Research Article

Phytochemical Analysis, *In Vitro* Anticancer Activity and HPTLC Fingerprint Profile of Seeds of *Abrus Precatorius* L.

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ABSTRACT

Cancer is a dreadful disease and any practical solution in combating this disease is of paramount importance to public health. In recent years, medicinal plants have attracted a lot of attention globally. Many herbs have been evaluated and are currently being investigated phytochemically to understand their anti-tumor actions against various types of cancers. *Abrus precatorius* L. - Family Fabaceae is a medicinal herb used for many diseases. The ethnomedicinal properties mentioned in the literature are antitumor, antifertility, antihelminthic, insecticidal, anti-inflammatory, antidiabetic and antibacterial. In the present investigation, four different extracts (*viz.* Aqueous, Ethanolic, Methanolic and Petroleum ether) of seeds of *Abrus precatorius* L. were analysed for their preliminary phytochemical constituents, whereas five different extracts (*viz.* Aqueous, Hydroalcoholic, Ethanolic, Methanolic and Petroleum ether) of seeds of *Abrus precatorius* L. were analysed for *in vitro* anticancer activity and HPTLC fingerprint. Preliminary phytochemical analysis of extracts of seeds of *Abrus precatorius* L. was carried out. Sulforhodamine B (SRB) assay was carried out for *in vitro* anticancer activity for five different extracts (*viz.* Aqueous, Hydroalcoholic, Ethanolic, Methanolic and Petroleum ether). HPTLC fingerprint profile was also developed and standardized for these extracts. The preliminary phytochemical analysis of extracts of seeds of *A. precatorius* L. showed presence of aleurone grains, amino acids, proteins, fats and fixed oils, tannins, alkaloids, steroids, glycosides, mucilage and flavonoids. *In vitro* anticancer activity was studied against 19 Human Cancer Cell Lines namely Cervix (ME180, SiHa), Leukemia (HL60, K562), Ovarian (A2780, Ovkar-3), Breast (MCF-7, MDA-MB-468, MDA-MB-435, MDA-MB-231, ZR-75-1, BT-474), Prostate (PC3, DU145), Colon (HT29, Colo205), Lung (A549), Hepatoma (HEPG2) and Oral (AW13516). Out of the 5 extracts and 19 cell lines used for studying anticancer activity, the Hydroalcoholic and Petroleum ether extracts of seeds of *Abrus precatorius* L. were active on Human Breast Cancer Cell Lines MCF-7 and Zr-75-1 respectively. The anticancer activity of Hydroalcoholic and Petroleum ether extracts of seeds of *Abrus precatorius* L. may be related to its flavonoid, terpenoid, alkaloid and protein contents. HPTLC fingerprint profile developed is unique to extracts of seeds of *Abrus precatorius* L.

Keywords: *Abrus precatorius* L., anticancer, Human Cancer Cell Lines, SRB assay, phytochemical analysis, HPTLC fingerprint profile

INTRODUCTION

Cancer is a major public health burden in both developed and developing countries. Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of mankind.[1] American Cancer Society and International Union against Cancer estimated that 12 million cases of cancer were diagnosed in 1997, with 7 million deaths worldwide; these numbers are expected to double by 2030.[2]

There are several medicines available in the market to treat the various types of cancer but no drug is found to be fully effective and safe. The major problem in the cancer chemotherapy is toxicity of the established drugs.[3] According to Cragg and Newman, over 50% of the drugs in clinical trials for anticancer properties were isolated from natural sources or are related to them.[4] The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. About 60% to 75% of FDA approved anticancer and anti-infectious drugs are of natural origin.[5]

From the earliest times, herbs have been prized for their pain-relieving and healing abilities and today developing countries still rely largely on the curative properties of plants. According to World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as primary healthcare system.[6] The use of naturally occurring molecules in the treatment of cancer has greatly contributed to the improvement of therapeutic efficacy of drugs used today in cancer chemotherapy.[7] Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer. Medicinal herbs are a significant source of herbal drugs. So far, pharmaceutical companies have screened more than 25,000 plants for anti-cancer drugs.[6]

