

Research Article

In vitro* anticancer activity of *Psoralea corylifolia* Linn. seeds on human cancer cell lines*Ria P Mathew^{1*}, Darshana Patil², Avinash Patil¹**

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Abstract

Medicinal herbs are increasingly gaining recognition as valuable complementary therapies for cancer. Numerous clinical studies have highlighted the positive impact of herbal medicines on cancer patient's survival, immune response and quality of life, particularly when used alongside conventional treatments. The present study has been conducted to assess the anticancer activity of *Psoralea corylifolia* Linn. seed aqueous extract and hydroalcoholic extract against 8 human cancer cell lines (MCF-7, HeLa, PC-3, K-562, HT-29, MDA-MB-231, DU-145 and Hep-G2). The SRB assay was used to evaluate the inhibition of cell growth. The results indicated that *Psoralea corylifolia* Linn. seed aqueous extract showed negligible anti-cancer property against all the cancer cell lines tested. However, *Psoralea corylifolia* Linn. seed 50% hydroalcoholic extract showed GI50 values of 34.6 μ g/ml, 46.1 μ g/ml, 30 μ g/ml and 28.9 μ g/ml against Breast cancer cell line (MCF-7), Cervical cancer cell line (HeLa), Prostate cancer cell line (PC-3) and Leukemia cancer cell line (K-562) respectively. Further, the 50% hydroalcoholic extract was altered with respect to the solvent used, method of preparation etc. and subsequently evaluated for its anticancer potential. The results revealed that *Psoralea corylifolia* Linn. soxhlet extract in absolute ethanol showed significant anticancer activity in all the above 4 cell lines with GI50 values <10 μ g/ml.

Keywords: *Psoralea corylifolia* Linn., Anticancer activity, Human cancer cell lines

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Introduction

Cancer is one of the leading causes of death worldwide. The number of cancer-related fatality is rising steadily. Cancer is a disease characterized by abnormal proliferation of cells due to disruptions in cellular modulation and obstruction of cell cycle progression. It thereby elicits malignant tumor cells formation with the possibility of becoming metastatic (1,2). For instance, prostate and colon cancers commonly metastasize to bones and the liver, respectively; lung cancer spreads to the adrenal glands, liver, brain and bones, while breast cancer frequently metastasizes to the lungs and bones (3,4).

Synthetic drugs are widely used in cancer treatment. However, they often cause severe toxic side effects both during and after treatment. Common side effects include hair loss, changes in skin color and texture, fatigue, nausea and infertility. In order to minimize these adverse effects, there is a growing preference for natural drugs derived from medicinal plants. Medicinal plants contain bioactive compounds that offer therapeutic benefits. Clinical research and phytochemical analyses have confirmed the antitumor potential of various herbal remedies against multiple

cancer types. Ethnomedicinal practices and traditional knowledge that are passed down through generations provides a valuable resource for identifying plants with anticancer properties (1,5). Herbal medicines target and inhibit key biochemical pathways involved in the transformation of normal cells into cancerous cells, aiding in treatment (6). Thus, numerous pharmaceutical companies are increasingly exploring and developing novel drugs from medicinal plants (7).

Psoralea corylifolia Linn. belonging to the family Fabaceae is a widely recognized traditional medicinal herb in China and India. It is extensively utilized in Ayurvedic medicine to cure several skin diseases including psoriasis, leprosy and leucoderma. Additionally, it exhibits numerous pharmacological properties such as antioxidant, anti-cancer, anti-inflammatory, hepatoprotective, anti-diabetic, anti-mycobacterial and anti-helminthic effects (7,8). This endangered plant, native to tropical and subtropical regions, holds great medicinal value and is listed in the Indian Pharmaceutical Codex as well as the Chinese, British and American pharmacopoeias. It is also listed in different traditional systems of medicine, including Ayurveda, Unani and Siddha. Ayurvedic texts describe the therapeutic properties of the *Psoralea corylifolia* Linn. (Bakuchi) plant in detail, highlighting its application in treating *Kushtha* (skin diseases), *Keshya* and *Tvachya* (skin and hair care), *Shwasa* and *Kasa* (bronchial asthma and cough), *Pandu* (anemia) and *Shotha* (oedema) (9).

