



Original article

Analysis of *Alpina officinarum* Hance, chemically and biologically

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ABSTRACT

Alpina officinarum Hance is one of the most commonly used herbs belongs to Family Zingiberaceae. The current work deals with the qualitative and quantitative chemical study of this plant rhizomes in addition to the investigation of its anticancer activities. The results of the qualitative analysis showed a variation of phytochemical contents in this plant. While quantitative analysis showed a very promising percentage of active materials and Pharmacopeial constants. Analysis of elements like Cu, Zn & Mg were variable chromium was the lowest (0.680 ppm). The active constituents showed the highest percentage of carbohydrate (20.25 ± 1.11) and the lowest was of lipid (2.79 ± 1.03), other constituents percentage ranged from 5.11 ± 1.31 to 18.26 ± 1.24 for protein and flavonoids respectively. The pharmacopeial constant determinations reported the highest in moisture content (11.02 ± 1.05). Total ash, water-soluble ash, and acid insoluble ash were varied in values (5.64 ± 1.31 to 2.01 ± 1.12).

The evaluation of the antitumor activities (*in vitro*) of the investigated plant rhizomes extract showed that; it exhibited a direct cytotoxic effect on the growth of some cell lines compared to the standard drug vinblastine sulphate. The activities were recorded against two cell lines; A-549 (Lung carcinoma) and CACO (colorectal carcinoma) with IC_{50} 6.72 ± 0.5 and 7.6 ± 0.3 $\mu\text{g/ml}$ respectively, these effects were better than the standard drug vinblastine sulphate (IC_{50} were 24.6 ± 0.7 & 30.3 ± 1.4 $\mu\text{g/ml}$). Moreover, the effect of the investigated extract was also promising on the other three cell lines (HCT-116 (Colon carcinoma, Hela (Cervical carcinoma) & Pc3 (prostate cancer) the best effect was on Hela with IC_{50} of 24.5 ± 1.1 $\mu\text{g/ml}$ better than vinblastine sulphate (59.7 ± 2.1 $\mu\text{g/ml}$).

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1. Introduction

Natural herbs have been and still in use as a source of remediation. Many biological compounds have been discovered from plants and have been used for the treatment of many diseases. However, more studies are still needed to evaluate the properties and side effects of the plants (Greenwell and Rahman, 2015).

Zingiberaceae is one of the largest plant families, it contains many important medicinal and nutritional plants which are widely distributed in several countries (Lakshmi et al., 2011). Plant species of family Zingiberaceae have many biological activities as; antiseptics, anti-allergic and anti-itching of *Curcuma longa*, *Curcuma zedoaria* and *Alpinia galanga* (Matsuda et al., 2004; Ye and Li, 2006).

Genus *Alpinia* is one of the most important Zingiberaceae members which comprises rhizomes. These rhizomes are used in folklore medicine for treating catarrh, rheumatism, bad breath, bronchial ulcers, cough, throat infections and digestive system problems (Victório et al., 2009). Litterateurs reported the presence of complex chemical profiles in *Alpinia* species (Basri et al., 2017).

Alpina Officinarum Hance, is belonging to *Alpina* Genus which has been used as food spices in both Chinese and Ayurvedic medicine since ancient times. It showed many biological activities cor-

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related to its wide range of chemical compounds (Basri et al., 2017).

The rhizomes of *Alpinia officinarum* Hance is the most important part of the plant, it was reported to be very effective on the digestive system disorders, and it also controls bacterial and fungal infections. Also, some compounds have been isolated from the rhizomes and proved to have some activities such as; anti-hepatotoxic and antioxidative, anti-dermatophyte (Housman et al., 2014).

Although folklore medicine has been used by mankind since ancient times, it still based on personal experience rather than scientific evidence. The pharmacological study of natural products is still not sufficient as well as the clinical trials involving herbal medicines. A particular problem impeding progress is the lack of standard methodologies for evaluating natural medicines. The present study aims to evaluate *Alpinia officinarum* rhizomes quantitatively and quantitatively to standardize its contents in addition to investigate its antitumor activity.

