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Comparative Assessment of the Volatile Profile, Antioxidant Capacity and Cytotoxic Potential of Different Preparation of *Millefolli Herba* Samples

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Abstract*: Millefolii herba is an available product on the Romanian market as mixture of stems, leaves and flowers of Achillea millefolium L. There were established its volatile compounds profile, total polyphenolic content (TPC), antioxidant capacity and effects on HCT 116 cell viability and programmed cell death. The infusion, hydroalcoholic extract and hydrodistillated essential oil were studied. A comparative analysis using static headspace (HS) and hydro-distillation (HD) GC/MS of the volatile components from Millefolii herba was realized: the essential oil contains chamazulene as the principal component (37.1%), while 1,8-cineole (46.8%) is the main constituent of headspace volatiles. The highest antioxidant capacity was found in essential oil, compared with hydroalcoholic extract, infusion and ascorbic acid. Yarrow hydroalcoholic extract reduced the HCT 116 cell viability and induced the apoptotic cell death in a dose and time dependent manner.*

Keywords*: Achillea millefolium L., GC/MS, total polyphenolic content, colon carcinoma cell viability*

1.Introduction

Achillea millefolium L. (commonly known as yarrow) belongs to *Asteraceae* family. It is a perennial flowering plant, being used in traditional medicine. Native to Eurasia, this wild plant, existing in many varieties and sub-species, doesn't require any particular conditions to grow. Yarrow plant has many medicinal uses [1], as infusion, tincture, compress, decoction, alcohol extract or essential oil and has many therapeutic activities: antibacterial, antifungal, antiparasitic, anti-inflammatory, antihypertensive, hemostyptic, gastro- and hepato-protective, antispasmodic, antioxidant, estrogenic, anticancer [2, 3]. It helps in the case of irregular menstruation, menopausal problems, cystitis and infections. These properties are due to the active biological compounds (chamazulene, caryophyllene, pinene, 1,8-cineole) found in *Achillea millefolium* L. Also, the plant is used as additive in the production of alcoholic beverages, food industry or cosmetics.

Various analytical methods are used to extract the volatile compounds from plant material. The most used technique for producing essential oils is hydro-distillation (HD) [4], but there are some other alternatives [5, 6]. HD is the most popular because it avoids the oil contamination with organic solvent, but the possible hydrolysis and oxidation of some compounds [7] can represent problem in the real volatile profile establishing. Compared to the traditional hydro-distillation, headspace (HS) is a method for analysis of volatiles in solid samples without major treatment, being rapid and easy to operate.

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Literature review of *Achillea millefolium* L. pattern showed that the essential oil can be distributed into several chemotypes, which were defined according to the major components [8]. The composition and quality of yarrow essential oil are also influenced by factors like development stage, the different parts of the analyzed plant, harvesting season, plant genetic type [9]. Dias *et al.* [10] studied the antitumor potential of *Achillea millefolium* L. against some carcinoma cell lines (breast, lung, cervical, colon and hepatocellular). The alcoholic extract of commercial yarrow showed a higher antitumor potential against colon carcinoma cell lines (HCT-15) than infusions and decoctions. The antitumor activity mechanism of the flavonoid casticin, derived from *Achillea millefolium* L. was also studied [11].

Some studies on Romanian *Achillea millefolium* L. were carried out only for the essential oil and revealed as main components borneol, 1,8-cineole and β-thujone [12,13] or chamazulene [14-17]. Also, only a small number of studies evaluated the antioxidant potential of the essential oil [16, 18].

To our knowledge, the headspace plant volatile profile, the antioxidant capacity of infusion and hydroalcoholic extract (the most consumed forms), as well as the antitumor activity of Romanian yarrow have not been yet reported in the literature. Therefore, in this study, the volatile compounds profile of the plant and the essential oil, the total polyphenolic content and the antioxidant capacity of *Achillea millefolium* L. commercialized on the Romanian market were established. Effects on HCT 116 (colon carcinoma) cell viability and programmed cell death (apoptosis) were also studied.

