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Jasonia montana Extracts Stimulate Cell Differentiation in HCT-116 and Caco-2 Cell Lines

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Abstract

Unlike differentiated cells, cancer stem cells (CSCs) can generate malignancy. Conventional chemotherapy results in CSCs spreading causing metastasis and relapse. Differentiation is an emerging approach providing a safer strategy to eradicate CSCs. *Jasonia montana* is a medicinal plant that occurs in Sinai Peninsula in Egypt. Here, we used two human colorectal cancer cell lines; HCT-116 and Caco-2 to investigate the ability of methanolic and petroleum ether extracts of *Jasonia montana* to differentiate colon CSCs. Tandem Mass–Spectrometry (MS/MS) revealed that quercitrin and 5-Hydroxycostic acid represent the major extracts ingredients. Cytotoxicity assay (SRB) illustrated that petroleum ether extract inhibited Caco-2 cells (IC50=10 µg/ml), whereas, methanolic extract inhibited HCT-116 cells (IC50=20 µg/ml). On treating HCT-116 and Caco-2 cells with methanolic and petroleum ether extract respectively in diluted doses to alleviate their cytotoxicity, qRT-PCR showed a significant increase in CK20 expression and a significant decrease in CD44 expression, a significant decrease in Sox-2 and NOTCH-1 expression and a significant increase in PTEN expression. Furthermore, qRT–PCR showed a significant decrease in miR-21-5P and miR-10b-5P expression and significant increase in miR-34a-5P and miR-145 expression. Our results suggest, for the first time, that *Jasonia montana* methanolic and petroleum ether extracts can induce colorectal CSCs differentiation.

Keywords: *Jasonia montana* extracts; colorectal cancer cells; differentiation; microRNAs

1. INTRODUCTION

Stem cells regulate tissue homeostasis by maintaining their self-renewal through stemness and differentiation signalling [1]. Cancer stem cells (CSCs) maintain tumours as they can evade immune responses contributing to disease recurrence, and metastasis and survive conventional anticancer treatments giving rise to therapeutic resistance [2]. As cancer stem cells can generate tumours, whereas differentiated cancer cells cannot, therefore, many studies have focused on targeting cancer stem cells to eradicate malignancies through a differentiation therapy approach [2]. Differentiation therapy is the promotion of a cell differentiation program thereby irreversibly changing the phenotype of cancer cells [3].

Notch is a well-known signalling pathway that plays a crucial role in tissue development and homeostasis. In particular, Notch is believed to be critical for the maintenance of undifferentiated stem/progenitor cell populations in the mammalian intestinal crypt [4]. Notch1 is one of four different receptors (Notch1, 2, 3, and 4) in the signalling pathway of the mammalian cells which is aberrantly activated in many cancers. Notch1 activation was stated as essential for adenoma development in adenomatous polyposis coli and multiple intestinal neoplasia in mice [5]. Similarly, Notch1 and HES1 were reported as significantly upregulated in human

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colon cancer. As it is overexpressed in many cancers, targeting Notch signaling may constitute a novel molecular therapy for cancer [6]. Recently, miRNAs have been reported to play critical roles in Notch signaling pathway. Several miRNAs have been shown to crosstalk with Notch pathway. For example, miR-1, miR-21, miR-34, miR-146, miR-199, and miR-200 can crosstalk with Notch signaling pathway [7]. Not only Notch can regulate several microRNAs expression; but also, Notch signaling elements including ligands, receptors, or effectors are subjected to regulation by microRNAs [8].

PTEN (Phosphatase and tensin homolog) is one of the most frequently inactivated tumor suppressors in cancer where variation in its expression levels and/or mutations is commonly observed in many human cancers including colorectal cancer [9]. PTEN indirectly regulates cellular differentiation as its main target is the cell growth and pro-survival signaling pathway: PI3K/AKT, which is considered a part of the molecular stemness program to which increased dedifferentiation is linked [10]. At the same time, PTEN is negatively regulated by Notch. In colorectal cancer, Koveitypour Z et al. reported that Notch signaling activates PI3/AKT pathway through activation of HES1 which is a negative regulator of PTEN [11]. On another level, PTEN is regulated by miRNA including miR-21, miR-200, miR-214, miR-126, and others [12].

