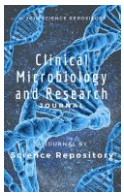

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Research Article

Evaluation of Anticancer Activities of *Clerodendrum formicarum* Gurke (Lamiaceae) and *Syzygium Cordatum* Hochst ex Krauss (Myrtaceae) Harvested in the City of Lubumbashi and its Surroundings in the Democratic Republic Of The Congo

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ABSTRACT

The main objective of this study was to extract the essential oils from *Clerodendrum formicarum and Syzygium cordatum* in order to determine the anticancer activity and establish the chemical constituents of that kind of essential oils. Hence, we have used hydrodistillation method to obtain the desired extracts or samples of essential oils. Indeed, the assessment of the anticancer activities showed that, at 100 µg/mL, the essential oils from *S. cordatum* inhibited the proliferation (98.2%) of breast cancer (MCF-7) cells. In the same context, the essential oils from *C. formicarum* also inhibited the proliferation (98.9%) of breast cancer (MCF-7) cells. We have additionally determined the chemical composition of the essential oils by GC-MS and identified 17 compounds in *C. formicarum* including caryophyllene (18.6%), (*E*)-3,7-dimethylocta-1,3,6-triene (7.6%), β-Myrcene (6.2%), and [1S-(1α, 7α, 8α)]-1,8a-dimethyl-7-(1-methylethenyl)-1,2,3,5,6,7,8,8a-octahydronaphthalene (5.6%) as major compounds. We have observed an unknown compound (25%) in the essential oils from *C. formicarum* (H-12) The chemical analysis showed 28 compounds in *S. cordatum* (HE-9) essential oils such as caryophyllene (7.2%) and cedrene (6.7%) as major compounds.

Introduction

Essential oils are volatile organic compounds found in plants. They are often extracted from flowers, leaves, roots, fruits, and seeds. The chemical constituents of essential oils are diverse; for example, alkaloids, terpenoids, and phenolic substances are found in different varieties of essential oils [1, 2, 3]. To the best of our knowledge, the antiproliferation of breast cancer (MCF-7) cells due to the action of essential oils from *S. cordatum*, and *C. formicarum* has not yet been reported in the literature. Recently, a review has highlighted several biological activities of *S. cordatum* such as antibacterial, antifungal, antidiarrheal, activity against sexually transmitted infections, antidiabetic, anticholinesterase, anti-inflammatory, antileishmanial, antioxidant, antiplasmodial and antiproteus [4]. Similarly, it has been reported that the biological activities of *Clerodendrum* genus include anti-inflammatory, antinociceptive, antioxidant, antihypertensive, anticancer, antimicrobial, antidiarrheal, hepatoprotective, hypoglycemic, hypolipidemic, memory enhancing, and neuroprotective [5].

Research on cancer drugs is currently vital because cancer is a real public health problem worldwide. It is the second leading cause of death in

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developed countries and it represents a major health problem in developing countries [6, 7]. The American Cancer Society estimates, in 2020, more than 1.5 million the number of new cases, and it estimates at 606,520 the number of patients who may die of their cancer the same year in the United States [8]. The Canadian society of cancer has reported that 225,800 new cases of cancer (including 29,800 cases of lung cancer, 27,400 cases of breast cancer, and 23,300 cases of prostate cancer) and 83,300 cancer deaths are likely to happen in Canada in 2020 [9].

In developing countries, cancer is emerging as a leading cause of death. In those countries, the number of new cases of cancer between the years 2000 and 2020 is estimated at 150 million [10]. It is, therefore, important for these countries to have cancer control plans using natural resources, for instance, natural resources of medicinal plant origin [11]. However, even in countries with cancer control plans already in place, the survival rate remains uncertain [12]. Biomedical cancer treatments are based on surgery, radiation therapy, immunotherapy, hormone therapy as well as chemotherapy. All of these treatments are costly for the majority of cancer victims in both developed and developing countries [13]. Our group is interested in the study of natural products to discover novel anticancer organic compounds. In that perspective, we have singularly undertaken the study of the essential oils extracted from *Clerodendrum formicarum*, and *Syzygium cordatum* to investigate their antiproliferative activity on breast cancer (MCF-7) cells, and their chemical composition as well.

Experimental Procedures

I Plant Materials

We have harvested the plant species from different sites in the city of Lubumbashi and its surroundings. The two plant species were identified by comparison with the reference herbarium of the National Institute for Agronomic Research (INERA) in Lubumbashi, and the Faculty of Agricultural Science of the University of Lubumbashi. All the samples studied were kept cool to maintain the plant materials as fresh as possible.