2. Material and methods

To fulfill the study goal, the work was divided into two parts, Phytochemical investigations (qualitatively & quantitatively analysis) and biological evaluations (acute toxicity or LD₅₀ and sub-chronic toxicity on liver and kidney function and *in-vitro* antitumor activity).

2.1. Phytochemical investigations:

2.1.1. Quantitative analysis

2.1.1.1. *Plant material*:. Rhizomes of *Alpinia officinarum* Hance, were obtained from herbal shops in Al-Kharj, Saudi Arabia, and identified by Dr. Jacob Thomas, Taxonomist, College of Science, King Saud University. The rhizomes were grounded to powder and kept for analysis.

2.1.1.2. *Phytochemical screening and extraction*. The phytochemical screening and chemical constituents' determination of *Alpinia officinarum* Hance, rhizomes were achieved as published methods in literatures (Tiwari et al., 2011; Awaad et al., 2018).

Extraction of 200 g air-dried powdered plant rhizomes was done using percolation methods as reported (Awaad et al., 2018) using ethanol (95%). The plant materials were soaked in aqueous alcohol for 2 days and the solvents then filtered on cotton, the marks lifted were re-extracted again till exhaustion (Awaad et al., 2016). The total alcohol extracts were reduced using a rotary evaporator at 25 °C and kept used for carrying the study.

2.1.2. Quantitative analysis

2.1.2.1. *Mineral and trace elements contents*. To evaluate mineral and trace elements contents and percentage, five grams of *Alpinia officinarum* powdered were placed in porcelain crucibles and heated in a muffle furnace to 500 °C for 24 h to get the plant free ash.

The crucible contains ash then placed in a desiccator at room temperature till it cooled. The ash then dissolved in concentrated sulphuric acid (10 ml), heated on the hot plate for 5 mins till the sulphuric acid fumes were ceased to evolve. Crucible contains sulphated ash was heated again in a muffle furnace at 400 °C for 4 h. 0.5 g of the obtained sulphated ash was dissolved in 50 ml of 5% HCl to get the solution for elements determination using Atomic Absorption Spectroscopy (AAS). The standard solution must be prepared for each element to drawn calibration curves for each of them to measure each element concentration (Manipur et al., 2018).

2.1.2.2. *Total fats contents*. Twenty grams of *Alpinia officinarum* dry powder was exhaustively extracted in Soxhlet using petroleum ether (60–40 °C) as a solvent for 12 h. The obtained extract was dried using reduced pressure at a temperature not exceeding 25 °C, weighed and calculated for total fat comparing to the dry weight (Sneh et al., 2013).

2.1.2.3. *Total protein contents*. Calculation of total protein concentration micro Kjeldahl method was used. One gram dried powder material was placed in the digestion flask, 10 g of powdered potassium sulphate, 0.5 g of copper sulphate and 25 ml of conc. sulphuric acid was added to it and digestion conducted by placing the flask in an inclined position and heating it below the boiling point of acid for about 5–15 min. The temperature was raised until the acid boiled briskly. A funnel was placed in top of the flask to restrict the circulation of air. Heating was continued until the solution became clear. The contents were cooled and diluted by adding 200 ml of water. Then, 0.5 g of granulated zinc and 50 ml of 40% NaOH solution were added to make the reaction strongly alkaline. The contents were mixed and at once attached to the distillation apparatus. In the receiving flask 25 ml. 0.1 N sulphuric acid was taken. After completion of the reaction. The flask with the mixture was removed, cooled and titrated against 0.1 N caustic soda solution using methyl red indicator (Saravanakumar et al., 2009).

2.1.2.4. *Total phenolic content*. The process was carried out using 2 mg of the total extract (previously prepared in Section 2.2.1) and adding to it 400 ml of Folin-Ciocalteu reagent and 2 ml of NaCO₃ (2%W/V). The mixture was left to stand at 200 °C for 30 min and the absorbance of the obtained color was measured at 760 nm using UV–vis Spectrophotometer. The concentration was calculated using gallic acid monohydrate standard curve. The total phenol content was calculated as gallic acid equivalent in mg/g (Manipur et al., 2018, Saravanakumar et al., 2009).