The cell differentiation process can also be regulated by microRNAs which target the stem cell fate-deciding components [13]. MiRNAs can be classified into two classes: the first class involves the pluripotent miRNAs which can promote the self-renewal and proliferation of stem cells but inhibit cell differentiation including miR-9, miR-137, miR-184, miR-200, miR-290, and, miR-302. The other class involves the pro-differentiation miRNAs which can promote cellular differentiation through inhibiting genetic and/or epigenetic stemness factors Sox2, Oct, and Nanog / Bmi-1, cell cycle transition or epithelial-mesenchymal transition including let-7, miR-122, miR-134, miR-145, miR-181, miR-296, and miR-470 [14].

Natural, chemical, and biological agents have been used to induce terminal ex vivo differentiation in some malignancies such as retinoic acid, sodium butyrate, and cytokines by influencing the signaling elements involved in the cellular differentiation pathway [3]. Natural agents can also be used to target miRNAs that are known to contribute to the processes of cellular differentiation [4]. *Jasonia montana* [=Chiliadenus montana (Vahl.) Brullo, Chrysocoma montana (Vahl.) Symb., Varthemia montana (Vahl.) Boiss.] is a medicinal plant belongs to family Compositae (Asteraceae) which comprises about 25000 species in 1300 genus. It occurs in the Mediterranean area and the Sinai Peninsula in Egypt [15]. The plant was reported to have a strong cytotoxic effect against HepG2 and MCF7 cells. Most interesting, when tested against HFB-4 normal cell line, *Jasonia montana* was non-toxic [16]. Such differential effect on cancer and normal cells adds an advantage if the plant is used for therapeutic purposes. Many other biological activities were also reported in the literature including the antidiabetic, antioxidant, and anti-inflammatory activities [17]. Nevertheless, up to our knowledge, the plant has not been adequately evaluated for its anti-cancer activity yet.

In this work, we investigated the ability of *Jasonia montana* extracts to induce cell differentiation hence can be used as differentiating therapy for cancer treatment. We examined the toxicity of methanolic and petroleum ether extracts of *Jasonia montana*, using two colorectal cancer cell lines; HCT-116 and Caco-2. We further assessed the ability of the plant extracts to induce stem cell differentiation by measuring the gene expression of elements regulating the cellular differentiation pathway and most important controlling miRNAs using quantitative real-time polymerase reaction (qRT-PCR).

2. EXPERIMENTAL

2.1. Plant Material:

Jasonia montana aerial parts were collected from west Alexandria and Hilly Areas from El-Arbaeen valley, Saint Catherine, South Sinai, Egypt. For this study, we used *Jasonia montana* collected from Saint Catherine, South Sinai, in January 2020 and identified by M. A. Gibaly (senior botanist at the National Research Center, Cairo, Egypt). Voucher specimens (7815) were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt.

2.2. Extract Preparation:

The plant was washed and subsequently oven-dried at 40 °C for 3 days until a constant weight was achieved. The dried samples were powdered and stored at 4 °C for further use. The extraction, using

methanol and petroleum ether, was conducted via maceration extraction (ME) by placing the lyophilized powder (5 gm) in a beaker containing 100 ml solvent to obtain a solid/liquid ratio (50 g/L). The beaker was placed in a thermo-stated water bath under continuous electromagnetic stirring for the required time period (Table 1).

Table 1: Extraction condition of different extracts.

Type of extract	Type of solvent	Temperature (°C)	Time (h.)
Methanolic extract	80% methanol	40	6
Petroleum ether extract	100% petroleum ether	61.32	5.45

2.3. Extract Purification:

The collected extracts were filtered through a Whatman paper filter n◦4, dried at 40 °C in a rotary evaporator and centrifuged at 8000 rpm for 10 min. The supernatants were concentrated under reduced pressure at 40 °C. The yield of extracts was measured and freeze-dried at -20 °C. All extracts were stored at 4 °C for further studies.

2.4. Tandem Mass – Spectrometry (MS/MS) Analysis:

The analysis was conducted using ABSCIEX Q TRAP 3200 mass-spectrometer (ABSCIEX, Germany) equipped with an electrospray ionization (ESI) interface. Serial dilutions of the two different extracts of *Jasonia montana* were prepared to reach concentrations 1 ug/ml and 100 ng/ml. By using (methanol 0.1% formic acid) as a solvent for positive mode and (methanol 1mM ammonium format) for negative mode. Each extract was injected into a mass-spectrometer at a flow of 10 µl/min. The mass spectrometer was operated with spray voltage set at 4.5 kV, at a temperature of 350 °C, and a Curtain gas flow of 10 L/h. Data acquisition was performed with analyst 4.0 software (ABSCIEX). Methanol for HPLC 99.9% (Riedel-deHaën, Honeywell, Germany), formic acid for mass spectrometry, 98%, ammonium format (Sigma-Aldrich, Steinheim, Germany).

