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Essential oil of *Ruta chalepensis* L. from Djibouti: Chemical Analysis and Modeling of *In Vitro* Anticancer Profiling

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Abstract: *Ruta chalepensis* L. (Rutaceae) is a tropical medicinal plant traditionally used in the Republic of Djibouti to treat several diseases, including tumors. In this study, the anticancer activities of this plant from Djibouti were investigated according to an in vitro evaluation method and statistical modeling. The results obtained will make it possible to complete the previous work already published on this genus of plant, in particular by using untested cancer cell lines, such as U87-MG, U2OS, RT4, PC3, NCI-N87, MRC-5, MIA-Paca2, K562, JIMT-T1, HEK293, HCT116, A549, and A2780. The main volatile compound turned out to be 2-undecanone (51.3%). Correlation modeling was performed from the principal component analysis (PCA) of IC_{50} of the essential oil and four active substances (vinblastine, doxorubicin, combrestatin A4, and monomethyl auristatin E) versus the cancer cell lines tested, which confirmed the effectiveness of the oil against 6 lines: U2OS, NCI-N87, MRC-5, MIA-Paca2, JIMT-T1, and HEK293. These data reveal promising prospects for good biomass management through the future exploitation of the *R. chalepensis* L. essential oil as a potential source of natural anticancer agents for targeted investigations.

Keywords: *R. chalepensis* L.; essential oil; chemical composition; cytotoxic activity; cancer; modeling

Citation: Abdoul-Latif, F.M.; Elmi, A.; Merito, A.; Nour, M.; Risler, A.; Ainane, A.; Bignon, J.; Ainane, T. Essential oil of *Ruta chalepensis* L. from Djibouti: Chemical Analysis and Modeling of *In Vitro* Anticancer Profiling. *Separations* **2022**, *9*, 387. https://doi.org/10.3390/separations9120387

Academic Editor: Stefania Garzoli

Received: 20 October 2022
Accepted: 17 November 2022
Published: 23 November 2022

1. Introduction

Nowadays, we are often faced with chronic health problems that have accompanied patients for several years; most diseases are not curable although they can be treated or better managed with the effective therapeutic arsenal we have [1–3]. Chronic diseases are present in 80% of patients who consult on an outpatient basis. According to the World Health Organization (WHO), nearly 40% of the population will be affected by chronic disease in the coming decades [4]. Almost all chronic diseases are more common in older people than in young adults, and in many cases, one person can suffer from several chronic diseases simultaneously [5]. The presence of multiple chronic diseases is associated with a decline in quality of life, personal health, mobility, and functional ability, as well as increases in hospitalization, use of health care resources, mortality, and costs. The use of multiple drugs, commonly referred to as polypharmacy, where one or more drugs can be used to treat each condition, is common [5,6]. However, polypharmacy is associated with adverse events such as mortality, adverse drug reactions, non-adherence to treatment, drug interactions, increased risk of hospitalization, and medication errors, which increase the complexity of treatment management for patients [7]. Despite the progress of pharmacology, the therapeutic use of medicinal plants remains present in some countries, especially in developing countries. According to the World Health Organization (WHO), nearly 80%

of the populations of developing countries in the African region use traditional medicinal plant-based medications [8–11].

The development of natural resources is a concern that is becoming increasingly important in many countries [12]. Thus, stakeholders recommend the evaluation of the safety and efficacy of plants that have traditional uses with a view to standardize their use and integrate them into conventional care systems [13]. The Republic of Djibouti, due to its geographical location, offers rich and diverse vegetation. A large number of aromatic and medicinal plants grow there spontaneously. Interest in these plants has continued to grow in recent years as their properties, due particularly to the essential oil fraction, can be used to treat certain chronic diseases [14–18].

In recent years, many studies have revealed the effectiveness of essential oils and their chemical constituents as a source of new bioactive natural products, including against cancer [19–23]. However, the mechanisms of action are still little studied or understood. Furthermore, their application in the pharmaceutical industry requires absolute pharmacodynamic specificity-selectivity.

On the other hand, the Rutaceae family, which includes more than 900 species divided into 150 genera, is distributed in tropical and temperate regions. The plants of this family are commonly used to treat certain microbial infections, inflammatory diseases, dermatological pathologies, and certain cancers [24–27]. Numerous studies have shown that the species of this family have anticancer properties against several cancerous lines. In particular, the two most abundant species of this family, such as *R. chalepensis* and *R. graveolens*, have shown positive effects in vitro, hence the results are promising for the treatment and prevention of cancer [28–30].