2.1.2.5. *Total flavonoids content*. Aluminum Chloride spectrophotometric technique was used to measure the flavonoid content of all plant extract. One gram of total extract (previously prepared in Section 2.2.1) was taken and add to it 0.1 ml of aluminum chloride (10%), 0.1 ml of potassium acetate (1 M) and 2.8 ml of distilled water. The reaction mixture was incubation at room temperature for 30 min. The absorbance was measured at 415 nm using UV–vis Spectrophotometer against the appropriate blank. Quercetin was used as standard (0.1 mg/ml) to calculate total flavonoid content mg/g dry weight (Kasetsart, 2009).

2.1.2.6. *Total estimation of carbohydrate*. Using boiling tube 50 mg of dry powdered rhizome placed in it and hydrolyzed after addition of 5 ml of 2.5 N HCl (on boiling water bath) for 5 h. the mixture then cooled and neutralized using sodium carbonate until releasing effervescence were stopped. It was then filtered using microfiltration processes, the filtrate was transferred to a volumetric flask completed to 100 ml using distilled water. Sample (0.2 ml) was moved in another flask and made up the volume to one ml with distilled water again. Using cuvette, the filtrate obtained was mixed with one ml of phenol and followed by 5.0 ml of sulphuric acid and kept at 25–30 °C for 20 min. The absorbance was recorded at 490 nm (Jain et al., 2017; Kasetsart, 2009).

2.1.2.7. *Estimation of total tannins*. The content of tannins in MEIC was evaluated as published (Polshettiwar and Ganjiwale, 2007). Using the colorimetric method for total tannins estimation is depending on a recording of the blue color made by the reduction of phospho tungsto molybdc acid by tannin like compound in alkaline medium, the procedure used was carried out as reported in published work (Padma et al., 2013).

2.1.2.8. Total ash content. One gram of plant powder sample was placed in a porcelain crucible then heated in a muffle furnace for about 24 h at 500 °C. The obtained ash then kept in a desiccator and weighed (Sneh et al., 2013).

2.1.2.9. Acid insoluble ash. After preparing the ash as mentioned before, the obtained ash was heated for 10 min with 20 ml of dilute hydrochloric acid, cool and filter (ash-less filter-paper). The ash-less filter was washed with hot water and reignited in a muffle furnace at 455 °C for 4 h and weight. Acid insoluble ash was calculated by reference to the air-dried plant.

2.1.2.10. Water-soluble ash. Total ash was prepared as mentioned before (Section 2.1.2.8), boiled in 20 ml of water for 10 min. The mixture then filtered on an ashless filter paper, washed three times using hot water and re-ignited in the muffle furnace for 2 h at 400 °C. The obtained ash was cooled and weighed again. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash (Alfy et al. 2012).

2.1.2.11. Moisture constants. Moisture content was determined using an oven. Five grams of sample material were placed in a porcelain crucible, weight and kept overnight in an air oven at 100–110 °C. It was then placed in a desiccator at room temperature till it cooled and weighed again. The procedure was repeated until three consecutive weights were the same. The loss in weight gave the moisture content (Al-Saleem et al., 2018).

2.2. Biological activities

2.2.1. Anticancer activity

2.2.1.1. Cell lines. Five cell lines; Hela (Cervical carcinoma), CACO (Colorectal carcinoma), Pc3 (prostate cancer), HCT-116 (colon carcinoma), and A-549 (Lung carcinoma) were used in this study.

2.2.1.2. In-Vitro anticancer activity. The anticancer activity of the investigated extract was done using clinically isolated five cell lines as published by Ünyayar et al. (2006). The anticancer activity was carried out in Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

2.2.2. Toxicity studies

2.2.2.1. Animals. Swiss albino mice of both sexes (35–38 g) were purchased from the animal house of Alazahar University, Cairo, Egypt. The animals were held in polypropylene cages and sustained under standard environmental conditions (Ooi et al., 2016).

