Anticancer Activity of Five Traditionally Used Medicinal Plants’ Extracts

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Abstract: Natural products play a critical role in cancer prevention and therapy today. There are numbers of anticancer agents from natural products used in the clinic. Fighting cancers with novel natural products, especially those extracted from plants, is a potential strategy to develop new anticancer drugs. In the following study, various extracts of well known medicinal plants named Holoptelea integrifolia (F), Operculina turpethum (R), Cardiospermum halicacabum L (S), Dilonix regia (F), Sesbania grandiflora seed have been studied for evaluating their anticancer activity. Our data showed that the cytotoxic activity of Operculina turpethum (R) ethanolic extract was relative high for all 6 cancer cell lines as compared to other extracts. The active compound and anticancer mechanism of these extracts are worth investigating in the future.

Keywords: Operculina turpethum, EtOH extract, Cell line cytotoxic activity, MTT bioassay.

INTRODUCTION

Cancer is simply elaborated as an uncontrolled division of normal physiological cells which leads to an abnormal tissue growth caused by the progressive increase in the number of dividing cells [1].

A free radical is any atom with the capability of independence existence which has at least one unpaired electron in its outermost shell [2]. The radicals are the byproduct of biological reactions or are originated from various exogenous factors. Reactive oxygen species (ROS), a type of free radicals have the important roles in normal in vivo cell biology, but high concentration of ROS can be greatly damaging to the biological cell membrane and DNA. Experimental, clinical and epidemiological findings have provided evidence of the involvement of these ROS in mutagenesis and carcinogenesis [3].

To protect the body against ROS, nature has provided the human cells a highly complex system to neutralize oxygen free radicals. Also some exogenous antioxidants are provided in the natural food such as β-carotene, α-tocopherol, ascorbic acid and zinc [4, 5]. As the development of life threatening diseases such as cancer is linked to the availability of these antioxidants, it is necessary to investigate various phytotherapeutic origins possessing antioxidant and free radical scavenging activities which can be potential anticancer agents.

Operculina turpethum L of family Convolvulaceae [6, 7] is commonly known as “Indian Jalap”. It is widely distributed in Bangladesh, India, Philippines, Africa and Australia. It is a stout perennial climber that exudes a milky juice on cutting. Various researchers have explored its anticancer, anti-proliferative [8], anti-inflammatory [9] anti-microbial and anti-diabetic [10, 11] properties. The ethanolic extract of Operculina turpethum is studied for its anticancer activity in our study.

Holoptelea integrifolia (Roxb)Planch. (Mughs, Indian Elm Tree), a roadside plant is a native of Asian tropical regions including India, China, Nepal and Sri Lanka [12]. The plant belongs to family Ulmaceae
and is well known in traditional medicine being used as anti-inflammatory [13], antioxidant, antimicrobial and anticancer agent [14, 15]. Considering the potential anticancer activity of phytochemicals in the leaves and stem bark [16], we investigate the anticancer potential of methanolic extract of *Holoptelea integrifolia* (Roxb)Planch fruit in this study.

**Delonix regia** (Bojer ex Hook) Raf, a native of Madagascar has now traveled the world and is commonly found through the tropical countries [17]. It is a member of the family *Leguminosae* “Gulmohr” locally cultivated in India and planted as an ornamental plant in parks and avenues etc. It is also found in Pakistan [18]. A flamboyant plant, it is well known for its worth seeing display of red-orange blood which covers the tree from May-June with great medicinal value [17].

**Cardiospermum halicacabum** L. belongs to the family *Sapindaceae*. It is found in the continents of America, Africa, Asia (India, Pakistan, and Bangladesh). It is a herbaceous climber having some common name such as heart vine, heart pea, heart seed and balloon vine. The plant has been studied for its anti-ulcer, anti-parasitic, analgesic, anti-pyretic, anti-malarial and anti-filarial activities. In this study, the ethylacetate extract of the plant has been studied for the finding of anticancer activity.

**Sesbania grandiflora** L. cultivated in gardens. It is known as *Agathi sesban* in English. It is distributed in Pakistan and India [19]. The plant contains rich in tannins, flavonoides, coumarins, steroids and triterpenes [20, 21]. The plant is used for the treatment of colic disorder, jaundice, poisoning condition, small-pox, eruptive fever, epilepsy and diabetes [22]. The ethanolic extract of *Sesbania grandiflora* L is studied for its anticancer activity in this study.

**MATERIAL AND METHODS**

**Plants Collection**

The plant materials were collected locally and were identified and authentified in Department of Botany, Faculty of Science, University of Karachi, Pakistan.

**Preparation of Extracts**

**Holoptelea integrifolia** (Roxb) [FAF]

The fruits of *Holoptelea integrifolia* (Roxb) were collected in flowering season (March-April) from University campus, cleaned, dried in the shade and ground to coarse powder. The powder was extracted with methanol in a soxhlet apparatus for 12-14 hours, and then evaporated on rotary evaporator to obtain oily condition.