In the present study the *Psoralea corylifolia* Linn. seed extract has been studied to explore its *in vitro* anticancer potential against the most common cancer types (lung, breast, cervical, leukemia, prostate and colon cancer).

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Materials and Methods

Collection and Authentication

Seeds of *Psoralea corylifolia* Linn. were procured from Unani drug shop, Bhivandi, Thane, Maharashtra, India and authenticated from Plant Drug Authentication Service, Agharkar Research Institute, Pune.

Drying

The collected seeds were washed under running tap water and blotted dry. The seeds were then shade dried, ground into powder, stored in an airtight container and used for further analysis.

Preparation of Extracts

Preparation of Aqueous extract

A 6:1 ratio of distilled water was added to the coarse powder, mixed thoroughly and refluxed for 2 hrs at 70-80 °C. The above step was repeated thrice. The extract was then filtered using Whatman filter paper and concentrated in a water bath at a temperature of 80°C. The prepared extracts were stored in airtight amber-colored bottles and kept in the refrigerator.

Preparation of Hydroalcoholic (50% ethanol) extract

A 4:1 ratio of hydroalcohol (50% ethanol) was added to the coarse powder, mixed thoroughly and macerated for 4 hrs. The mixture was refluxed for 2 hrs at 70-80 °C. The above step was repeated thrice. The extract was then filtered using Whatman filter paper and concentrated in a water bath at a temperature of 80°C. The prepared extracts were stored in airtight amber-colored bottles and kept in the refrigerator.

Preparation of extracts: PR60, PR70, PR100 and PS100

Hydroalcohol with different composition of ethanol ie. 60% ethanol, 70% ethanol and absolute ethanol were added separately to the powder in a ratio of 3:1. The mixture was mixed thoroughly and macerated for 4 hrs. Thereafter the mixture was refluxed for 2 hours. The above step was repeated thrice. The extract was then filtered using Whatman filter paper and concentrated in a water bath at a temperature of 80°C. The prepared extracts were stored in airtight amber-colored bottles and kept in the refrigerator. The resultant extracts were labelled as PR60 (*Psoralea corylifolia* Linn. reflux extract of seed with 60% ethanol), PR70 (*Psoralea corylifolia* Linn. reflux extract of seed with 70% ethanol) and PR100 (*Psoralea corylifolia* Linn. reflux extract of seed with absolute ethanol). The PS100 extract (*Psoralea corylifolia* Linn. soxhlet extract of seed with absolute ethanol) was prepared using a Soxhlet apparatus. Coarse powder of *Psoralea corylifolia* Linn. seeds were subjected to hot successive continuous extraction in a Soxhlet apparatus using absolute ethanol in the ratio of 1:4 for 6 hrs at 70-80 °C. The extract was then concentrated in a water bath at a temperature of 80°C. The prepared extracts were stored in airtight amber-colored bottles and kept in the refrigerator. The resultant extracts were labelled as PS100.

In vitro Anticancer Activity

This was a preliminary *in-vitro* study conducted to evaluate the anti-cancer property of *Psoralea corylifolia* Linn. seeds and compare it with positive control drug Adriamycin (ADR). The study was carried out at Anti-cancer drug Screening Facility, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, Maharashtra, India.

Human Cancer Cell Lines

Various Human Cancer Cell Lines used for *in vitro* SRB Assay were Breast Cancer Cell Lines (MCF-7, MDA-MB-231), Cervical Cancer Cell Line (HeLa), Prostate Cancer Cell Lines (PC-3, DU-145), Leukemia Cell Line (K-562), Colon Cancer Cell Line (HT-29) and Liver Cancer Cell Line (Hep-G2). The cell lines were grown in an appropriate medium that contains 10% fetal bovine serum and 2 mM L-glutamine.

Sulforhodamine B (SRB) Assay

The *in vitro* SRB assay was carried out on various human cancer cell lines at the Tata Memorial Centre – Advanced Centre for Treatment, Research, and Education in Cancer (ACTREC), Navi Mumbai, Maharashtra, India. This antiproliferative SRB assay was employed to evaluate the inhibition of cell growth. SRB assay is a reliable and sensitive method for measuring the drug-induced suppression of cell proliferation. It is a simple test system for assessing the impact of test compounds on the proliferation of cultured cancer cells (10). This assay has been used since its development in 1990 (11).