2.5. Human Colorectal Cancer Cell Lines:

Human Colon cancer cell lines Caco-2 and HCT-116 were obtained frozen in liquid nitrogen (–180 °C) from VACSERA (the Egyptian Company for Production of Vaccines, Sera and Drugs). The tumor cell line was maintained as mono-layer cultures in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin.

2.6. Cell viability assay by SRB (Sulforhodamine-B): Cytotoxicity was determined using sulforhodamine-B (SRB) method according to Skehan et al. [18] Cells were seeded in 96- well microtiter plates at a concentration of 3 × 103 cells/well. They were left to attach for 24 h before incubation with drugs. The cells were treated for 48 h with different concentrations (0, 12.5, 25, 50, and 100 µg/ml) of *Jasonia montana*. The optical density (O.D) of each well was measured spectrophotometrically at 570 nm using an ELISA microplate reader (TECAN SunriseTM, Germany). The mean values were estimated as the percentage of cell viability as follows: O.D (treated cells) / O.D (control cells) × 100. The IC_{50} value (the concentration that produces 50% inhibition of cell growth) of each drug was calculated using dose-response curve-fitting models (Graph-Pad Prism software, version 5).

2.7. Cell Treatment:

Human Colon cancer cell lines HCT-116 and Caco-2 were treated for 48 h with methanolic and petroleum ether extracts respectively in diluted doses of each extract (the dilution is four times the IC_{50} values; i.e., 5 µg/ml and 2.5 µg/ml).

2.8. Bioinformatic Tools:

The measured parameters were selected using three bioinformatics databases including MiRTarBase (https://www.bio.tools/mirtarbase), microRNA.org (https://www.microrna.org) and Targetscan (http://www.targetscan.org) are used to select the miRNAs that are associated with different differentiation pathways. The interrelation between selected parameters is illustrated in Figure 1.

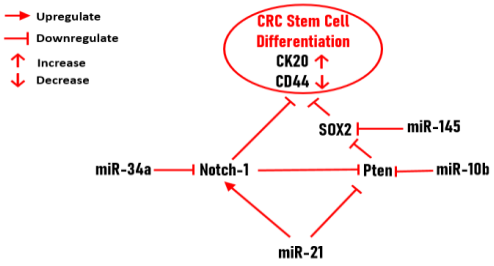

Figure 1: A Diagrammatic representation of the interrelation between variuos measured parameters

2.9. Quantitative Real-Time Polymerase Chain Reaction assay (qRT-PCR):

QRT-PCR was performed to determine the expression levels of our genes of interest (PTEN, Notch-1, Sox-2, CD 44 and CK20), as well as four miRNAs; miR-145, miR-10b, miR-21, miR-34a. Extraction of total RNA from cells including miRNAs was done using miRNeasy Mini Kit, Catalog no. 217004 (Qiagen, Germany). The protocol followed the manufacturer's instructions. The quality and quantity of extraction products were measured by Nanodrop™ (Thermo Fisher, UK). The miscript RNA reverse transcription kit miScript II RT Kit catalog no. 218161 was used for cDNA synthesis from total RNA and miRNA (Qiagen, Germany). Quantification of the target genes and miRNAs was performed using the miScript SYBR Green PCR Assay kit (Qiagen, Germany) using a reference gene (GAPDH and RNU6B) as the normalizer. Fold change is calculated according to Schmittgen and Livak [19]. The primers for mRNA and miRNAs were obtained from (Qiagen, Germany). The primers for mRNA and miRNAs were obtained from (Qiagen, Germany).

2.10. Statistical Methods:

Data was analyzed using GraphPad PRISM advanced statistics version 7. Numerical data were expressed as mean and standard deviation. For not normally distributed quantitative data, comparison between two groups was done using the Mann-Whitney test (non-parametric t-test). All tests were two-tailed. A p-value < 0.05 was considered significant.