II Extraction of Essential Oils

We have used hydrodistillation method to extract essential oils. Indeed, 2000g of fresh plant materials were introduced into a 6 L flask containing 3 L of water and the mixture was distilled for 4 h. The condensed vapor or organic phase (essential oils) was separated from the hydrosol by decantation. The yields of essential oils were moderate (0.14% for *C. formicarum*), and 0.07% for *S. cordatum*). We noticed that the odors of the essential oils (HE-12) from *C. formicarium* were weak, while the essential oils (HE-9) from *S. cordatum* had very strong odors.

III Anticancer Activity Tests

Breast cancer MCF-7 cells (ATCC, Rockville, MD) were routinely grown in suspension in 90% RPMI 1640 (Roswell Park Memorial Institute) (Sigma, Saint Louis, MO) containing L-glutamine (2 nM), antibiotics (100 IU penicillin/mL, 100 µg streptomycin/mL), and supplemented with 10% (v/v) fetal bovine serum (FBS) at 37°C in a 5% CO_2 humidified atmosphere. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10% FBS. The cell viability assay was performed according to the procedure as described by Roy *et al*. (2007). In brief, triplicate cultures of 1×10^4 MCF-7 cells in the RPMI medium (100 µL) in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ) were incubated at 37°C in a 5% CO_2 humidified atmosphere. Each sample of essential oils was tested at different concentrations (0.1, 1, 10 and 100 µg/mL) and the aminosteroid RM-581 (reference) was used as a positive control. They were first solubilized in DMSO, diluted at appropriate multiple concentrations with culture medium, added to each well and incubated for 3 days. Control cells were treated with the medium and DMSO only (final DMSO concentration < 0.5%). Following each treatment, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS); (Cell Titer 96 Aqueous, Promega, Madison, WI) were added to each well and the culture was incubated for 4 h. MTS is converted to water-soluble colored formazan by dehydrogenase enzymes present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). The results are expressed as cell proliferation and the control (basal proliferation of culture medium + DMSO) is fixed at 100 %.

IV GC/MS Analysis of Essentials Oils

Extract oil solutions were prepared in dichloromethane with concentrations between 3390 and 4840 ppm (weight/volume). Each solution was injected (0.5µL) into a GCMS from Agilent (GC model 6890 and MS model 5973). The GC column used was a DB-5MS with an inside diameter of 0.25mm, a film thickness of 0.25µm and a length of 30m. The carrier gas was helium at a constant rate of 1mL/min. Injector split flow mode was used with a split ratio of 50:1 and a split flow of 50mL/min. Inlet temperature was 250ºC. Gradient temperature in the oven started at 50ºC, holding for 1 minute, then increased by 5ºC/min to 260ºC and held for 5 minutes. Total time taken for the process was 48 minutes. Solvent delay was 3 minutes and acquisition range mass started at 20amu and finished at 350amu with a scanning rate of 4.33scan/sec. Mass spectrum of each chromatogram peak was searched against the NIST databank.

Table 1: Cytotoxic effect of essential oils (HE-12, HE-9) on breast cancer cells (MCF-7) proliferation.

	First assay	SD	Second assay	SD	Average	SD
CTL	99.99	13.17	99.98	12.85	99.99	0.01
RM-581 (0,2 µg/mL)	103.70	4.99	108.76	10.82	106.23	3.58
RM-581 (2 µg/mL)	84.56	13.63	74.71	7.40	79.63	6.96
HE-12 (0,1 µg/mL)	106.39	10.35	92.68	19.12	99.53	9.69
HE-12 (1 µg/mL)	125.97	18.54	108.02	2.93	116.99	12.69
HE-12 (10 µg/mL)	126.79	16.26	107.56	6.20	117.17	13.60
HE-12 (100 µg/mL)	1.69	2.41	0.50	0.36	1.09	0.84
HE-9 (0,1 µg/mL)	114.42	13.70	99.31	12.13	106.86	10.69
HE-9 (1 µg/mL)	122.16	11.67	100.33	16.52	111.24	15.44
HE-9 (10 µg/mL)	111.48	17.07	105.35	13.76	108.42	4.34
HE-9 (100 µg/mL)	3.40	3.00	0.20	0.18	1.80	2.26

Results and Discussion

As a part of our ongoing research in natural products, we report herein our preliminary results, which displayed that essential oils (HE-12, HE-9) inhibited the proliferation of breast cancer (MCF-7) cells (Table 1, Figure 1). In fact, at only 100 µg/mL essential oils (HE-12) from *C. formicarum* killed 98.9 % of breast cancer (MCF-7) cells, and essential oils (HE-9) from *S. cordatum* killed 98.2 % of breast cancer (MCF-7) cells as well. These findings encouraged us to undertake further investigations.