Abrus precatorius L. belonging to family Fabaceae is a leguminous climber popularly known as Rati in Hindi, Crab's eye in English, Gunja in Sanskrit. The plant has been used in Hindu medicines from very early times, as well as in China and other ancient cultures.[8] The seeds have been used to treat fever, malaria, headache, dropsy and to expel worms. A decoction of the seeds is applied

for abdominal complaints, conjunctivitis, trachoma and malarial fever. Central Africans use powdered seed as an oral contraceptive. It is also used to lower high blood pressure and relieve severe headache.

The seed has purgative properties and is used as an emetic, tonic, aphrodisiac and for nervous disorders.[9]

Abrus –saponins I and II, abrisapogenol, β-amyrin, squalene, abricin, abridine, cycloartenol, campesterol, cholesterol and sitosterol have all been found in the seeds. Proteins – abrins I, II and III, *Abrus precatorius* agglutinin (APA) I and (APA) II are present in the seeds. Alkaloids and nitrogen compounds – precatorine, trigonelline, choline and abrine are present in the seeds. Flavonoids and anthocyanins – abrectorine, dimethoxycentaureidin-7-o-rutinoside, precatorins I, II and III and xyloglucosyl-delophinidin and p-coumaroyl-galloyl-glucosyl-delphinidin have been isolated from the seeds.[10]

The seeds possess various pharmacological activities such as antidiabetic,[11] antioxidative,[12] antiviral,[13] antihelminthic, antidepression,[14] memory enhancing,[15] antimicrobial,[16-22, 11] anti-inflammatory,[14,23-25] antiarthritic,[23,25-27] anticancer,[23,28-33] antifertility,[14,34-41] antimalarial,[14,42] antiallergic,[25,43] antiasthamatic,[44] anticataract,[45] antiinsecticide,[46] antitoxicity activity,[47-49]

Plants synthesize a wide variety of chemical compounds, which can be classified by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites.[50]

Among the 120 active compounds isolated from the higher plants widely used in modern medicine, 80 percent showed a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived.[51]

The Indian Ayurvedic system treasures a host of medicinal plants that have been shown to possess cytotoxic and cytostatic effects on tumour cell lines.[52]

However, there are few experimental studies, which validate the possible antitumor properties of plants.[53] Several medicinal plants have been screened based on the integrative approaches on drug development from Ayurveda.[54]

The inclusion of TLC fingerprints in modern pharmaceutical herbal monographs is now a standard practice.[55]

A number of studies were carried out in various medicinal plants for the phytochemical characterization using TLC.[56-57]

Therefore, in response to the quest for search of anticancer agents, present research work was designed to investigate anticancer potential of seeds of *Abrus precatorius* L. along with the phytochemical analysis and development of HPTLC profile for various extracts.

MATERIALS AND METHODS

Collection and Authentication

Seeds of *Abrus preactorius* L. were collected from Vikramgad, Taluka Jawhar, (M. S.), India and authenticated from Plant Drug Authentication Service, Agharkar Research Institute, Pune, (M. S.), India.

Drying

The seeds collected were washed under running tap water, blotted dry and kept for drying in oven at temperature 40 ± 2°C for five days. The dried seeds were powdered and stored in air tight container.

Preliminary Phytochemical Analysis

Powdered seeds were extracted with Water, Ethanol, Methanol and Petroleum ether.

The extracts were filtered and subjected to preliminary qualitative tests for the presence of various phytochemicals as described by Khandelwal[58] and Kokate.[59]

In vitro Anticancer Activity

Preparation of Extracts

Extracts were prepared using various solvents as described by Anonymous.[60]

Preparation of Aqueous extract

Distilled water was added to the powder in a ratio of 6:1. It was mixed thoroughly and refluxed for 2 hrs at 80°C.

The above step was repeated 3 times. The extract was filtered and concentrated using Vacuum Rotary Evaporator. Extract prepared was stored in air tight amber coloured bottle.

