World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Cytotoxicity against four cell lines of human cancer by the fractionated extract of *Dovyalis caffra*, exhibiting high oxylipin signature, and by jasmine oil

Seham MA Moustafa[1*], Khaled Mahmoud[2], Bassem M Menshawi[2], Gamila M Wassel[2], Marwa M Mounier[2]

[1]Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt
[2]Department of Pharmacognosy, National Research Centre, Dokki, Cairo 12622, Egypt

Received: 07-02-2015 / Revised: 23-02-2015 / Accepted: 25-02-2015

ABSTRACT

The methanol extract of *Dovyalis caffra* branches has been recommended by us, in a previous work, for further work on the bases of exhibiting promising in vitro cytotoxicity against four cell lines of human cancer namely: breast (MCF-7), colon (HCT-116), hepatocellular (HepG2) and lung (A-549) carcinomas. On further fractionation of the crude extract, the aqueous methanol fraction showed low LC_{50} as well as evident selectivity index (SI) values towards the four carcinoma cell lines mentioned above. This fraction (aqueous methanol) was subjected to GC/MS analysis to elucidate its constituting phytochemicals. The results showed that the majority of these constituents represented oxygenated fatty acid- derived oxylipins that are assumed to furnish the hexa- and octa- decanoid pathways of jasmonate (JA) biosynthesis. The LC_{50} and SI values were also determined for investigating possible effects of pure jasmine oil against the four carcinoma cell lines under study. The results showed pronounced anticancer activities of jasmine oil, particularly with hepatic and breast cell lines.

Keywords: Cytotoxicity, Cancer, LC_{50}, SI, GC/ MS, Oxylipins, *Dovyalis caffra*, Jasmine oil

INTRODUCTION

Herb science and the use of medicinal plants is a kind of technology of huge prehistorically, historical, and continuing importance to medicine [1]. In recent years, small molecular weight products from plants (phytochemicals) have been tested for use as multitargted anticancer agents for cell cycle arrest, inhibition of cell growth, proliferation and metastatic effects as well as for promoting apoptosis and cell death [2]. In an earlier work [3], we screened the methanol extracts of 200 wild and cultivated species in Egypt for their in vitro cytotoxicity against four human cancer cell lines of breast (MCF-7), colon (HCT-116), hepatocellular (HepG 2), and lung (A-549). In this respect, we selected the extract of *Dovyalis caffra* (branches), as one of those exhibiting most promising cytotoxicity. This extract was further subjected to schematic fractionation for detecting the most anticancer bioactive fraction with respect to the LC_{50} and selectivity index (SI) values. The SI values were determined, as compared to their in vitro cytotoxicity with those of normal human skin cell line (BJ-1). *Dovyalis caffra* (Hook. f. and Harv.) Sim (Salicaceae), commonly known as kei apple, is a medium size spiny tree or shrub, drought, frost, and salt tolerant (Figure 1). The plant is usually 3-5 m in height, but sometimes reaches 8 m with a much branched crown. The plant may be grown close together to form a good hedge or fence. The fruits are often eaten fresh, made into jam, used in desserts or pickled. It is a traditional food plant in Africa, where the fruits have potential to improve nutrition, boost food security, foster rural development and support sustainable land care [4]. The plant is native to Africa, but has been introduced to many regions of warm temperate climates including Egypt. It is among the collections of the Botanic garden, Faculty of Science, Ain Shams University, Cairo, Egypt.

Analysis of the aqueous methanol extract of *Dovyalis caffra* (branches) by GC/MS elucidated the occurrence of decanoids and decanoid derivatives as major constituting phytochemicals. Since such metabolites are assumed to furnish the jasmonate pool, the cytotoxicity of jasmine oil (JO) against the four cancer cell lines under study was also investigated. In this connection, jasmonates are recognized as being signals in plant responses, for most biotic and abiotic factors, to control defense gene expression, growth, and fertility

***Corresponding Author Address: Seham MA Moustafa,** *Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt; E-mail: sehammoustafa@yahoo.com*

throughout the plant kingdom and have been studied extensively in *Arabidopsis thaliana* [5].

