Cytotoxic Effect of *Boswellia Sacra* on Human Cancer Cell Lines

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Abstract

Natural compounds are used as pharmaceutical agents in modern drug development because they exhibit antioxidant, antimicrobial and anti-tumor properties. The extracts of *Boswellia sacra* have been used in the present study in order to determine the in vitro cytotoxic effect they might demonstrate against human cancer cell lines. The compounds of the extracts were identified with analytical chemistry techniques and the viability of human cancer cell lines after treatment with the *Boswellia sacra* extracts was performed with MTT and clonogenic assays. Analytical methods identified 10 major boswellic acids present in the extracts of *Boswellia sacra*. Human pancreatic cancer PANC1, human colon cancer HCT116 and human lung adenocarcinoma MOR cell lines exhibited high sensitivity at lower concentrations of the extracts than the human breast cancer MCF7 and MDA-MB-231 and the human prostate cancer LNCaP and SerBob cell lines that required higher concentration of both the natural extracts for the induction of cell death. Natural extracted compounds require safety and efficacy studies to determine the potential they have to be used as therapeutic agents.

Keywords: Cytotoxicity • *Boswellia sacra* • Extracts • Cell viability • Cancer

Introduction

Cancer is the leading cause of death worldwide and the number of deaths is continuously rising. Tumor cells have lost their normal regulation of proliferation and cell death. Carcinogenesis is a series of events resulting in excessive cell division and tumor formation. The tumor affects the surrounding tissues and may spread to other parts of the body through the blood circulation [1].

Natural drugs have been used in traditional systems of different cultures for centuries. In several parts of the world, consumption of plants due to their health benefits has been part of tradition folk medicine. Plant-derived compounds can be characterized as potential sources for antioxidants, antibiotics and anti-cancer agents. Substances that exhibit antimutator properties belong to various chemical groups and can be obtained through extraction from the plant or with biotechnological tools to produce plant-derived compounds [2].

In modern drug development, pure natural compounds are used as pharmaceutical agents and whole plants or plant parts are used as herbal remedies. That provides potential for the natural compounds to act synergistically. Moreover, natural compounds are commonly pleiotropic, act on more than one target, making them potential candidates for the development of anticancer drugs [3].

*Boswellia sacra* belong to Burseraceae family and originate in Oman and Yemen. Frankincense is an aromatic resin derived from the exuded gums from the *Boswellia* trees and has been used for centuries for their aroma properties in perfumes and fumigants. Chemical constituents of *Boswellia* essential oils vary significantly due to time of harvest, climate, storage conditions and methods of preparation. The extracts have the ability to regulate immune cytokine production and leukocyte infiltration. Moreover, extracts exhibit antibacterial, anti-carcinogenic and anti-neoplastic properties. Major chemical components of *Boswellia sacra* extract are boswellic acids that provide the anti-inflammatory activity [4].

The aim of the present study is the in vitro screening of cytotoxic activity of the extracts from *Boswellia sacra* on a variety of human cancer cells.

Materials and Methods

Extraction and isolation of boswellic acids from *boswellia sacra* resin

Five grams of *Boswellia sacra* resin (supplied as free sample from Oman) were finely ground, 50 ml of methanol were added and the sample was placed on a roller shaker for 5 days in the dark. The extract was filtered and the remaining solid was rinsed with 20 ml of methanol. The liquids were combined and evaporated under vacuum (IKA RV6, Germany). The resulting yellow oil was dissolved and diluted in 200 ml LC-MS grade methanol.

LC-MS analysis

Boswellic acids were identified by liquid chromatography-mass spectroscopy (1260 Infinity Series HPLC, 6120 Quadrupole MSD, Agilent Technologies Inc., Richardson, TX, USA) equipped with a reverse phase column, Zorbax Eclipse Plus C18 (5 μm, 250 mm × 4.6 mm, Agilent Technologies, Santa Clara, CA, USA) maintained at 40°C. Chromatograms were integrated and analyzed using OpenLAB Chemstation (version M8301AA, Revision C.01.07 Agilent Technologies Inc., Richardson, TX, USA). The mobile phases used were: (A) H2O: Methanol 50:50 containing 5 mM ammonium acetate (Sigma-Aldrich, USA) and (B) Methanol: 2-propanol (Sigma-Aldrich, USA) 80:20 containing 5 mM ammonium acetate. The separation was carried out at 0.5 ml/min flow rate with the following gradient: 30%-50% of B in 5 min, 80% in 40 min, and then 100% of B at 50 min, which was maintained for 10 min. The injection volume was 20 μL and UltraViolet (UV) detection was carried out at 230, 250 and 280 nm. Mass spectrometry analysis was performed, as mentioned above, with a 6120 Series Quadrupole System (Agilent Technologies, USA) equipped with an electrospray ionization source (ESI) operating in negative mode. Identification of boswellic acids was performed by scan analysis with a 100 m/z-1000 m/z scan range.

