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First Report on Comparative Essential Oil Profile of Stem and Leaves of Blepharispermum hirtum Oliver and Their Antidiabetic and Anticancer Effects

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Abstract: The current research was designed to explore the Blepharispermum hirtum Oliver (Asteraceae) stem and leaves essential oil (EO) composition extracted through hydro-distillation using gas chromatography-mass spectrometry (GC-MS) analysis for the first time. The EOs of the stem and leaves of B. hirtum were comparatively studied for the in vitro antidiabetic and anticancer potential using in vitro α-glucosidase and an MTT inhibition assay, respectively. In both of the tested samples, the same number of fifty-eight compounds were identified and contributed 93.88% and 89.07% of the total oil composition in the EOs of the stem and leaves of B. hirtum correspondingly. However, camphene was observed as a major compound (23.63%) in the stem EO, followed by β-selinene (5.33%) and β-elemene (4.66%) and laevo-β-pinene (4.38%). While in the EO of the leaves, the dominant compound was found to be 24-norursa-3,12-diene (9.08%), followed by β-eudesmol (7.81%), β-selinene (7.26%), thunbergol (5.84%), and caryophyllene oxide (5.62%). Significant antidiabetic potential was observed with an IC50 of 2.10 ± 0.57 μg/mL by the stem compared to the EO of the leaves of B. hirtum, having an IC50 of 4.30 ± 1.56 μg/mL when equated with acarbose (IC50 = 377.71 ± 1.34 μg/mL). Furthermore, the EOs offered considerable cytotoxic capabilities for MDA-MB-231. However, the EO of the leaves presented an IC50 = 88.4 ± 0.5 μg/mL compared to the EO of the stem of B. hirtum against the triple-negative breast cancer (MDA-MB-231) cell lines with an IC50 = 123.6 ± 0.8 μg/mL. However, the EOs were also treated with the human breast epithelial (MCF-10A) cell line, and from the results, it has been concluded that these oils did not produce much harm to the normal cell lines. Hence, the present research proved that the EOs of B. hirtum might be used to cure diabetes mellitus and human breast cancer. Moreover, further studies are considered to be necessary to isolate the responsible bioactive constituents to devise drugs for the observed activities.

Keywords: Blepharispermum hirtum; Asteraceae; GS-MS analysis; essential oils; triple-negative breast cancer; α-glucosidase

1. Introduction

Medicinal plants and their products serve as both traditional and commercial alternative innovative remedies [1]. Due to the efficacy and lower adverse effects, the demand for herbal therapies has increased. Plant based natural products, comprising essential oils (EOs) have gained attention due to their usage in foodstuff, cosmetics, and pharmaceutical...
productions. Constituting a range of several lipophilic and extremely volatile constituents, obtained from an extensive range of diverse chemical classes, EOs are attributed to multiple health benefits: analgesic, anti-inflammatory, antioxidant, antimicrobial, anticancer, and antidiabetic [2].

Diabetes mellitus is considered as a world health issue, linked to two key features including insufficient insulin secretion or insensitivity to their action. Their high rate of prevalence reflects its severity, and according to the projected statistics of WHO, more than 422 million people have diabetes, 1.5 million deaths are directly attributed to diabetes each year, and the prevalence ratio will increase to 693 million by 2045 [1,3]. The diabetes might lead to several other complications such as polyuria, polydipsia, impaired vision, and skin infections [4]. Therefore, strategies need to be developed to combat these drawbacks. In this context, α-glucosidase (EC 3.2.1.20) has become a promising target for the treatment of diabetes mellitus. The inhibition of these carbohydrates’ key metabolic enzymes slows down carbohydrate digestion, resulting in the low absorption of glucose, leading to normalizing the blood glucose levels. Hence, the investigation of new anti-diabetic agents using natural sources is currently of need because of their non-cytotoxic effects [5–7].

Increased interest of users concerning pharmacologically effective plant-based natural products (NPs) as substitute therapies to treat cancer has increased the attention of scientists worldwide [4]. However, an intensifying significance has been observed recently that EOs act as an anticancer medication to overcome the development of multidrug resistance and critical harmful effects linked with available antitumor remedies [5]. Therefore, due to the key role of EOs in cytotoxic therapy, the EOs of unreported plants might be used as a complementary remedy [6].