2.Materials and methods

Plant material: Commercially available mixture of stems, leaves and flowers of *Achillea millefolium* was purchased (10 packed tea of 50 g) from the main national supplier of herbal teas (PLAFAR). The products of this supplier have controlled origin and they are fulfilling all the national requirements. They also meet the quality criteria imposed by the European standards. On the package of herbal tea was specified that the content is *Millefolii herba*. Prior to use the mixture was manually grounded.

Infusion: A volume of 50 mL boiled water was poured over 2 g of dry plant and left for 20 min to obtain the infusion. The solution reaching the room temperature was filtrated. The residue from the filter was rinsed three to four times with small volumes of distilled water, and the filtered infusion was adjusted to 50 mL.

Hydroalcoholic extract: Five grams of finely cut plant were maintained in a bottle in darkness with 75 mL (50 %) ethanol for 7 days, stirring it 3-4 times a day. After that, the solution was filtered and adjusted to 100 mL with 50% ethanol.

Essential oil: 100 g of *Millefolii herba* samples were hydrodistilled in a Clevenger-type apparatus for 4 h [19]. The essential oil was dried over anhydrous Na_2SO_4, stored in a dark glass bottle and kept at 4 °C until analysis.

Headspace analysis: 1.0 g of dry plant was placed in a 20 mL headspace vial sealed with silicone rubber septum and aluminum cap. The vial was heated to 80°C for 10 min before the injection. A volume of 500 µL of the headspace gas was injected into the column of the GC/MS.

Cell cultivation and treatment: HCT 116 cells were maintained in a humid atmosphere containing 5% CO_2 and grown as monolayers in McCoy's 5A medium w: L-glutamine, w: 2.2 g/L $NaHCO_3$ supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin/streptomycin. Cells were seeded (150,000 cells/mL) in 12-well plates and 96 well plates with a volume of 2 mL and 200 µL per well, respectively, depending on the experiment. 24 hours later indicated amounts of hydroalcoholic extracts were added to the culture. So the concentration of the extract used for treatment would be 0.25 %, 0.5 %, 1 %, 2 %, and 2.5 % regarding treatments of 10 µL, 20 µL, 40 µL, 80 µL, 100 µL, respectively. The same concentrations were used for the apoptosis quantification experiment. Based on the literature data regarding the cytotoxic activity of some extracts with similar composition against HCT 116 cell lines [10], this type of activity was tested on hydroalcoholic extract of *Achillea*.

Head space volatiles and essential oil composition: The essential oil samples diluted in hexane (1:10) (1 µL injection) and headspace gas (500 µL) were analyzed by GC/MS using a Thermo Electron system - Focus GC chromatograph coupled with a Polaris Q ion trap mass detector, both controlled with Xcalibur® software [20]. A DB-5MS capillary column (25 m × 0.25 mm; 0.25 µm of film thickness) was used with helium 6.0 as the carrier gas (1 mL/min). Both headspace and liquid samples were analyzed under the same chromatographic conditions. The GC oven temperature program was: the initial temperature 60 °C (3 min), then increased to 200 °C at 10 °C/min and after that at 12 °C/min to the final temperature of 240 °C (2 min). The ion source and interface temperatures were 200 °C and 250 °C, respectively. The detector operated in the electron impact mode (70 eV) and the detection was performed in the range of m/z 35–300 (full scan mode). Separated compounds were identified according to their retention indices and based on mass spectrum provided by electronic libraries (Wiley, NIST) and literature [21]. Alkanes, C8-C20 in hexane (Sigma Aldrich Co., St. Louis, USA) were used as reference points in the calculation of retention indices (RI) in GC/MS analysis.

Total polyphenolic content: Total polyphenolic content (TPC) of the essential oil, infusion and hydroalcoholic extract samples was determined according to Folin-Ciocalteu (F-C) method using Folin-Ciocalteu reagent and gallic acid, GA (Sigma Aldrich Co., St. Louis, USA) as standard [22]. The absorbances were measured at 760 nm. TPC was expressed as GA equivalent (GAE) in mg/100 mL extract or GAE mg/g dry material. The calibration equation was $A = 0.0863\ C_{GA}$ (µg/mL) + 0.0157 ($R^2 = 0.9986$).