Detailed information is reviewed or mentioned on jasmonates (JAs) and their precursors, the octadecanoids and hexadecanoids, with emphasis on their occurrence, biosynthesis, related genes, regulation, and signal transduction pathways [6-12]. Programmed cell death (PCD) could be also induced in grapevine by exogenously added methyl jasmonate (MeJA), where both lipoxygenase activity and functional octadecanoid biosynthesis were required [13]. It has been reviewed [14] that MeJA and related JAs were found to inhibit in vitro cancer cell proliferation and induced cell death in a wide range of human cancer cell types [15-18].

Thus, the present work intended to assess, by GC/ MS analysis, the phytochemical constituents that might be responsible of the anticancer activity in the fractionated extract of *Dovyalis caffra* branches. Possible effects of jasmine oil, in this context, have been also investigated.

MATERIALS AND METHODS

Materials: Samples of *Dovyalis caffra* plant were collected from Orman Botanic Garden, Giza, Egypt. A voucher specimen representing each collection was kept in the herbarium of the National Research Centre (NRC), Cairo, Egypt. The plant is also represented in the Botanic garden of the Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt and kept in the herbarium of the same department. Pure jasmine oil was purchased from Al-Quorashi, Cairo, Egypt. Human lung carcinoma (A-549 cell line) and skin normal human cell line (BJ-1) "A telomerase immortalized normal foreskin fibroblast cell line" were obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden. Human breast carcinoma (MCF-7 cell line), colon carcinoma (HCT-116 cell line), and hepatocellular carcinoma (HepG2 cell line) were obtained from Vacsera (Giza, Egypt).

Methods: Preparation of the plant extract, cell cultures and performance of the cell viability assays were carried out according to the procedure described by [19], with slight modification as described in details by [3].

Determination of LC_{50} and SI values of the plant fractionated extract and jasmine oil: The LC_{50} values were calculated using probit analysis by means of the SPSS computer program (SPSS for windows, statistical analysis software package /version 9/ 1989 SPSS Inc., Chicago, USA). The selectivity index (SI) showed cytotoxic selectivity against cancer cells versus normal cells (BJ-1, skin human normal cell line). Fractionation of the methanol extract of *Dovyalis caffra* branches was done according to the procedure described by [20] with slight modification as shown, herein, in scheme 1. The emerging fractions were dried under vacuum either at 40°C in a rotator evaporator (in case of solvents other than water) or freeze dried (in case of water fractions). The dry fractions were kept away from light, heat and moisture (in a -20°C deep freezer). Natural jasmine oil was solubilized in DMSO (dimethyl sulfoxide), and then a down series dilutions ranging from 1000-12.5 ppm were tested for their possible cytotoxicity against the four human tumor cell lines under investigation (MCF-7, HCT-116, HepG2, A-549).

GC/ MS analysis: On the bases of preliminary experimentation, the aqueous methanol fraction was subjected to GC/ MS analysis for phytochemical identification. The apparatus used was model 7890A/ 5975 B, USA, with the following characteristics and conditions: Sample inlet GC; Injection source GC ALS; Spectrometer; Oven equilibration time 0.5 min; Maximum temperature 280 degrees C; Oven program 40ºC for 3 min, 10ºC/ min to 150 ºC for 3 min, and then 10ºC/ min to 220 ºC for 6 min, and then 15ºC/ min to 260 ºC for 15 min; run time 47.667 min and 2 min (post run). The database of the used GC/ MS (NIST, USA) consisted of more than 62000 patterns. The spectra of unknown components were compared with those of the known components inherent in the ACAL library (Analytical Chemistry Assuit University Lab). The names, molecular weights and structures of the components of the test materials were ascertained according to the IUPAC International Chemical Identifier (IUPAC Standards InChI) approved by the International Union of Pure and Applied Chemistry in collaboration with the National Institute of Standards and Technology (NIST), version 1.04 released on September, 2011.

