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RESEARCH ARTICLE

CYTOTOXIC EFFECT OF FLOWER EXTRACTS OF THREE SPECIES OF *ALOE* GROWING IN YEMEN ON CANCER CELL LINE: *ALOE RUBROVIOLACEAE, ALOE VERA* AND *ALOE SABAEA*, AGAINST ELEVEN TYPES OF CANCER CELL LINES

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ABSTRACT

Background and aims: Natural products, in particular plant extracts, have opened up great chance in the area of drug progress owing to their chemical variety. The *Aloe* genus has long been known to be used for medicinal uses in countless parts of the world. This study was planned to inspect the phytochemicals and anti-cancer capabilities of *Aloe rubroviolaceae, Aloe vera* and *Aloe sabaea* flowers.

Materials and Methods: Three types of ethanolic extracts of plants traditionally used in Yemen to treat a variety of diseases have been tested *in vitro* for their potential anticancer activity on different human cancer cell lines. The cytotoxic activity of the ethanolic extracts of tested plants was determined using eleven strains of human cancer cells, namely: MCF-7 (breast cancer), PC-3 (prostate cancer), HEP-2(human epithelial carcinoma), MNFS-60 (myelogenous leukemia), CACO (intestinal cancer), A-549 (lung adenocarcinoma), HeLa (cervical cancer), RD (rhabdomyosarcoma**)**, HepG2 (hepatocellular carcinoma), HCT-116 (colon cancer), and CHO-K1(Chinese hamster ovary). A colorimetric sulforhodamine B assay was applied to assess the *in vitro* cytotoxic activity of various extracts. Growth inhibition of 50% (IC50) for each extract was calculated from the optical density of treated and untreated cells. Doxorubicin, a broad-spectrum anticancer drug was used as a positive control.

Results: More interesting cytotoxic activity was observed for *Aloe vera* extract more than *Aloe sabaea and Aloe rubroviolaceae, extract*.

Conclusions: This study presents an initial screening for anti-proliferative activity of a variety of *Aloe* species flowers extracts on diverse cancer cell lines. Different extracts of *Aloe* species significantly inhibit the growth of various cancer cell lines in a concentration-dependent manner. Advance researches are necessary to understand the possible mechanism(s) of action of these extract on a variety of cancer cells and separation of active phyto-chemicals.

Keywords: *Aloe* species, cytotoxic activities, flower extracts, *in vitro*, Yemen.

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INTRODUCTION

Aloe is a genus that contains more than 550 species of flowering succulent plants. *Aloe vera*, or "true *Aloe"* is the most widely known species. It is described this since it is grown as a standard source for various pharmaceutical purposes. These species, as *Aloe rubroviolaceae*, *Aloe vera*, and *Aloe sabaea* are grown or harvested from the wild for similar applications[1-3]. The genus is inhabitant to tropical regions, South Africa, Madagascar, Jordan, the Arabian Peninsula

including Yemen and is endemic to a variety of islands in the Indian Ocean such as Yemeni Socotra, Reunion, Mauritius, and the Comoros Islands. A few species have also been adapted in other areas such as the India, Mediterranean, South and North America, Australia, and the Hawaiian Islands[4]. Most varieties of *Aloe* have a rosette of large, thick, fleshy leaves. The flowers of the *Aloe* are tubular, often yellow, orange, pink, or red, and bush, densely clumped and drooping, at the apex of simple or branching stems devoid of leaves. Many varieties of *Aloe* appear without stems, with the rose growing directly at ground level; further types may have a branched or un-branched stalk from which the thickset leaves grow. They show a discrepancy in color from gray to light green, and are sometimes striped or speckled. Some species of *Aloe* inhabitant to South Africa are tree-like (*arborescent*)[5]. *Aloe* species are often grown as an ornamental plant in both gardens and pots. Many types of *Aloe* are very ornamental and are appreciated by collectors of succulents. *Aloe vera* is used internally and externally on humans as a folk or alternative medicine. *Aloe* species is known for its medicinal and cosmetic properties. About 75% of *Aloe* species are used locally for medicinal uses in addition to other herbal plants[6-9]. In Yemen, recent researches investigated the effect of herbal plants on viral and bacterial agents and protozoa in which traditional medicine and flavors are used in Yemen where a large number of people rely on herbal plants to treat their diseases[10,11].