NMR spectroscopy

The sample was prepared by adding 5 mg of *Boswellia sacra* to 0.5 ml...
of deuterated solvent (chloroform-d1) in 5 mm NMR tubes. 1H-NMR spectra waws obtained using a Bruker Avance spectrometer at 400 MHz proton frequency (AV-III-HD, 400, Rheinstetten, Germany). All chemical shifts were reported in parts per million (ppm) relative to the deuterated chloroform used. Spectra was automatically corrected for phase and baseline distortions using TOPSPIN (version 3.5p5, Bruker Biospin, Spring, TX, USA).

**Fourier transform infrared spectroscopy (FT-IR)**

The Fourier Transform Infrared Spectrometer was used to detect surface functional groups at the range of 340 cm⁻¹-4000 cm⁻¹. The Infrared spectrum of Boswellia Sacra was in solid powder form and they were compared to the IR spectrum of a commercial capsule of Boswellia Sacra. The model used to obtain the spectrums is Shimadzu IRspirit equipped with QATR-S accessory.

**Differential scanning calorimetry**

Thermograph of Boswellia Sacra powder was obtained and evaluated using a DSC 3+ (Metller, Toledo) with STARE Software, calibrated with Indium. The method used has the following parameters: Temperature Range 20°C-450°C, Heating Rate 10°C/min, Nitrogen flow rate 50 mL/min. The sample size is 3 mg for the powder sample and 4.3 mg for the commercial capsule.

The same method was repeated with a change in the heating rate, to 5°C/ minute in order to increase the sensitivity.

**Cell culture**

Commercial cancer cell lines were purchased from ECACC (European Collection of Authenticated Cell Cultures) (Salisbury, UK) representing a wide range of cancer types (Table 1). Cells were cultured in a humidified cell incubator at 37°C and 5% CO₂, and passaged when cells reached 80% confluence. RPMI 1640 Catalog#R0883 was obtained from Sigma-Aldrich, Darmstadt, Germany, and L-glutamine Catalog #G7513-100 ML was purchased from Sigma-Aldrich, Darmstadt, Germany and FBS Catalog #FB-1001/500 was obtained from BioSera, Nuaille, France. MEM Non-essential amino acids (NEAA) Catalog#M7145-100 ML was purchased from Sigma-Aldrich, Darmstadt, Germany. Sodium pyruvate Catalog#S836 was purchased from Sigma-Aldrich, Darmstadt, Germany and HEPES Catalog#H0887-100 ML was purchased from Sigma-Aldrich, Darmstadt, Germany.

Several main boswellic acids were present in the alcoholic extract. The mass spectra of all identified boswellic acids are provided in the Supporting Information. 1H NMR experiments were also performed in the extract in order to obtain the spectra. The Infrared spectrum of Boswellia Sacra extract was obtained using a Bruker Avance spectrometer at 400 MHz proton frequency (AV-III-HD, 400, Rheinstetten, Germany). All chemical shifts were reported in parts per million (ppm) relative to the deuterated chloroform used. Spectra was automatically corrected for phase and baseline distortions using TOPSPIN (version 3.5p5, Bruker Biospin, Spring, TX, USA).

**Cell viability analysis**

MTT assay was performed to assess cell viability. The viable cells were seeded at a density of 2 x 10⁴ (200 μl/well) in 96-well plate and incubated at 37°C and 5% CO₂ for 24 h to form a cell monolayer. After 24 h of incubation, supernatant on the monolayer was aspirated and 20 μl of 5 mg/ml MTT in PBS was added to each well and incubated for 3 h at 37°C and 5% CO₂. Supernatants were discarded and 100 μl of DMSO was added and the plates were incubated for 5 min 37°C and 5% CO₂ to solubilize the formazan crystals and absorbance was measured at 560 nm and the reference wavelength was at 605 nm.

