BIOACTIVITIES AND CHEMOPROFILE COMPARISONS OF CHENOPODIUM AMBROSIODES L. AND CHENOPODIUM BOTRYS L. GROWING IN KASHMIR, INDIA

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ABSTRACT

Objectives: The objectives of the study were the gas chromatography-mass spectrometry (GC-MS) identification and comparison of the chemical constituents, evaluation of the antifungal and anticancer activities of two species of genus Chenopodium, for example, Chenopodium ambrosioides (Ca) and Chenopodium botrys (Cb) growing in Kashmir, Himalayan region.

Methods: The hydrodistilled essential oil of Ca and Cb was subjected to GC-MS analysis and antifungal activity for minimum inhibitory concentration (MIC) determination against different human pathogenic fungal strains using broth microdilution assay in 96-well microtiter plates as per the protocol of the clinical and laboratory standards institute (2008 M27-A3). The anti-proliferative activity of the essential oils was also evaluated against the two cell lines MCF-7 (human mammary carcinoma cells) and A549 (Human lung adenocarcinoma epithelial cells).

Results: A total of 34 compounds identified in Ca with α-terpinene (37.17%), isoascaridole (20.48%), and ascaridole (14.83%) as the key compounds. The key compounds of Cb were shoyobunol (18.91%), and hedycaryol (9.51%), germacrene-D-4-ol (8.57%), with 65 identified compounds. Both the species were found to have comparable antifungal activities against human pathogenic fungi with MIC values in the range of 0.031 mg/ml-0.256 mg/ml for Ca and 0.031 mg/ml-0.126 mg/ml in case of Cb. Maximum antiproliferative activity was observed at 125 µg/ml concentration in A549 cell line, while as the oils inhibited the growth of MCF-7 cell line at a lower concentration of 31.25 µg/ml.

Conclusion: The essential oils of Ca and Cb were found to have potent anticancer and antifungal activities and can have potential use as a natural fungicide.

Keywords: Gas chromatography-mass spectrometry, Chenopodium ambrosioides, Chenopodium botrys, Ascaridole, α-Terpenene, Shoyobunol.

INTRODUCTION

The genus Chenopodium belongs to family Chenopodiaceae and includes about 150 species which are annual herbs or bushy plants growing in semiarid to arid regions and have resistance to adverse climate [1]. The chemical investigations have been carried out on the essential oils of Chenopodium species [2] and are reportedly found to contain numbers of compounds such as ecdysteroids [3], flavonoids [4], sesquiterpenes [5], and saponins [6]. In India, the genus is reportedly represented by 21 species, and some are cultivated for vegetables and grains [7]. Chenopodium species have significance due to their broad area of medicinal applications in traditional medicine as anthelmintic, antispasmodic, emmenagogue, stomachic, for pain in amenorrhea, diaphoretic, abortifacient and for catarrh, asthma, and migraine [8,9]. Chenopodium album is reported to possess antileishmanial activity against Leishmania donovani in inbred BALB/c mouse [10].

Chenopodium ambrosioides (Ca) is an aromatic herb widely distributed throughout India and is found growing in Kashmir, Central Punjab, West Bengal, Bihar, Maharashtra, the Deccan, and eastern Ghats [11] in moist undisturbed waste places as a weed [12]. The extract and essential oil of this plant species are used as anthelmintic due to the presence of ascaridole [13]. The essential oil of Ca is known to possess allelopathic activity [14], and its aqueous extract is considered a blood purifier, stimulant, and cures hyperthermia [15].

Chenopodium botrys (Cb) is a strongly aromatic medicinal plant growing in dry sandy areas in the temperate Himalayas from Kashmir to Sikkim [16]. Cb finds use in multiple therapeutic applications such as expectorant, anticonvulsant, antibacterial, and tonic [17]. In Kashmir Himalayas, an ethnomedicinal survey indicates that the seed decoction of the plant is used relieves a headache due to gallbladder troubles, for treating tapeworm infestation in children [18], anthelmintic, diuretic, liver diseases, and laxative [19]. In Tibetan medicine, Cb is used to treat stomach and liver problems [20].

As part of the research program on the phytochemical screening of the medicinal plants from Kashmir, investigations were carried out on the essential oil isolated from the two Chenopodium species, for example, Ca and Cb by gas chromatography-mass spectrometry (GC-MS) analysis and evaluated the antifungal and anti-proliferative activity as no previous studies on the oils of these two plant species growing in Kashmir has been reported. The study will help in finding the phytochemicals from the two plant species growing in temperature Himalayan region and to evaluate their antifungal and anticancer efficacies.