3. RESULTS

3.1. Quercitrin and 5-Hydroxycostic acid are the major components of both *Jasonia montana* methanolic and petroleum ether extracts:

On studying *Jasonia montana* extracts using ABSCIEX Q TRAP 3200 mass spectrometers using positive mode ESI (Figure 2), the resolution mass spectrum in both methanolic and petroleum ether extract had a prominent molecular ion peak $[M]_+$ at *m/z* 449.0 which represent a similar molecular weight of one of the flavonoids (Quercitrin). Other minor components in both extracts with molecular ion peak $[M]_+$ at *m/z* 239.0 were detected which represent a similar molecular weight of one of the terpenes (Chiliadenol A). Also, the essential oil (1,8-cineole) was detected in a minor amount in both methanolic, petroleum ether extract with molecular ion peak $[M]_+$ at *m/z* 155.2.

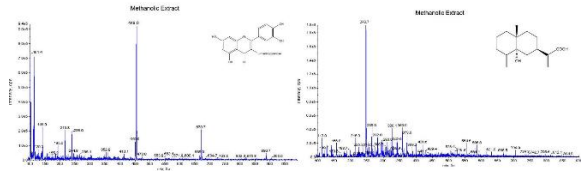

Figure 2: Positive ESI mode mass spectra of the two extracts of *Jasonia Montana* using ABSCIEX Q TRAP 3200 mass spectrometer. A: methanolic extract, B: petroleum ether extract

On the other hand, when using negative mode ESI (Figure 3), the resolution mass spectrum of both extracts had a prominent molecular ion peak $[M]^-$ at *m/z* 249.1 which represents a similar molecular weight of one of the Acids previously isolated from *Jasonia montana* known as 5-Hydroxycostic acid. Other minor components were detected in both extracts with molecular ion peak $[M]^-$ at *m/z* 329.2 which represent a similar molecular weight of one of the flavonoids (3,3`-dimethoxyquercetin). So, the biological activity detected from both methanolic and petroleum ether extract in our study it may be due to the effect of Quercitrin, 1,8-cineole, and 5-Hydroxycostic acid on biological cells.

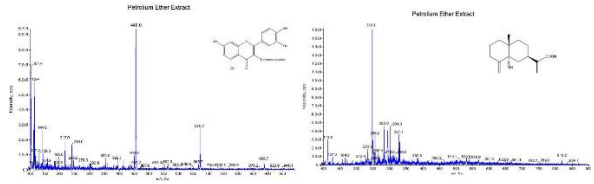

Figure 3: Negative ESI mode mass spectra of the two extracts of *Jasonia montana* using ABSCIEX Q TRAP 3200 mass spectrometer. A: methanolic extract, B: petroleum ether extract

3.2. Methanolic and petroleum ether extracts of *Jasonia montana* showed strong cytotoxic activity:

On treatment with both extracts, a concentration-dependent decrease in the cellular growth rate was obtained as shown in Figure 4. The methanolic extract inhibited the growth of HCT-116 and Caco-2 cells at IC_{50} = 20 and 83 µg/ml, whereas, petroleum ether extract inhibited the growth of both cells at IC_{50} = 60 and 10 µg/ml respectively.

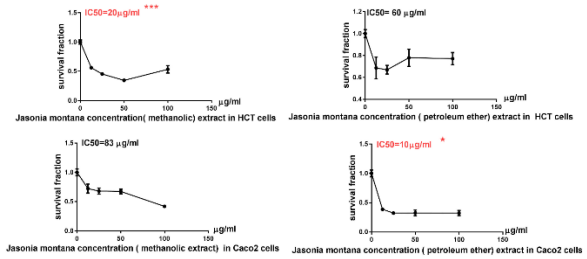

Figure 4: The cytotoxicity of *Jasonia montana* methanolic and petroleum ether extracts on HCT-116 cells (upper panel) and Caco-2 cells (lower panel) (Experiments were performed in triplicate.). Significance was adjusted at $p<0.05$.

3.3. *Jasonia montana* extracts affected significantly the expression of CD44 and CK20 in both colorectal cancer cells:

As illustrated in Figure 5, our extracts affected some differentiation which markers favor cell differentiation. A very highly significant decrease in the expression of CD44 was obtained when HCT-116 and Caco-2 cells were treated with methanolic and petroleum ether extract respectively (p = 0.0003 and 0.001). Also, a very highly significant and a highly significant increase in the expression of CK20 when HCT-116 and Caco-2 cells were treated with both extracts (p = 0.001 and 0.0143 respectively).