Results and Discussion

In the present study, the results of the anti-cancer activity of aqueous and hydroalcoholic (50% ethanol) extracts of *Psoralea corylifolia* Linn. seeds are depicted in Table 1. The aqueous extracts displayed negligible anti-cancer activity (GI50 > 80µg/ml & Non evaluable TGI) against all the selected human cancer cell lines when compared with positive control drug Adriamycin. However the hydroalcoholic (50% ethanol) extract showed enhanced anti-cancer activity against 4 human cancer cell lines namely Breast cancer cell line - MCF-7 (GI50 = 34.6 µg/ml & TGI > 80µg/ml), Cervical Cancer Cell Line - HeLa (GI50 = 46.1 µg/ml & TGI > 80 µg/ml), Prostate Cancer Cell Line - PC-3 (GI50 = 30.0 µg/ml & TGI = 65.1 µg/ml), Leukemia Cell Line - K-562 (GI50 = 28.9 µg/ml & TGI > 80 µg/ml) when compared with the aqueous extract.

On the basis of the results of hydroalcoholic (50% ethanol) extract the extracts were altered with respect to the concentration, solvent composition and method of extraction and subsequently the extracts made were evaluated for their anticancer potential against the above 4 cell lines. The results indicate that the PR60 extract showed significant anticancer activity against Leukemia Cell Line (K-562) whereas the PR70 and PR100 extracts showed significant anticancer activity against 3 human cancer cell lines namely Breast Cancer Cell Line (MCF-7), Prostate Cancer Cell Line (PC-3) and Leukemia Cell Line (K-562). On the other hand, the PS100 extract showed significant anticancer activity against all the 4 cell lines under study. The LC50, TGI and GI50 values for the PR60, PR70, PR100 and PS100 extracts are as given in Table 2. The effect of all the above extracts on human cancer cell lines have been depicted in Figure 1 and 2. The microscopic images depicted in Figure 2, clearly shows the adverse effects of *Psoralea corylifolia* Linn. seed extracts (PR60, PR70, PR100 and PS100) on the cellular morphology of HeLa, K-562, MCF-7 and PC-3 cells. The findings have shown that the Soxhlet extract of *Psoralea corylifolia* Linn. seeds in absolute ethanol (PS100) has promising cytotoxic activity against HeLa, K-562, MCF-7 and PC-3 cells. Thus the dataset in this study shows the potential of *Psoralea corylifolia* Linn. as an anticancer agent which can be used further for drug development and designing in the pharmaceutical industry.

In developed countries, mortality rates from stroke and coronary heart disease are declining, while cancer has emerged as the primary impediment to life expectancy. In 2019, more than 10 million people died from cancer, which is nearly twice the number from 1990 (12). The COVID-19 pandemic in 2020 hampered cancer diagnosis and treatment due to health care setting closures. This potentially increased the late-stage cases and mortality (13).

Lung cancer (12.4% of all cancers) was the most frequently diagnosed cancer in 2022, followed by breast (11.6%), colorectal (9.6%), prostate (7.3%), and stomach (4.9%) cancers. Lung cancer was also the leading cause of cancer death, causing an estimated 1.8 million fatalities (18.7%), followed by colorectal (9.3%), liver (7.8%), breast (6.9%), and stomach (6.8%) cancers (14).

Breast cancer is one of the predominant cancers in women. Female breast cancer is the second leading cause of global cancer incidence with an estimated 2.3 million new cases and the fourth leading cause of cancer mortality worldwide causing 6,66,000 deaths in 2022. On the other hand, Cervical cancer is the fourth most prevalent cancer in both incidence and mortality among women globally and ranks in the top five cancers for both incidence and mortality in India. In transitioning countries, mortality rates from female breast, cervical and ovarian cancers exceeded those from lung cancer (14). Among women, breast and cervical cancers dominated as the most frequently diagnosed cancers globally (12).

Prostate cancer was recorded as the second most frequently diagnosed cancer and the fifth leading cause of cancer death among men in 2022. Acute lymphoblastic leukemia was more prevalent among children, particularly in Latin America and Asia, while acute myeloid leukemia was common in adults and children in higher Human Development Index (HDI) settings (14). This indicates the significant impact of Breast cancer, Cervical cancer Prostate cancer and Leukemia on mortality worldwide. Due to the high population density Asia is found to have the heaviest cancer burden. Hence efforts are crucial to address its escalating burden, particularly in transitioning countries experiencing rising incidence and high mortality rates (15).