RESULTS

GC/ MS analysis of the aqueous methanol extract fraction of the branch extract: The most interesting seven components were further highlighted in the chromatograph of the fractionated extract of *Dovyalis caffra* branches. The constituents of interest with their retention time (RT) values are shown in the chromatograph in Figure 2. The values of RT, molecular weight (MW), and concentration (%) are presented in Table 1. The components of interest in the aqueous methanol extract fraction of *Dovyalis caffra* branches (Figure 2 and Table 1) are: Hexadecanoic

acid (1, 49%), Methyl 11-(3-pentyl-2-oxiranyl) undecanoate (1.15%), 2-monopalmitin (10.99%), 1, 3, 12-Nonadecatriene (4.86%), 2-Mono stearoyl glycerol (6.46%), Octadecyl dimethylamine (5.82%), and 2-tetradecanone (25.46%).

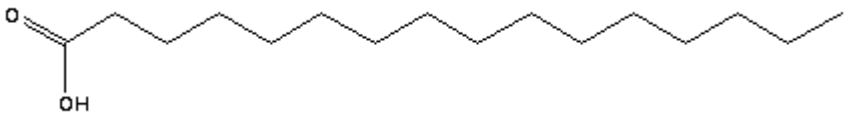
Cytotoxicity of jasmine oil against the four human tumor cell lines: Table 2 shows cytotoxicity of jasmine oil, as indicated by LC_{50}, and selectivity according to SI values towards breast (MCF-7), colon (HCT-116), hepatocellular (HepG2) and lung (A-549) human carcinoma cell lines. These results indicate that the determined LC_{50} and SI values are promising for possible in vitro treatment of the studied tumor cell lines with jasmine oil, particularly HepG2 and MCF-7 (Table 2).

DISCUSSION

Recently, small molecular weight phytochemicals have been tested for bioactivity against cancer. Such activities include cell cycle arrest, inhibition of cell growth, proliferation and metastatic effects as well as promotion of apoptosis and cell death [2]. In our earlier work [3], the methanol extracts of 200 wild and cultivated plant species in Egypt have been screened for their possible in vitro cytotoxicity against four human cancer cell lines of breast (MCF-7), colon (HCT-116), hepatocellular (HepG 2), and lung (A-549).

The extract of *Dovyalis caffra* branches has been selected among the most promising plant extracts for further use as anticancer agents. Thus, in the present work, the crude methanol extract of *Dovyalis caffra* branches was further subjected to schematic fractionation, where the aqueous methanol fraction proved to be most bioactive. Consequently, this fraction was further analyzed by GC/ MS to identify its phytochemical constituents (Figure 2 and Table 1). These phytochemicals and their significance will be briefly mentioned in the following:

1. Hexadecanoic acid

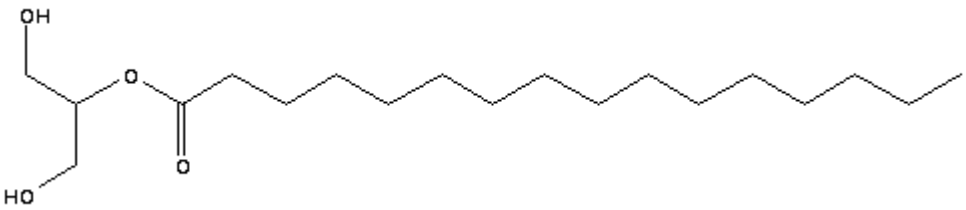


Other workers showed in vitro selective cytotoxic activity of this compound to human leukemic cells, without cytotoxicity to normal human dermal fibroblast cells [21], NCI-H23 non-small cell lung carcinoma cell line and HS578T breast cell line [22, 23], and pancreatic β-cells [24].

2. Methyl 11-(3-pentyl-2-oxiranyl) undecanoate (Oxiraneundecanoic acid, 3-pentyl-, methyl ester)

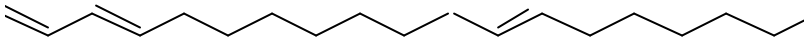
As far as the authors are aware, no studies have been shown for the anticancer activity of this compound. However, undecanoic acid is a medium-chain fatty acid that has been used in the treatment of dermatophytoses in humans [25].