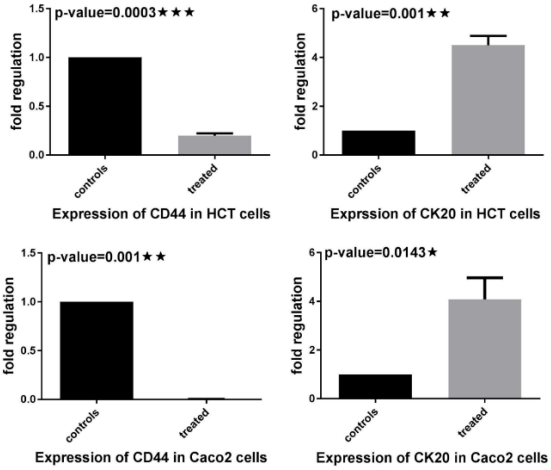

Figure 5: The effect of *Jasonia montana* methanolic and petroleum ether extracts on the expression of CD44 and CK20 in HCT-116 cells (upper panel) and Caco-2 cells (lower panel). Significance was adjusted at $p<0.05$.

3.4. *Jasonia montana* extracts significantly affected the expression of Sox-2, Notch-1, PTEN, and their regulating miRNAs:

As illustrated in Figures 6 and 7, our extracts affected a number of differentiation regulatory molecules and their controlling miRNAs, which finally enhances cell differentiation. Notch-1 and Sox-2 expression were significantly downregulated (p = 0.0003 and 0.014); ((p = 0.014 and 0.004), respectively, while that of PTEN was significantly upregulated (p = 0.0003 and 0.001) respectively, in both extract-treated HCT-116 and Caco-2 cells. On the other hand, miR-34a-5P and miR-145 expression were significantly upregulated (p = 0.014); ((p = 0.007 and 0.0006) respectively, while that of miR-10b-5P and miR-21-5P were significantly down-regulated (p = 0.0017 and 0.001); (p = 0.0004 and 0.0125), respectively, in both cell types.

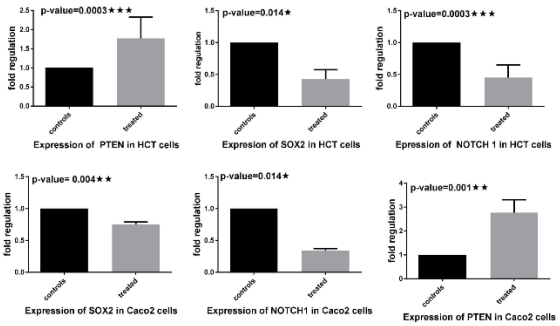

Figure 6: The effect of *Jasonia montana* methanolic and petroleum ether extracts on the expression of Notch-1, Sox-2 and PTEN in HCT-116 cells (upper panel) and Caco-2 cells (lower panel). Significance was adjusted at $p<0.05$.

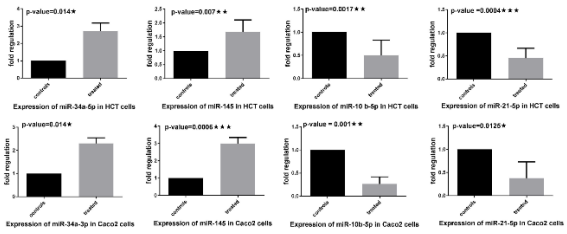

Figure 7: The effect of *Jasonia montana* methanolic and petroleum ether extracts on the expression of miR-34a-5P, miR-145, miR-10b-5P and miR-21-5P in HCT-116 cells (upper panel) and Caco-2 cells (lower panel). Significance was adjusted at $p<0.05$.

4. DISCUSSION

Differentiation therapy is a promising and safer alternative to conventional cancer therapy. Such therapy targets a cancer hallmark, which is loss of differentiation, that forces cells to self-renewal and

proliferation. It aims at inducing cancer cell differentiation to remove them from the proliferative compartment. Retinoic acid was the first drug-induced differentiating agent used to treat acute promyelocytic leukemia. Now, several solid tumors are also treated using this approach [20].