*Blepharispermum hirtum* Oliver (family: Asteraceae) is a naturally growing tree, about 2 m in height, and is endemic to Dhofar (Oman). The plant has very broad and soft leaves with a basic inflorescence containing a capitulum of white flowers. The genus *Blepharispermum* comprises 15 species, all of which are shrubs, except for *B hirtum*. *Blepharispermum* species are distributed over different regions of Africa, the Arabian Peninsula, and India [7]. Decoctions as well as the root powder of *B. subsessile* have been used by local practitioners in India for the treatment of various health ailments used in nervous disorders, while the whole plant is used in diarrhea, stomach ache, rheumatic affections, skin diseases, eye troubles, anti-inflammatory diseases, and irregular menstruation [8–10]. Recently, Fatope et al., [9] reported ent-kaurene diterpenoids with larvicidal and antimicrobial activity. It also has promising potential to resist microbes and antifeedant significance [9]. The genus *Blepharispermum* is an affluent basis for many bioactive ingredients including dimethyl isoencecalin and 5-hydroxy-6-acetyl-2-hydroxymethyl-2-methyl chromene [10].

Furthermore, the reported literature of EOs and the traditional uses of the plants growing in Oman have been noticed to have promising potential to cure diabetes and cancer [11,12]. Natural products derived from plants and plant products that have been traditionally used to treat various diseases including cancer and diabetes have advantages in drug discovery [13]. Thus, the current study was designed to profile the constituents of the EOs and determine the in vitro antidiabetic and cytotoxic significance of *B. hirtum*. Hence, the recent study will update the literature on the genus *Blepharispermum* and report on the EOs of *B. hirtum* for the first time.

2. Materials and Methods

2.1. General Instrumentation

The MDA-MB-231 and MCF-7 cell lines were acquired from the American Type Culture Collection (ATCC) and MCF-10A was purchased from the Iranian Biological Resource Center (IBRC) (Tehran, Iran). GC-MS was conducted on a gas chromatography-mass spectrometer (GC-MS-QP2010, Shimadzu Kyoto, Japan). The α-glucosidase enzyme (EC 3.2.1.20, Sigma-Aldrich, Darmstadt, Germany) and spectrophotometer (xMark™ Microplate Spectrophotometer, Bio-Rad, Hercules, CA, USA) were used for the α-glucosidase activity.
High-Speed Multifunctional Grander (Grand Household, Code. GR-SCG350H) was used for the grinding of the plant. Analytical grade reagents were used in the current study.

2.2. Collection and Identification of Plant Materials

The whole plant material of *B. hirtum* (8.7 kg) was collected from Salalah, the Dhofar region of Oman (April–May 2020). After identification by the plant taxonomist (Syed Abdullah Gilani, Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa, Oman), the leaves (4.0 kg) were separated from the stems (4.2 kg) and placed under shade at room temperature for dryness. The dried samples were ground into fine powder (50–300 mesh) using a stainless-steel blender. A voucher specimen of *B. hirtum* (BHO-03/2020) was deposited in the herbarium of the Natural and Medical Sciences Research Center, University of Nizwa, Oman.

2.3. Essential Oils Extraction

The essential oils extracted through hydro-distillation from the leaves and stem of the *B. hirtum* yielded 1.2 g (0.052%) and 0.95 g (0.045%), respectively, using a Clevenger-type apparatus (three times for at least 6 h) and were observed to be yellow-colored [14,15]. A known quantity of the EOs was collected, dried over anhydrous sodium sulfate (Na₂SO₄), and kept in the refrigerator at 4 °C until further GC-MS analysis and in vitro antidiabetic and cytotoxic assays.