The investigation of natural products as potential anticancer agents marks a significant milestone in medical research, blending modern scientific approaches with the principles of traditional medicine. These natural products play a pivotal role in the discovery of new anticancer drugs, providing researchers with opportunities to identify novel bioactive compounds for cancer treatment (16). An increasing number of studies indicate that *Psoralea corylifolia* Linn. exhibits strong anticancer activity with minimal side effects, making it a promising candidate for anticancer drug development. Specifically, *Psoralea corylifolia* Linn. has demonstrated anticancer effects in numerous studies (17).

The seeds of *P. corylifolia* exhibit cytotoxic activity against SNU-1 and SNU-16 stomach carcinoma cell lines, with IC₅₀ values of 53 and 203 µg/ml, respectively. This activity has been attributed to the presence of the cytotoxic coumestan derivative, psoralidin (18). In another study, psoralidin isolated from the seeds of *Psoralea corylifolia* Linn. was found to be highly cytotoxic against HT-29 (colon) and MCF-7 (breast) cancer cell lines, with IC₅₀ values of 0.3 and 0.4 µg/ml. Conversely, in the same study, angelicin displayed only marginal cytotoxicity, while psoralen was found to be inactive against A549 (lung) and HepG2 (liver hepatoma) cell lines (19, 20).

Ethanol, methanolic, chloroform, and aqueous extracts of *P. corylifolia* seeds were evaluated against the tumor cells of mice and were found to enhance antibody complement-mediated cytotoxicity during tumor development (21). Bakuchiol is a monoterpenoid phenol isolated from *P. corylifolia* seeds (22). When compared with resveratrol, bakuchiol demonstrated superior efficacy in inhibiting tumor cell growth in the human lung adenocarcinoma A549 cell line (23). Additionally, psoralidin and neobavaisoflavone, in combination with TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), exhibited anti-cancer effects by promoting apoptosis in LNCaP prostate cancer cells (24). Similarly, psoralidin showed significant anti-cancer activity against human lung cancer (A549) cells by reducing cell viability in a dose- and time-dependent manner (25,26).

Table 1: *In vitro* anticancer activity of *Psoralea corylifolia* Linn. seeds on human cancer cell lines

| Human cancer cell lines | Tissue of Origin | Drug concentrations in µg/ml | Extracts | | |
|-------------------------|------------------|------------------------------|----------|------|------|
| | | | PWE | PHA | ADR |
| -MCF 7 | Breast | LC50 | NE | >80 | <10 |
| | | TGI | NE | >80 | <10 |
| | | GI50 | >80 | 34.6 | <10 |
| HeLa | Cervix | LC50 | NE | >80 | <10 |
| | | TGI | NE | >80 | <10 |
| | | GI50 | >80 | 46.1 | <10 |
| PC-3 | Prostate | LC50 | NE | >80 | <10 |
| | | TGI | NE | 65.1 | <10 |
| | | GI50 | >80 | 30.0 | <10 |
| K-562 | Leukemia | LC50 | NE | >80 | >80 |
| | | TGI | NE | >80 | >80 |
| | | GI50 | >80 | 28.9 | <10 |
| HT-29 | Colon | LC50 | NE | >80 | <10 |
| | | TGI | NE | >80 | <10 |
| | | GI50 | >80 | 60.6 | <10 |
| MDA-MB-231 | Breast | LC50 | NE | NE | <10 |
| | | TGI | NE | NE | <10 |
| | | GI50 | >80 | >80 | <10 |
| DU-145 | Prostate | LC50 | NE | NE | >80 |
| | | TGI | NE | NE | <10 |
| | | GI50 | >80 | >80 | <10 |
| Hep-G2 | Liver (Hepatoma) | LC50 | NE | NE | 57.7 |
| | | TGI | NE | NE | <10 |
| | | GI50 | >80 | >80 | <10 |

Keywords:

- Values highlighted indicate positive results

PWE: *Psoralea corylifolia* Linn. aqueous seed extract; PHA: *Psoralea corylifolia* Linn. hydroalcoholic (50% ethanol) seed extract; ADR: Adriamycin, Positive control compound; LC50: Concentration of drug causing 50% cell kill; TGI: Concentration of drug causing total inhibition of cell growth; GI50: Concentration of drug causing 50% inhibition of cell growth; NE: Non- evaluable data