2.2.2.2. Extracts preparation. Ten grams of the previously prepared dried alcohol extract of the plant rhizomes were dissolved in distilled water (freshly) using an emulsifying agent (Tween 80) (El-Meligy et al., 2017).

2.2.2.3. Acute and sub-chronic toxicity test. The oral median lethal dose (LD50) of the total alcohol extract of *Alpinia officinarum* was determined as described by (Carocho et al., 2013). The investigated extract was evaluated up to 4000 mg/kg. To investigate the sub-chronic toxicity, the alcohol extract (400 mg/kg) administrated orally for 35 days and side effects on liver and kidney functions were evaluated (El-Meligy et al., 2017) was used urea and Serum creatinine were determined as measures of kidney functions (El-Meligy et al. (2017)).

2.3. Statistical analysis

All results were presented as mean ± S.E. Assessments between means were approved out using a one-way ANOVA test and by the Tukey HSD test by SPSS, type 14 (SPSS, Chicago, IL). Variances at $p = 0.005$ were reflected statistically important

3. Results and discussion

3.1. Phytochemical investigations

3.1.1. Qualitative analysis

The screening *Alpinia officinarum* rhizomes phytochemically showed the presence of variable active constituents; carbohydrates, flavonoids, sterols, proteins, triterpenes, and tannins. These phytochemical groups play a very important role in the biological activities of this plant (Kokate et al., 2004).

3.1.2. Quantitative analysis

Total alcohol extract yielded 20 g of gummy residue. Various techniques like chromatographic analysis, UV spectrophotometric

Table 1
Concentrations of various elements in *Alpinia officinarum* rhizome.

Element	Cr	Mn	Fe	Ni	Cu	Zn	Mg	Ca	Na	K
Percentage ppm	0.680	72.30	85.50	21.02	0.753	9.331	569.5	438.8	152.8	3570

Table 2
Calculated concentration of *Alpinia officinarum* Active phytochemical groups.

Phytochemical groups %	mg/g w/w of extract					
	Carbohydrates	Lipids	Protein	Flavonoids	Phenolic	Tannins
Percentage	16.87 ± 1.09	15.15 ± 0.13	18.16 ± 1.04	5.10 ± 1.01	2.71 ± 1.13	20.17 ± 1.11

Each value represents the mean of three repetition ± SE.

Table 3
Quantitative estimation of pharmacopoeial constants in *Alpinia officinarum* rhizome.

Parameter	Moisture	Total Ash	Acid insoluble ash	Water soluble ash
Percentage	11.02 ± 1.05	5.64 ± 1.31	2.01 ± 1.12	3.11 ± 1.01

Each value represents the mean of three repetition ± SE.

Table 4
Effect of *Alpinia officinarum* Hance rhizome extract on liver and kidney markers.

Parameter	Normal Control	Total alcohol extract
ALT (U/L)	59.15 ± 1.30	58.21 ± 1.10
AST (U/L)	48.60 ± 1.20	47.91 ± 1.30
Total bilirubin (mg/dL)	1.70 ± 0.60	1.69 ± 0.20
Total protein (g/dL)	8.70 ± 0.80	8.50 ± 0.50
Albumin (g/dL)	3.90 ± 0.30	3.80 ± 0.40
Urea (mg/dL)	37.66 ± 1.40	36.53 ± 1.50
Creatinine (mg/dL)	0.49 ± 0.60	0.50 ± 0.50

Alcohol extract of *Alpinia officinarum* Hance rhizome (400 mg/kg) were administered to rats for 35 days, n = 10, sera were collected and different enzymes were measured.

analysis, and Atomic absorption spectrometric determination of heavy metals were carried out.

The ethanol extraction of *Alpinia officinarum* rhizomes has been phytochemically screened to confirm the presence of its active components used for all types of chemical components percentage. The results are mentioned in Tables 1–3.

