants were assessed.[9-11] So, we highlighted two medicinal plants in Yemen, *Myrtus communis* and *Flemingia grahamiana*. Our previous studies reported the antibacterial and antioxidant activities of *F. grahamiana* and *M. communis*.[12,13]

And, this study aimed to identify the chemical compounds of *Flemingia grahamiana* Wight & Arn and *Myrtus communis* L. extracts by GC-MS and evaluate their anticancer activity on MCF-7 cell lines using the MTT method.

Materials and Methods

Plant materials

The pods of *F. grahamiana* and leaves of *M. communis* were obtained from Yafa area, Yemen in October 2019. And they were authenticated.[12] (Authentic No. of *Flemingia grahamiana* and *Myrtus communis* are MACH- 01264 and MACH- 01265). The selected parts of plants were dried at room temperature. The leaves were ground to powder and the pods were rubbed to get Warrus powder

Preparation of the extracts

The methanol extracts of leaves and Warrus powder were prepared by a Soxhlet device and the aqueous extracts by a magnetic stirrer as described in.[12-14]

GC-MS analysis

GC-MS study carried out at CIL, Panjab University Jalandhar- India. Analysis was performed on a GC-MS TSQ 8000 by Thermos Fisher Scientific Inc., (Waltham, USA) equipped with TG 5MS (30 m x 0.25 mm, 0.25 µm) column was used for separation. The temperature of the injector was 250°C. Oven temperature was controlled to 80°C for 2 min. and increased to 260°C at a rate of 10°C/min and programmed to

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the final temperature for 10 min. Helium was the carrier gas used at flow rates of 1 ml/min. MS spectra were obtained at range width m/z 50-650, injection Volume 1 µL, run time were 24.08 and 24.11 for *F. grahamiana* and *M. communis*, respectively. Scans were 7080 and 7088 for, *F. grahamiana* and *M. communis*, respectively. Peaks were identified by comparing data with NIST library.[15]

Anticancer activity
Cell culture
MCF-7 breast cancer cells were provided by National Center for Cell Science (NCCS), Pune. Cells were grown in Eagles Minimum Essential Medium (EMEM) with 10% FBS at 37°C, 5% CO_2 and 95% air in a humid incubator. Monolayer cells were detached using trypsin/EDTA (ethylenediamine tetra-acetic) then reseeded. The medium is changed twice a week.[16]

MTT assay
Viable cells were calculated using a hemocytometer, and plated in 96-well microtiter plates were (5×10^3 cell/well) and incubated for 24 h. Culture medium was used to dilute extracts stock solution (50 mg/mL in methanol or water). The required final extracts concentrations were 2.5, 5, 10, 15 and 25 µg/ml. The medium was replaced by a fresh medium containing final concentrations of extracts. Cells were re-incubated at 37°C, 5% CO_2 for a further 48 h, then 20 µl of MTT reagent (5 mg/mL in PBS) was added to each well. After incubated for an additional, 4 h, the medium containing MTT was discarded, and replaced by100 µl of DMSO to dissolve MTT (Formazan crystals) and left for 10 min. at room temperature. Finally, the absorbance was measured at 570 nm using a microplate reader.[17] The cell inhibition % was calculated as the following.
Cell Inhibition % = 100- Absorbance $_{(sample)}$ / Absorbance $_{(control)}$)] x100.

Statistical analysis
The absorbance results were expressed as mean ± SD of three replicates of each concentration and analyzed by One- way ANOVA (SPSS software). The IC_{50} values were calculated by linear regression using Excel Microsoft.