**Clonogenic assay**

The viable cells were plated at a density of 50-100 (3 ml/well) in 8-well plates and treated with varying concentrations of the natural substances at 37°C and 5% CO₂ for at least 1 week. At this time point, supernatants were removed and the cells were washed for two times with PBS. Cells were fixed in fixative solution (75% methanol and 25% acetic acid) for 5 min at room temperature. The fixative was discarded and the cells were stained with 0.5% crystal violet for 3 h at room temperature. Cells were then washed under running tap water to remove the dye. Plates were scanned with Gel Doc and colonies were counted with ImageJ Plugin for colony measurement. The survival fraction was calculated as the percentage of treated over untreated cells.

**Statistical analysis**

Data are expressed as mean ± SD for each triplicate and the measurement of blank was used for the correction of the experimental values. T-test was performed to compare the untreated controls with the treated samples and differences were considered statistically significant at a p-value ≤ 0.05. The measurements were used to determine the IC50 values. All the calculations were performed at the Microsoft Excel 2016.

**Results**

**Identification of boswellic acids**

Several main boswellic acids were present in the alcoholic extract. The following Table 1 present the UV chromatogram of the extract of identified compounds.

The mass spectra of all identified boswellic acids are provided in the Supporting Information. Upper left numbers correspond to molecules listed in (Table 2 and Figure 1). 1H NMR experiments were also performed in the extract in order to confirm the presence of boswellic acids which are expected to have cytotoxic effect (Figure 2). Four boswellic acids were identified in the extract:

- 3-O-acetyl-11-keto-β--boswellic acid: 1H NMR (400 MHz, chloroform-d); 5.55, 5.30, 2.41, 2.08, 1.31, 1.22, 1.18, 1.14, 0.94, 0.82, 0.80
- 3-O-acetyl-β--boswellic acid: 1H NMR (400 MHz, chloroform-d); 5.30, 5.13, 2.09, 1.25, 1.18, 1.04, 0.92, 0.89, 0.82, 0.79
- 11-keto-β--boswellic acid: 1H NMR (400 MHz, chloroform-d); 5.55, 4.07, 1.34, 1.31, 1.15, 1.11, 0.92, 0.80, 0.79
- β--boswellic acid: 1H NMR (400 MHz, chloroform-d); 5.12, 4.07, 1.31, 1.14, 1.04, 0.89, 0.81, 0.75

The results were in accordance with data reported in the literature [5].

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**Table 1.** ECACC cell lines and culture medium suitable for each cell line.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Type</th>
<th>Culture medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF7</td>
<td>Human breast adenocarcinoma</td>
<td>RPMI 1640 + 10% FBS + 2% L-Glutamine + 2% NEAA</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Human breast adenocarcinoma</td>
<td>RPMI 1640 +15% FBS + 2% L-Glutamine + 2% NEAA</td>
</tr>
<tr>
<td>HCT116</td>
<td>Human colorectal carcinoma</td>
<td>DMEM + 10% FBS + 2% L-Glutamine</td>
</tr>
<tr>
<td>MOR</td>
<td>Human lung adenocarcinoma</td>
<td>RPMI 1640 + 10% FBS + 2% L-Glutamine</td>
</tr>
<tr>
<td>PANCl</td>
<td>Human pancreatic carcinoma</td>
<td>DMEM + 10% FBS + 2% L-Glutamine</td>
</tr>
<tr>
<td>LNCAp</td>
<td>Human prostate adenocarcinoma (hormone sensitive)</td>
<td>RPMI 1640 + 10% FBS + 2% L-Glutamine + 2% sodium pyruvate + 2% HEPES</td>
</tr>
<tr>
<td>SerBob</td>
<td>Human prostate cancer (hormone refractory)</td>
<td>Keratinocyte-SFM + 2ng/ml leukaemia inhibitory factor + 2ng/ml stem cell factor + 100ng/ml cholaer toxin + 1ng/ml granulocyte macrophage colony stimulating factor + 10% FBS</td>
</tr>
</tbody>
</table>
Figure 1. UV chromatogram of *Boswellia sacra* extract.

Figure 2. $^1$H-NMR spectrum of *Boswellia sacra*. Zoomed areas of the spectrum are available as supporting information.