3. 2-Monopalmitin (hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester)



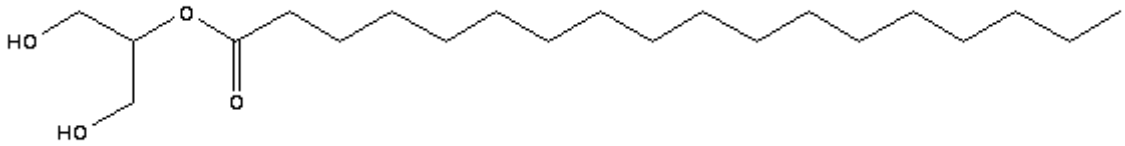
2- Monopalmitin is a member of the chemical class known as monoacylglycerols. In cancer, monoacylglycerol (MAG) serves as a critical node for the lipid signaling pathways in both physiological and disease contexts. So, the therapeutic potential of MAG lipase inhibitors has been studied with respect to controlling fatty acid release for the synthesis of protumorigenic signaling lipids [26]. 2-Monopalmitin was also among the main components for treating different human cancers by the extracts of bitter melon [27] and *Arum palaestinum* Boiss [28].

4. 1, 3, 12- Nonadecatriene



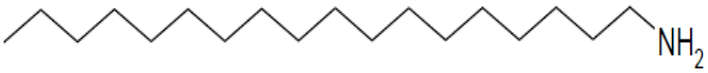
This phytochemical has been recorded as one of the major chemical constituents of the fatty acid pool of many plants. In this respect, 1, 3, 12- nonadecatriene was identified in the plant extracts as one of the biologically active compounds that might serve in treatments of many diseases including cancer (hepatoprotective) and that acts as a potential antioxidant. This compound was mainly recorded in the extracts of seeds and different plant parts of *Cassia auriculata* [29, 30].

5. Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, (2- Monostearoylglycerol)



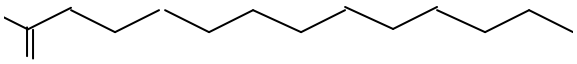
Hydroxy fatty acids (HFAs) have antibiotic, anti-inflammatory, and anticancer activities and therefore can be applied for medicinal uses [31]. Octadecanoic acid (2-hydroxy-1-(hydroxymethyl) ethyl ester) has been detected by many authors in the extracts of many plants (e.g. [32, 33]. This compound has been found to reduce prostate cancer in a cell line-dependent manner [34]. From another point of view, [35] found that *Nocardia cholesterolicum* and *Flavobacterium DS5* converted oleic acid to 10 hydroxy stearic acid and linoleic acid to 10-hydroxy-12(Z)-octadecanoic acid, via octadecanoic intermediates. Some of the new fatty acids were patently used, for e.g., 7, 10-dihydroxy-8(E)-octadecenoic acid as an antibacterial, particularly against food-borne pathogens, whereas the tetrahydrofuranyl moiety is known in anti cancer drugs.

6. Octadecyldimethylamine (1-Octadecanamine)



Octadecylamine is a basic chemical and solely used as an intermediate for the production of daughter products such as ethoxylates, amine derivatives, amides, etc. [36]. It is also used for the synthesis of many nanoconjugates for different targets. In this connection, octadecylamine- retinoic acid conjugate is applied for enhancing the cytotoxic effects of 5- fluorouracil (5-FU) using the low density lipoprotein (LDL) receptor targeted-nanostructured lipid carriers, and thus reduces 5-FU dose in colorectal cancer [37]. Lipid nanoparticles with different oil/ fatty ratios were used as carriers of buprenorphine (opoid dependent) and its prodrugs for injection against chronic pains [38]. A sialic acid–octadecylamine conjugate (SA–ODA) was synthesized and anchored on the surface of pixantrone (Pix)-loaded liposomes to achieve an improved anticancer effect [39]. This was based on that sialic acid is a critical element for tumor development and its receptors are highly expressed on the tumor-associated macrophages (TAMs) that play important roles in the growth and metastasis of tumors.