Jasonia montana is a common medicinal plant with therapeutical activities attributed to different groups of effective substances [21]. Referring to the literature, more investigation is required to evaluate the plant in the field of cancer therapy. In this work, two different *Jasonia montana* extracts using methanol and petroleum ether were prepared and characterized by Tandem Mass – Spectrometry (MS/MS) analysis. We investigated the cytotoxic activity of each extract using the SRB assay. Finally, two colorectal cancer cell lines; HCT-116 and Caco-2 cells were treated with diluted doses, to alleviate the cytotoxic effects of the extracts, for 48 hours and the gene expression of a number of stem cell differentiation signaling molecules, as well as their regulating miRNAs, were investigated to evaluate the ability of such extract to induce stem cell differentiation.

Our results revealed that the IC_{50} values obtained on treating HCT-116 and Caco-2 cells with methanolic and petroleum ether extract are 20 and 10 µg/ml respectively. Our two extracts, following the American National Cancer Institute, can be considered as strong cytotoxic agents as they achieve an IC_{50} value lower than 30 µg/ml [22-24].

Characterization of methanolic and petroleum ether extracts using Tandem mass- spectrometry (MS/MS) analysis showed that Quercitrin and 5-Hydroxycostic acid are major components in both methanolic and petroleum ether extracts of *Jasonia montana* to which biological effects are most probably attributed as reported previously [25-27]. Quercetin, which is the most abundant bioflavonoid compound, is mainly present in the glycoside form of quercitrin. It was reported to have a moderate cytotoxicity on both HCT-116 cells (IC_{50} 47 µg/ml) [28] and Caco-2 cells (IC_{50} 79 µg/ml) [29]. Although different studies indicated that quercitrin is a potent antioxidant, the action of this compound is not well understood. Previous studies reported that quercitrin has antiproliferative and apoptotic effects on colon cancer cells [30]. Others revealed that quercitrin has antiproliferative and apoptotic effects on lung cancer cells by modulating the immune response through significant increases in caspase-3 activity, loss of MMP, and increases in the apoptotic cell population [31]. After confirming its anticarcinogenic effects in vivo, quercitrin could be a novel and strong anti-cancer agent. While 5-Hydroxycostic acid is one of the eudesmane-type sesquiterpenes which has anti-inflammatory properties, the previous study confirms its antiangiogenic effect and ability to suppress breast cancer cell migration through VEGF- and Angiopoietin 2-mediated signaling pathways [32]. Some eudesmanolids were reported to have strong toxicity against HCT-116 cells (IC_{50} 5.6 and 6.7 µM) [33]. Chiliadenol-A, 1,8-cineole, and 3,3`-dimethoxyquercetin were also detected in our extract with a minor amounts which is consistent with Hammerschmidt et al. [34]; Soliman et al. [25] and El-Bsoumy et al. [27].

CK20 (cytokeratin 20) is a differentiation marker by which the differentiated intestinal epithelium essentially can be distinguished from the undifferentiated counterpart. On the contrary, CD44, the prominent CSC marker, was shown to be expressed by colorectal cancer-initiating cells which also represent the undifferentiated cell characteristics and morphology [35]. Our data reported for the first time that *Jasonia montana* extracts were able to induce cancer stem cell differentiation in both HCT-116 and Caco-2 cells evidenced by the significant increase in CK20 expression (p = 0.001 and 0.0143) and the significant decrease in CD44 expression (p = 0.0003 and 0.001) in *Jasonia montana* extracts – treated cells.

Then, a number of signaling molecules contributing to CSC differentiation were investigated. First, we investigated the Sox-2 expression in the *Jasonia montana* extracts – treated cells. Sox2 is a transcription factor involved in the maintenance of the undifferentiated status in pluripotent stem cells. Aberrant expression of Sox-2 has been demonstrated in human colorectal cancer [36]. Also, Sox-2 was reported to be associated with a down-regulated expression of the differentiation marker CDX2 in CRC patients [37]. Our results showed that the extracts were significantly able to inhibit Sox-2 expression in both HCT-116 and Caco-2 cells (p = 0.014 and 0.004 respectively). Second, we investigated Notch signaling in the *Jasonia montana* extracts – treated cells. In the normal state, Notch