2.4. GC-MS Analysis

The chemical constituents in the stem and leaf samples of the understudy plant were determined through the Perkin Elmer Clarus (PEC) 600 GC System (Perkin Elmer, Waltham, MA, USA) using gas chromatography-mass spectrometry (GC/MS) analysis. The GC/MS instrument was coupled with an Rtx-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) at 260 °C, connected to a PEC 600 mass spectrometer (MS). Electron multiplier (EM) voltage was achieved from autotune with 70 eV ionizing energy (IE). The carrier gas was helium (99.9999%) with a flow rate of 1 mL/min, while temperatures of 260 °C and 280 °C were used for the injection, transfer line, and ion source, respectively, during the whole analysis. The oven temperature was kept at 60 °C, holds for 1 min, at a flow rate of 4 °C/min–260 °C, and stood for 4 min. The essential oil solution (1 µL) was injected with a split ratio of 10:1. The complete chromatographic data were obtained by accumulating the full-scan mass spectra in the range of 45–550 amu. Furthermore, the total processing time of the GC/MS analysis was 55 min.

Identification of the Components

The essential oils extracted from the leaves and stems of *B. hirtum* were identified by their respective chromatogram peaks obtained for each compound through GC-MS analysis. Some of the compounds were identified by comparing their mass spectra with the MS library database (NIST 2011 v.2.3). Compound identification was also made possible by comparing their retention times (Rt) with those of the pure authentic samples and by means of their retention index (RI), relative to the series of n-hydrocarbons [14–16].

2.5. In Vitro α-Glucosidase Inhibitory Assay

Evaluation of the α-glucosidase inhibitory significance of the essential oils of the tested samples proceeded at 37 °C using 0.5 mM phosphate buffer (pH 6.8) [9,10]. High to low doses of the tested samples including (60, 30, 15, 7.8, 3.90, and 1.95 µg/mL), respectively, were incubated with the enzyme (2 U/mL) in phosphate buffer for 15 min at 37 °C. After adding the 25 µL substrate, p-nitrophenyl-a-D-glucopyranoside (0.7 mM, final), a spectrophotometer was used to track the changes in absorbance at 400 nm for 30 min. DMSO-d₆ (7.5 percent final) was used as a positive control. As a reference standard, acarbose (IC₅₀ = 377.7 1.34 µg/mL) was employed. Furthermore, the IC₅₀ was calculated by using EZ-fit software, as explained in the statistical analysis section by Equations (2) and (3).
2.6. In Vitro Cytotoxic Potential

In vitro cytotoxicity capacity of EOs was determined by performing an MTT (yellow tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay by using an aggressive breast cancer cell line (MDA-MB-231) [17]. Human breast normal cell line MCF-10A was kept as a control in the study. Cells were cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% FBS and 1% antibiotics (100 U/mL penicillin). The cells were seeded in a 96-well plate at a density of 1.0 × 10^4 cells/well and incubated for 24 h at 37 °C in 5% CO₂. The medium was discarded, and both cell lines were treated with different concentrations (3, 10, 30, 100, and 300 µg/mL) of plant EOs [18] after 48 h of incubation (Maher et al. [19]). A total of 20 µL of MTT solution (5 mg/mL) was pipetted into each well and incubated for another 4 h. The medium was later discarded, and the formazan precipitate was dissolved in DMSO. The absorbance of the mixtures was determined using a microplate reader at 570 nm. All experiments were performed in triplicate and the cytotoxicity was expressed as a percentage of cell viability compared to the untreated control cells [18]

\[
\text{% Viability} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\] (1)

2.7. Statistical Analysis

Excel and the SoftMax Pro package were used as the applications to examine the results for biological activity. The following formula was used to determine the % inhibition.

\[
\text{% Inhibition} = 100 - \left( \frac{O_{D_{\text{test compound}}}}{O_{D_{\text{control}}}} \right) \times 100
\] (2)

All of the tested substances’ IC₅₀ values were calculated using EZ-FIT (Perrella Scientific, Inc., Amherst, MA, USA). All experiments were carried out in triplicate to reduce the likelihood of mistakes, and differences in the results are reported as the standard error of mean values (SEM).

\[
SE = \frac{s}{\sqrt{n}}
\] (3)

The cytotoxic activity was estimated via IBM SPSS Statistics 26 software and utilized to analyze the dose response and computation of IC₅₀.