Antioxidant capacity: Free radical scavenging capacity was assessed according to the original 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [23] with minor modifications. The results of DPPH assay were expressed as percentage inhibition (I%), efficient concentration value (EC_{50}) and ascorbic acid equivalent antioxidant capacity (AEAC). To all samples (0.1-5% for infusion, 0.02-0.4% for essential oil and 0.1-1.7% for hydroalcoholic extract) 0.05% ethanolic solution of DPPH was added. The absorbances were measured at 517 nm against ethanol as blank. A mixture of ethanol and 0.05% DPPH was used as control sample.

For AEAC procedure, mixtures of DPPH (0.05%) and different volumes of suitable ascorbic acid working solutions (0.2-0.8 mL of 10 µg/mL and 0.1-0.5 mL of 100 AA/mL) were prepared. The volume of each mixture was brought to 2 mL with ethanol. The concentration of ascorbic acid varied between 0.1 and 2.5 mg/100 mL. Radical scavenging capacity expressed as the percentage inhibition (I%) was calculated using the equation $I\% = [(A_{control} - A_{sample})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control sample and A_{sample} is the absorbance of the sample containing essential oil or infusion of yarrow. The I% value was calculated for the same concentration of the extracts (0.1%, v/v). The parameter EC_{50} represents the sample concentration required to obtain a 50% antioxidant effect. EC_{50} was graphically estimated as the substrate concentration necessary to decrease the initial absorbance (at 517 nm) of the purple DPPH by 50%. The AEAC parameter was calculated using the formula: $AEAC\ (mg\ AA/100\ mL) = [EC_{50(AA)}/EC_{50(sample)}] \times 100$.

All absorbances were measured with a UV-Vis spectrophotometer (Jasco V-530) equipped with 1.00 cm quartz cells, running Spectra Manager as software. The results of all spectrophotometric measurements were expressed as the average of three independent measurements.

Cell viability and proliferation assay: To study the effects of yarrow on the HCT 116 (colon carcinoma) cell viability WST1 cell proliferation reagent was used. WST-1 is a tetrazolium salt and it gets cleaved to formazan by mitochondrial dehydrogenases in metabolically active cells gets. The formazan dye intensity is then measured at wavelengths 420 - 480 nm.

Cells were seeded in 96 well microplates and allowed to grow for 24 hours. After treatment of cells with indicated amounts of yarrow extract, the cell viability and cells proliferation were analyzed applying WST1 reagent according to manufacturer's protocol. WST-1 is a ready-to-use colorimetric assay for the nonradioactive quantification of cellular proliferation, viability and cytotoxicity. 10 µL of WST1 reagent was added into each well of the 96 well plates. Following 4h incubation at 37 °C, the

optical density (OD) was measured at a test wavelength of 450 nm and a reference wavelength of 650 nm using ELISA reader (Bio-Rad, CA, USA). Percent viability (V%) was calculated as $[OD_{sample}/OD_{control}] \times 100$.

Apoptotic cell death analysis: Apoptotic cell death was evaluated by an Annexin-V affinity assay by flow cytometry. HCT 116 cells were seeded at 150×10^3 per 1 mL in 12-well plates and treated with yarrow extract. Cell death response of treated and untreated control cells was assessed by FITC conjugated Annexin-V (Alexis Biochemicals). FITC-Annexin-V staining was performed according to the manufacturer's protocols. Cells were quantified by flow cytometer (FACS Canto, Becton Dickinson) on FlowJo software. WST1 reagent (Roche) was used in cell viability assay to indicate metabolically active cells. FITC conjugated Annexin V dye was used to determine apoptotic cell death activity in cells. DPBS without Ca and Mg, trypsin 0.25%/EDTA 0.02% in PBS, McCoy's 5A medium w: L-glutamine, w: 2.2 g/L $NaHCO_3$ (PAN Biotech) supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin/streptomycin was used in cell culture studies.