7. 2-Tetradecanone



Tetradecanone is among the bioactive phytochemicals exhibiting antioxidant, anti-inflammation and antibacterial properties in the essential oil fractions of many plants including *Ficus capensis* [40]. 2-tetradecanone is a sex pheromone of *Hoplia equina* LeConte (an important pest of cranberries). The Using bucket traps baited with pheromone load were efficient in male capture and captures of nontarget arthropods, including pollinators [41]. Tetradecanone derivatives are among chemicals containing specific branched chain and terminal groups that have been prepared and applied as anti-cancer and immune boosting agents (Patent WO, 2001) [42]. For curing and inhibiting cancer growth, a lipoplex was designed, which comprised: (a) cationic liposome, and (b) expression vector containing saxatilin gene of sequence information (derived from *Agkistrodon saxatilis*). The cationic liposome of the invention was preferably one selected from the group consisting of liposome containing lysine-aspartate-tetradecanol and lysine-glutarate-tetradecanol conjugates (Patent US 2005) [43].

In the present work, attention was attracted for the predominant occurrence of hexadecanoids, octadecanoids and their derivatives in the prominently cytotoxic extracts of *Dovyalis caffra* (branches) against the cancer cell lines under study. The metabolic pool of hexa- and octa- decaoids and related compounds is augmented to furnish jasmonate production as an end product [6]. Jasmonates (JAs) are originally found as major constituents in jasmine oil (JO), representing signals in plant responses with most biotic and abiotic factors [5]. JA and its volatile methyl ester (MeJA) represent oxygenated fatty acid derived cyclopentanones collectively called oxylipins with their related metabolites. Detailed information is reviewed on JAs and their precursors, the octadecanoids and hexadecanoids, with emphasis on their occurrence, biosynthesis, related genes, regulation, and signal transduction pathways [6-12, 44]. Therefore, in the last part of the present work, pure jasmine oil (JO) was solubilized in DMSO and tested at different concentrations (1000- 12.5 ppm) for possible cytotoxic effects against the breast (MCF-7), colon (HCT-116), hepatocellular (HepG2) and lung (A-549) human carcinoma cell lines under investigation. In this context, the determined LC_{50} and SI values indicated that JO is promising for in vitro treatment of the studied tumor cell lines, particularly HepG2 and MCF-7 (Table 2). These results could be further reinforced by those of other authors who showed that MeJA and related JAs have been found to inhibit in vitro cell proliferation and to induce cell death in a wide range of human cancer cell types [16-18]. Recently, most of JA activities against cancer have been reviewed [14], in an attempt to get an integrated view and better understanding of its multifaceted modes of action. Furthemore, the intermediates in the biosynthesis of JAs were also proved to be active in at least some of JA-induced processes [45]. The synthetic levels of JA and 12- oxophytodienoic acid (OPDA) and other intermediate oxylipins varied considerably among plant species, giving rise to the suggestion that their relative and absolute concentrations (the oxylipin signature) might provide an additive flexibility to the multifunctional jasmonate signaling system [46]. Octadecanoid and oxadecanoid alterations of gene expression and the oxilipn signature have also been discussed by [47]. The *Arabidopsis opr3* mutant is defective in the isoform of OPDA reductase required for JA biosynthesis. Treating *opr3* plants with exogenous OPDA powerfully up-regulated several genes and disclosed two distinct downstream signal pathways [48]. Thus, the authors were able to investigate the effects of OPDA on gene expression without having to consider OPDA conversion to JA. In the present work, the cytotoxic effect of JO against the four human tumor cell lines under investigation might be undersigned by one or more of the following known inferences of JAs: 1. the action of JAs to inhibit mitosis concomitant with protein modulation [49], and programmed cell death (PCD) [13], 2. induction of hypersensitive-like response by MeJA in absence of virulent pathogen [50], 3. the crucial responses of JA signals upon plant subjection to stress factors and pathogenic effects [13, 51, 52], and 4. the JA-mediated regulation of secondary metabolites [53]. However, identifying the appropriate role of JO in cytotoxicity against cancer cell lines needs further work.

Figure 1. Branches of *Dovyalis caffra* showing its spiny nature. The nomenclature of the plant is derived from the occurrence of the spines where *Dovyalis* in Greek means spear, whereas *caffra* is derived from Kaffraria (Eastern Cape, South Africa; the plant origin).