It is estimated that in 2018, there were 18.1 million new cases of cancer and 9.6 million deaths worldwide. Approximately 17% of females and 20% of males will develop cancer at certain point whereas 9% of females and 13% of males will expire from it. In 2008, roughly 12.7 million cancers were detected and in 2010 only nearly 7.98 million persons died. Cancers account for about 16% of deaths. The mainly common as of 2018 are lung cancer (1.76 million deaths), colorectal cancer (860,000), stomach cancer (780,000), liver cancer (780,000) and breast cancer (620,000)[12,13]. Consistent with the limited Yemeni cancer studies, the mainly common types of cancer among Yemeni adults and children were leukemia (33.1%), lymphoma (31.5%), central nervous system tumors (7.2%), and bone tumors (5.2%)[14-18]. The potential for natural products to be used as a source of anti-cancer agents was accepted in the 1950s by the United States (the National Cancer Institute) is directed by the late Dr. Jonathan Hartwell. NCI has made it a most important assistance to the finding of new naturally occurring anticancer agents in the course of its holding and grant support, together with an important program for plant and marine groups. Anti-cancer drugs, such as indole alkaloids. Vincristine, podophyllotoxin derivatives, vinblastine, teniboside and etoposide are prominent chemical treatments of plant origin obtained either directly through isolation or derived from lead structures[19]. *Aloe* species that originated in the Arabian Peninsula are well recognized for their medicinal use and outside of this species. The traditional uses of *Aloe* species include wound and burn healing and topical treatment of skin diseases[20]. Several researchers have also uncovered the role of *Aloe* species in treating eye infections, stomach ailments, constipation and malaria[21,22]. The antimicrobial effect of *A. perryi* has also been reported[23]. Therefore, screening of higher plants for anticancer agents has been pursued on an international level[24]. Yemen is characterized by its vast area, where variations in climate appear due to differences in elevations, which results in a great diversity of its plants. Yemen's botanicals are known for their use in folk and traditional medicine[25,26]. Thus, this study aims to explore the antiproliferative potential of several *Aloe* species flowers against several human cancer cell lines.

MATERIALS AND METHODS

Sample collection and Identification: The flowers of *A. Rubroviolaceae* and *A. Vera* were collected from saber area in the city of Taiz and Badan mountains (Ibb) respectively, while flowers of *A. Sabaea* were collected from Miatam and Aldlel villages (Ibb)**.** The taxonomy work and identification of the plants was confirmed by Professor: Abdul Walli Al-Kholidy, Department of Botany, Faculty of Agriculture, Sana'a University, Yemen. The flowers of all plants were collected during the flowering stage in November 2017. A voucher specimen (CCD 25) was prepared and deposited in the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

Preparation of Samples: The flowers of the three plants were dried separately in the air and ground to a coarse powder. The powder was then stored in airtight containers at room temperature until use.

Preparation of ethanol extracts: The dried flower powder was extracted separately by soaking in sufficient amount of ethanol (99%) with repeated shaking for 1 week and filtered. Each flower filtrate was evaporated and dried separately under reduced pressure at 45°C using a rotary evaporator (Buchi Rotavapor R-200, Serial No. 05009474, Switzerland) and the process was repeated twice until the extraction was complete.

Phytochemical screening: First phytochemical screening were performed standard methods described by by Altemimi *et al.*,[27]. Then, TLC technology was used to identify the components of ethanolic extracts of plant flowers using a TLC plate coated with 60 F254 silica gel, 20×20 cm (Merck, Germany). The first developed chromatograms were examined under UV light (VilberLourmat, French) at wavelengths of 254 nm and 365 nm. Then each chromatogram was analyzed for the presence of biologically active components by spraying with appropriate reagents[27].