This paper aims to communicate the research on the medicinal plant *R. chalepensis* L. by studying the chemical composition of its essential oil, as well as its cytotoxic activity in cancer cell lines. This plant is one of the pharmacological and commercially significant medicinal plants in Djibouti and is a plant that is still utilized in traditional medicine. Furthermore, it contains significant amounts of secondary metabolites, including alkaloids, flavonoids, phenols, amino acids, furanocoumarines, and saponines, which have well-targeted pharmaceutical properties [31].

2. Material and Methods

2.1. Origin of the R. chalepensis L. Plant

Several *R. chalepensis* L. plants are found in the Foret de Day National Park of the Republic of Djibouti (11°45′03.6″ N 42°41′13.2″ E). The aerial parts were air-dried and partially powdered. The species was identified by the first author in collaboration with the ESTK-USMS team (Superior School of Technology of Khenifra, University of Sultan Moulay Slimane) and was classified in the herbarium of the Medicinal Research Institute (CERD) of Djibouti with the accession number RC105-2019.

2.2. Essential Oil: Extraction and Chemical Composition

The hydrodistillation method, using a Clevenger laboratory apparatus, was used to extract the essential oil from the aerial parts of the *R. chalepensis* L. plant. After 3 h of distillation, the pure distilled oil was stored in amber glass bottles in a freezer at −4 °C. The operation was repeated several times to collect the maximum amount of oil [32].

Gas chromatography coupled with a mass spectrometer equipped with a flame ionization detector (FID) (Agilent Technologies 7820, Santa Clara, CA, USA) was used to identify the chemical composition of the molecules present in the *R. chalepensis* L. essential oil (Figure 1) [33]. All components of the essential oil were quantified and identified by the relative peak percent area, retention time, and mass fragmentation pattern using the NIST mass spectral library.

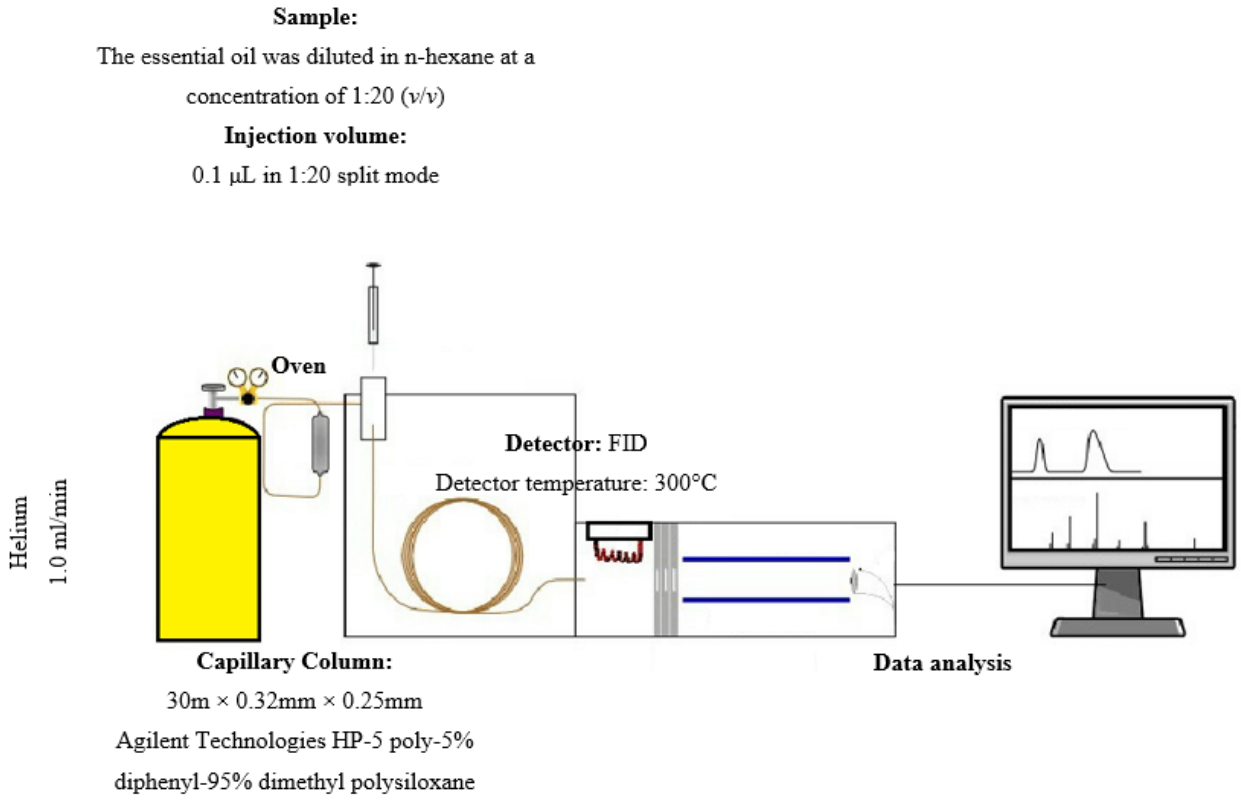


Figure 1. GC-MS analysis.