**Operculinaturpethum** [OTB-EtOH]

The plant materials (root) were purchased from herbal market, authenticated, washed, dried and cut into small pieces and made coarsed and then processed in electrical grinder (Monilax, Japan). The plant materials were extracted with 70% ethanol using rotary evaporator under controlled conditions. The extracted solvent was subjected to distillation to obtain yellowish brown crude residue followed by separation with EtOAc to obtain OTB-EtOH.

**Sesbania Grandiflora** [SG-EtOH]

The whole plant materials were dried by using an oven at 40°C for 7 days. The plant was grinded into powder. The powdered material was soaked in ethanol in a glass container for 7 days. The extract was concentrated by evaporation and dried to solid in an oven.

**Dilonix regia** [DR-EtOH]

The plant materials (flowers) were collected from Karachi University campus. Voucher specimen (No.00133) was deposited in the Herbal Museum of The Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. After authentication, the plant materials (flowers) were washed, dried and coarse material powder used for extraction. The plant materials were perculated with ethanol for 4-6 days then decanted perculate was filtered off through filter paper (Whatmann No.1) followed by evaporation under reduced pressure and controlled temperature.

**Cardiospermum halicacabum** L [CH-EtOAc]

The whole plant was collected in June. The plant was recognized by Prof. Dr. Ghazla.H.Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.

Air dried whole plant was powdered and perculated in ethanol at room temperature for 20 days. The filtrate was filtered through Whatmann No.1 filter paper and concentrated on rotary evaporator under controlled conditions. Semi solid brown ethanol extract was partitioned with water and ethylacetate to separate lipophilic and hydrophilic components from the extracts. Lipophilic EtOAc extract (CH-EtOAc) was taken for this study.
Chemicals and Reagents

The purified chemicals used during research work were commercially purchased from Oxoid (England) and Merck (Germany) and Sigma (USA).

Method (Cytotoxicity Assays)

The hepatocellular carcinoma cell line BEL-7404, lung cancer cell line H460, human epidermoid carcinoma cell line KB-3-1, prostatic cancer cell line DU145, breast carcinoma cell line MDA-MB-435, and colon cancer cell line HCT-116 were used to determine cytotoxicity of the 5 extracts (FAF, DR-EtOH, SG-EtOH, OTB-EtOH and CH-EtOAc). Human primary embryonic kidney cell line HEK293 was used as non-cancer cell line (control).

MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide) assay was used for the study.

Cells (5 x 10^3/well) were seeded in 96-well plates and cultured overnight then various concentrations of plant extract (FAF, DR-EtOH, SG-EtOH, OTB-EtOH and CH-EtOAc) were added respectively. After 72 hours of incubation, 20 μl MTT solution (4 mg/ml) was added to each well and incubated for an additional 4 hours and then the supernatant was discarded. Subsequently, each well was dissolved in 100 μl of dimethylsulfoxide (DMSO). The absorbance was determined using an OPSYS microplate Reader from DYNEX Technologies, Inc. (Chantilly, VA) at a wave length of 570 nm. All the experiments were repeated 3 times and mean was calculated.

Statistical Analysis

All data were represented as mean ± SD. The mean ± SD was calculated from three experiments performed in triplicate each time. The IC_{50} (concentrations required to inhibit growth by 50%) were calculated from survival curves using the Bliss method. Student’s t-test was used for the statistical analysis. The statistical significant was determined at p < 0.05.

RESULTS AND DISCUSSION

The selected plants for this study are already well-known for their use in multiple medical ailments but the exploratory work to determine their effective role in human tumorous conditions still needs to be carried out. In this study, phytochemical analysis of the plants is performed to precisely determine the distinct phytochemical constituents present in their extracts. The knowledge of these phytoconstituents presence will assist the future research regarding the physiological effects of these specific chemicals.

Holoptelea integrifolia, whose fruit extract is chosen for our cytotoxic activity, is a globally famous traditional plant being used as an astringent, anthelmintic, acid gastritis lessening agent and wound healing promoter [23]. Stem bark of the plant has phytoconstituents named holoptelin-A and B, friedlin, epifredlin, hederagenin, 1,4-naphthalenedione that exhibits remarkable anti-bacterial activity [24]. Operculina turpethum linn, possesses anti-ulcer, anti-proliferative, anti-microbial activities [8, 10]. Its root extract contains glycosides (Scopoletin, turpethinic acid A, B, C, D and E) [6]. But the anticancer activity of root extract has not been studied till date.