Table 2. Molecular mass and retention time of compounds identified in the methanolic extract of *Boswellia sacra*.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RT (min)</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 11-keto-ursolic acid</td>
<td>24.9</td>
<td>469</td>
</tr>
<tr>
<td>2 11-keto-$\beta$-boswellic acid</td>
<td>29.0</td>
<td>469</td>
</tr>
<tr>
<td>3 3-O-acetyl-11-keto-$\beta$-boswellic acid</td>
<td>36.5</td>
<td>511</td>
</tr>
<tr>
<td>4 3-$\alpha$-hydroxy-8,24-dien-trucallic acid</td>
<td>39.8</td>
<td>455</td>
</tr>
<tr>
<td>5 3-O-acetyl-oleanolic acid</td>
<td>40.7</td>
<td>497</td>
</tr>
<tr>
<td>6 3-O-acetyl-ursolic acid</td>
<td>44.7</td>
<td>497</td>
</tr>
<tr>
<td>7 $\alpha$-boswellic acid</td>
<td>46.8</td>
<td>455</td>
</tr>
<tr>
<td>8 $\beta$-boswellic acid</td>
<td>48.6</td>
<td>455</td>
</tr>
<tr>
<td>9 3-O-acetyl-$\alpha$-boswellic acid</td>
<td>51.1</td>
<td>497</td>
</tr>
<tr>
<td>10 3-O-acetyl-$\beta$-boswellic acid</td>
<td>52.0</td>
<td>497</td>
</tr>
</tbody>
</table>

According to a FT-IR experiment some characteristic peaks are interpreted and the results are in accordance with the structural information of boswellic acid (Figure 3 and Table 3).
<table>
<thead>
<tr>
<th>Wavenumber (cm(^{-1}))</th>
<th>Functional group</th>
<th>Compound type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2921, 2866</td>
<td>C-H stretching</td>
<td>Alkane</td>
</tr>
<tr>
<td>1733</td>
<td>C=O Stretching</td>
<td>Aldehyde</td>
</tr>
<tr>
<td>1717</td>
<td>C=O Stretching</td>
<td>Carboxylic Acid</td>
</tr>
<tr>
<td>1700</td>
<td>C=O Stretching</td>
<td>Aryl Acid</td>
</tr>
<tr>
<td>1457</td>
<td>C-H Bending</td>
<td>Alkane</td>
</tr>
<tr>
<td>1378</td>
<td>COO- Symmetric Stretching</td>
<td>Carboxylates</td>
</tr>
<tr>
<td>1241</td>
<td>C-CO-C stretching</td>
<td>Aryl Aketone</td>
</tr>
<tr>
<td>989</td>
<td>C=C bending</td>
<td>Alkene</td>
</tr>
</tbody>
</table>

Table 3. Infrared absorption frequencies.

Based on literature, the main confirmed compound is 11-keto-\(\beta\)-Boswellic acid (KBA) with an endotherm peak around 190°C (Figure 4).

Inhibition of cell proliferation after treatment with *Boswellia sacra*

Cell viability was calculated with MTT assay and the results after 24 h and 48 h of treatment in Figure, with various concentrations of *Boswellia sacra* on the seven different human cancer cells Figure 4. The viability was calculated as the percentage of the treated cells over the untreated and the cytotoxicity was calculated as the percent difference of viability. The values of cytotoxicity were then used to calculate the IC50 of the extracts on the different cancer cell lines (Table 4).

<table>
<thead>
<tr>
<th>IC50 (\mu g/ml)</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF7</td>
<td>111.9</td>
<td>180.83</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>194.27</td>
<td>40.08</td>
</tr>
<tr>
<td>HCT116</td>
<td>115.46</td>
<td>174.95</td>
</tr>
<tr>
<td>MOR</td>
<td>248.89</td>
<td>177.3</td>
</tr>
<tr>
<td>PANC1</td>
<td>11.95</td>
<td>34.2</td>
</tr>
<tr>
<td>LNCaP</td>
<td>120.68</td>
<td>90.64</td>
</tr>
<tr>
<td>SerBob</td>
<td>200.56</td>
<td>113.22</td>
</tr>
</tbody>
</table>

Table 4. Cancer cell lines IC50 concentrations for *Boswellia sacra* extracts according to MTT assay.

Inhibition of single cancer cell growth by *Boswellia sacra*

After counting the clones, survival rate was calculated as the percentage of the treated samples over the untreated. The survival fraction of the investigated cell lines after treatment with *Boswellia sacra* is shown in (Table 5).
and chemotherapeutic properties [6]

Discussion

Plant-derived medicines have made a great contribution towards human health and natural compounds extracted from plants may have therapeutic potential. Isolation and identification of active compounds from plants, followed by bioassay, has demonstrated that some extracts have antibiotic, antioxidant and chemotherapeutic properties [6].