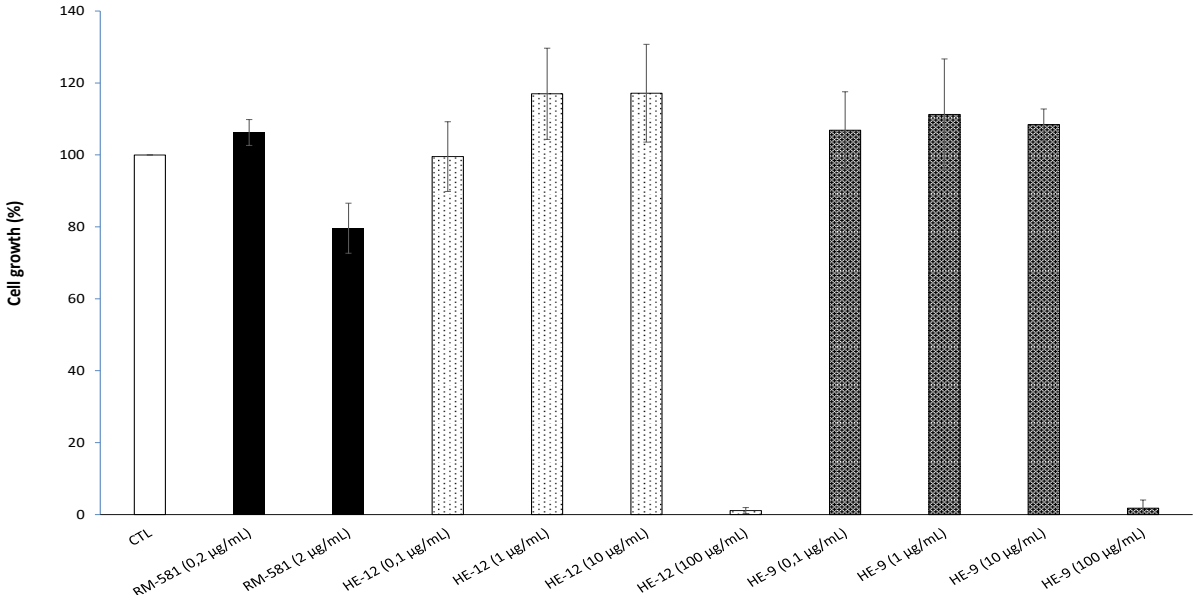

Figure 1: Cytotoxic effect of essential oils (HE-12 and HE-9) on breast cancer (MCF-7) cell proliferation.

The cytotoxic effect of essential oils (HE-12, HE-9) on breast cancer (MCF-7) cell proliferation could be due to the synergy of organic substances because the essential oils (HE-12, HE-9) were a mixture of several known and unknown organic compounds (Tables 2 & 3). That is why further investigations are needed to reveal the structures of the unknown compounds.

Table 2: Chemical composition of essential oils (HE-12) from *C. formicarum*.

Pic	RT (min)	Composition	%	Identification
1	6.58	α-pinene	2.4	GC/MS
2	7.75	β-pinene	0.9	GC/MS
3	8.03	β-myrcene	**6.2**	GC/MS
4	9.04	1-methyl-4-(1-methylethyl)benzene	1.6	GC/MS
5	9.33	(E) 3,7-dimethyl-1,3,6-octatriene	**7.6**	GC/MS
6	9.64	(Z) 3,7-dimethyl octa-1,3,6-triene	0.4	GC/MS
7	19.25	4-bis(1-methylethenyl)-l-ethenyl-1-methyl-2-cylohexane	1.7	GC/MS
8	20.06	caryophyllene	**18.6**	GC/MS
9	20.76	4-methylene-1,1,7-trimethyl decahydro-lH-cycloprop[e]azulene	1.4	GC/MS
10	20.97	α-caryophyllene	**3.5**	GC/MS
11	21.39	4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene-2-bopropenyl	2.9	GC/MS
12	21.46	epizonarene	2.3	GC/MS
13	21.82	[1S-(1α,7α,8aα)]-8a-dimethyl-7-(1-methylethenyl)-, - 1,2,3,5,6,7,8,8a-octahydro naphthalene	**5.6**	GC/MS
14	22.00	α-limonene	**4.2**	GC/MS
15	22.15	(1S-cis), 1,4-dimethyl-7-(1-ethylethylidene),1,2,3,4,5,6,7,8-octahydro azulene	**3.7**	GC/MS
16	22.54	(-)-α-panasinsene	1.2	GC/MS
17	24.05	1-methyl-6-methylenebicyclo [3.2.0] heptane	**4.0**	GC/MS

