**In vitro** anti-proliferative activities of *Aloe perryi* flowers extract on human liver, colon, breast, lung, prostate and epithelial cancer cell lines

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**Abstract:** Natural products, especially plant extracts have offered vast opportunities in the field of drug development due to its chemical diversity. The genus *Aloe* has for long been used for medicinal purposes in different parts of the world. The present study was designed to investigate the phytochemicals and anti-cancer potential of *Aloe perryi* flowers. The phytochemical analysis revealed the presence of carbohydrates, glycosides, phytosterols, phenols, flavonoids and proteins. While alkaloids and saponins were absent. The percentage inhibition of various extracts (viz. petroleum ether, chloroform, ethyl acetate, butanol and aqueous) of *A. perryi* flowers on seven human cancer cell lines (HepG2, HCT-116, MCF-7, A549, PC-3, HEp-2 and HeLa) has been evaluated using MTT assay. All the extracts significantly inhibit the proliferation of cancer cells in a concentration-dependent manner. The petroleum ether extract was most active, where the inhibition was recorded as 92.6%, 93.9%, 92%, 90.9%, 88.9%, 82% and 85.7% for HepG2, HCT-116, MCF-7, A-549, PC-3, HEp-2 and HeLa cells, respectively. The results also revealed that HCT-116 cells were more sensitive among all the cell lines studied.

**Keywords:** *Aloe perryi*, phytochemicals, cancer cell lines, cytotoxicity, viability assay.

**INTRODUCTION**

Cancer is symbolized by an unregulated cell growth. The most common types of cancers being lung cancer, stomach cancer, liver cancer, colorectal cancer and breast cancer (WHO, 2010). Globally cancer continues to fig. among the leading causes of death with approximately 8.2 million cancer related deaths in 2010 (WCR, 2014). In the next two decades the demographics of population will change, this means that even if the current cancer rate remains the same, the incidence of 12.7 million new cases in 2008 will mount to 21.4 million by 2030 (GLOBOCAN, 2010). With such alarming figs. in the expected rise of cancer cases, several approaches are looked upon to develop an effective cure for this deadly disease (Neidle and Thurston, 2005). Although various advancements in the prevention and treatment of cancer have occurred, the successful treatment remains a challenge. Chemotherapy is a common treatment that has proved useful in a number of cancer cases including breast, colorectal, pancreatic, osteogenic sarcoma, testicular, ovarian and certain lung cancers (DeVita Jr and Chu, 2008). However, poor selectivity and toxicity limits the use of chemotherapy. Therefore, the advancements in identifying new drugs with higher selectivity and less toxicity have gained momentum. Consequently, the exploration of herbal therapies to identify novel hits and leads has increased (Vickers, 2004).

For thousands of years, plants are an important source of medicine in pharmaceutical biology. As per WHO, even today, 80% of population relies on traditional medicine (Yates, 2002). The genus aloe (family Asphodelaceae) is a collection of flowering succulents consisting of over 500 known species including *Aloe vera*, *Aloe barbadensis*, *Aloe ferox*, *Aloe chinensis*, *Aloe indica*, *Aloe perryi* etc. *A. perryi*, also known as Perry’s aloe is endemic to Island of Socotra in Yemen. Its natural habitat is the dry rocky areas. Chemically Aloe possesses various pharmacologically important compounds such as essential oils, alkaloids, amino acids, anthroquinone glycosides, glycoproteins, vitamins, minerals and lectins (Al-Dubai and Al-Khulaidi, 1996; Atherton, 1997; Bazeb, 2002; Jia et al., 2008; Naser 2005). Aloe species originating from the Arabian Peninsula are well documented for their medicinal usage and out of this *A. perryi* has the widest usage. Traditional uses of *A. perryi* include wound healing, burns and topical treatment of skin diseases (Al-Fatimi et al., 2005). Several researchers have also revealed the role of *A. perryi* in the treatment of eye infections, stomach ailments, constipation and malaria (Mothana et al., 2012; Mothana et al., 2009). Antimicrobial effect of *A. perryi* is also reported (Awadh Ali et al., 2001).

However, despite the widespread usage of *A. perryi* in traditional medicine, our literature survey reveals only a few reports on its cytotoxicity (Mothana et al., 2012; Mothana et al. 2009). Furthermore, no report was available on the phytochemicals present and cytotoxic...
potential of flowers of *A. perryi*. Thus, this study aims to explore the anti-proliferative potential of various extracts (viz. petroleum ether, chloroform, ethyl acetate, butanol and aqueous) of *A. perryi* flowers against several human cancer cell lines (HepG2, HCT-116, MCF-7, A549, PC-3, HEP-2 and HeLa) and their screening for phytochemicals present.