The major classes of chemical compounds that are present in Boswellia are the boswellic acids. The distribution of boswellic acids varied between the different species of Boswellia and Boswellia sacra appeared to have higher content of boswellic acids comparing to Boswellia serrata and Boswellia papyrifera [7]. In the present study, we have identified 10 boswellic acids that were detectable in the methanol extracts of Boswellia sacra, including those that have been proven to have anti-proliferative and cytotoxic functions against human cancer cell lines.

The aim of this study was to investigate the cytotoxic effect of the extracts of Boswellia sacra on human cancer cell lines. Specific cancer types, such as triple negative breast cancer that is characterized by lack of expression of estrogen receptor, progesterone receptor and human epidermal growth factor 2 (HER2), still lack targeted therapy options and are restricted to in vivo studies as well as improvements in formulation have been contacted [20, 21].

The identification of 10 boswellic acids in the methanol extracts of Boswellia sacra was achieved by several analytical techniques and 3-O-acetyl-β-boswellic acid, 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid (AKBA) isolated from the extracts of Boswellia serrata have been shown to exhibit anti-cancer activity in vitro. AKBA exhibited the greater anti-proliferative activity against human leukemia, human prostate, and human meningioma cell lines and in animal models, among the rest of the boswellic acids [8-15].

Boswellic acids from Boswellia sp. resins have been recognized as compounds that trigger various biological activities, including anti-cancer and anti-inflammatory functions. β-boswellic acid from the extracts of Boswellia cateri has been shown to be cytotoxic against human neuroblastoma cell lines. 3-O-acetyl-β-boswellic acid, β-boswellic acid, 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid (AKBA) isolated from the extracts of Boswellia serrata have been shown to exhibit anti-cancer activity in vitro. AKBA exhibited the greater anti-proliferative activity against human leukemia, human prostate, and human meningioma cell lines and in animal models, among the rest of the boswellic acids.

Cytotoxicity was not the same at all cancer cell lines that have been used in that study. Human pancreatic cancer PANC1 and human colon cancer HCT116 cell lines exhibited greater sensitivity to lower concentrations of Boswellia sacra and their viability percentage was lower than the rest of the human cancer cell lines. The human lung cancer and human breast cancer cell lines have been shown to be more resistant to Boswellia sacra and their proliferation.

The cancer cell lines that were more sensitive to lower concentrations of the extracts, PANC1, MCF7 and HCT116, appeared to have lower IC50 values at 24 h of treatment comparing to 48 h. On the other hand, the cancer cell lines that had higher IC50 values at 24 h exhibited much lower values comparing to 48 h. Even the triple negative breast adenocarcinoma MDA-MB-231 cell line was less resistant to the cytotoxicity of the extracts at 48 h. The results are in agreement with previous studies that showed the Boswellia sacra extracts to induce breast cancer cell-specific cytotoxicity, including the MDA-MB-231 cell line, and that the extracts might be effective for advanced breast cancer [18].

The results from the clonogenic assay revealed that inhibition of colony formation was also great at low concentrations against the human pancreatic cancer and human colon cancer cell lines, but there was also observed a higher inhibition of colony formation against the two human prostate cancer cell lines. Both biological assays exhibited that the triple negative human breast cancer cell line MDA-MB-231 was more resistant to the treatment with the extracts of Boswellia sacra, than the hormone sensitive human breast cancer cell line MCF7. Our results are in accordance with the report by (p.1) that MCF7 viability was greater reduced than MDA-MB-231 with Boswellia sacra essential oil extracts [4].

Nowadays, many scientists all over the world are testing boswellic acids for their potential cytotoxic function and they are modifying these structures by synthesing new derivatives [19]. This group of compounds has gained interest and new in vivo studies as well as improvements in formulation have been contacted.

Conclusion

We have demonstrated that the extracts of Boswellia sacra have tumor cytotoxicity in multiple cancer cell types. Human breast cancer cell lines appeared to be more resistant to the extracts of the Boswellia sacra powder and required higher concentrations to inhibit cell viability. Human pancreatic cancer cell line exhibited the highest sensitivity at low concentrations of the extracts and human colon cancer and human lung adenocarcinoma demonstrated similarly high sensitivity to the treatments. Human prostate cancer cell lines exhibited medium sensitivity against Boswellia sacra extracts. Safety and toxicity studies for the validation of the in vitro results will be required and future pre-clinical and clinical studies to determine the safety and efficacy of Boswellia sacra natural compounds as therapeutic agents for treating various types of cancer.

Acknowledgments

None

References