Table 1: The Cytotoxic Effects of Various Plant Extracts (FAF, DR EtOH, SG EtOH, OTB EtOH and CH EtOAc) in the BEL-7404, H460, KB-3-1, DU145, MDA-MB-435, HCT-116 and HEK293 Cell Lines

<table>
<thead>
<tr>
<th></th>
<th>FAF</th>
<th>DR EtOH</th>
<th>SG EtOH</th>
<th>OTB EtOH</th>
<th>CH EtOAc</th>
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<tbody>
<tr>
<td>BEL-7404</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>63.05 ± 15.88*</td>
<td>&gt;300</td>
</tr>
<tr>
<td>H460</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>66.39 ± 14.43*</td>
<td>139.03 ± 11.00*</td>
</tr>
<tr>
<td>KB-3-1</td>
<td>242.15 ± 13.23</td>
<td>123.28 ± 6.65</td>
<td>91.26 ± 9.86</td>
<td>27.90 ± 5.78**</td>
<td>55.35 ± 3.43**</td>
</tr>
<tr>
<td>DU145</td>
<td>132.60 ± 7.45</td>
<td>261.23 ± 14.10</td>
<td>77.58 ± 1.39</td>
<td>58.38 ± 2.36**</td>
<td>68.66 ± 6.91**</td>
</tr>
<tr>
<td>MDA-MB-435</td>
<td>&gt;300</td>
<td>181.26 ± 4.30</td>
<td>&gt;300</td>
<td>99.72 ± 16.66</td>
<td>190.65 ± 28.66</td>
</tr>
<tr>
<td>HCT-116</td>
<td>132.09 ± 2.38</td>
<td>&gt;300</td>
<td>286.88 ± 17.80</td>
<td>45.85 ± 8.88**</td>
<td>74.18 ± 9.29**</td>
</tr>
<tr>
<td>HEK293</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>107.65 ± 3.48</td>
<td>228.29 ± 15.55</td>
</tr>
</tbody>
</table>

IC_{50}: concentration that inhibited cell survival by 50%. Data are mean ± SD of three independent experiments performed in triplicate.
A serial concentrations used in various plant extracts were 0, 0.3, 1, 3, 10, 30, 100, 300 μg/ml, respectively.
*P < 0.05, **P < 0.01; Statistical difference and very significant difference (lower), respectively, were calculated by comparing the IC_{50} of the extract with that of HEK293.
phytocompounds named alkaloids, phenols, flavonoids and glycosides [27].

*Sesbania grandiglora* L., whose extracts are used in colic disorders, jaundice, small-pox [22] and rich contain tannins, flavonoides and triterpens [19, 20].

To analyze the cytotoxic effect of these plants extracts denoted by (FAF, DR-EtOH, SG-EtOH, OTB-EtOH and CH-EtOAc) on hepatocellular carcinoma cell line BEL-7404, lung cancer cell line H460, human epidermoid carcinoma cell line KB-3-1, prostatic cancer cell line DU145, breast carcinoma cell line MDA-MB-435, colon cancer cell line HCT-116 and human primary embryonic kidney cell line HEK293, MTT assay was used.

Significant anticancer activity of the selected plant extracts was revealed. The result of our study showed that the cytotoxic activity of OTB-EtOH (Table 1 Figure 1D) was relative high for all 6 cancer cell lines as compared to other extracts. The IC₅₀ values were found to be <100 µg/ml in all 6 cancer lines. The IC₅₀ values of OTB-EtOH were 27.90± 5.78 µg/ml and 45.85 ± 8.88 µg/ml.

![Figure 1](image_url): The cytotoxic effects of various plant extracts (A) FAF, (B) DR EtOH (C) SG EtOH, (D) OTB EtOH (E) CH EtOAc, in the BEL-7404, H460, KB-3-1, DU145, MDA-MB-435, HCT-116 and HEK293 cell lines as determined by MTT assays. A serial concentrations used in various plant extracts were 0, 0.3, 1, 3, 10, 30, 100, 300 µg/ml, respectively.
μg/ml for KB-3-1 and HCT-116 cells, respectively (Table 1 Figure 1D). While in the non-cancer cell HEK293, the IC$_{50}$ value of OTB-EtOH was 107.65±3.48 μg/ml (p < 0.01). CH-EtOAc (Table 1 Figure 1E) showed some cytotoxicity in most of cancer cell lines except for hepatocellular carcinoma cell line BEL-7404. However, the cytotoxicities of FAF (Table 1 Figure 1A) and DR-EtOH (Table 1 Figure 1B) were relatively weak as compared to the other extracts. In all 6 cancer cell lines, IC$_{50}$ values of these 2 extracts were > 100 μg/ml. Our future research will be aimed at isolating the active components from these extracts. The distinct mechanisms of antitumor activity of the isolated components will be further explored using in vivo pharmacokinetic and pharmacodynamics driven approach to develop safe and specific anticancer agents that would hopefully have a potential to eradicate some types of cancer.

REFERENCES