METHODS

Plant material

The fresh aerial parts of Ca and Cb were collected locally in the month of August 2017. The plants were identified by taxonomist at Centre for Biodiversity and Taxonomy, University of Kashmir and Voucher specimens of Ca (KASH-2628) and Cb (KASH-2629) have been deposited in Kashmir University herbarium. The light yellow colored essential oils were isolated by carrying out the hydrodistillation of
the fresh aerial parts of the two plant species for 3 h using Clevenger apparatus [21]. The isolated essential oils were dried by placing over anhydrous sodium sulfate.

**GC-MS analysis**

GC-MS analysis of the Chenopodium essential oils was carried out using GC-MS ShimadzuQP-2010 system with an Rt-5 column (30 m × 0.25 mm id × 0.25 µm film thickness). The injector temperature was 260°C. Oven temperature program was held at 50°C for 2 min, heating at 3°C and keeping the temperature constant at 210°C for 10 min and from 210°C to 250°C at 6°C/min with a hold time of 31 min. Carrier gas used was helium. Pressure 69 kPa, total flow 137.3 mL/min, column flow 1.21 mL/min, linear velocity 39.9 cm/s, purge flow 3.0 mL/min, split ratio: 110:0; ion source temperature 230°C; and interface temperature 270°C. Injection volume was 0.3 µl. The MS scan parameters include El ionization voltage of 70 eV and spectra were recorded in the mass range of 40–650 µ.

The identification of the compounds in the essential oils was based on mass spectral comparisons with those of NIST 11 (National Institute of Standards and Technology; US and WILEY 8) library [22,23].

**Cell culture and in vitro antiproliferative assay**

Human cancer cell lines (MCF-7 and A549) were purchased from National Centre for Cell Science (Pune, India). The cells were grown in Dulbecco's modified eagle's medium, supplemented with 10% fetal bovine serum, and 1% penicillin-streptomycin at 37°C in a humidified incubator containing 5% CO₂. The essential oils were prepared in dimethyl sulfoxide (DMSO) and cells were treated with the fresh medium before treatment with oils. Both the oils were prepared in dimethyl sulfoxide (DMSO) and cells were treated with different concentrations (15.6, 31.25, 62.5, 125, 250, and 500 µg/mL) of the oil. After 12 h treatment, cell growth was evaluated using 96-well microtiter plates.

**Evaluation of antifungal activity**

The antifungal activities of Chenopodium essential oils (in terms of their minimum inhibitory concentration [MIC]) against different human pathogenic fungal strains were evaluated by broth microdilution assay using 96-well microtiter plates. For this purpose, MCF-7 and A549 cells were seeded at 10⁴ cells/well and allowed to grow overnight. Next day, the media were replaced with 200 µl of the fresh medium before treatment with oils. Both the oils were prepared in dimethyl sulfoxide (DMSO) and cells were treated with different concentrations (15.6, 31.25, 62.5, 125, 250, and 500 µg/mL) of the oil. After 12 h treatment, cell growth was evaluated by MTT assay. MTT solution of 50 µl (5 mg/ml of PBS) was added to each well, and the plates were incubated for 3 h at 37°C in the dark. The media were aspirated, and 150 µl of MTT solvent (DMSO) was added to each well to solubilize the formazan crystals. The absorbances of the microtiter plate reader or by naked eye visualization.

**RESULTS**

The essential oils with light yellow color obtained by hydrodistillation of the aerial parts of Ca and Cb growing in Kashmir were obtained in the yield of 0.35% w/v and 0.23% w/v, respectively, of the fresh weight basis.

The GC-MS analysis of Ca from Kashmir Himalayan region, results in the identification of 34 compounds (Table 1) accounting to the total percentage of 95.78% of the total oil composition with highest percentage of monoterpene hydrocarbons (52.54%) followed by oxygenated monoterpenes (38.95%), esters (1.37%), oxygenated sesquiterpenes (0.41%), and miscellaneous compounds (2.51%). The major compounds of the essential oil of Ca are α-terpinene (37.17%), isosaccaridole (20.48%), ascaridole (18.43%), and p-cymene (11.76%).

The essential of Cb consists of 65 identified compounds (Table 2), comprising (94.32%) of the total oil with the dominance of oxygenated sesquiterpenes (49.82%), followed by sesquiterpenes (25.74%), monoterpene hydrocarbons (11.56%), esters (3.71%), and others (3.49%). The major compounds from this species are shobunol (18.91%), hedyacol (9.51%), germacrene-D-4-ol (8.57%), 8-cadinene (4.90%), cardin-4-en-10-ol (4.42%), geranyl acetate (3.56%), limonene (3.47%), and 1-methyl-5-methylene-1,6-cyclohexadiene (2.85%).