Results and Discussion

The results obtained by GC–MS analysis of methanol extracts of *Flemingia grahamiana* (Warrus powder) and *Myrtus communis* (leaves) are presented in Tables 1 & 2, respectively with retention time (RT), peak area (%), name of compounds and molecular weight (MW), and Figures 1 &2. Twenty-one and twenty compounds were identified in methanol extracts of *F. grahamiana and M. communis*, respectively.
GC–MS analyses, showed that *F. grahamiana* is composed of contained Cyclohexasiloxane, dodecamethyl- (21.24%), Benzoic acid, 2-(dimethylamino) ethyl ester (11.79%), Cycloheptasiloxane, tetradecamethyl (11.06%), 2-Pentanol,3-methyl- (7.38%), as major compounds. The Most chemical components of *M. communis* were Phenol, 3,4-dimethoxy-(24.41%), 3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- (17.44%), 2-Propanamine,N-(1-methylethyl)-N-nitroso-(8.92%), and Durohydroquinone (6.36%).
According to Chryssavgi *et al*.,[18] leaves of *M. communis* contain several chemical compounds, for example, 1,8-cineole, linalyl acetate, *α*-Terpineol, that agree with our results, but also different in other compounds. Several studies indicated that the chemical compounds of medicinal plant change as a function of the region. This may be due to environmental factors including temperature, geography, day length, nutrients, etc. [19] More studies are likely to be required at the level of gene expression to find out the reasons for phytochemical diversity, variance in the number of phytochemicals and differences in plant medicinal possibility from region to region. [20]
Among identified phytochemical compounds that have a wide range of Pharmacological Activities Cyclohexasiloxane, dodecamethyl- which has antimicrobial and anti-fungal properties.[21-23] cycloheptasiloxane, tetradecamethyl compound, which has potent antibacterial, anti-fungal, sterilizer agents, cosmetics agent, Moisturizers, antiperspirants, and deodorants.[21,22] Also, *β*-caryophyllene showed pharmacological activity, including anticancer and analgesic properties, [24] antibacterial, antifungal, and anticancer, [25] anticancer, antioxidant, and antimicrobial.[26]
Sitarek *et al*.,[27] suspected the essential oils of *L. sibiricus* have antimicrobial, anticancer, anti-inflammatory, and antioxidant due to the main compounds identified (E)-*β*-caryophyllene and germacrene D. However, this may be due to the synergistic effect of the oil components. Hexadecanoic acid, methyl ester also showed anti-inflammatory, anticancer hypocholesterolemic, hepatoprotective, nematicide, insectifuge, anti-acne, antihistaminic, anti-eczemic, and anti-androgenic. [28]
The biological effects of the Eucalyptol compound were antimicrobial antimalarial and anti-leishmanial activities,[29] anti-inflammatories.[30] Linalyl acetate has properties of antioxidant and anticancer activities[31] Durohydroquinone was reported to have antimicrobial activities.[32] *α*-Terpineol have several Pharmacological activities such as anticonvulsant, sedative antinociceptive, anticancer and antifungal. [33] 4,4,8-Trimethyltricyclo [6.3.1.0 (1,5)] dodecane 2,9-diol which has anti-inflammatory activities.[34]

Anticancer activity
The effect of plant extracts in growth inhibition MCF-7 cells was evaluated by MTT assay. The results showed that all the plant extracts inhibited breast cancer cells' growth in dose-dependent (Table 3 and Figures 3). The highest percentages of inhibition were 52.01% and 53.38% for methanol and aqueous extracts of *M. communis* at 25 µg/ml concentration with IC_{50} 22.10 ± 0.22 and 20.49 ± 0.29 µg/ml, respectively. Methanol and aqueous extracts of *F. grahamiana*, showed moderate anticancer activity and need concentrations of more than 25 µg/mL to inhibit the growth of 50% of cells. (Table 3 and Figure 3).
Methanol and hot aqueous extracts of *Myrtus communis* were evaluated for their anti-tumor effects on Epithelial 5637 and MCF-7 cell lines and IC_{50} values were as >50 µg/ml.[11] Romeilah,[35] reported that LC_{50} values of *Myrtus communis* essential oil were 104.55 and 137.01 µg mL^{-1} against HL-60, and NB4 cell lines, respectively. *In vitro* cytotoxicity of several extracts of *M. communis* was evaluated against MDA-MB-231 and MCF 7 cell lines by MTT and SRB methods. The extracts showed cytotoxic potential in breast cancer with IC_{50} values ranged were 7 - 138 µg/ml.[36] According to Tretiakova *et al*.,[1] the EC_{50} values of *M. communis* were range 3.11 to 50 (µM) of viable cells of each cancer cell type. And the IC_{50} values of *M. communis* essential oil were 58.30, 104.55 and137.01 µg ml^{-1} against EACC, HL-60 and NB4 cell lines respectively.[32] The calculated IC_{50} values vary widely between studies. This high variability is most likely due to differences in the chemical nature of plant between geographical areas, as well as differences in the extent and mechanism of their action.[37]
According to Gumula *et al*.,[38] the compounds isolated from leaves of *F. grahamiana* were examined against MCF-7 cells line. These compounds possess the significant anti-tumor activity and IC_{50} values were ranging from 7.6 to 317.40 µM/ml. The phylogenetic tree revealed that Flemingia and Rhodamnia species are neatly related to Ashwagandha, implying the anticancer properties of Rhodamnia and Flemingia from Sumatra.[39] There are no other reports regarding the anticancer activities of *F. grahamiana*