Cytotoxicity assay: The cytotoxicity of the extracts was tested against eleven types of cancer cell lines purchased from AcceGen (USA, New Jersey): Human hepatocellular carcinoma (HepG2), human colon cancer (HCT-116), human breast cancer (MCF-7), human lung adenocarcinoma (A-549), human prostate cancer (PC3), human epithelial carcinoma (HEp-2) and humancervical cancer (HELA), myeloid leukemia (M-NFS- 60), human epithelial colorectal adenocarcinoma (CACO-2), chinese Hamster Ovary (CHO-K1), and

rabdomyosarcoma (RD). Cells were obtained from the American Type Culture Collection (ATCC). Tumor cells were propagated in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/ml gentamicin. All cells were maintained at 37°C in a humidified 5% CO_2 and sub-cultured twice a week.

Evaluation of cellular cytotoxicity: The tested cell lines were seeded in a 96-well plate at a cell concentration of 1×10^4 cells per well in 100 µl of growth medium. Fresh medium containing different concentrations of plant extract was added 24 hours after sowing. Serial two-fold dilutions of the test chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtitre plates (Falcon, NJ, USA) using a multichannel pipette. Microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for 48 h. Three wells were used for each test sample concentration. Control cells were incubated without test sample and with or without DMSO. At the end of the incubation period, the production of viable cells was determined by a colorimetric method. Briefly, media was aspirated and crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the dishes rinsed with tap water until all excess stains were removed. Glacial acetic acid (30%) was then added to all wells and mixed well, and the absorbance of the plates was then measured after gentle shaking on a microplate reader (TECAN, Inc.), using a test wavelength of 590 nm. The absorbance was proportional to the number of surviving cells remaining in the culture plate. All results were corrected for background absorbance detected in the wells without adding dye. The treated samples were compared with the cellular control in the absence of the tested compounds. All experiments were performed in triplicate. The effect on cell growth was calculated as the difference in the absorbance ratio in the presence and absence of the tested extracts and shown in the dose-response curve. The concentration at which cell growth was inhibited to 50% of the control (IC50) was obtained from the dose-response curve.

Statistical analysis

The percentage cell viability was calculated using the Microsoft Excel®. Percentage cell viability was calculated as follows (absorbance at 590 nm):

$$\% \text{ Cell viability} = \frac{\text{AbsC x AbsT}}{\text{AbsC}} \text{X100}$$

Where; AbsC=mean Abs of test metabolite, AbsT=mean Abs control.

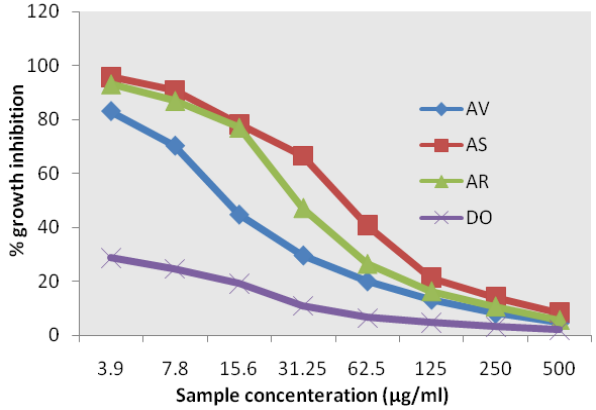

Figure 1: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *A. Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the HCT-116cell line.