MATERIAL AND METHODS

Chemicals and consumables

Dulbecco’s Modified Eagle’s Medium (DMEM) an all other chemicals, solvents and reagents were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. Trypsin-EDTA solution, and antibiotic/antimycotic solution and Fetal bovine serum (FBS) were purchased from GIBCO® Invitrogen, Life Technologies, USA. Culture wares and consumable used in this study were procured from Nunc, Denmark.

Cell culture

Human hepatocellular carcinoma (HepG2), human colon cancer (HT-116), human breast cancer (MCF-7), human lung adenocarcinoma (A-549), human prostate cancer (PC3), human epithelial carcinoma (HEp-2) and human cervical cancer (HeLa) cell lines were cultured in DMEM, supplemented with 10% FBS, 0.2% sodium bicarbonate, and 1% antibiotic/antimycotic solution. Cells were grown in 5% CO₂ at 37°C in high humid atmosphere.

Plant material and extraction

The fresh flowers of *A. perryi* were collected during the flowering stage in May-June, 2013, from Island of Socotra, Yemen. A plant taxonomist in the Department of Botany, Faculty of Science, Sana’a University, Yemen authenticated the flowers and a voucher specimen (#4469) is deposited in the herbarium Pharmacognosy Department, Sana’a University.

The *A. perryi* flowers dried under shade, powdered coarsely and stored in airtight container for further use. The powdered *A. perryi* flowers (1.5 kg) were extracted with methanol (3 × 10L) at room temperature using cold maceration procedure. The combined methanol extract was concentrated under reduced pressure to obtain a thick gummy mass. It was suspended in water and successively extracted with petroleum ether (A), chloroform (B), ethyl acetate (C), n-butanol (D), and remaining water soluble fraction (E).

Phytochemical screening

The crude extracts of *A. perryi* were subjected to preliminary screening for the presence of active secondary metabolites (alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, flavonooids and proteins). Each plant extract was tested with specific chemical reagents according to the standard procedures (Tiwari et al., 2011).

Experimental design

Different cell lines tested in this study were used to evaluate the cytotoxic effects of *A. perryi* flowers extract. All the cells were treated with different concentrations (1.56-50 µg/mL) of extract for 24 h. After treatment, the anti-proliferative activities was determined using 3-(4,5-dimethylthiazol-2-yl),5-biphenyl tetrazolium bromide (MTT) assay. Untreated control sets were run under identical condition.

Drug solutions

The extracts were not completely soluble in aqueous medium; therefore, the stock solutions of all the extracts were prepared in dimethylsulphoxide (DMSO) and diluted in culture medium to reach the desired concentrations. The concentration of DMSO in culture medium was not more that 0.1% and this medium was used as control.

Cytotoxicity evaluation using viability assay

For cytotoxicity assay, the tested cell lines were seeded in 96-well plate at a cell concentration of 1×10⁴ cells per well in 100 µl of growth medium. Fresh medium containing different concentrations of the extracts was added after 24 h of seeding. Serial two-fold dilutions of the extracts were added to confluent cell monolayers dispensed into 96-well, flat-bottomed micro titer plates (Falcon, NJ, USA) using a multi channel pipette. The micro titer plates were incubated at 37°C in a humidified incubator with 5% CO₂ for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. After the end of incubation period, the viable cells yield was determined by a colorimetric method. In brief, the media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Micro plate reader (TECAN, Inc.), using a test wavelength of 590 nm. The absorbance is proportional to the number of surviving cells in the culture plate. All the results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell viability of each tested compound was calculated (Mosmann, 1983; Wilson, 2000).

Data analysis

The percentage cell viability was calculated using the Microsoft Excel®. Percentage cell viability was calculated as follows:

\[
\% \text{Cell viability} = \left( \frac{\text{Mean Abs of control} - \text{Mean Abs of test metabolite}}{\text{Mean Abs control}} \right) \times 100
\]
Where: Abs: absorbance at 590 nm.

The 50% cell inhibitory concentration (IC$_{50}$), the concentration required to kill or cause visible changes in 50% of intact mammalian cells, was estimated from graphic plots. STATA statistical analysis package was used for the dose response curve drawing in order to calculate IC$_{50}$.

RESULTS

Table 1 represents the various phytochemicals present in different extracts of *A. perryi*. The petroleum ether and chloroform extracts contain glycosides, phytosterols and proteins and amino acids. The ethyl acetate extract contains phytosterols and flavonoids. The butanol extract contains phenols and flavonoids. The aqueous extract contains glycosides, phytosterols and flavonoids.

Fig. 1: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against HepG2 cell line.