The atomic absorption spectrometric analysis showed that the plant rhizomes contain essential elements like Cu, Zn & Mg and the other heavy metals were variable but within the international permitted limits (Table 1). The percentage of chromium was the lowest (0.680 ppm), while Potassium was the highest (3570 ppm). This variation of elements in *Alpinia officinarum* rhizomes increases its medicinal and nutritive value.

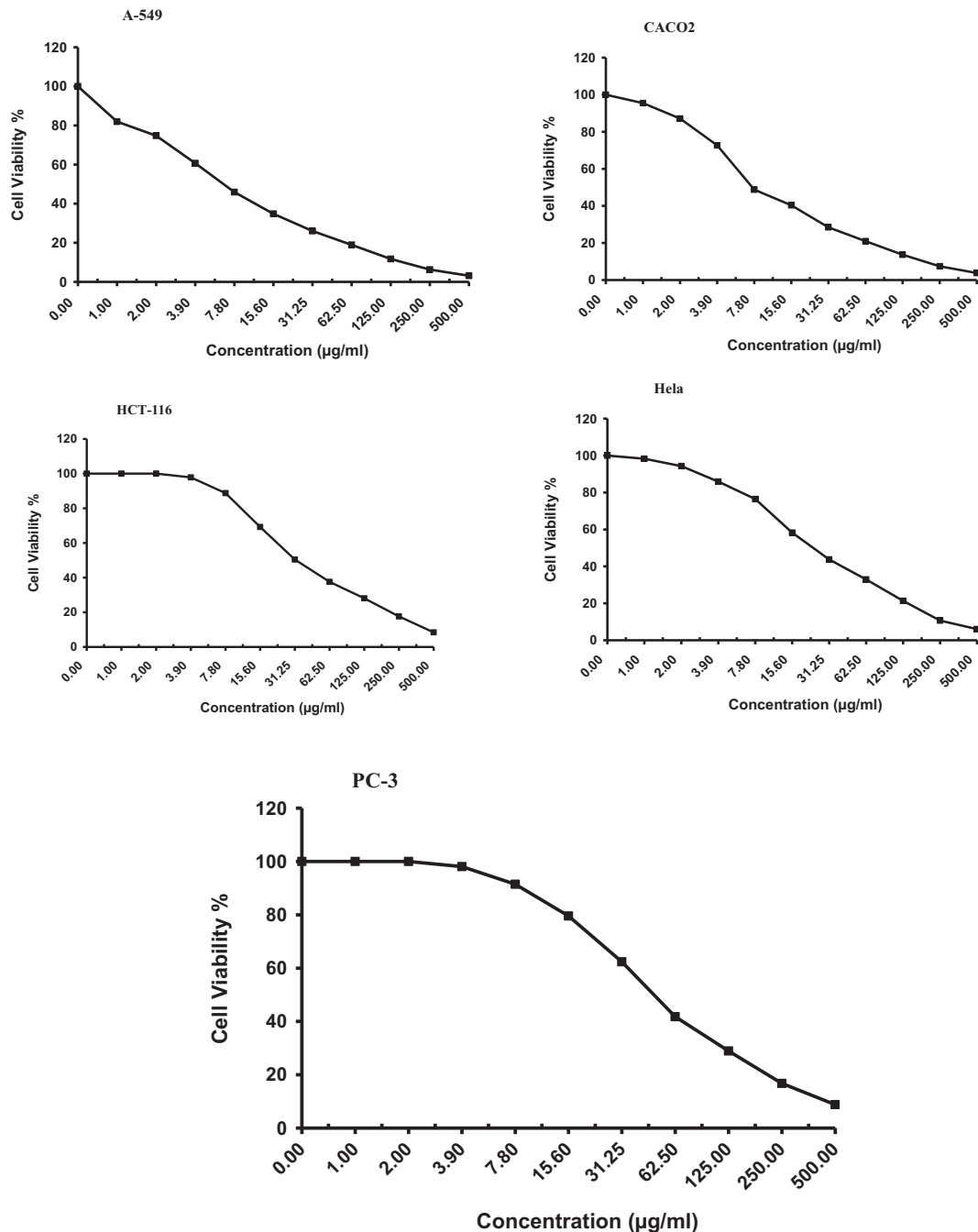


Fig. 1. The cytotoxic effect of *Alpinia officinarum* Hance on A-549, CACO, HCT-116, HeLa, and Pc3 cell lines.