Scheme 1: The scheme of fractionation

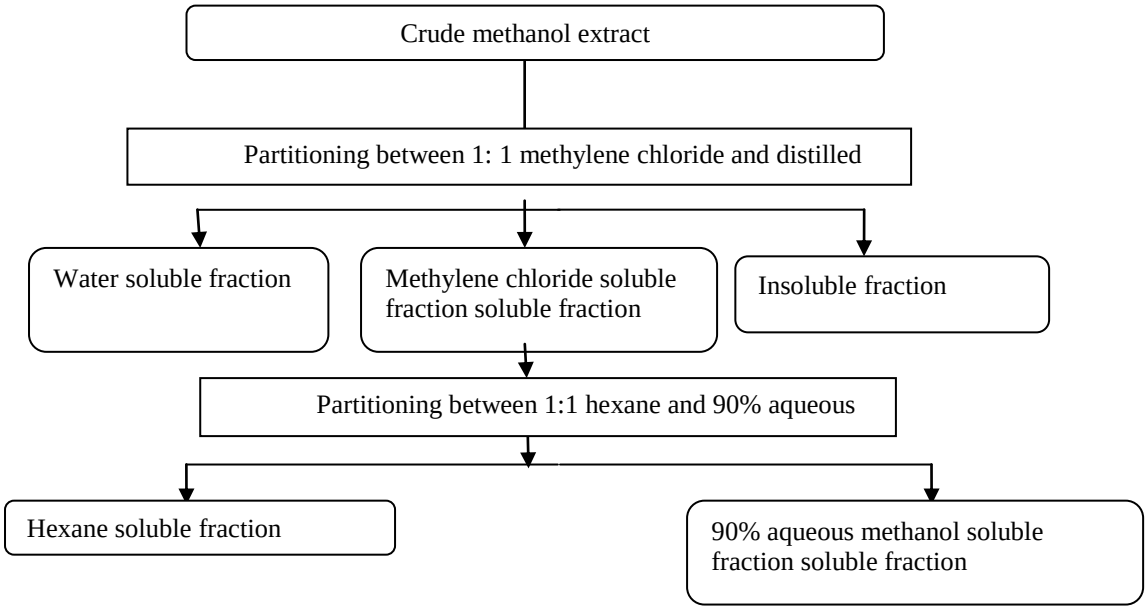


Table 1: The components of interest in the GC/ MS analysis of the aqueous methanol extract fraction of *Dovyalis caffra* branches, showing nomenclature, molecular formula (MF), molecular weight (MW), Retention time (RT), and percent.

Analyte / Parameter	**MF**	**MW**	**RT**	**% of total**
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240	23.614	1.49
Methyl 11-(3-pentyl-2-oxiranyl)undecanoate	$C_{19}H_{36}O_3$	312.266	25.01	1.15
2-Monopalmitin	$C_{19}H_{38}O_4$	330.270	32.418	10.99
1,3,12-Nonadecatriene	$C_{19}H_{34}$	262.266	34.557	4.86
2-Monostearoylglycerol	$C_{21}H_{42}O_4$	258.308	34.996	6.46
Octadecyldimethylamine	$C_{20}H_{43}N$	297.340	45.874	5.82
2-Tetradecanone	$C_{14}H_{28}O$	212.214	46.941	25.46

Table 2. LC_{50} (the concentration required to kill 50% of the cell population) values for jasmine oil on human tumor cancer cell lines of breast (MCF-7), colon (HCT- 116), hepatocellular (HepG2), and lung (A-549). Values of LC_{50} (± standard errors) are calculated using SPSS statistical program. The selectivity index (SI) values for the same cell lines were determined, as compared to their in vitro cytotoxicity with those of normal human skin cell line (BJ-1). Jasmine oil was solubilized in DMSO.