Table 2: In vitro anticancer activity of *Psoralea corylifolia* Linn. seeds on human cancer cell lines

| Human Cancer cell lines | Tissue of Origin | Drug concentrations in μ g/ml | Extracts | | | | |
|-------------------------|------------------|-----------------------------------|----------|------|-------|-------|-----|
| | | | PR60 | PR70 | PR100 | PS100 | ADR |
| -MCF 7 | Breast | LC50 | NE | 72 | 73 | 54 | NE |
| | | TGI | 73 | 44 | 41 | 16 | <10 |
| | | GI50 | 30 | 16 | 10 | <10 | <10 |
| HeLa | Cervix | LC50 | >80 | 73 | 71 | 62 | NE |
| | | TGI | 65 | 55 | 52 | 24 | <10 |
| | | GI50 | 47 | 38 | 33 | <10 | <10 |
| PC-3 | Prostate | LC50 | >80 | 66 | 63 | 54 | NE |
| | | TGI | 61 | 43 | 40 | 23 | <10 |
| | | GI50 | 33 | 20 | 17 | <10 | <10 |
| K-562 | Leukemia | LC50 | >80 | 73 | 64 | 51 | <10 |
| | | TGI | 40 | <10 | <10 | <10 | <10 |
| | | GI50 | <10 | <10 | <10 | <10 | <10 |

Keywords:

- Values highlighted indicate positive results

PR60 - *Psoralea corylifolia* Linn. reflux extract of seed in 60% ethanol; PR70 - *Psoralea corylifolia* Linn. reflux extract of seed in 70% ethanol; PR100 - *Psoralea corylifolia* Linn. reflux extract of seed in absolute ethanol; PS100 - *Psoralea corylifolia* Linn. Soxhlet extract of seed in absolute ethanol; ADR - Adriamycin, Positive control compound; LC50: Concentration of drug causing 50% cell kill; TGI: Concentration of drug causing total inhibition of cell growth; GI50: Concentration of drug causing 50% inhibition of cell growth; NE: Non-evaluative data

Conclusion

Based on the results of the present study, it was concluded that the soxhlet extract of *Psoralea corylifolia* Linn. seeds in absolute ethanol (PS100) exhibited significant *in vitro* anticancer activity with GI50 less than 10 μ g/ml in all the 4 cell lines namely Breast Cancer Cell Line (MCF-7), Cervical Cancer Cell Line (HeLa), Prostate Cancer Cell Line (PC-3) and Leukemia Cell Line (K-562). This may be due to the effect of the secondary metabolites present in the extract. The results give suggestive evidence that the *Psoralea corylifolia* Linn. seeds exhibit some correlation between the claimed ethnomedicinal uses and the cell proliferative activity. Apparently, the promising active principle in *Psoralea corylifolia* Linn. seed inhibits Breast Cancer, Cervical Cancer, Prostate Cancer and Leukemia indicating need to investigate the underlying mechanism by which this activity was exhibited. Further, the extracts need to be screened against different cell lines apart from the selected cell lines to confirm the activity.