2.3. Cytotoxicity Activity

All cancer cell lines (U87-MG, U2OS, RT4, PC3, NCI-N87, MRC5, MIA-Paca2, K562, JIMT-T1, HEK293, HCT116, A549, and A2780) were obtained from the standardized collections of the European Collection of Cell Cultures (ECACC, Porton Down, UK), the American Type Culture Collection (ATCC, Rockville, MD, USA), and the German Collection of Microorganisms and Cell Cultures of the Leibniz Institute (DSMZ, Braunschweig, Germany). Ethical approval for this study was granted by the ethics committee of ESTK-USMS (Morocco) and genetic information was archived under accession number: 2019-1215-0003.

Cells were cultured in their appropriate media, supplemented with 10% fetal bovine serum, 1% glutamine, and 1% penicillin/streptomycin, and maintained at 5% CO_2. Cultures were checked daily using an inverted microscope to assess the absence of microbial particles.

R. chalepensis L. essential oil was tested in different concentrations (0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 µg/mL); 10 µL of each concentration was added to 90 µL of culture medium containing the cancer cells. After incubation for 72 h, 100 µL of CellTiter Glo reagent was added to each well and incubated for 15 min. Luminescence was recorded using a spectrophotometer. The concentration–response curves were generated with EXCEL software and the 50% inhibition concentration values (IC_{50}) were calculated from polynomial trend curves of the fourth degree [34].

Vinblastine, doxorubicin, combrestatin A4, and monomethyl auristatin E (Sigma-Aldrich, St. Louis, MO, USA) were used as a positive control.

2.4. Statistical Analysis

The calculations of all tests were statistically evaluated using the XLSTAT software 2016 (associated with EXCEL). Values are presented as mean ± uncertainty at a 5% significance level of three replicates for each experiment using the Student's *t*-test.

Principal component analysis (PCA) is a mathematical tool to study the correlation between several parameters [35]. The objective was to visualize and summarize the information concerning the effectiveness of the samples tested, including the essential oil,

vinblastine, doxorubicin, combrestatin A4, and monomethyl auristatin E, showing the different values obtained in relation to cancer cell lines to have a summary representation allowing easier interpretation. More precisely, PCA allowed the grouping of the cell lines studied, which were linked by the active product.

Coding was performed according to the obtained IC_{50} value of each sample in the cancerous cell lines. The numeric values of this model are shown in Table 1.

Table 1. Numerical coding of the 50% inhibition concentration (IC_{50}).

Concentration (µg/mL)	[0; 1]	[1; 5]	[5; 10]	[10; 20]	[20; 100]	>100
Code	5	4	3	2	1	0

3. Results

The yield of essential oil obtained for *R. chalepensis* L. was 0.08%. GC-MS was used to determine the chemical analysis of the constituents present in the extracted *R. chalepensis* L essential oil, which are presented in Table 2. The GC-MS results showed that 28 compounds represent 99.9% of the overall composition of the essential oil, with 2-undecanone being the most abundant compound at a percentage of 51.3%. Moreover, two other compounds, octyl acetate and 2-acetoxytetradecane, were moderate constituents of the essential oil at 17.3% and 7.1%, respectively.

Table 2. Chemical composition of the *R. chalepensis* L. essential oil.