Conclusion

This study found out that *F. grahamiana* and *M. communis* contain a variety of phytochemical compounds. Methanol and aqueous extracts of *M. communis* and *F. grahamiana* showed good anticancer activity against MCF-7cell lines. Future works are required for pharmacological evaluation of both plants and experiment their effects against different cancer cell lines *in vivo* and *in vitro*.

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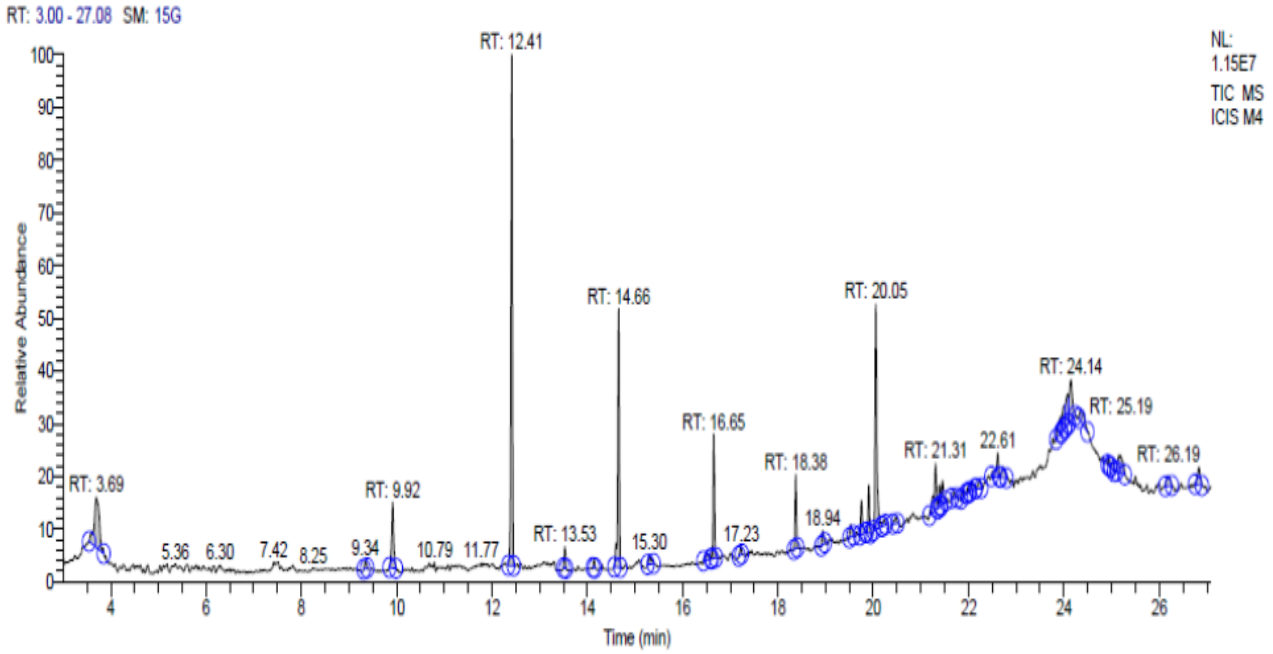

Figure 1: GC-MS Chromatogram of *F. grahamiana* (Warus).