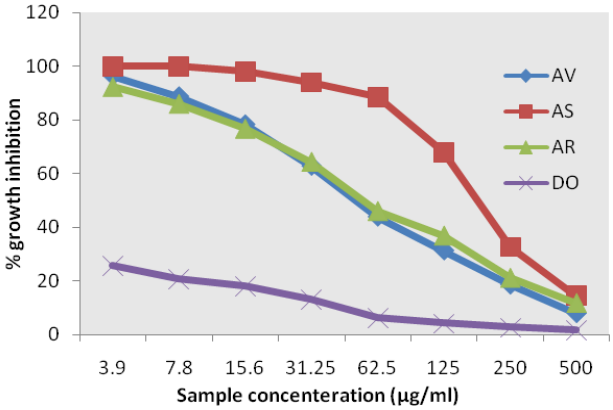

Figure 2: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *Aloe Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the HpeG2 cell line.

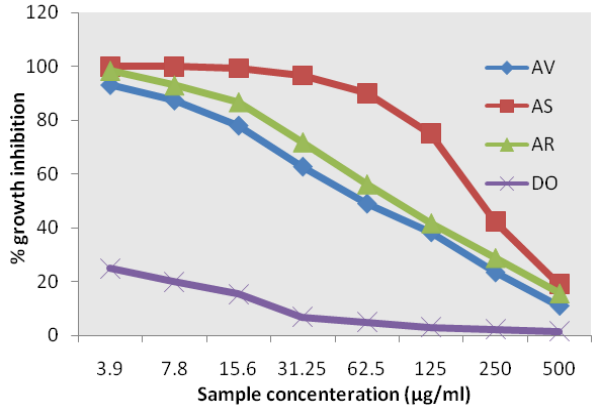

Figure 3: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *A. Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the MCF-7 cell line.

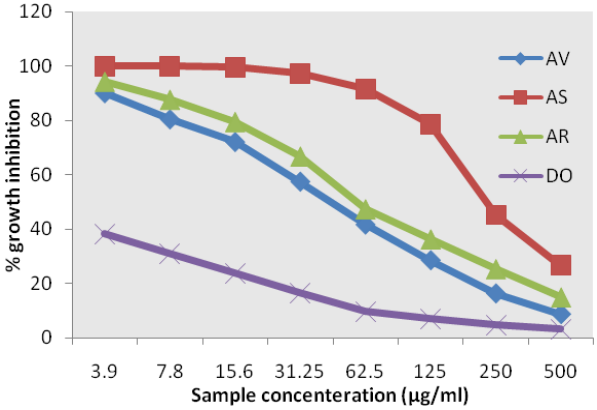

Figure 4: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *Aloe Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the PC3 cell line.

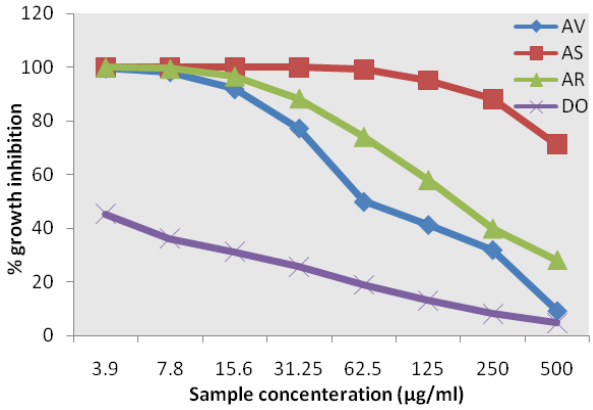

Figure 5: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *A. Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the HEP-2 cell line.

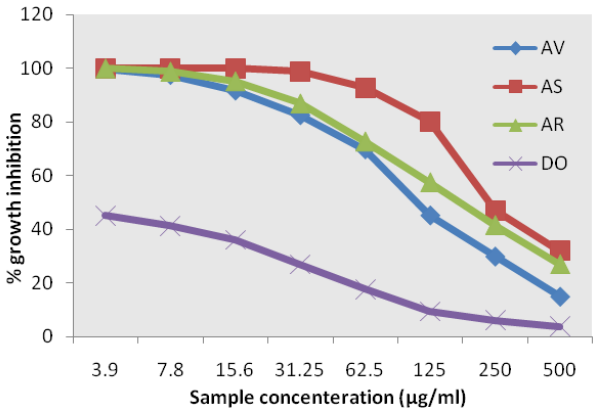

Figure 6: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *Aloe Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the MNFS-60 cell line.