Pic	RT	Compounds	Relative Percentage (%)
1	8.57	2-nonanone	2.8
2	9.52	Cyclohexene, 3,4-diethenyl-3-methyl-	1.0
3	10.5	2-decanone	0.8
4	10.82	Octyl acetate	17.3
5	11.55	Piperitone	0.4
6	11.92	Chloromethyl octyl ether	0.2
7	12.13	2-undecanone	51.3
8	12.26	2-undecanonol	1.9
9	12.38	Stearic acid	0.3
10	12.67	2-acetoxytridecane	1.0
11	13.15	2-dodecanone	2.8
12	13.68	1-Methyl-2-decalone	0.4
13	13.93	Cis-caryophyllene	1.1
14	14.02	2-acetoxytetradecane	7.1
15	14.49	Bicyclosesquiphellandrene	0.3
16	14.58	5-ketobornyl acetate	0.6
17	14.74	Germacrene D	0.9
18	14.9	2-tridecanone	1.0
19	15.59	B- elemol	1.2
20	15.71	Nerolidol	0.3
21	16.03	Caryophyllene oxide	0.9
22	16.41	Cubenol	0.5
23	16.72	T-cadinol	0.1
24	16.97	Viridiflorol	0.4
25	17.05	3-Heptenoic acid, 7-phenyl-, ethyl ester,	1.8
26	18.63	Dihydrosafrol	2.1
27	18.74	Piperonyl acetone	0.4
28	21.57	3-Methyl-2-butenoic acid, cyclobutyl ester	1.0
		Total (%)	**99.9**

RT: Retention time.

The cytotoxic properties of the *R. chalepensis* L. essential oil were carried out according to the spectroscopic method of luminescence. The effect of the essential oil was determined according to the viability of each cancer cell line, hence, the essential oil was tested at different concentrations ranging from 5×10^{-3} µg/mL to 10 µg/mL. Cell viability expressed as a function of the logarithm of the concentration is shown in Figure 2, in addition to polynomial trend curves of the fourth degree. From this figure, the 50% inhibition concentrations (IC_{50}) values were determined, which are presented in Table 3.

Table 3. IC_{50} (µg/mL) values for products tested (*R. chalepensis* L. essential oil and its active ingredients).

Cell Line	Essential Oil	Vinblastine	Doxorubicin	Combrestatin A4	MMAE
U87-MG	6.03 ± 0.49	2.00 ± 0.04	99.61 ± 2.34	9.00 ± 0.50	0.21 ± 0.03
U2OS	5.45 ± 0.76	-	-	-	-
RT4	>100	-	36.29 ± 1.20	-	0.50 ± 0.01
PC3	8.97 ± 0.17	-	2.09 ± 0.03		0.36 ± 0.03
NCI-N87	2.31 ± 1.28	-	-	-	1.65 ± 0.07
MRC-5	7.85 ± 0.13	-	39.88 ± 1.22	-	-
MIA-Paca2	4.84 ± 0.04	-	-	-	4.36 ± 0.2
K562	3.08 ± 0.59	20.00 ± 0.12	-	5.00 ± 0.30	3.12 ± 0.2
JIMT-T1	6.66 ± 0.15	-	-	-	-
HEK293	1.39 ± 0.27	-	-	-	-
HCT116	>100	35.00 ± 0.84	-	2.00 ± 0.10	2.07 ± 0.02
A549	8.22 ± 0.73	-	56.60 ± 0.84	20.00 ± 0.10	0.46 ± 0.05
A2780	>100	-	-	-	0.45 ± 0.01