Regarding the quantity of organic compounds in the essential oils, they are only uncalibrated percentages, and not rounded to the nearest percentage. Indeed, GC-MS analysis identified 17 compounds in *C. formicarum* including caryophyllene (18.6%), (Z)-3,7-dimethylocta-1,3,6-triene (7.6%), β-myrcene (6.2%), and [1S- (1α, 7α, 8α)]-1,8a-dimethyl-7-(1-methylethenyl)-1,2,3,5,6,7,8,8a-octahydronaphthalene (5.6%) as major compounds. We have observed an unknown compound (25%) in the essential oils from *C. formicarum* (H-12). In the same context, GC-MS analysis showed 28 compounds in *S. cordatum* (HE-9) essential oils such as caryophyllene (7.2%) and cedrene (6.7%) as major compounds.

Table 3: Chemical composition of essential oils (HE-9) from *S. cordatum.*

Pic	TR (min)	Composition	%	Identification
1	11.32	(Z) 3,7-dimethyl octa-1,3,6-triene	0,6	GC/MS
2	14.45	1,8-dimethyl-1,2,3,4-tétrahydronaphthalene	2.2	GC/MS
3	17.62	4-carene	0.2	GC/MS
4	18.91	α-cubebene	0.9	GC/MS
5	19.29	1,2,3-trimethylindene	1.1	GC/MS
6	20.06	caryophyllene	**7.2**	GC/MS
7	20.27	3-methyl-6-(1-methylethylidene)cyclohexene	0.3	GC/MS
8	20.77	4-methylene-1,1,7-trimethyl-1H-cycloprop[e]azulene	1.7	GC/MS
9	20.97	α-caryophyllene	1.2	GC/MS
10	21.07	humulene	0.3	GC/MS
11	21.12	p-fluoroethylbenzene	0.4	GC/MS
12	21.97	cedrene-V6	**6.7**	GC/SM
13	22.14	(1S-cis) 1,4-dimethyl-7-(1-methylethylidene)-1,2,3,4,5,6,7,8-octahydroazulene	2.3	GC/MS
14	22.37	3-methyl-Pyrido[3,4-d]pyrimidin-4(3H)-one	0.2	GC/MS
15	22.48	4,7-dimethyl-1-(1-methylethyl)-1,2,4a,5,8,8a-hexahydro naphthalene	1.7	GC/MS
16	22.54	α-panasinsen	1.0	GC/MS
17	23.20	4-methylene-2,8,8-trimethyl-2-vinyl bicyclo[5.2.0]nonane	0.8	GC/MS
18	23.49	β-elemene	2.8	GC/MS
19	23.90	2-methyl-1,5-(4H)-dihydropyrido-(2,3-b)-1,4-diazepine-4-one	0.5	GC/MS
20	24.04	1-bopropenyl-3-propenylcyclopentane	1.1	GC/MS
21	24.57	caryophyllene	0.7	GC/MS
22	25.07	8,9-dehydronéoisolongifolene	2.3	GC/MS
23	25.18	(2R-cis) 1,2,3,4,4a, 5,6,7-octahydro-α,α,4a, β-tetramethyl naphthalenemethan-2-ol	1.9	GC/MS
24	25.44	2,6,6,9-tetramethyl tricyclo [5.4.0.0(2,8)] undec-9-ene	1.8	GC/MS
25	25.51	thujopsene	0.9	GC/MS
26	25.71	1,7-dimethyl-4-(1-methylethenyl)-octahydro-1,4-methano-1H-indene	**3.6**	GC/MS
27	25.79	neoisolongifolene	0.8	GC/MS
28	26.02	β-vatirenene	0.8	GC/MS

Conclusion

Cancer is a global public health problem. That is why, research for the discovery of new anticancer drugs with new modes of action is essential. In this regard, we have singularly undertaken the study of the essential oils extracted from *Clerodendrum formicarum* (HE-12) and *Syzygium cordatum* (HE-9), and the resulting observation exhibited that these plant species have potential organic compounds endowed with properties against breast cancer cells (MCF-7). These preliminary results encourage us to continue research on Congolese medicinal plants because they have not yet been documented to a significant extent. These plants constitute huge amounts of underexploited research materials from a scientific point of view. Congolese flora offers researchers a unique opportunity to make new discoveries for science, medicine, industry and the economy. This investigation reveals that monoterpenes are the main compounds of the essential oils from *C. formicarum* (HE-12) and *S. cordatum* (HE-9), and it is important to mention that monoterpenes are interesting organic compounds because of their bioactive potential [13].

Conflicts of Interest

None.

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