Cell lines	**Jasmine oil**	
	LC_{50}	SI
MCF-7	35 ± 1.3	1.1
HCT- 116	79.1 ± 2.4	0.5
HepG2	23.9 ± 1.6	1.5
A- 549	90.1 ± 2.1	0.4
BJ- I	37 ± 1.7	------

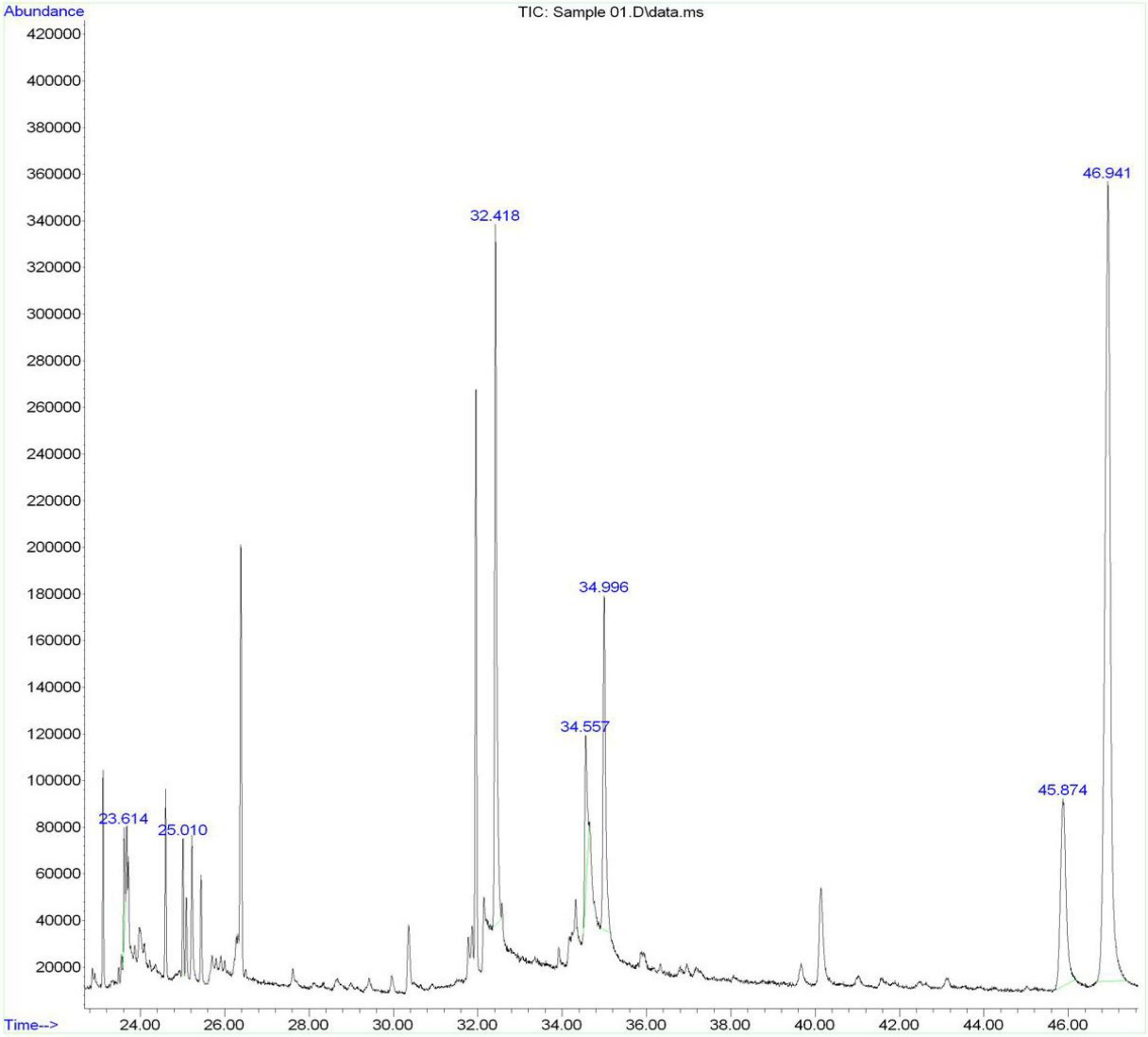


Figure 2. Chromatograph of the GC/ MS analysis of the aqueous methanol fraction of *Dovyalis caffra* branches showing predominant hexadecanoids, octadecanoids and their derivatives.

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