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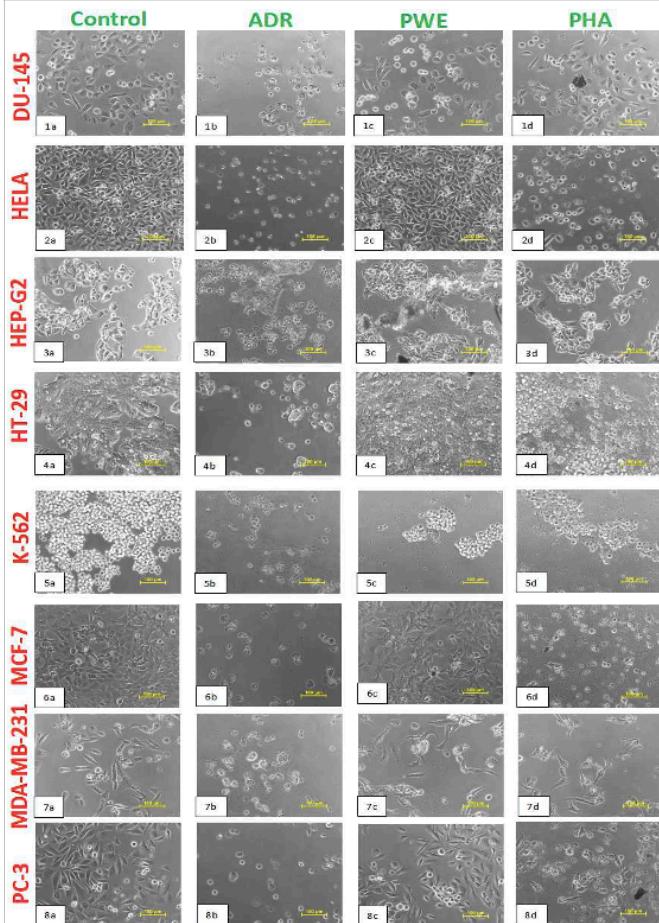
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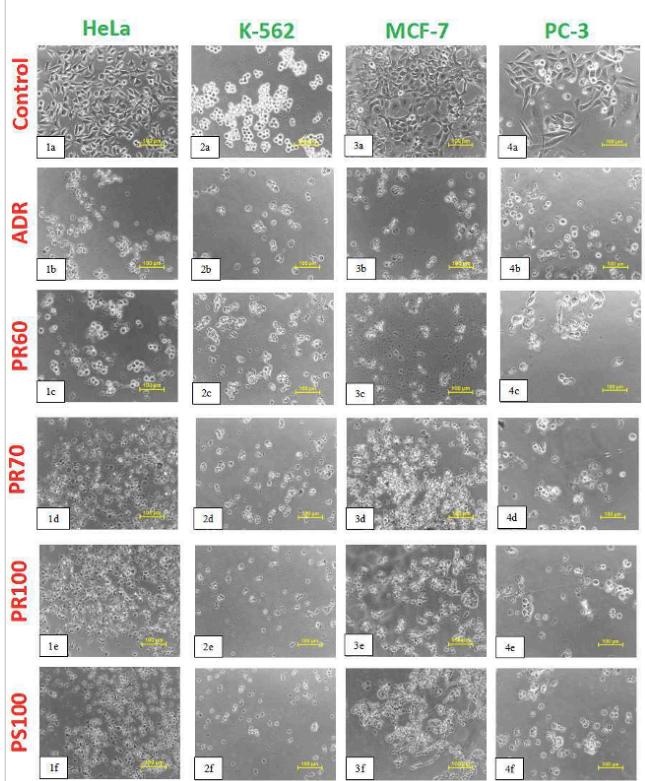
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Figure 1: Morphological changes of human cell lines representing cancer of the Breast (MCF-7, MDA-MB-231), Cervix (HeLa), Prostate (PC-3, DU-145), Leukemia (K-562), Colon (HT-29) and Liver (Hep-G2) in response to the treatment of positive control compound - Adriamycin and *Psoralea corylifolia* Linn. seed extract.



Keywords: 1a, 2a, 3a, 4a, 5a, 6a, 7a, 8a: Control cell lines without any treatment; 1b, 2b, 3b, 4b, 5b, 6b, 7b, 8b: Cell lines treated with Positive control compound - Adriamycin; 1c, 2c, 3c, 4c, 5c, 6c, 7c, 8c: Cell lines treated with *Psoralea corylifolia* Linn. seed aqueous extract; 1d, 2d, 3d, 4d, 5d, 6d, 7d, 8d: Cell lines treated with *Psoralea corylifolia* Linn. seed hydroalcoholic (50% ethanol) extract

Figure 2: Morphological changes of human cancer cell lines representing cancer of the Cervix (HeLa), Leukemia (K-562), Breast (MCF-7) and Prostate (PC-3) in response to the treatment of positive control compound - Adriamycin and *Psoralea corylifolia* Linn. seed extracts.



Keywords: 1a, 2a, 3a, 4a: Control cell lines without any treatment; 1b, 2b, 3b, 4b: Cell lines treated with Positive control compound - Adriamycin; 1c, 2c, 3c, 4c: Cell lines treated with PR60 extract; 1d, 2d, 3d, 4d: Cell lines treated with PR70 extract; 1e, 2e, 3e, 4e: Cell lines treated with PR100 extract; 1f, 2f, 3f, 4f: Cell lines treated with PS100 extract