Table 1: Chemical compounds of methanolic extract of *F. grahamiana* (Warrus) by GC-MS

No.	RT (min)	Area %	Molecular formula	Compound name	Mol. wt.
1	3.69	7.38	$C_6H_{14}O$	2-Pentanol,3-methyl-	102
2	9.34	0.42	$C_{11}H_{24}$	Decane, 3-methyl-	156
3	9.92	3.95	$C_{10}H_{30}O_5Si_5$	Cyclopentasiloxane, decamethyl-	370
4	12.41	21.24	$C_{12}H_{36}O_6Si_6$	Cyclohexasiloxane, dodecamethyl-	444
5	13.53	0.80	$C_{15}H_{24}$	α-Cubebene	204
6	14.14	0.32	$C_{15}H_{24}$	β- Caryophyllene	204
7	14.66	11.06	$C_{14}H_{42}O_7Si_7$	Cycloheptasiloxane, tetradecamethyl	519
8	15.29	0.75	$C_{15}H_{24}$	Germacrene D	204
9	16.51	0.73	$C_{15}H_{24}O$	12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8 tetramethyl-, (1R,3E,7E,11R)-	220
10	18.38	2.82	$C_{18}H_{54}O_9Si_9$	Cyclononasiloxane, octadecamethyl	667
11	18.94	0.71	$C_{27}H_{54}O_4Si_2$	1-Monolinolein, 2TMS derivative	498
12	19.52	0.75	$C_{19}H_{38}O$	2-Nonadecanone	282
13	19.75	1.75	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	270
14	19.91	1.89	$C_{12}H_{36}O_4Si_5$	Trisiloxane,1,1,1,5,5,5-hexamethyl-3,3 bis[(trimethylsilyl)oxy]-	384
15	20.05	11.79	$C_{11}H_{15}NO$	Benzoic acid, 2-(dimethylamino)ethyl ester	193
16	21.31	3.38	$C_{14}H_{42}O_5Si_6$	1,1,1,3,5,7,7,7-Octamethyl-3,5-bis(trimethylsiloxy) tetrasiloxane	458
17	21.39	1.11	$C_{27}H_{52}O_4Si_2$	9,12,15-Octadecatrienoicacid,2,3-bis[(trimethylsilyl)oxy] propyl ester,	496
	25.19	2.32		(Z,Z,Z)-	
18	21.45	1.34	$C_{18}H_{34}O_2$	6-Octadecenoic acid, (Z)-	282
19	21.69	0.43	$C_{19}H_{34}O_6$	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	358
20	24.08	2.36	$C_{20}H_{16}N_2O_2$	Benzamide, N,N'-1,4-phenylenebis-	316
21	24.14	3.63	$C_{23}H_{40}$	n-Heptadecylbenzene	316.