receptors regulate the proliferation of crypt progenitor cells and the differentiation of colonic epithelial cells. However, aberrantly activated Notch1 contributes to cancer cell stemness and CRC development. Targeting Notch pathway, therefore, seems to be an interesting therapeutic approach [38]. Here, we elucidate that *Jasonia montana* extracts have a significant ability to inhibit Notch1 expression in both HCT-116 and Caco-2 cells (p = 0.0003 and 0.014 respectively). Interestingly, in agreement with our finding, quercetin that we illustrated as one of the two major extracts components, has been reported to induce Notch-inhibiting activity in a leukemia cell line [39]. Accordingly, the inhibition of Notch1 expression in both cell lines can be attributed, in particular, to quercetin. Third, we investigated the effect of *Jasonia montana* extracts on PTEN expression. It was reported that loss of PTEN function resulted in increased intestinal stem cells with altered differentiation [40]. However, such an effect is believed to occur through PI3K/Akt axis and Sox2, Oct3/4, and Nanog [10][41]. In our work, *Jasonia montana* extracts – treated cells showed a significant increase in PTEN expression in comparison to untreated cells (p = 0.0003 and 0.001). It's worth noting that quercetin was reported by Cao Y et al. to upregulate PTEN in a human tubular epithelial cell line [42]. As Notch1, PTEN, and Sox2 are mutually affected by each other, further study is required to address whether they are independently affected by the extracts.

We further investigated a number of miRNAs that regulate CSC differentiation. MiR-21 is an oncogenic miRNA overexpressed in most epithelial cancers. To confirm its role, Yu Y et al. reported that miR-21 knockdown induced differentiation and reduced migration and invasion of colon cancer cells [43]. In our study, HCT-116 and Caco-2 cell treatment with *Jasonia montana* extracts significantly inhibited the expression of miR-21-5P (p= 0.0125) which should contribute to CSC differentiation as demonstrated in both cell lines. This is in line with Cao Y et al. who reported that treatment with quercetin inhibited miR-21-5P in a human tubular epithelial cell line [42]. Following Wu Y et al. and Xiong Y et al., miR-21-5P can repress PTEN [44] and crosstalk with Notch1 [45], respectively, in colorectal cancer, both of which contribute to enhancing cell differentiation. From such a perspective, *Jasonia montana* extracts – induced inhibition of miR-21 might also contribute to CSC differentiation through upregulating PTEN and downregulating Notch1 expression. We also studied the tumor suppressor miRNA; miR-34a-5P which directly targets Notch-1 [46], and the oncogenic miRNA; miR-10b-5P which targets PTEN [47-48]. We recorded a significant inhibition of miR-10b-5P (p = 0.0012). Our finding, in agreement with Cheng Y et al., suggests that the overexpressed PTEN, in *Jasonia montana* extracts – treated cells, most probably have been achieved by a negative feedback action [48]. Unexpectedly, our results showed significant inhibition of miR-34a-5P (p = 0.0143). This finding suggests that Notch1 was not affected by the inhibition of miR-34a and confirms that Notch1 has been directly inhibited by quercetin. MiR-145 is a tumor suppressor miRNA downregulated in colorectal cancer. Quercetin was reported to inhibit miR-145 in human ovarian carcinoma cells [49] and endometrial stromal cells [50]. In our study, *Jasonia montana* extracts significantly inhibited miR-145 in HCT-116 and Caco-2 cells (p = 0.0006, 0.007 respectively) most probably through quercetin. Yu Y et al. reported that administration of miR-145 induces differentiation of colorectal CSC through inhibiting Sox-2 [51]. To assess whether Sox-2 was inhibited directly by quercetin or as a result of miR-145 inhibitory action, additional research is required.

5. CONCLUSION

In addition to their strong cytotoxic effect, our report suggests, for the first time, that *Jasonia montana* methanolic and petroleum ether extracts can be used in diluted doses as colorectal CSC differentiation-inducing agents. Investigating such extracts *in vivo* as well as other cancer models is kindly recommended.

6. CONFLICTS OF INTEREST:

All authors declare that they don't have any conflict of interest.

7. LIST OF ABBREVIATIONS:

CRC: Colorectal cancer
CSC: Cancer stem cells
qRT-PCR: Quantitative real-time polymerase chain reaction:
SRB: Sulforhodamine
miRNA: Micro-ribonucleic acid
ELISA: Enzyme-limked immunosorbent assay
MS/MS: Mass spectrometry/mass spectrometry, i.e.; tandem mass spectrometry)
CK20: Cytokeratin-20

PTEN: Phosphatase and TENsin homolog
Notch-1: Notch homolog 1, translocation-associated (Drosophila)
Sox-2: SRY (sex determining region Y)-box 2
PI3K: Phosphatidylinositol-3-Kinase
Akt: protein kinase B

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