Anticancer activity of the essential oils was determined using MTT assay. Results of the antiproliferative activity showed maximum growth inhibition at 125 µg/ml against A549 cell line (Fig 1) while as the oils inhibited the growth of MCF-7 cell line at a lower concentration of 31.25 µg/ml.

**DISCUSSION**

GC-MS analysis of the essential oils of the two species reveals a total of 92 identified compounds from both the species with only seven compounds being common to the two species. The results indicate that there are greater qualitative and quantitative differences between the oils of the two species and also between the oils of the same species which are previously investigated. These differences can be due to the difference of chemotypes and variability of the subspecies and difference in climatic and geographical areas of the plant growth.

Seven different chemotypes: Ascaridole, α-terpenene, α-pinene, p-cymene, carvacrol, limonene, and α-terpinyl acetate are reported to occur in Ca [26]. The results of the present investigation of the oil
composition on Ca are different from the previous reports, and the present oil has been found to belong to α-terpenene chemotype. The Ca essential oil analyzed from different parts of India is also reported to belong to α-terpenene chemotype, with oil from Chandigarh (India) containing the major constituents as α-terpinene (47.37%), p-cymene (25.77%), cis-ascaridole (14.75%) [27], the oil from Uttarakhand containing α-terpinene (44.68%), p-cymene (21.28%), ascaridole (17.89 %) [28], and the major constituents of oil from Southern Hills of India were found to be α-terpinene (63.6%), p-cymene (19.5%), and ascaridole (6.2%) [29]. Further, the Ca essential oil from Nigeria is also reported to belong to α-terpenene chemotype with major constituents as α-terpenes (63.6%), p-cymene (26.4%), and ascaridole (3.9%) [30]. However, the oil from for Iran has been found to be rich in α-terpenes (15.90%), camphor (12.40%), and trans-ascaridole (6.38%) [30], and the Mexican chemotype contains limonene (31.50%), and trans-pinocarveol (26.70%) as the major constituents [31]. The Ca oil from Brazil is found richer in Z-ascaridol (61.4 %) and E-ascaridol (18.6%) [32] and the oil from China has also been found to contain Z-ascaridole (27.27%), p-cymene (19.05%), and isascaridole (14.15%) [33]. The chemotype of Ca from Togo (Bangladesh) is also reported to be ascaridole type with ascaridole content of 51.12% [34].

The present results of the oil composition of Cb show much greater differences than the previously analyzed samples from different parts of the world. The Cb from Greece has been found to possess elemol acetate (16.3%), elemol (14.1%), and botrydrol (11.1%) [35]. The Cb from Iran reportedly contains α-eudesmol (15.5%), epi-α-muurolol (11.3%), and cubenol (10.5%) [36]. Another research studies from Iran revealed the presence of γ-terpineol (5.2%), p-cymene (19.0%), and isascaridole (7.0%) as the main components of the oil [30] and further one more investigation from two different localities of Iran is reportedly known to possess; juniper camphor (16.5% and 25.7%), elemol (14.3% and 13.4%), and α-cadinol (8.2% and 11.6%) [37]. Mahboubi et al. [38] have identified 2,3-dehydro-4-oxo-β-lonone (22.4 %), 7-epi-α-muurolol (11.5%), and elemol (7.4%) as the key compounds of Cb from Iran.

Our results show that the Chenopodium essential oils have a potent antiproliferative activity on A549 and MCF-7 human cancer cell lines. The oil treatment showed a concentration-dependent antiproliferative activity of the human cell lines studied. The Ca oil treatment showed a concentration-dependent antiproliferative activity on A549 and MCF-7 human cancer cell lines. The Cb essential oil from Nigeria has an IC50 value of 9.45 µg/ml observed after 24 h.