Ethical approval

Ethical approval was obtained from the Medical Research and Ethics Committee of the College of Medicine and Health Sciences, Sana'a University with reference number (121) on 13/02/2017.

RESULTS

Cytotoxicity of ethanolic extracts of flowers from three species of *Aloe* against eleven cancer cell lines (MCF-7, PC-3, HEP-2, MNFS-60, CACO, A-549, HELA, RD, HepG2, HCT-116, and CHO-K1) using the Crystal Violet Staining Assay and evaluated according to American Cancer Institute guidelines[28].

Figure 1 to Figure 11 and Table 1 show the results and reveal that *A. Vera* flower extract showed significant dose-dependent cytotoxic activity against four cell lines (CACO, A-549, RD, HCT-116) with IC50 values of 14.6±0.65, 12±0.50 and 14.7±1.88 and 14±0.46, respectively. However, *A. Sabaea* flower extract exerts a very low cytotoxic activity against cell lines, compared to *A. Vera* extract. IC50 indicated that the most cytotoxic effect of *Aloe Vera* extract was against the A-549 cell line (IC50=43.6±3.07).

On the other hand, *A. Rubroviolaceae* flower extract showed little cytotoxic activity against three cell lines (CACO, A-549, HCT-116) with IC50=30.1±0.95, 26.5±1.05 and 29.7±0.78, respectively. In the present study, the cytotoxic activity was mainly observed in the extract of *A. Vera* flowers, which showed the highest cytotoxic activity followed by the *A. Rubroviolaceae* flower extract, which showed moderate cytotoxic activity, while the *A. Sabaea* flower extract showed the lowest cytotoxic activity.

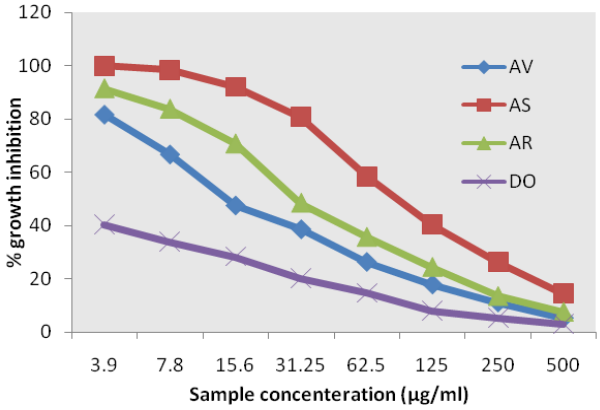

Figure 7: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *A. Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the CACO cell line.

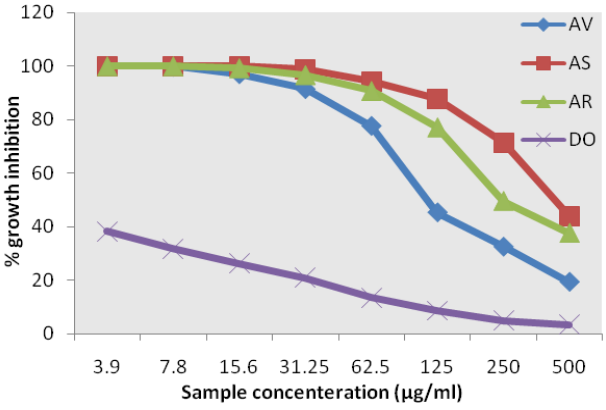

Figure 8: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *Aloe Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the CHO-K1 cell line.

DISCUSSION

Since the foundation of human history, innate products have been used for medicinal uses to treat a diversity of diseases including cancer[29]. Many chemoprophylaxis are molecules derivative from plant resources or their synthetic analogues[30]. Vegetable lands were the most a significant foundation and at this time, around 60% of the drugs used to cancer treatment have been extracted from natural products, as vinblastine and vincristine from *Catharanthus roseus*[31], Camptothecin from Camptothica acuminate[32], Taxol and docetaxel from Taxus Previfolia[33]. Fruits and vegetables are also known to reduce the risk of cancer in humans[34,35]. Some isolated compounds showed anticancer potential with low toxicity compared to conventional drugs.