Fig. 2: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against HCT-116 cell line.

Fig. 3: Percentage of inhibition of *Aloe perryi* flowers extracts (A): Petroleum ether; (B) Chloroform; (C): ethyl acetate; (D) Butanol; (E): Aqueous against MCF-7 cell line.

DISCUSSION

Natural products are being used since the beginning of human history for the medical purposes to treat various diseases including cancer (Al-oqail et al., 2013). Many chemo preventive medicines are the molecules derived from the plant materials or their synthetic analogues (Solowey et al., 2014). Plant territory has been the most important source and currently, ~60% of drugs used to treat the cancer have been isolated from natural products (Gordaliza, 2007), such as vincristine and vinblastine from *Catharanthus roseus* (Johnson et al., 1963), camptothecins from *Camptotheca acuminata* (Wall et al., 1963), significantly inhibit the proliferation of cancer cells in a concentration-dependent manner. Moreover, the highest percentage of petroleum ether (50 µg/ml) show the highest inhibition in proliferation in all cell lines, where the inhibition was recorded as 92.6%, 93.9%, 92%, 90.9%, 88.9%, 82% and 85.7% for HepG2, HCT-116, MCF-7, A549, PC-3, HeP-2 and HeLa cells, respectively. Furthermore, the results obtained, for all the cell lines also revealed that there was no significant effect on percentage inhibition at 1.56 µg/ml treated cells. Additionally, it can be clearly observed that A-549, PC-2 and HeP-2 cells exposed to 1.56 µg/ml of all solvent extracts did not show any significant difference in the percentage inhibition in the proliferation (figs. 4-6). On the other hand, HeLa cells also showed no inhibition when cells were treated with a concentration ranging from 3.125 to 12.5µg/ml of ethyl acetate and aqueous extract of *A. perryi* (fig. 7). The results clearly showed that HCT-116 cells were more sensitive among all the cell lines studied. On the other hand among the extracts, the highest percentage of inhibition for petroleum ether extract was found in HCT-116 cell line with the (IC$_{50}$=5.61 µg/ml), followed by HeLa (IC$_{50}$=5.83 µg/ml), A-549 (IC$_{50}$=7.54 µg/ml), MCF-7 (IC$_{50}$=7.88 µg/ml), HepG2 (IC$_{50}$=8.2 µg/ml), PC-3 (IC$_{50}$=9.51 µg/ml) and HeP-2 (IC$_{50}$=10.1 µg/ml) (table 2).
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1966), taxol and docetaxel from Taxus brevifolia (Wani et al., 1971). Vegetables and fruits have also been known to reduce the risk of cancer in humans (Chen et al., 2006; Moon et al., 2011). Some of the isolated compounds have exhibited anticancer potential with the low toxicity as compared to conventional drugs, eg. meisoindigo, isolated from the Chinese plant Indigofera tinctoria and flavopiridol, isolated from the Indian tree Dysoxylum binectariferum (Saklani and Kutty, 2007).

With the hypothesis that A. perryi flower extract might contain various antitumor agents that could be incredibly useful in killing the different human cancer cells, this study, was designed to screen the phytochemicals present and examine the effects of petroleum ether, chloroform, ethyl acetate, butanol and aqueous extracts of A. perryi on different human cancer cell lines, i.e. hepatocellular carcinoma (HepG2), colon cancer (HT-116), breast cancer (MCF-7), lung adenocarcinoma (A-549), prostate cancer (PC3), epithelial carcinoma (HEp-2) and cervical cancer (HeLa).