| S. No. | Name                                      | Molecular formulae | Molecular mass  | RT   | Area (%)   |
|-------|-------------------------------------------|--------------------|----------------|------|------------|
| 1     | Myrcene                                   | C10H16             | 38.697         | 0.21 | 13.96      |
| 2     | α-Terpine                                 | C10H16             | 17.197         | 0.24 | 37.17      |
| 3     | p-Cymene                                  | C10H16             | 17.197         | 0.24 | 37.17      |
| 4     | Limonene                                  | C10H16             | 17.197         | 0.24 | 37.17      |
| 5     | γ-Terpine                                 | C10H16             | 17.197         | 0.24 | 37.17      |
| 6     | Terpinolene                               | C10H16             | 17.197         | 0.24 | 37.17      |
| 7     | Thujol                                    | C10H16             | 17.197         | 0.24 | 37.17      |
| 8     | Cyclooctanone                             | C10H16             | 17.197         | 0.24 | 37.17      |
| 9     | 2,6-dimethyl-2,4-Heptadiene               | C10H16             | 17.197         | 0.24 | 37.17      |
| 10    | 1,2,3,1',2',3'-Hexamethyl-bicyclopentyl-2,2'-diene | C10H16 | 17.197 | 0.24 | 37.17      |
| 11    | Phellandral                               | C10H16             | 17.197         | 0.24 | 37.17      |
| 12    | p-Cymen-8-ol                              | C10H16             | 17.197         | 0.24 | 37.17      |
| 13    | 4-Acetyl-2-Carene,                        | C10H16             | 17.197         | 0.24 | 37.17      |
| 14    | 2,5,5-Trimethyl-3-hexyn-2-ol              | C10H16             | 17.197         | 0.24 | 37.17      |
| 15    | Ascaridole                                | C10H16             | 17.197         | 0.24 | 37.17      |
| 16    | cis-Pipertone oxide                       | C10H16             | 17.197         | 0.24 | 37.17      |
| 17    | 1,1,4,4-tetramethylcylohexane             | C10H16             | 17.197         | 0.24 | 37.17      |
| 18    | trans-Ascaridol glycol                    | C10H16             | 17.197         | 0.24 | 37.17      |
| 19    | γ-Terpineol                               | C10H16             | 17.197         | 0.24 | 37.17      |
| 20    | Isoascaridole                             | C10H16             | 17.197         | 0.24 | 37.17      |
| 21    | (Z2)-Hexenyltiglate                      | C10H16             | 17.197         | 0.24 | 37.17      |
| 22    | Fragranyl acetate                        | C10H16             | 17.197         | 0.24 | 37.17      |
| 23    | α-Limonene diepoxide                      | C10H16             | 17.197         | 0.24 | 37.17      |
| 24    | Allyl hexanoate                           | C10H16             | 17.197         | 0.24 | 37.17      |
| 25    | Hexyltiglate                              | C10H16             | 17.197         | 0.24 | 37.17      |
| 26    | Germacrene D                             | C10H16             | 17.197         | 0.24 | 37.17      |
| 27    | 2,2,3,4-tetramethyl-3-cyclopenten-1-one   | C10H16             | 17.197         | 0.24 | 37.17      |
| 28    | Ocetylglacte                              | C10H16             | 17.197         | 0.24 | 37.17      |
| 29    | Genyl tiglate                             | C10H16             | 17.197         | 0.24 | 37.17      |
| 30    | 1,3-dimethyl-3-vinylcyclohexene-1         | C10H16             | 17.197         | 0.24 | 37.17      |
| 31    | Shoybunol                                 | C10H16             | 17.197         | 0.24 | 37.17      |
| 32    | 1-pentadecanol                            | C10H16             | 17.197         | 0.24 | 37.17      |
| 33    | n-Hexadecanol                             | C10H16             | 17.197         | 0.24 | 37.17      |
| 34    | Phytol                                    | C10H16             | 17.197         | 0.24 | 37.17      |

Total identified %: 95.78

Grouped constituents
- Monoterpene hydrocarbons: 52.54
- Oxygenated monoterpenes: 38.95
- Esters: 1.370
- Oxygenated sesquiterpenes: 41.0
- Others: 2.51