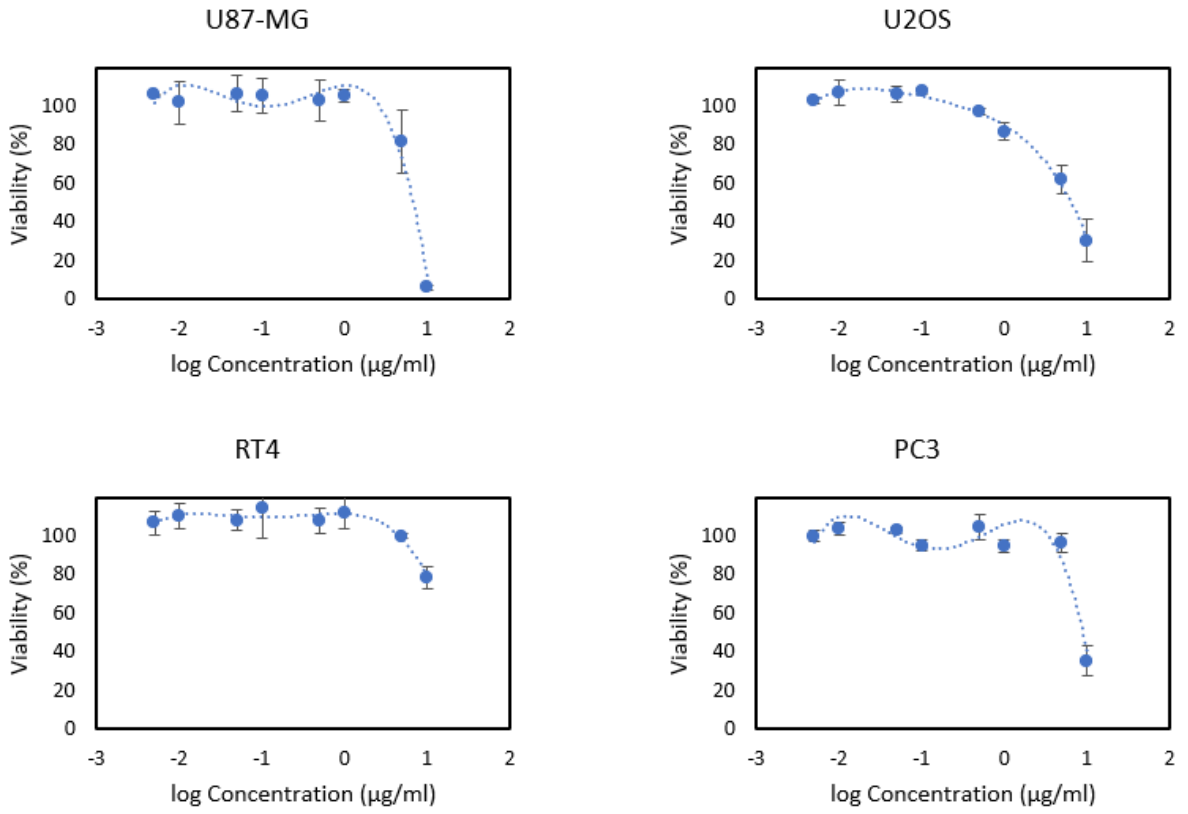
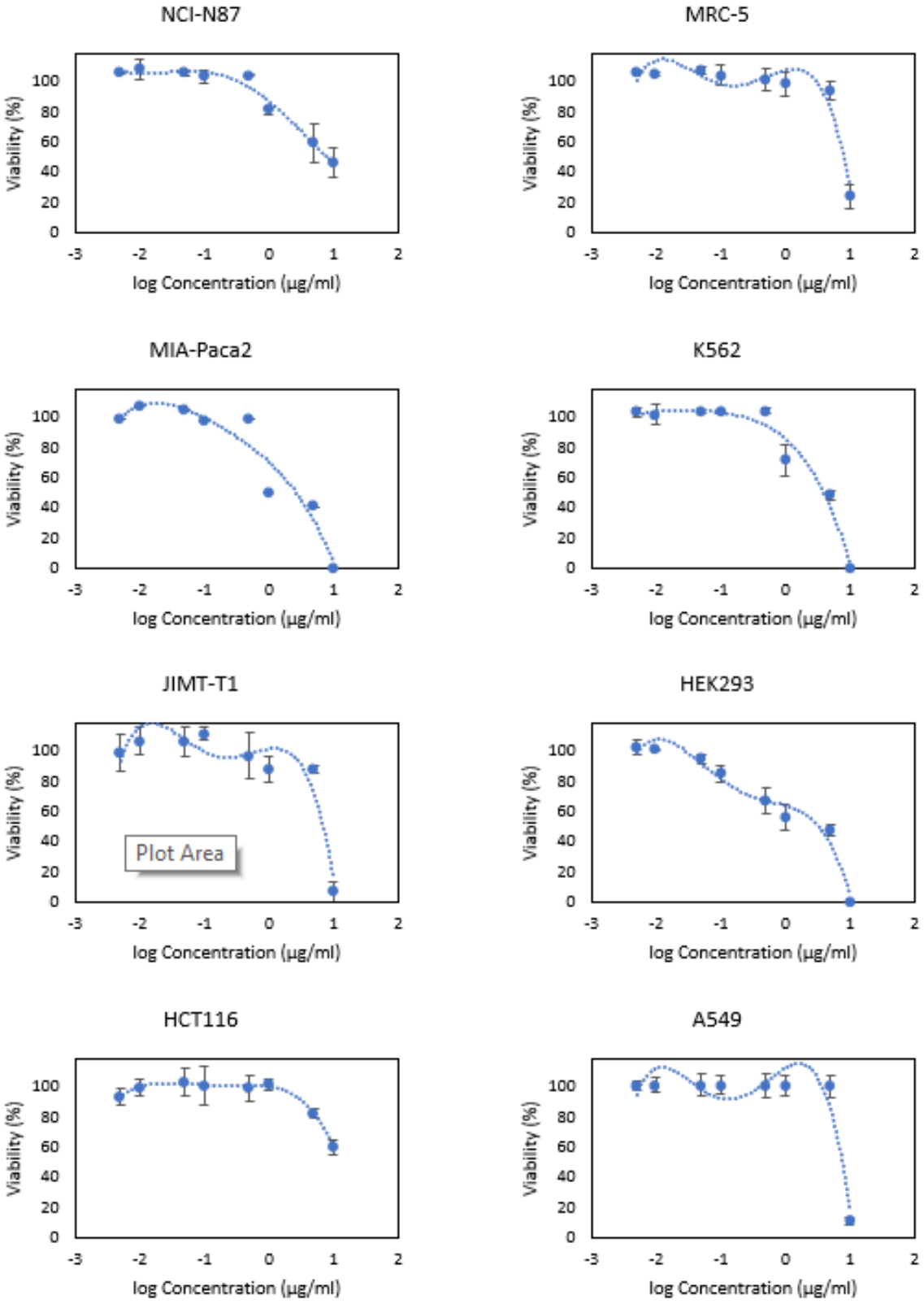