3. Results and Discussion

3.1. Composition of Essential Oil

The role of essential oils in the therapy of human health complications from ancient times to date cannot be denied. The promising potential attributed to EOs is due to the presence of valuable ingredients. In the current study, through the GC-MS analysis, fifty-eight compounds were identified in the EOs of the stems and leaves of *B. hirtum* (Table 1). The compounds identified in the stem through GC-MS screening contributed 93.88% of the total oil composition among which camphene was noticed as the dominant compound having 23.63%, followed by β-selinene with 5.33%, β-elemene (4.66%), and laevo-β-pinene (4.38%) (Table 1 and Figure 1). While the same number of compounds were identified in the EOs of the leaves of the *B. hirtum* sample, which contributed 89.07% of the total composition, with major compounds of 24-norursa-3,12-diene at 9.08%, followed by β-eudesmol (7.81%), β-selinene (7.26%), thunbergol (5.84%), and caryophyllene oxide (5.62%) (Figures 1 and 2). The compound camphene was earlier reported in the EOs of *Piper cernuum*, as presented by Girola et al. [20], while β-selinene was previously noticed in *Litsea cubeba* and *Lanthana camara*, as stated by Si et al. [21] and Sarma et al. [22], respectively. Furthermore, our data consented to the outcomes elaborated by Quassinti et al. [23] in *Hypericum hircinum* and *Ferulago macrocarpa*, as described by Sajjadi et al. [24]. In addition, our data are also supported by the outcomes earlier reported by Akpulat et al. [25] and Hulley et al. [26] in the EOs of some plants belonging to the family Asteraceae, which might be due to the presence of the common chemical ingredients. However, our findings do not match the
EOs reported by Mejia et al. [27] in *Brassica nigra* and also with the literature documented by Oroojalian et al. [28] in some Apiaceae species. Many factors are responsible for the variation among the contents present within a plant species including the difference in plant family and environmental gradients [29].

Table 1. The GC-MS analysis of the EOs of *Blepharispermum hirtum* Oliver.

| S. No. | Compounds                                      | RT<sub>min</sub> (min) | RI<sub>cal</sub> | RI<sub>rep</sub> | % Stem | % Leaves |
|-------|-----------------------------------------------|------------------------|-----------------|-----------------|--------|----------|
| 1     | 5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane      | 7.44                   | 927             | 920             | 0.12   | 0.03     |
| 2     | 3-Thujene                                      | 7.65                   | 935             | 928             | 3.11   | 0.06     |
| 3     | Camphene                                       | 7.88                   | 944             | 935             | 23.63  | 2.19     |
| 4     | 2,4(10)-Thujadiene                            | 8.39                   | 963             | 957             | 0.53   | 0.03     |
| 5     | Sabine                                         | 8.91                   | 982             | 964             | 2.21   | 0.14     |
| 6     | Laevo-β-Pinene                                 | 9.01                   | 986             | 978             | 4.38   | 0.12     |
| 7     | β-Myrcene                                      | 9.37                   | 999             | 981             | 0.91   | 0.25     |
| 8     | α-Phellandrene                                 | 9.75                   | 1013            | 997             | 0.39   | 0.05     |
| 9     | 3-Carene                                       | 9.92                   | 1019            | 1005            | 0.11   | 0.04     |
| 10    | p-Cymene                                       | 10.30                  | 1033            | 1011            | 1.46   | 0.14     |
| 11    | D-Limonene                                     | 10.42                  | 1037            | 1018            | 2.79   | 0.46     |
| 12    | γ-Terpinene                                    | 11.25                  | 1067            | 1047            | 1.51   | 0.11     |
| 13    | Linalool                                       | 12.34                  | 1106            | 1082            | 0.49   | 0.32     |
| 14    | Perillen                                       | 12.40                  | 1108            | 1086            | 0.04   | 0.08     |
| 15    | α-Campholenal                                   | 13.10                  | 1134            | 1102            | 0.42   | 0.31     |
| 16    | 2,9-Dimethyl-5-decyne                          | 13.10                  | 1136            | 1103            | 0.31   | 0.03     |
| 17    | L-Pinocarveol                                   | 13.46                  | 1147            | 1108            | 0.85   | 0.84     |
| 18    | cis-Verbenol                                   | 13.52                  | 1149            | 1110            | 0.25   | 0.36     |
| 19    | trans-Verbenol                                 | 13.61                  | 1153            | 1128            | 0.71   | 2.51     |
| 20    | p-Mentha-1,5-dien-8-ol                         | 14.18                  | 1174            | 1148            | 0.56   | 0.69     |
| 21    | Terpinen-4-ol                                  | 14.47                  | 1185            | 1175            | 0.67   | 0.46     |
| 22    | Myrtenol                                       | 15.00                  | 1205            | 1174            | 0.45   | 0.49     |
| 23    | Levoverbenone                                  | 15.34                  | 1218            | 1191            | 0.26   | 0.86     |
| 24    | cis-Cardveol                                    | 15.54                  | 1226            | 1208            | 0.08   | 0.37     |
| 25    | Bornyl acetate                                 | 17.26                  | 1292            | 1269            | 1.23   | 0.83     |
| 26    | α-Terpiny acetate                              | 18.78                  | 1354            | 1322            | 0.91   | 0.97     |
| 27    | Copaene                                        | 19.48                  | 1383            | 1376            | 0.47   | 0.44     |
| 28    | β-Bourbonone                                   | 19.71                  | 1392            | 1386            | 0.84   | 1.57     |
| 29    | β-Elemene                                      | 19.84                  | 1398            | 1398            | 4.66   | 4.52     |
| 30    | Caryophyllene                                  | 20.54                  | 1428            | 1421            | 3.73   | 4.35     |
| 31    | Humulene                                       | 21.32                  | 1462            | 1454            | 1.31   | 1.55     |
| 32    | Alloaromadendrene                              | 21.49                  | 1469            | 1459            | 0.39   | 0.59     |
| 33    | γ-Muurolene                                    | 21.80                  | 1483            | 1471            | 1.05   | 1.01     |
| 34    | Germacrone D                                   | 21.94                  | 1489            | 1480            | 3.26   | 1.31     |
| 35    | β-Selinene                                     | 22.08                  | 1495            | 1509            | 5.33   | 7.26     |
| 36    | α-Selinene                                     | 22.26                  | 1503            | 1500            | 2.92   | 4.63     |
Table 1. Cont.