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| S. No | Compound name | Molecular formulae | Molecular mass | RT | Area % |
|-------|---------------|--------------------|----------------|----|--------|
| 1     | 5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane | C_{10}H_{16} | 136 | 7.193 | 0.06 |
| 2     | α-Pinenol    | C_{10}H_{16} | 136 | 7.671 | 1.28 |
| 3     | Sabinele     | C_{10}H_{16} | 136 | 9.170 | 1.78 |
| 4     | β-Pinenol    | C_{10}H_{16} | 136 | 9.345 | 1.13 |
| 5     | Myrcene      | C_{10}H_{16} | 136 | 9.901 | 2.79 |
| 6     | 3,7,7-trimethylbicyclo[4.1.0]hept-2-ene, | C_{10}H_{16} | 136 | 10.238 | 0.20 |
| 7     | β-Phellandrene | C_{10}H_{16} | 136 | 10.533 | 0.28 |
| 8     | 3,7,7-trimethylbicyclo[4.1.0]hept-3-ene, | C_{10}H_{16} | 136 | 10.631 | 0.17 |
| 9     | α-Terpineol  | C_{10}H_{16} | 136 | 10.992 | 0.27 |
| 10    | Limonene     | C_{10}H_{16} | 136 | 11.560 | 3.47 |
| 11    | γ-Terpineol  | C_{10}H_{16} | 136 | 12.810 | 0.07 |
| 12    | 4-Terpineol  | C_{10}H_{16} | 154 | 13.391 | 0.11 |
| 13    | 6-2-Carene   | C_{10}H_{16} | 136 | 14.025 | 0.06 |
| 14    | Linalool     | C_{10}H_{16} | 154 | 14.797 | 0.34 |
| 15    | Nonanal      | C_{10}H_{16} | 142 | 14.973 | 0.59 |
| 16    | Terpenin-4-ol | C_{10}H_{16} | 154 | 18.420 | 0.10 |
| 17    | Isocaryophyllene | C_{10}H_{16} | 204 | 19.141 | 0.23 |
| 18    | α-Elemene    | C_{10}H_{16} | 204 | 25.167 | 0.15 |
| 19    | α-Cubebene   | C_{10}H_{16} | 204 | 25.323 | 0.46 |
| 20    | α-Copaene    | C_{10}H_{16} | 204 | 25.833 | 0.16 |
| 21    | Geranyl acetate | C_{10}H_{16} | 196 | 27.355 | 3.56 |
| 22    | (E)-Camphene  | C_{10}H_{16} | 204 | 28.274 | 0.41 |
| 23    | (E)-Caryophyllene | C_{10}H_{16} | 204 | 28.920 | 2.56 |
| 24    | Germacrene D | C_{10}H_{16} | 204 | 29.323 | 0.33 |
| 25    | Cadina-3,5-diene | C_{10}H_{16} | 204 | 30.113 | 0.18 |
| 26    | α-Humulene   | C_{10}H_{16} | 204 | 30.369 | 1.01 |
| 27    | (E,E), α-Farnesene | C_{10}H_{16} | 204 | 31.092 | 0.26 |
| 28    | β-Muurolene  | C_{10}H_{16} | 204 | 31.254 | 1.10 |
| 29    | 3,7,7-trimethylbicyclo[4.1.0]hept-3-ene, | C_{10}H_{16} | 204 | 31.488 | 2.85 |
| 30    | Dehydro aromadendrene | C_{10}H_{16} | 204 | 31.768 | 1.16 |
| 31    | α-Zingiberene | C_{10}H_{16} | 204 | 32.128 | 0.72 |
| 32    | 3,7,7-trimethylbicyclo[4.1.0]hept-2-ene, | C_{10}H_{16} | 204 | 32.242 | 0.68 |
| 33    | 3,7,7-trimethylbicyclo[4.1.0]hept-3-ene, | C_{10}H_{16} | 204 | 32.592 | 0.18 |
| 34    | γ-Cadinene   | C_{10}H_{16} | 204 | 32.834 | 2.29 |
| 35    | δ-Cadinene   | C_{10}H_{16} | 204 | 33.139 | 4.90 |
| 36    | 6-epi-shoyubonol | C_{10}H_{16} | 222 | 33.357 | 2.50 |
| 37    | Cubebol      | C_{10}H_{16} | 222 | 33.531 | 1.33 |
| 38    | α-Cadinol    | C_{10}H_{16} | 222 | 33.741 | 0.86 |
| 39    | Hedycaryol   | C_{10}H_{16} | 222 | 34.471 | 9.51 |
| 40    | Z-Nerolidol  | C_{10}H_{16} | 222 | 34.833 | 0.46 |
| 41    | Germacrene D-4-ol | C_{10}H_{16} | 222 | 35.499 | 8.57 |
| 42    | β-Eudesmol   | C_{10}H_{16} | 222 | 35.805 | 0.53 |
| 43    | (E)-Oplopanone | C_{10}H_{16} | 220 | 36.323 | 0.36 |
| 44    | β-Caradecadiene | C_{10}H_{16} | 220 | 37.095 | 0.33 |
| 45    | Epicubanol   | C_{10}H_{16} | 222 | 37.235 | 0.45 |
| 46    | γ-Eudesmol   | C_{10}H_{16} | 222 | 37.437 | 0.47 |
| 47    | Er-3,9-dien-1-ol | C_{10}H_{16} | 220 | 37.858 | 0.68 |
| 48    | Er-4-en-10-ol | C_{10}H_{16} | 220 | 37.948 | 0.27 |
| 49    | Sabinene     | C_{10}H_{16} | 222 | 40.115 | 18.91 |
| 50    | Cedrol       | C_{10}H_{16} | 220 | 40.990 | 0.20 |
| 51    | Cedro-4-en-10-ol | C_{10}H_{16} | 220 | 41.895 | 0.25 |
| 52    | Cedro-5-en-10-ol | C_{10}H_{16} | 220 | 43.365 | 0.22 |
| 53    | Cedronyde    | C_{10}H_{16} | 220 | 43.828 | 0.74 |
| 54    | Hexadecanol  | C_{10}H_{16} | 242 | 46.323 | 0.39 |
| 55    | (E)-β-Farnesene | C_{10}H_{16} | 204 | 47.684 | 0.19 |
| 56    | 18-Oxolauron-17-yl acetate | C_{10}H_{16} | 346 | 48.428 | 0.15 |
| 57    | Cedro-4-en-10-ol | C_{10}H_{16} | 220 | 51.735 | 0.50 |
| 58    | Abietadiene  | C_{10}H_{16} | 272 | 52.565 | 0.09 |
| 59    | Phytol       | C_{10}H_{16} | 296 | 53.489 | 0.26 |
| 60    | Isocuberyl   | C_{10}H_{16} | 346 | 59.150 | 0.17 |