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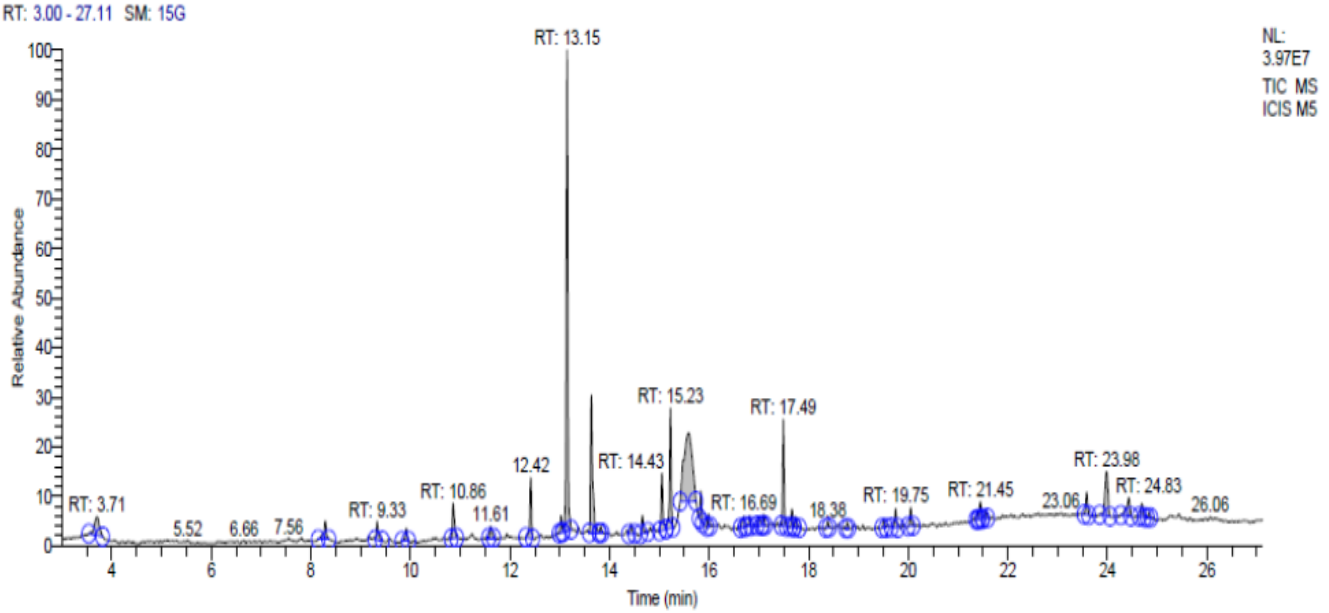

Figure 2: GC-MS chromatogram of *M. communis* leaves.

Table 2: Chemical compounds of methanolic extract of *M. communis* (leaves) by GC-MS

No.	RT (min)	Area %	Molecular formula	Compound name	Mol. Wt.
1	3.71	2.90	$C_6H_{14}O$	2-Pentanol,3-methyl-	102
2	8.29	1.49	$C_{10}H_{18}O$	Eucalyptol/1,8-Cineole	154
3	9.33	1.28	$C_{12}H_{20}O_2$	Linalyl acetate	196
4	10.86	2.24	$C_{10}H_{18}O$	α-Terpineol	154
5	11.61	0.79	$C_{10}H_{16}$	(+)-3-Carene	136
6	13.02	0.77	$C_{13}H_{22}O_2$	3-Cyclohexene-1-methanol, α,α,4-trimethyl-, propanoate	210
7	13.15	24.41	$C_8H_{10}O_3$	Phenol, 3,4-dimethoxy-	154
8	13.63	8.92	$C_6H_{14}N_2O$	2-Propanamine,N-(1-methylethyl)-N-nitroso-	130.
9	13.83	0.30	$C_{14}H_{26}O$	2-Nonenal,-2pentyl-	210
10	15.23	6.36	$C_{10}H_{14}O_2$	Durohydroquinone	166
11	15.59	17.44	C13H20O2	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	208
12	17.00	0.68	$C_{15}H_{20}O$	Ar-turmerone	216
13	17.10	0.56	$C_{12}H_{22}O$	4a(2H)-Naphthalenol,octahydro-4,8a-dimethyl-,(4α,4aα,8aβ)-	182
14	17.66	0.80	$C_{14}H_{28}$	3-Heptene,2,2,3,5,5,6,6heptamethyl-	196
15	18.38	0.29	$C_{19}H_{34}O_6$	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	358
16	18.77	0.26	$C_{11}H_{18}O_2$	2(3H)-Benzofuranone,hexahydro4,4,7atrimethyl	182
17	19.51	0.47	$C_{15}H_{26}O_2$	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane2,-9diol	238
18	19.75	1.10	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester/[Palmitic acid, methyl ester;]	270
19	20.05	0.91	$C_{11}H_{15}NO_2$	Benzoic acid, 2 (dimethylamino) ethyl ester	193
20	21.56	0.52	$C_{27}H_{46}O_2$	Eicosanoic acid, phenylmethyl ester	402