Preparation of extracts using different solvents

Different Solvents (Hydroalcoholic extract (50:50), Ethanol, Methanol and Petroleum Ether) were added separately to the powder in a ratio of 4:1. The mixture was mixed thoroughly and macerated for 4 hrs.

The mixture was refluxed for 2 hours. The above step was repeated 3 times.

The extract was filtered and concentrated using Vacuum Rotary Evaporator. Extracts prepared were stored in air tight amber coloured bottles and kept in refrigerator.

Human Cancer Cell Lines

Various Human Cancer Cell Lines used for *in vitro* SRB Assay are Cervix Cancer Cell Lines (ME180, SiHa), Leukemia Cell Lines (HL60, K562), Lung Cancer Cell Line (A549), Breast Cancer Cell Lines (MCF7, MDA-MB-468, MCF-7, MDA-MB-468, MDA-MB-435, MDA-MB-231, ZR-75-1, BT-474), Prostate Cancer Cell Lines (PC3, DU145), Hepatoma Cell Line (HEP G2), Colon Cancer Cell Lines (HT29, Colo205), Ovarian Cancer Cell Lines (A2780, Ovkar-3) and Oral Cancer Cell Line (AW13516).

Sulforhodamine B (SRB) Assay

In vitro SRB Assay of the prepared extracts was performed on the various Human Cancer Cell Lines at Tata Memorial Centre – Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai, (M. S.), India.

The antiproliferative SRB assay was performed to assess growth inhibition. This is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye.[61] The microtiter plates were taken out after 48 hours incubation of the cells with test materials and gently layered with chilled 50% Trichloroacetic acid (TCA) in all the wells to produce a final concentration of 10%. The tissue culture plates were incubated at 4°C for one hour to fix the cells attached to the bottom of the wells. The supernatant was then discarded. The plates were washed five times with distilled water to remove TCA, growth medium, low molecular weight metabolites, serum proteins etc. Plates were air dried, SRB dye was added to each well of the plates and incubated at room temperature for 30 minutes. The unbound SRB was removed quickly by washing the wells five times with 1 % Acetic acid and then air dried. 100µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 540 nm.

HPTLC Fingerprint

A qualitative densitometric HPTLC analysis was performed with Aqueous, Hydroalcoholic, Ethanolic, Methanolic and Petroleum ether extracts for the development of characteristic fingerprint profile. 10 µl of extract was spotted on pre-coated silica gel 60 F_{254} HPTLC plates (Merck) with the help of CAMAG Linomat V applicator. The plate was developed in glass twin trough chamber (20 cm × 10 cm) pre-saturated with mobile phase N-Butanol: Acetic acid: Water (4:4:1) for Aqueous, Hydroalcoholic, Ethanolic and Methanolic extracts and N-Hexane: Ethyl acetate (1:1) for Petroleum ether extract. While developing different solvent systems for maximum separation of phytoconstituents, it was observed that the same solvent system did not work for all the extracts due to difference in polarity as Water being highly polar and Petroleum ether being highly non-polar. Hence, for Aqueous, Hydroalcoholic, Ethanolic and Methanolic extracts same solvent system was optimized whereas a separate solvent system was developed for Petroleum ether extract. The plate was derivatized using Anisaldehyde-Sulphuric acid reagent and scanned using CAMAG TLC Scanner 3. The plate was visualized using CAMAG TLC Visulaizer, Documentation and Evaluation system.

RESULTS

In recent years, medicinal plants have attracted a lot of attention globally. Since long time evidence has accumulated to demonstrate promising potential of medicinal plants used in various traditional, complementary and alternative systems especially for cancer treatment.[62] Hence, the present research work was undertaken to evaluate anticancer potential and to carry out phytochemical analysis of seeds of *Abrus precatorius* L. The preliminary phytochemical screening of the seed powder was carried out using various solvents *viz.* Petroleum ether, Ethanol, Methanol and Water. These extracts when subjected to various qualitative phytochemical analysis showed the presence of tannins, alkaloids, steroids, aleurone grains, amino acids, glycosides, mucilage, proteins, flavonoids, fats and fixed oils (Table 1).