Total identified %

Grouped constituents

(Contd...)
using MTT assay [39]. There are no previous reports regarding the antiproliferative activity of Cb essential oils. The work demonstrates that Ca and Cb exert antiproliferative activity against A549 and MCF-7 cell lines, but the constituents responsible for the activity and their mechanism of antiproliferative activity needs to be evaluated.

Ca and Cb possess good antifungal activity against the tested fungal strains and the antifungal activities of the essential oils, of the two species, are comparable. MIC<sub>80</sub> results depict that Ca is more active against C. krusei while Cb shows potent antifungal activity against C. krusei and C. parapsilosis strains. It is not possible to compare the antifungal activity of the two Chenopodium essential oils as no previous investigations have been carried out on the antifungal activity of Ca and Cb essential oils. However, the antifungal activity of Ca against different Aspergillus species has been reported, and it has been proposed that ascaridole is the principal fungitoxic compound [32].

The present data along with the absence of ascaridole in Cb having comparable antifungal activity with Ca suggest that the antifungal activity may also involve other major and minor oxygenated sesquiterpene compounds that have a synergistic effect.

CONCLUSION

The chemoprofiling of the two species of the genus Chenopodium by GC-MS reveals that a-terpenene and schubumol are the principal compounds of Ca and Cb, respectively, while Ca is dominated by sesquiterpene hydrocarbons followed by oxygenated monoterpenes, Cb is dominated by oxygenated sesquiterpenes followed by sesquiterpenes hydrocarbons. Both the oils show a moderate antiproliferative activity against the human cancer cell lines of varied origin. Moreover, oil extracts from both the species show potent antifungal activity against the tested human fungal pathogens.

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AUTHORS’ CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors Bashir Ahmad Ganai and Khaliquz Zaman Khan designed the study. Shameem A. Shameem carried out the literature survey, the isolation and chemical characterization of the oils, wrote the protocol, and the first draft of the manuscript. Authors, Abdul Haseeb Shah, Hafsa Qadri, and Ajaz A. Waza, managed the antifungal and anticancer study and wrote the respective protocols. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare to have no conflict of interest.

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Table 2: (Continued)

| Strains          | Chenopodium ambrosioides | Chenopodium botrys |
|------------------|--------------------------|-------------------|
| MIC<sub>80</sub> (mg/ml) | MIC<sub>80</sub> (mg/ml) |
| Candida albicans | 0.252                    | 0.126             |
| Candida glabrata | 0.126                    | 0.126             |
| Candida parapsilosis | 0.126            | 0.031             |
| Candida kefyr    | 0.063                    | 0.126             |
| Candida tropicalis | 0.063                | 0.063             |
| Candida dubliniens | 0.063              | 0.063             |
| Candida krusei   | 0.031                    | 0.031             |

*MIC<sub>80</sub> was determined following the standard Clinical Laboratory Standards Institute protocol and was performed in triplicates. MIC: Minimum inhibitory concentration.
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