Statistical analysis: All the experimental data were expressed as means of three measurements/determinations ± the standard deviation values (SD). Statistical significance of responsive differences among differentially treated cell populations in biological studies were assessed with unpaired Student's t-test at $p < 0.01$.

3.Results and discussions

Essential oil yield: The average yield of the essential oil obtained on the basis of three succesive extractions of *Achillea millefolium* was 0.3 ± 0.06 % (mL essential oil/100 g herba). This value is in agreement with data existing in literature of 0.3 - 1.4% *Achillea millefolium* depending on the extraction procedure, the climate, the soil type, the vegetative stage or the stage of the full bloom [24].

The volatile compounds in Millefolii herba: The results of GC/MS analysis of the volatile compounds performed on both solid sample and essential oil (Table 1) showed that most compounds identified belong to monoterpene hydrocarbons and oxygenated monoterpenes categories. The two methods, HS-GC/MS and HD-GC/MS were found to be complementary, leading to important information on the content of *Millefolii herba,* available for population consumption. Using the hydrodistillation as extraction method, the chromatographic technique led to identification of some other compounds, namely sesquiterpenoids (71.7%).

Table 1. Composition of *millefolii herba* head space volatiles and essential oil

No	RI exp.*	RI lit.**	Compound	HS % (± SD)	HD % (± SD)
Monoterpene hydrocarbons					
1	909	906	Santolina triene	1.6 (0.06)	n.d.
2	928	924	α-Thujene	0.8 (0.05)	0.3 (0.02)
3	936	932	α-Pinene	3.8 (0.07)	1.0 (0.03)
4	952	946	Camphene	7.3 (0.09)	0.2 (0.01)
5	976	969	Sabinene	1.1 (0.04)	4.1 (0.05)
6	981	974	β-Pinene	1.9 (0.05)	6.4 (0.09)
8	1006	1002	α-Phellandrene	0.1 (0.01)	0.1 (0.01)
9	1019	1014	α-Terpinene	0.3 (0.01)	1.0 (0.03)
10	1027	1020	*p*-Cymene	4.6 (0.07)	0.6 (0.02)
11	1031	1024	Limonene	0.7 (0.02)	0.4 (0.01)
13	1062	1054	γ-Terpinene	n.d.	2.2 (0.05)
17	1090	1086	α-Terpinolene	0.1 (0.01)	0.5 (0.01)
Oxygenated monoterpenes					
7	998	999	Yomogi alcohol	1.0 (0.04)	0.1 (0.01)
12	1036	1026	1,8-Cineole	46.8 (0.29)	3.3 (0.06)
14	1063	1056	Artemisia ketone	1.9 (0.08)	n.d.
15	1072	1065	*trans*-Sabinene hydrate	1.8 (0.06)	n.d.
16	1084	1080	Artemisia alcohol	2.9 (0.07)	n.d.