Analysis of different phytochemical contents of *Alpinia officinarum* rhizomes (Table 2) revealed the percent of variable concentrations the highest was in carbohydrate (20.25 ± 1.11) while the lowest was in lipid (2.79 ± 1.03), other constituents percentage ranged from 5.11 ± 1.31 to 18.26 ± 1.24 for protein, phenolic, flavonoids, and tannins respectively. The occurrence of this variable percentage of these active compounds indicated that this plant has very good potential to be a source of natural ruminant.

Determination of some pharmacopeia constants (Table 3) reported that moisture content was very high (11.02 ± 1.05) this

means the plant is not highly dried. Other constants such as; acid insoluble ash, Total ash, water-soluble ash and were varied in values (5.64 ± 1.31 to 2.01 ± 1.12) this means that the plan is rich in active principles.

3.2. Biological evaluations

3.2.1. Test for toxicity

Admiration of *Alpinia officinarum* Hance, rhizome alcohol extract orally to rats didn't show any sign of toxicity or death over 24 h period. Therefore it considered safe for human use (Ooi et al., 2016).

The sub-chronic toxicity also proved the non-toxic nature of the investigated rhizome extract. The administrated daily therapeutic dose (400 mg/kg) didn't show any alteration in liver and kidney function markers (Table 4). It means that the plant extract is neither hepatotoxic nor nephrotoxic (Rysz et al., 2017).

3.2.2. Anticancer activity

Activities of *Alpinia officinarum* Hance extract was evaluated on five clinically isolated cancer cell lines *in-vitro*. The results showed

Table 5
The IC₅₀ values of *Alpinia officinarum* Hance, rhizome extract on cell lines.

Cell line	IC ₅₀ (µg/ml)	
	Total extract	Vinblastine Sulphate
A-549 (Lung carcinoma)	6.72 ± 0.50	24.60 ± 0.70
CACO (Intestinal carcinoma)	7.6 0 ± 0.30	30.30 ± 1.40
HCT-116 (Colon carcinoma)	32.30 ± 3.10	3.50 ± 0.20
Hela (Cervical carcinoma)	24.50 ± 1.10	59.70 ± 2.10
Pc3 (prostate cancer)	50.00 ± 2.40	21.20 ± 0.90