Figure 2. *Cont.*



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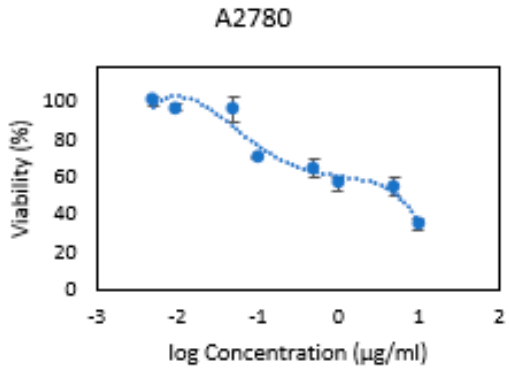

Figure 2. Cytotoxicity curves of the *R. chalepensis* L. essential oil in 13 cancer cell lines.

The results obtained from the IC_{50} of *R. chalepensis* L. essential oil in the 13 cancer cell lines are interesting, particularly in the NCI-N87 and HEK-293 lines, which showed an IC_{50} of 2.31 µg/mL and 1.39 µg/mL, respectively. On the other hand, the *R. chalepensis* L. essential oil did not show any effect in RT4, HCT116, and A2780 cell lines.

As we have described, principal component analysis (PCA) is a data analysis tool to determine the correlation between tested samples and cancer cell lines. We studied the essential oil and four active substances (vinblastine, doxorubicin, combrestatin A4, and monomethyl auristatin E) versus 13 cancer cell lines (U87-MG, U2OS, RT4, PC3, NCI-N87, MRC5, MIA-Paca2, K562, JIMT-T1, HEK293, HCT116, A549, and A2780).

To deepen the study, the modeling of the IC_{50} values was done by principal component analysis, according to the following approximations:

1 Tests for cytotoxic activity are independent.
2 Efficacy was coded according to the obtained IC_{50} values (Table 4).

Table 4. New parameters for numerical coding of the efficacy of the products tested against cancer cell lines.

Cell Line	Essential Oil	Vinblastine	Doxorubicin	Combrestatin A4	MMAE
U87-MG	3	4	1	3	5
U2OS	3	0	0	0	0
RT4	0	0	1	0	5
PC3	3	0	5	0	5
NCI-N87	4	0	0	0	4
MRC-5	3	0	1	0	0
MIA-Paca2	4	0	0	0	4
K562	4	1	0	3	4
JIMT-T1	3	0	0	0	0
HEK293	5	0	0	0	0
HCT116	0	1	0	4	4
A549	3	0	1	1	5
A2780	0	0	0	0	5

Table 3 gives the coded information of the 50% inhibition concentrations, hence the correlation between the products tested with each other is displayed in Figure 3 according to two axes F1 and F2, with retained variabilities of 43.41% and 32.00%, respectively, corresponding to the total of 75.42% of the information. The correlations between the tested products and the cancer cell lines are shown in Figure 4.