18	1104	1098	*cis*-Sabinene hydrate	0.8 (0.04)	n.d.
19	1111	1101	α-Thujone	4.8 (0.10)	0.3 (0.01)
20	1123	1112	β-Thujone	0.5 (0.02)	0.4 (0.01)
21	1128	1118	*cis*-*p*-Menth-2-en-1-ol	0.4 (0.01)	0.2 (0.01)
22	1146	1136	*trans*-*p*-Menth-2-en-1-ol	0.5 (0.01)	n.d.
23	1154	1141	Camphor	9.8 (0.06)	0.9 (0.03)
24	1174	1165	Borneol	4.8 (0.09)	1.1 (0.02)
25	1184	1174	Terpinen-4-ol	0.4 (0.01)	1.5 (0.03)
26	1196	1186	α-Terpineol	0.6 (0.03)	1.1 (0.03)
27	1214	1207	*trans*-Piperitol	0.4 (0.01)	n.d.
28	1240	1235	*cis*-Chrysanthenyl acetate	n.d.	0.6 (0.02)
29	1292	1284	Bornyl acetate	0.1 (0.01)	n.d.
Sesquiterpenes					
30	1358	1345	α-Cubebene	n.d.	0.1 (0.01)
31	1385	1374	α-Copaene	0.1 (0.01)	0.3 (0.02)
32	1397	1387	β-Cubebene	n.d.	0.4 (0.02)
33	1434	1417	β-Caryophyllene	0.1 (0.01)	11.8 (0.21)
34	1469	1452	Humulene	n.d.	1.9 (0.06)
35	1498	1484	Germacrene D	n.d.	4.8 (0.09)
36	1534	1522	δ-Cadinene	n.d.	1.1 (0.06)
44	1760	1730	Chamazulene	n.d.	37.1 (0.30)
Oxygenated sesquiterpenes					
37	1596	1577	Spathulenol	n.d.	2.8 (0.06)
38	1604	1582	Caryophyllene oxide	n.d.	5.3 (0.09)
39	1615	1592	Viridiflorol	n.d.	1.4 (0.03)
40	1643	1627	1-*epi*-Cubenol	n.d.	0.7 (0.02)
41	1653	1630	γ-Eudesmol	n.d.	1.1 (0.05)
42	1659	1638	τ-Cadinol	n.d.	0.8 (0.04)
43	1676	1652	α-Eudesmol	n.d.	2.0 (0.08)
			Total identified	100.0	97.9
			Monoterpenes	22.3 (0.48)	16.8 (0.33)
			Oxygenated monoterpenes	77.5 (0.93)	9.5 (0.23)
			Sesquiterpenes	0.2 (0.02)	57.5 (0.77)
			Oxygenated sesquiterpenes	0.0 (0.00)	14.1 (0.37)

Note: the percentage composition of the essential oils was computed from GC peak areas without any correction factors; n.d. – not detected.
*Retention index (RI) relative to standard mixture of *n*-alkanes on DB-5MS column

The main components of *Achillea millefolium* identified using HS techniques were 1,8-cineole (46.8%), camphor (9.8%), camphene (7.3%), α-thujone (4.8%), borneol (4.8%), *p*-cymene (4.6%) and α-pinene (3.8%) from a total of twenty-nine compounds. In the essential oil obtained by hydrodistillation, thirty-six compounds were identified by GC/MS representing 97.9% of the total analyzed oil, the main components being chamazulene (37.1%), β-caryophyllene (11.8%), β-pinene (6.4%), caryophyllene oxide (5.3%), germacrene D (4.8%), sabinene (4.1%) and 1,8-cineole (3.3%).

HD is able to extract some oxygenated monoterpenes and oxygenated sesquiterpenes that were not detected using HS technique. This can be explained by the fact that HD involves more steps and longer analysis times, leading to chemical changes. Furthermore, the results presented in Table 1 show that HS technique is much more efficient than HD for terpenes extraction. Thus, one of the limitations of HD is represented by the potential losses of the some terpenes like 1,8-cineole or camphor.

Chamazulene, which is responsible for the blue colour of the oil, is a derivative of azulene which is formed from matricine during distillation procedure (by a complex series of chemical reactions: dehydrogenation, dehydration, ester hydrolysis) [25] being commonly considered an artefact of the hydrodistillation itself. The occurrence of chamazulene in the essential oils distinguishes between *Achillea millefolium* species of different ploidity levels, the considerable content of proazulene being closely related to tetraploid plants [26]. As expected, the results in the Table 1 show that matricine does not appear when HS-GC/MS technique is used, as long as the initial heating temperature was kept

at 80°C. At a temperature around 150°C chamazulene is present in a large quantity, meaning that matricine has produced it [27]. Therefore, the 80 °C temperature used seems to be optimal to keep the matricine untransformed, the obtained volatile profile of the yarrow being very close to the real one. Moreover, infusions are prepared using hot water and thus one can also have an image of the volatile compounds profile of yarrow infusion drinking for curative purposes.