| S. No. | Compounds                        | RT<sub>min</sub> | RI<sub>cal</sub> | RI<sub>rep</sub> | % Stem | % Leaves |
|-------|----------------------------------|------------------|------------------|------------------|--------|---------|
| 37    | Cubebol                          | 22.64            | 1521             | 1512             | 0.33   | 0.99    |
| 38    | δ-Cadinene                       | 22.82            | 1529             | 1514             | 1.59   | 2.93    |
| 39    | Elemol                           | 23.38            | 1554             | 1535             | 0.63   | 1.42    |
| 40    | Germacrene D-4-ol                | 23.99            | 1582             | 1570             | 0.09   | 0.31    |
| 41    | Caryophyllene oxide              | 24.19            | 1991             | 1575             | 2.89   | 5.62    |
| 42    | Humulene 1,2-epoxide             | 24.743           | 1617             | 1596             | 0.61   | 1.16    |
| 43    | γ-Eudesmol                       | 24.786           | 1619             | 1627             | 0.58   | 1.18    |
| 44    | Cubenol                          | 25.09            | 1634             | 1631             | 0.13   | 0.38    |
| 45    | tau-Cadinol                      | 25.341           | 1946             | 1637             | 0.77   | 1.78    |
| 46    | β-Eudesmol                       | 25.57            | 1657             | 1644             | 2.73   | 7.81    |
| 47    | Benzyl Benzoate                  | 27.77            | 1767             | 1765             | 0.12   | 0.54    |
| 48    | α-Phellandrene, dimer            | 28.32            | 1794             | 1801             | 0.43   | 0.76    |
| 49    | m-Camphorene                     | 31.11            | 1945             | 1960             | 0.09   | 0.31    |
| 50    | Cembrene A                       | 31.41            | 1961             | 1970             | 0.32   | 0.58    |
| 51    | p-Camphorene                     | 31.68            | 1978             | 1977             | 0.08   | 0.41    |
| 52    | Geranyl-α-terpinene              | 32.21            | 2007             | 1990             | 0.05   | 0.13    |
| 53    | Verticillol                      | 32.70            | 2036             | 2036             | 0.29   | 0.38    |
| 54    | Cembrene B                       | 34.55            | 2046             | 2161             | 0.25   | 0.29    |
| 55    | Thunbergol                       | 34.71            | 2156             | 2173             | 3.32   | 5.84    |
| 56    | 24-Norursa-9(11),12-triene       | 46.48            | 2156             | 3042             | 1.23   | 3.17    |
| 57    | 24-Noroleana-3,12-diene          | 46.645           | 3013             | 3057             | 1.55   | 4.03    |
| 58    | 24-Norursa-3,12-diene            | 47.198           | 3060             | 3105             | 3.46   | 9.08    |