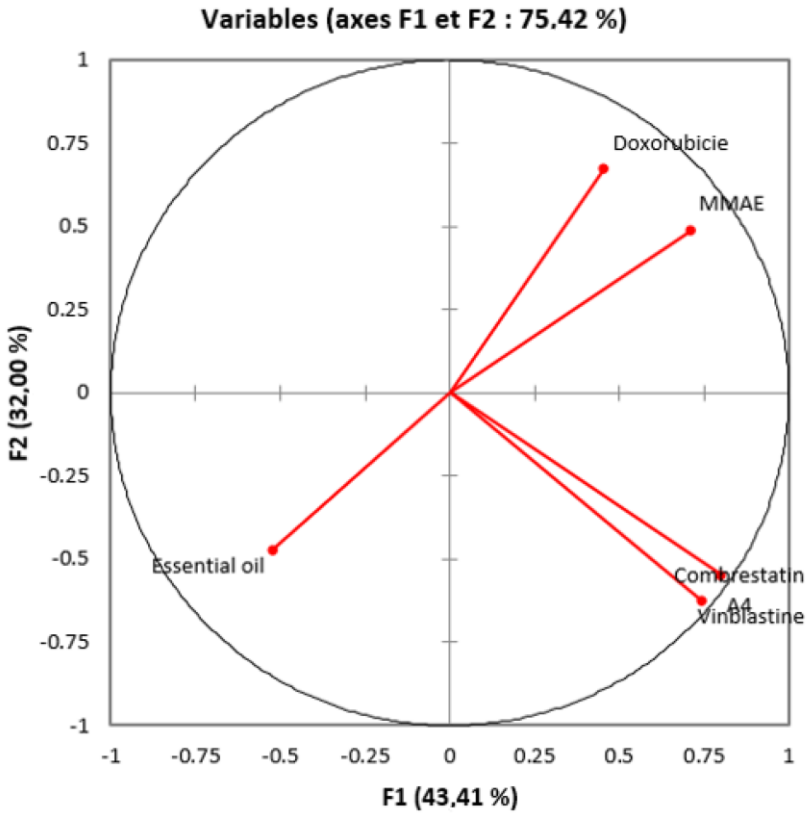

Figure 3. Correlations between the samples tested.

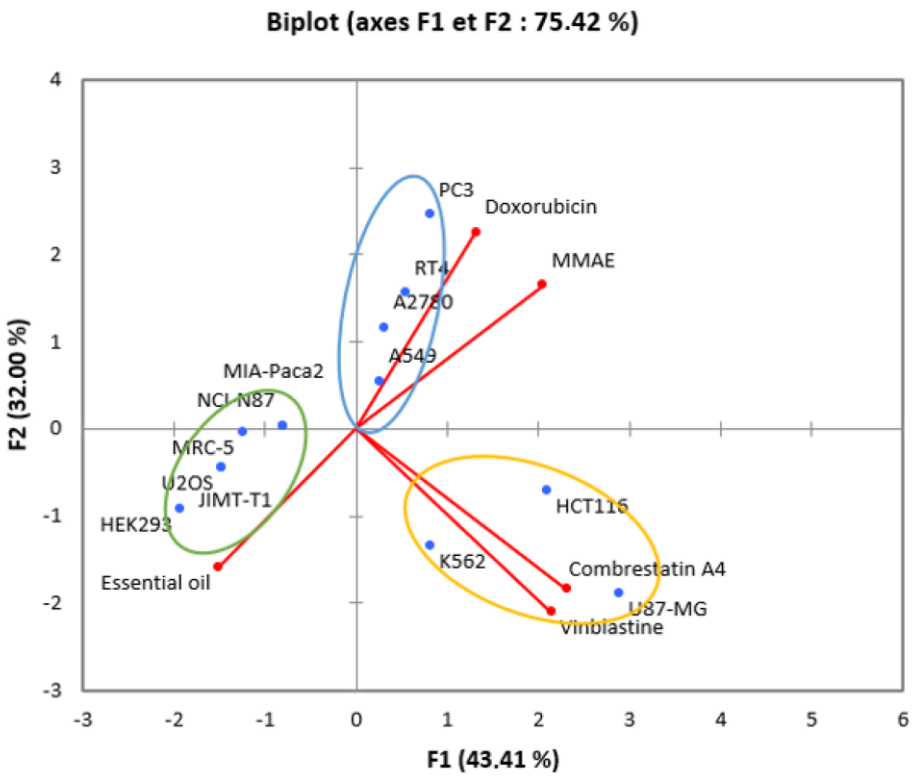

Figure 4. Biplot of the correlation between samples tested and cancer cell lines.

The interpretation of the data obtained makes it possible to divide the products tested and the cancer cell lines into three groups:

Group 1: the effectiveness of the essential oil against cancer cell lines U2OS, NCI-N87, MRC-5, MIA-Paca2, JIMT-T1, and HEK293.
Group 2: the effectiveness of the active substances Vinblastine and Combrestatin A4 against the cancer cell lines U87-MG, K562, and HCT116.
Group 3: the effectiveness of the active substances Doxorubicin and Monomethyl Auristatin E against the cancer cell lines RT4, PC3, A549, and A2780.