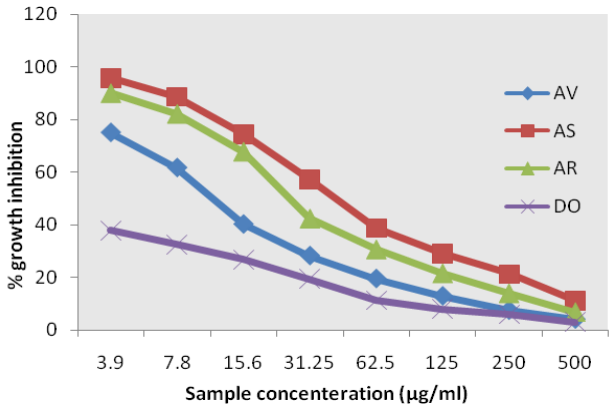

Figure 9: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *A. Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the A-549 cell line.

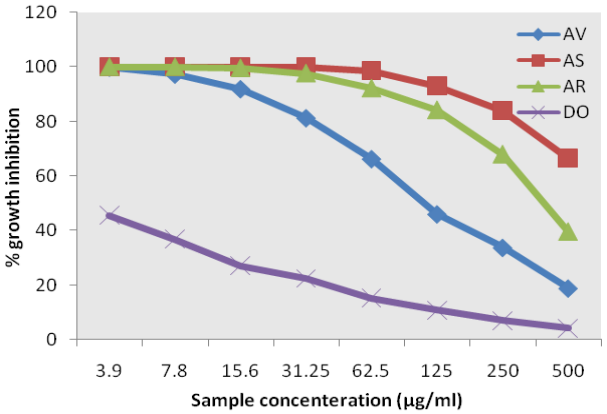

Figure 10: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *Aloe Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the HELA cell line.

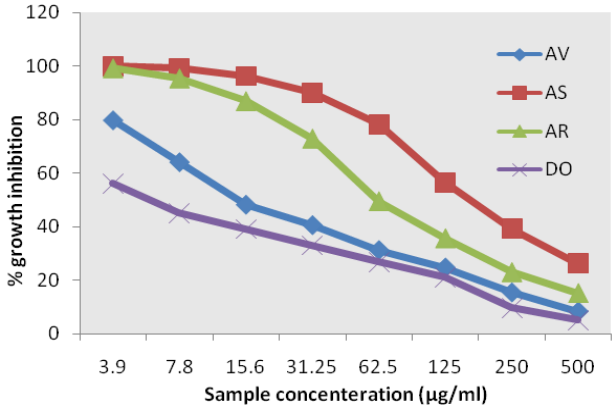

Figure 11: Percentage inhibition of cell growth of *A. Vera* (*AV*), *A. Sabaea* (*AS*), *Aloe Rubroviolaceae* (*AR*) extracts and Doxorubicin (DO) against the RD cell line.

Table 1: *In vitro* cytotoxic activities of *A. Vera* (AV), *A. Sabaea* (AS), *A. Rubroviolaceae* (AR) extracts against various carcinoma cell lines.

Cell Lines	*A. Vera*	*A. Sabaea*	*A. Rubroviolaceae*	Doxorubicin
MCF-7	60.3±5.17	>100	89.5±14.9	0.35±0.02
PC-3	46.1±6.07	>100	58.3±1.57	1.68±0.15
HEP-2	62.3±3.19	>500	>100	3.58±0.11
MNFS-60	>100	>100	>100	1.99±0.12
CACO	14.6±0.65	92±5.81	30.1±0.95	1.71±0.03
A-549	12±0.50	43,6±3.07	26.5±1.05	0.95±0.16
HELA	>100	>500	>100	3.56±0.12
RD	14.7±1.88	> 100	61.8±4.44	6.07±0.22
HepG2	52.4±4.79	> 100	55.7±3.09	0.36±0.02
HCT-116	14±0.46	51.3±4.08	29.7±0.78	0.49±0.04
CHO-K1	>100	>100	>100	0.84±0.06