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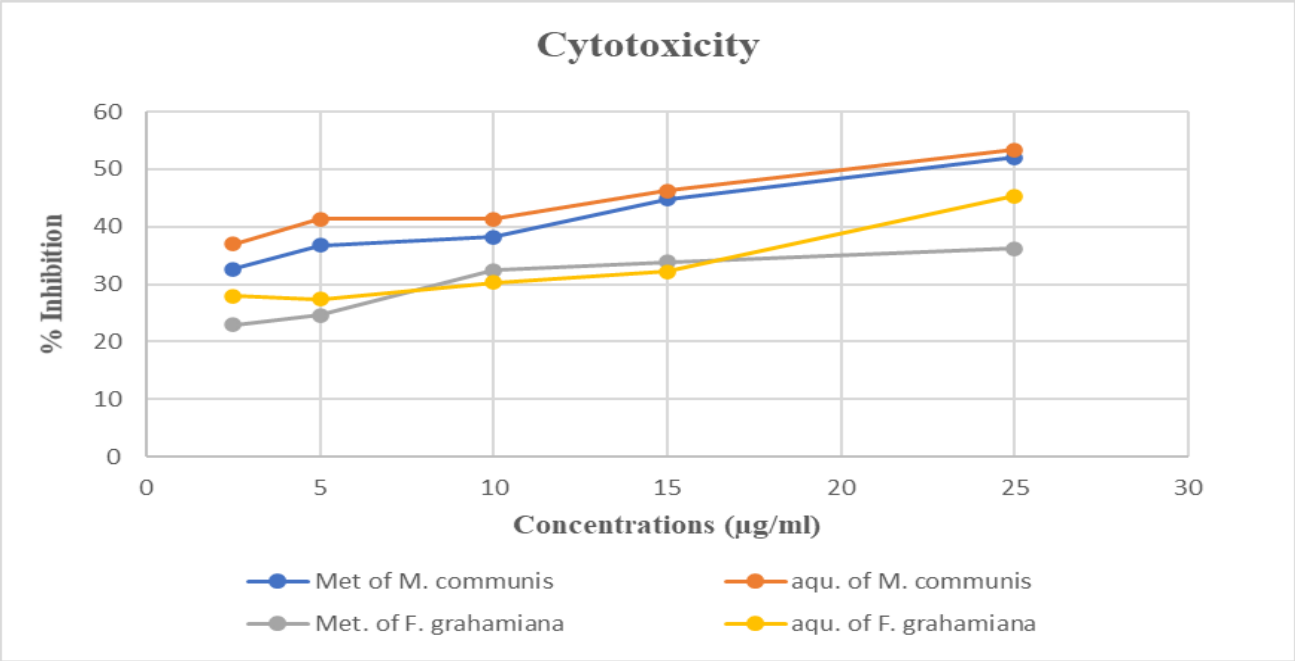

Figure 3: Percentage of cell growth inhibition of methanol and aqueous extracts of *F. grahamiana* and *M. communis* against MCF-7 cell lines.

Table 3: Percentage of cell growth inhibition and IC_{50} of extracts of *F. grahamiana* and *M. communis* against MCF-7 cell lines by MTT assay

Concentrations (µg/ml)	Extracts (% of growth inhibition)			
	Myrtus communis		*Flemingia grahamiana*	
	Methanol extract	aqueous extract	Methanol extract	aqueous extract
2.5	32.69	36.96	22.95	27.94
5	36.72	41.39	24.64	27.46
10	38.25	41.39	32.45	30.32
15	44.85	46.22	33.90	32.22
25	52.01	53.38	36.23	45.32
		(IC_{50} ± SD)		
	22.10 ± 0.22	20.49 ± 0.29	-	-

Results are mean ± SD (n = 3). - = IC_{50} hasn't calculated because % growth inhibition less than 50% at 25 µg.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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