4. Discussion

The medicinal plant *R. chalepensis* L. is known for its richness in secondary metabolism products, and particularly in essential oils and alkaloids [36]. It is used therapeutically in the Republic of Djibouti for various purposes and several diseases, particularly as a febrifuge, local antivenom, and for bowel diseases.

The constituents of the *R. chalepensis* L. essential oil have been studied by many researchers and, as mentioned here, 2-undecanone was found to be the predominant component (51.3%). In the literature, however, the content of 2-undecanone varies greatly between different studies and regions. For example, the 2-undecanone content has been reported to constitute 38.1%, 52.5%, 66.5%, and 89.9% of the essential oil from Argentina [37], Iran [38], Turkey [39], and Tunisia [40], respectively. After 2-undecanone, octyl acetate was the second major component in our study. It was found that the yield and composition of essential oils are considerably dependent on the season of plant collection, topographic parameters, climatic factors, and operating conditions for obtaining the oil [41–43].

In the present study, the in vitro cytotoxic effect of *R. chalepensis* L. in cancer cell lines U87-MG, U2OS, RT4, PC3, NCI-N87, MRC-5, MIA-Paca2, K562, JIMT-T1, HEK293, HCT116, A549, and A2780 was evaluated. The results showed that *R. chalepensis* L. oil was highly cytotoxic in HEK293 and NCI-N87 cells, followed by K562, MIA-Paca2, U2OS, U87-MG, JIMT-T1, MRC-5, A549, and PC3 cells, respectively. Several authors have proven that essential oils with a high content of 2-undecanone have interesting anticancer activities against dozens of cancer cell lines (Hep G2, MCF-7, PC-3, HGC-27, and CLS-145); moreover, it was found to have the ability to induce apoptosis in cells, which is also consistent with the results of our studies [44–48].

To deepen our work, the anticancer profile of the essential oil was studied according to modeling and coding of the concentrations of inhibition of 50% in parallel with active substances, such as Vinblastine, Doxorubicin, me Combrestatin A4, and Monomethyl Auristatin E. The results made it possible to group the effectiveness of each substance and the essential oil against certain cancer cell lines. Three groups are well defined: the first group includes the effect of the essential oil in U2OS, NCI-N87, MRC-5, MIA-Paca2, JIMT-T1, and HEK293 cell lines; the second group includes the effect of Vinblastine and Combrestatin A4 in U87-MG, K562, and HCT116 cell lines; and the third group includes the effect of Doxorubicin and Monomethyl Auristatin E in RT4, PC3, A549, and A2780 cell lines. It is known that each active substance has a mechanism of action in cancer cells and according to the results obtained, groups 1 and 3 are well correlated inversely; on the other hand, group 2 has no correlation with group 1 and 3.

These favorable results concerning the cancerous activity of the *R. chalepensis* L. essential oil confirms the interest of the use of the flora of the Republic of Djibouti in the treatment of several types of cancer. This has added value to the positive findings of our previous work on the other plants of the region, including *Tagetes minuta*, *Lavandula coronopifolia*, *Cymbopogon schoenanthus*, *Nepeta azurea*, *Ocimum basilicum*, and *Ocimum americanum* [14–16].

5. Conclusions

To date, no information is available on the in vitro scolicidal activity of the *R. chalepensis* L. essential oil on its anticancer activities in cancer cell lines (HEK293, NCI-N87, K562, MIA-Paca2, U2OS, U87-MG, JIMT-T1, MRC-5, A549, and PC3). The results presented in this study offer important new data in this regard. Further studies are now needed to

identify the mechanism of the activity of the *R. chalepensis* L. essential oil and to determine if it would be a viable in vivo remedy to eradicate cancer cells.

Author Contributions: Conceptualization, F.M.A.-L. and T.A.; methodology, A.E.; software, A.M., M.N., A.R., A.A. and J.B.; validation, F.M.A.-L., T.A. and A.A.; formal analysis, A.R. and J.B.; investigation, F.M.A.-L.; resources, F.M.A.-L.; data curation, T.A.; writing—original draft preparation, A.A.; writing—review and editing, F.M.A.-L.; visualization, T.A.; su-pervision, A.A.; project administration, F.M.A.-L.; funding acquisition, F.M.A.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data from this research are mentioned in this paper.

Conflicts of Interest: The authors declare no conflict of interest.

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