Previous studies [28] have revealed that most of the monoterpene hydrocarbons are lost through infusion and maceration and the analysis of volatile and semi-volatile compounds does not reveal major differences between these two. In addition, Fierascu *et al.* [29] have found that the *A. millefolium* hidroalcoholic extract (ethanol) contains only 41 compounds, while in the essential oil 82 components were identified (unspecified amounts). However, there were only a few compounds that have remained in similar proportions in all the three extracts (infusion, tincture and essential oil).

The literature review highlighted that the essential oils originating from various countries of Europe belong mainly to two distinct chemotypes, named chamazulene [30] and camphor and 1,8-cineole [31-33]. Comparing the composition of the essential oil determined in this study with those reported in the literature showed that Romanian yarrow belongs to chamazulene chemotype. In addition, the HS analysis emphasized a high content of 1,8-cineole.

Antioxidant capacity: The antioxidant capacity of all three extract types are given in Table 2. The strongest DPPH scavenging activity was obtained for the essential oil and its TPC value was found to be higher than those of infusion and hydroalcoholic extracts and even of ascorbic acid itself. This may be due to the fact that small molecules (like monoterpenes) have better access to the radical site and have higher apparent antioxidant capacity with DPPH test [34].

Table 2. Total phenol content and antioxidant capacity based on dpph assay of yarrow herb extracts (AA was used for comparison)

Sample	I%	EC50, %	AEAC, mg AA/100 mL	TPC, mg GAE/100 mL
Infusion	**6.7 ± 0.1**	**1.1 ± 0.1**	**36.6 ± 0.3**	**26.2 ± 0.1**
Hydroalcoholic extract	**8.9 ± 0.2**	**0.8 ± 0.1**	**53.3 ± 0.6**	**141.1 ± 1.9**
Essential oil	**37.3 ± 0.3**	**0.2 ± 0.02**	**200.2 ± 1.5**	**1093.2 ± 2.5**
Ascorbic acid	**13.3 ± 0.2**	**0.4 ± 0.03**	**-**	**-**

In the case of essential oil the GC/MS analysis revealed the presence of large amounts of terpenoids. Most of them, like α- and β-pinene, camphene, limonene, 1,8-cineole, borneol [35], sabinene, α-phellandrene [36], α- and γ-terpinene, α-terpinolene [37], *p*-cymene [38], α- and β-thujone, champhor [39], terpinen-4-ol, humulene [40], α-terpineol [41], β-caryophyllene [42], chamazulene [43], have been reported to possess antioxidant properties. The results in Table 2 confirm the high antioxidant capacity of the essential oils, on the basis of DPPH assay.

In the case of infusions and macerates (in 70% ethanol), the polarity of these solvents permits the extraction of polar compounds such as polyphenols. Polyphenols represent the major constituents responsible for the antioxidant activity of water and hydroalcoholic extracts [10].

The percentage inhibition (I%) of the essential oil were found to be higher than those ones of infusion and macerate while the efficient concentration value (EC50) were found to be lower. This because the percentage inhibition (I%) and efficient concentration value (EC50) are in reverse proportionality. It is interesting to note that the Folin-Ciocalteu assay led to high values of the antioxidant capacity expressed in gallic acid equivalent and ascorbic acid equivalent, respectively. These results are consistent with literature data according to which terpenes interfere with the the Folin-Ciocalteu assay [44].

Essential oils are not water soluble and so even a strong cup of tea will only contain 10 - 15% of the essential oil. On the other hand, considering that the necessary daily intake of AA is 70 - 80 mg [45], a tea cup infusion of 4 g *Achillea millefolium* in 100 mL of boiling water is an important dietary source of bioactive compounds, containing the equivalent of 36 mg of AA and a TPC of 26 mg (6.5 mg GAE/g leaves).

Effects of yarrow extract on cell viability and apoptotic cell death in HCT 116 (colon carcinoma) cell line: The literature reports a strong relationship between TPC and reduction of cell viability suggesting that the TPC value could be used as a predictive tool for the cytotoxic effects of a bioactive plant in vitro [10]. Therefore, the study of effects of yarrow extract on the cell viability and apoptotic cell death in HCT 116 (colon carcinoma) cell line was carried out. As was previously proved [10], in order to obtain better results in the present study hydroalcoholic extracts were used.