Total % of the identified compounds: 93.88 % (% Stem) and 89.07 % (% Leaves).

RI<sub>cal</sub> = Retention index calculated. RI<sub>rep</sub> = Retention index obtained from database (NIST, 2011). RT = Retention time (min).

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**Figure 1.** The GC chromatogram of the essential oils of the stem of *B. hirtum.*
3.2. In Vitro Antidiabetic Significance

It is very clear that in recent times, natural products have been considered as untapped diamonds because of their invaluable medicinal use and lower side effects. The recent studies were designed to keep these key features of new drug candidates. The reported pharmacotherapeutic importance of EOs in the treatment of diabetes encouraged us to identify anti-diabetic agents via evaluating natural resources [30]. Therefore, in the current studies, two samples of EOs extracted from B. hirtum were subjected to, due to their crucial role in the inhibition of the key anti-diabetic targeted enzyme, α-glucosidase. Interestingly, both samples displayed overwhelming anti-diabetic potential with very high potency IC50 = 2.10 ± 0.57 µg/mL (stem) and 4.30 ± 1.56 µg/mL (leaves) when compared with the marketed drug acarbose IC50 = 377.71 ± 1.34 µg/mL (Figure 3). This invaluable high potency of these natural products further showed and strengthened their role as anti-diabetic agents. The stem EOs contained camphene in a higher quantity (23.63%) compared to the leaves (2.19%), which has a significant role in curing diabetes, as reflected in the literature stated by Mishra et al. [31] and Hachlafi et al. [32], and this might be the reason for which the EOs of the stem depicted a significant capacity to act as an antidiabetic agent.

In addition, our findings were in agreement with the data reported by Majouli et al. [33] for Hertia cheirifolia and Ceylan et al. [34], which documented the significance of Thymus spathulifolius due to the presence of common constituents and the same technique used in the mentioned plant species and understudy plant samples. However, our results did not match the previously described outcome of Ahmad [35] for M. spicata and Basak et al. [36], which revealed the significance of the EOs of Laurus nobilis. Variation in the capacity of the plants mainly depends upon the chemical ingredients that might be altered due to the edaphic, climatic, quality, and availability of water, as stated by Shah et al. [37], and is also affected by the elemental and other ingredients present in the water available for plants.

Figure 2. The GC chromatogram of the essential oils of the leaves of B. hirtum.

Figure 3. The in vitro antidiabetic significance of B. hirtum essential oils: (A) Stem, (B) leaves, and (C) standard acarbose.
3.3. In Vitro Cytotoxicity Capacity

The cytotoxic potential of the tested samples of *B. hirtum* EOs was evaluated from low to high doses using human breast cancer cell line MDA-MB-231 compared to human normal breast epithelial cell lines; Michigan cancer foundation (MCF) MCF-10A was used as a control in the experiment. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to determine the decrease in the cancer cell viability induced by cytotoxic agents. For MDA-MB-231, the IC$_{50}$ values, % inhibition, and viability of the tested EOs are presented in Table 2. Our findings show that the EOs of the leaves and stem have promising capabilities against MDA-MB-231 cells with IC$_{50}$ values of 88.4 ± 0.5 and 123.6 ± 0.8 µg/mL, respectively. To determine whether the cytotoxic effects of the oils were selective for malignant cells in comparison to the non-malignant cells, the non-tumorigenic MCF-10A cells were screened through the tested EOs from low to high doses (3, 10, 30, 100, and 300 µg/mL) in a similar manner as the cancer cells. After the MTT assay, the results of the % inhibition and viability for the MCF-10A cell lines by essential oils are presented in Table 3. The results showed that these cells were less susceptible to the actions of the essential oil, particularly at a higher dose of 300 µg/mL. The data in this study revealed that the triple negative MDA-MB-231 cells, which bear an aggressive phenotype, responded more favorably to EOs, and showed greater cytotoxicity. The significant potential for cytotoxicity was observed when non-tumorigenic MCF-10A cells were exposed to this plant’s EOs, which suggested that EOs have the potential in offering promising treatment for patients with breast cancer. Some valuable constituents were noticed in the understudy plant samples due to which they offered promising potential cancer therapy. Our findings agreed with the report described by Ortiz et al. [38] of the plant species belonging to the genus *Santalum* and Furtoda et al. [39] in the *Blepharocalyx salicifolius*. Our findings also favor the study of Loizzo et al. [40], who described the significance of the EOs of some plants of the family Lamiaceae and Lauraceae.