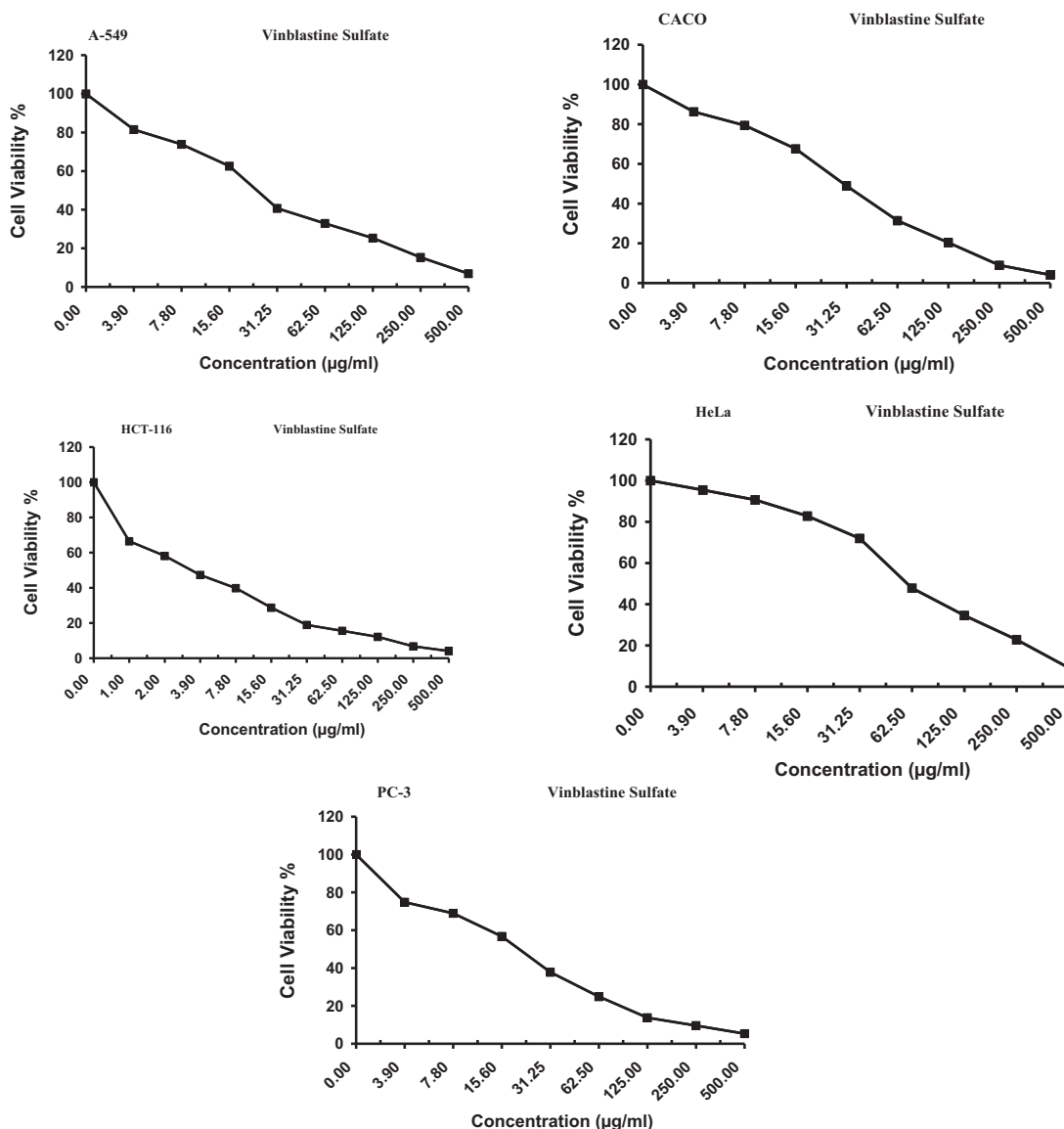


Fig. 2. The cytotoxic effect of Vinblastine Sulfate as Reference Standard on A-549, CACO, HCT-116, Hela, and Pc3 cell lines.

dose depending cytotoxic effect (Fig. 1 & Table 5) when compared with the effect of vinblastine sulphate as a standard drug (Fig. 2 & Table 5).

The highest anticancer activity of *Alpinia officinarum* Hance, was recorded on A-549 (Lung carcinoma) and CACO (colorectal carcinoma), this was clear in their IC₅₀ which were 6.72 ± 0.5 and 7.6 ± 0.3 µg/ml for A-549 and CACO respectively, this effects were better than the standard drug vinblastine sulphate 24.6 ± 0.7 & 30.3 ± 1.4 µg/ml for A-549 and CACO respectively.

Furthermore, the investigated plant extract showed promising effect on the other three cell lines (HCT-116 (Colon carcinoma, Hela (Cervical carcinoma) & Pc3 (prostate cancer), IC₅₀ of *Alpinia officinarum* on Hela (Cervical carcinoma) was better (24.5 ± 1.1 µg/ml) than vinblastine sulphate (59.7 ± 2.1 µg/ml).

The lowest effect was recorded for Pc3 (prostate cancer) with IC₅₀ of 50 ± 2.4 µg/ml which can be concedes promising when compared with standard drug vinblastine sulphate (IC₅₀ 21.2 ± 0.9 µg/ml). The effect of this plant as an anticancer can be attributed to the presence of phenolic compounds in it (Rysz et al., 2017).

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