Cytotoxic activity is expressed as IC50 (µg/mL)±SD (n=3), which is the concentration of extract at which 50% of cell growth was inhibited relative to cells incubated in the presence of<0.1% DMSO vehicle control. All cell lines were treated with doxorubicin as a positive control. MCF-7 (breast cancer), PC-3 (prostate cancer), HEP-2(human epithelial carcinoma), MNFS-60 (myelogenous leukemia), CACO (intestinal cancer), A-549 (lung adenocarcinoma), HeLa (cervical cancer), RD (rhabdomyosarcoma), HepG2 (hepatocellular carcinoma), HCT-116 (colon cancer), and CHO-K1(Chinese hamster ovary).

Meisoindigo, isolated from the Chinese plant Indigofera tinctoria and flavopiridol, isolated from the Indian Dysoxylum binectariferum tree[35]. Regarding the results of the current study, cytotoxicity evaluation showed that low concentration of *A. rubroviolaceae*, *A. vera* and *A. sabaea* extracts significantly inhibited cell proliferation of CACO, A-549, RD and HCT-116 cell lines with a decrease in IC50. The results are in agreement with those of Al-Oqail *et al.*,[29], who achieved a dose-dependent response at diverse concentrations on HEp2, MCF-7, WISH and *Vero* cells. The current study results also showed that CACO, A549, RD, and HCT-116 cells were most sensitive to *A. Vera* among all studied cell lines and among the extracts, the highest inhibition of ethanol extract was found in the A549 cell line with (IC50=12 µg/ml), followed by with HCT-116 (IC50=14 mcg/mL), RD (IC50=14.7 µg /mL), and CACO (IC50=14.6 µg/mL).This type of variability between different cell lines was also reported by Heo *et al.*,[37],

who reported the anticancer effects of the plant extract on HEK-293, HCT-116, HeLa, MCF-7, Hep3B, SNU-1066 and SNU-601 cell line. In an additional study, the discrepancy cytotoxic response of diverse cancer cell lines (HeLa, HepG2, MCF-7, CACO-2, and L929) was also reported and it was concluded that the plant extract efficiently inhibited cell proliferation depending on the concentration of the extract, also as types of cells[38]. The current results are also consistent with previous findings, that plant extracts reduce cell viability in human breast cancer (T47D) cells, due to the sensitivity of cancer cells to lethal flavonoids[39]. Furthermore, the growth-inhibitory consequence of components of *A. Vera* species in plants has also been confirmed in human uterine cancer (HeLa), melanoma (B16F10), human gastric cancer (MK-1)[38] and in other human cancer cell lines[40-43]. This growth-inhibitory activity may be a consequence of the plant extracts' ability to inhibit DNA synthesis as measured by incorporation of thymidine tritate into cells[44], leading to cell death[45].

CONCLUSION

In conclusions, this study presents the phytochemical analysis and preliminary examination of the anti-proliferative activity of extracts of different types of *Aloe* species on different cancer cell lines. We showed that different extracts of *A. rubroviolaceae*, *A. vera* and *A. sabaea* significantly inhibited the growth of different cancer cell lines (CACO, A-549, RD, HTC-116) in a concentration-dependent manner. Among all extracts, *A. vera* extract showed the greatest activity and A-549, HCT-116 and RD cells were the most sensitive. Additionally, the existence of phytochemicals such as phenols, flavonoids, phytosterols, proteins and glycosides has been approved. Advance inspections are needed to recognize the possible mechanism(s) of action of these extracts on different tumor cells and to isolate the active phytochemicals.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the design, implementation, statistical analysis and manuscript drafting. All authors read and approved the final manuscript.

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