Table 2. The % viability and inhibition of *B. hirtum* essential oil on the breast cancer cell line MDA-MB-231.

| Tested Samples | Conc. (µg/mL) | % Viability | % Inhibition | IC$_{50}$ (µg/mL) |
|----------------|--------------|-------------|--------------|------------------|
| Leaves         |              |             |              |                  |
| 3              | 94.33        | 5.66        |              |                  |
| 10             | 82.41        | 17.58       |              |                  |
| 30             | 71.09        | 28.90       |              |                  |
| 100            | 47.85        | 52.14       |              |                  |
| 300            | 26.26        | 73.73       |              |                  |
| Stem           |              |             |              |                  |
| 3              | 96.49        | 3.50        |              |                  |
| 10             | 85.43        | 14.56       |              |                  |
| 30             | 72.65        | 27.34       |              |                  |
| 100            | 53.04        | 46.95       |              |                  |
| 300            | 37.05        | 62.94       |              |                  |

Table 3. The % viability and inhibition of *B. hirtum* essential oil on the normal breast cell line MCF-10A.

| Tested Samples | Conc (µg/mL) | % Viability | % Inhibition | IC$_{50}$ (µg/mL) |
|----------------|--------------|-------------|--------------|------------------|
| Leaves         |              |             |              |                  |
| 3              | 96.29        | 3.70        |              |                  |
| 10             | 93.04        | 6.99        |              |                  |
| 30             | 89.34        | 10.65       |              |                  |
| 100            | 86.96        | 13.03       |              |                  |
| 300            | 78.07        | 23.99       |              | >300             |
### Table 3. Cont.

| Tested Samples | Conc (µg/mL) | % Viability | % Inhibition | IC<sub>50</sub> (µg/mL) |
|----------------|-------------|-------------|--------------|-----------------------|
| Stem           | 3           | 97.50       | 2.49         |                       |
|                | 10          | 95.28       | 4.71         |                       |
|                | 30          | 89.93       | 10.06        |                       |
|                | 100         | 85.10       | 14.89        |                       |
|                | 300         | 79.22       | 20.77        | >300                  |

### 4. Conclusions

The comparative analysis of the B. hirtum stem and leaves EOs revealed that the understudy plant is an affluent source of responsible bioactive chemical constituents that are intended to produce as useful properties as the plant. Fifty-eight constituents were observed in the EOs of the stem and leaves of B. hirtum and contributed 93.88% and 89.07% of the total amount, respectively. Camphene was observed as a major compound (23.63%), followed by β-selinene (5.33%), β-elemene (4.66%), and laevo-β-pinene (4.38%) in the stem EO. While the 24-norursa-3,12-diene (9.08%), β-eudesmol (7.81%), β-selinene (7.26%), thunbergol (5.84%), and caryophyllene oxide (5.62%) were noted as the dominant constituents. Considerable potential to cure diabetes was offered by the tested samples compared to the standard. Moreover, the EOs of B. hirtum produced significant cytotoxicity effects against the breast cancer cell line MDA-MB-231 and were non-toxic to the normal cell line MCF-10A. The occurrence of the chemical constituents and promising α-glucosidase and cytotoxic activities of the EOs validate their pharmaceutical and nutraceutical importance. Hence, the analysis revealed that B. hirtum EOs can be used as an alternative promising natural remedy to cure diabetes mellitus and cancer.

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