Treating HCT 116 cells with yarrow extract reduced cell viability in a dose- and time-dependent manner (Figure 1). Treatment with higher amounts of the extract for 24 h and 48 h resulted in a decrease in cell viability ($p < 0.01$).

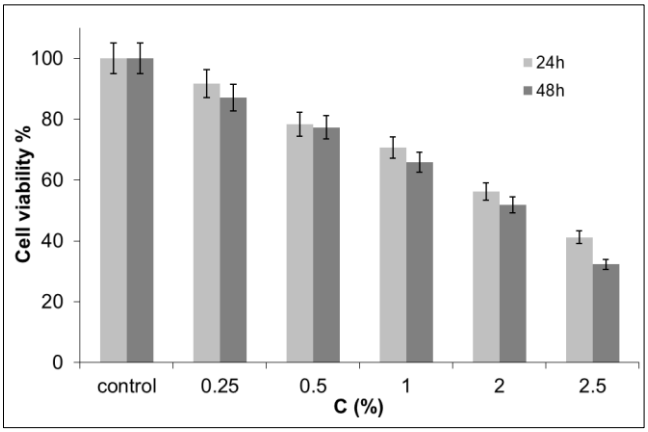

Figure 1. WST1 assay performed to demonstrate the effect of yarrow extract on HCT 116 proliferation and viability

The obtained results by flow cytometry indicated that yarrow extract led to the apoptotic cell death growing with the dose and time increasing (Figure 2)

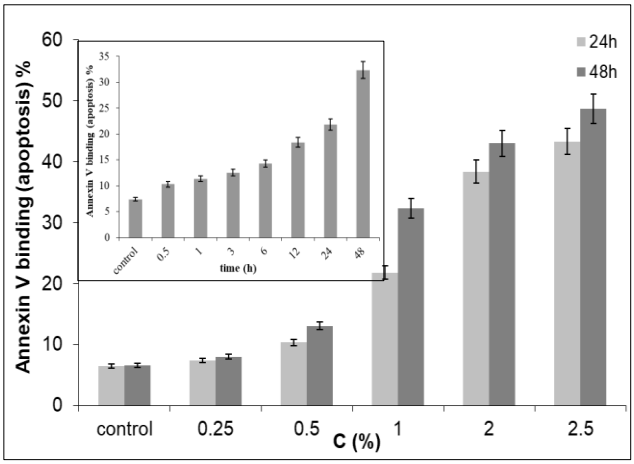

Figure 2. HCT 116 cells treated with indicated amounts of yarrow extract for 24 h or 48 h and HCT 116 cells treated with 40 µL of yarrow extract (1 %) at indicated time points (inset)

Cells were stained with FITC-Annexin-V and subjected to FACS analysis ($p < 0.01$) to demonstrate the effect of yarrow extract inducing apoptotic cell death. Apoptotic activity enhanced with the time increasing ($p < 0.01$) (Fig. 2 inset).

3.Conclusions

The present study performed on *Achillea millefolium* L. commercialized on the Romanian market showed a quasi-complete pattern of its volatile compounds using hydro-distillation or headspace extraction techniques. As it was expected, some volatile components have been modified during the HD process. Thus, HS-GC/MS analysis revealed that the yarrow samples have a significantly content of 1,8-cineole. HD-GC/MS showed that the oil extract has a considerable content of chamazulene. These facts ensure that yarrow has a high medicinal potential with multiple health benefits depending on the formulations. For different samples the oil extract has higher radical scavenging activity and TPC than the infusion or alcoholic extracts. Furthermore, treatment of HCT 116 colon carcinoma cells with yarrow extract induced the apoptotic cell death in a time and dose dependent manner in these cells. Apoptosis plays an important role in cellular homeostasis. Alterations in apoptotic mechanisms have been associated with cancer pathogenesis and drug resistance [46]. Considering our findings, further studies to evaluate effects of *Achillea millefolium L.* extract in apoptotic pathways are of importance, which would shed a light on its potential as a therapeutic candidate in cancer prevention and treatment.

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