The phytochemical screening of various extracts of A. perryi revealed the presence of some secondary metabolites such as glycosides, phytosterols, phenols, flavonoids and proteins and amino acids as shown in table 1. These phytochemicals detected are known to have medicinal importance as anticancer, antibacterial, analgesic, anti-inflammatory, antitumor and antiviral agents (Cai et al. 2004, Miliauskas et al. 2004). The cytotoxicity evaluation revealed that increasing the concentration of A. perryi extract greatly inhibit the cell proliferation of different cell lines in a concentration dependent manner, when cells were treated with 1.56 to 50 µg/ml for 24 h. Our results are in well accordance with those of Al-Oqail et al. (2013), who obtained a dose-dependent response of different concentrations on HEp2, MCF-7, WISH and Vero cells. Our results also demonstrated that HCT-116 cells were more sensitive among all the cell lines studied and among the extracts, the highest percentage of inhibition for petroleum ether extract was found in HCT-116 cell line with the (IC50=5.61 µg/ml), followed by HeLa (IC50=5.83 µg/ml), A-549 (IC50=7.54 µg/ml), MCF-7 (IC50=7.88 µg/ml), HepG2 (IC50=8.2 µg/ml), PC-3 (IC50=9.51 µg/ml) and HEp-2 (IC50=10.1 µg/ml). These kind of variation among different cell lines have also been reported previously by Heo et al. (2014), who have reported anticancer effects of plant extract on HEK-293, HCT-116, HeLa, MCF-7, Hep3B, SNU- 1066 and SNU-601 cell lines. In other study, differential cytotoxic response towards different cancer cell lines (HeLa, HepG2, MCF-7, CACO-2 and L929) have also been reported and concluded that the plant extract effectively inhibit the proliferation of cells depending on the extract concentration as well as cell types (Elsayed et al., 2015). Our results are also in
agreement with the previous findings, where plant extracts decrease the cell viability in human breast cancer (T47D) cells, due to the sensitivity of cancerous cells towards the death flavanoids (Abdolmohammadi et al., 2008). Furthermore, growth inhibitory effect of certain constituents of plant also have been shown in human uterus carcinoma (HeLa), murine melanoma (B16F10) cells, human gastric adenocarcinoma (MK-1) (Fujika et al., 1999) and in other human cancer cell lines (Kim et al., 2002; Kumi-Diaka and Butler, 2000; Farshori et al., 2013; Farshori et al., 2014). This growth inhibitory activity might be due to the ability of plant extracts to inhibit the DNA synthesis as measured by the incorporation of tritiated thymidine into cells (Worthen et. al., 1998), which leads to cell death (Watson & Preedy, 2010).

**CONCLUSION**

In conclusions, this study provides a phytochemical analysis and preliminary screening for anti-proliferative activity of various *A. perryi* extracts on different cancer cell lines. We have shown that different extracts of *A. perryi* significantly inhibit the growth of various cancer cell lines (HepG2, HT-116, MCF-7, A-549, PC3, HEp-2 and HeLa) in a concentration-dependent manner. Among the all extracts, petroleum ether have shown more activity and HCT-116 cells were more sensitive, followed by HeLa, A-549, MCF-7, HepG2, PC-3 and HEp-2 cells. In addition, the presence of phytochemicals such as phytosterols, phenols, flavonoids, proteins and glycosides has been confirmed. Further investigations are required to understand the possible mechanism(s) of action of these extract on various cancer cells and isolation of active phytochemicals.

**ACKNOWLEDGEMENT**

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**REFERENCES**


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**Table 1:** Phytochemical analysis of various extracts of *Aloe perryi* flower.

<table>
<thead>
<tr>
<th>Chemical Constituents</th>
<th>Chemical Tests</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Glycosides</td>
<td>Modified Borntrager’s Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer’s Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Phytosterols</td>
<td>Libermann Burchard’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>Ferric Chloride Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkaline Reagent Test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Proteins &amp; Amino Acids</td>
<td>Xanthoproteic Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Froth Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* A: Petroleum ether; B: Chloroform; C, Ethyl acetate; D, n-Butanol; E, Aqueous; +, present; -, Absent.

**Table 2:** *IC50* values obtained for each of the extracts in different human cancer cell lines.

<table>
<thead>
<tr>
<th>Tumor Cell Lines</th>
<th>IC50 of Petroleum Ether extract (µg)</th>
<th>IC50 of Chloroform extract (µg)</th>
<th>IC50 of Ethyl Acetate extract (µg)</th>
<th>IC50 of Butanol Extract (µg)</th>
<th>IC50 of Aqueous extract (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepG-2</td>
<td>8.2</td>
<td>17.3</td>
<td>18.5</td>
<td>16.9</td>
<td>21.5</td>
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<td>HCT-116</td>
<td>5.61</td>
<td>14.1</td>
<td>19.8</td>
<td>10.3</td>
<td>17.5</td>
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<tr>
<td>MCF-7</td>
<td>7.88</td>
<td>22.9</td>
<td>&gt;50</td>
<td>12.7</td>
<td>27.4</td>
</tr>
<tr>
<td>A-549</td>
<td>7.54</td>
<td>13.0</td>
<td>18.8</td>
<td>18.8</td>
<td>22.6</td>
</tr>
<tr>
<td>PC-3</td>
<td>9.51</td>
<td>21.7</td>
<td>&gt;50</td>
<td>19.7</td>
<td>29.4</td>
</tr>
<tr>
<td>HEp-2</td>
<td>10.1</td>
<td>18.7</td>
<td>29.1</td>
<td>26.7</td>
<td>40.1</td>
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<td>HeLa</td>
<td>5.83</td>
<td>12.1</td>
<td>&gt;50</td>
<td>11.1</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>
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