Table 1: Preliminary Phytochemical Analysis of extracts of seeds of *Abrus preactorius* L.

Sr. No.	Tests	ME	EE	PE	AE
1.	Acid compounds	ND	ND	ND	ND
2.	Amino acids	ND	+	+	+
3.	Anthraquinones	ND	ND	ND	ND
4.	Aleurone grains	+	+	+	+
5.	Alkaloids	+	+	+	+
6.	Carbohydrates	+	ND	ND	+
7.	Essential oil	ND	ND	ND	ND
8.	Fats and fixed oils	+	+	+	ND
9.	Flavonoids	+	+	ND	+
10.	Glycosides	+	+	ND	+
11.	Mucilage	ND	+	+	ND
12.	Proteins	+	ND	+	+
13.	Resins	ND	ND	ND	ND
14.	Saponins	ND	ND	ND	+
15.	Starch	ND	ND	ND	ND
16.	Steroids	+	+	+	ND
17.	Tannins	+	ND	+	+

Key: ME – Methanolic extract, EE – Ethanolic extract, PE – Petroleum ether extract, AE – Aqueous extract.

The results showing anti-cancer activity of 5 extracts of *Abrus precatorius* L. on 19 different Human Cancer Cell Lines are presented in Table no. 2, 3, 4 and 5. Out of the 5 extracts and 19 cell lines used for studying anticancer activity, the Hydroalcoholic and Petroleum ether extracts of seeds of *Abrus precatorius* L. were active against Human Breast Cancer Cell Lines MCF-7 and Zr-75-1 respectively.

Chromatographic fingerprint can successfully demonstrate both "similarities" and "differences" between various samples and the authentication and identification of herbal medicines can be accurately conducted even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples.

Once the method was developed and optimized, the plates were observed under UV 254 nm and 366 nm and well separated bands were observed.

Table 2: *In vitro* anticancer activity of extracts of seeds of *Abrus precatorius* L. using SRB Assay

Cell Lines / Extracts	Human Cervix Cancer Cell line						Human Leukemia Cell line						Human Ovarian Cancer Cell Line					
	ME180			SiHa			HL60			K562			A2780			Ovkar - 3		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	>80	>80	>80	>80	>80	>80	26.6	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydroalcoholic	>80	>80	>80	>80	>80	>80	>80	>80	31.3	>80	>80	>80	>80	>80	>80	>80	>80	>80
Ethanolic	>80	>80	>80	>80	>80	>80	>80	>80	35.4	>80	>80	72.9	>80	>80	>80	>80	>80	>80
Methanolic	>80	>80	>80	>80	>80	>80	>80	>80	38.0	>80	>80	>80	>80	>80	>80	>80	>80	>80
Petroleum ether	>80	>80	>80	>80	>80	>80	>80	>80	44.6	>80	>80	>80	>80	>80	>80	>80	>80	>80

Table 3: *In vitro* anticancer activity of extracts of seeds of *Abrus precatorius* L. using SRB Assay

Cell Lines / Extracts	Human Breast Cancer Cell Line																	
	MCF – 7			MDA – MB - 468			BT474			MDA – MB - 435			Zr – 75 - 1			MDA – MB - 231		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	50.8	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydroalcoholic	75.1	47.4	19.7	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Ethanolic	>80	72.1	28.3	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	49.0	>80	>80	>80
Methanolic	>80	70.6	34.7	>80	>80	>80	>80	>80	>80	>80	>80	>80	76.2	55.1	34.1	>80	>80	>80
Petroleum ether	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	60.3	34.8	>10	>80	>80	>80

Table 4: *In vitro* anticancer activity of extracts of seeds of *Abrus precatorius* L. using SRB Assay

Cell Lines / Extracts	Human Prostate Cancer Cell Line						Human Colon Cancer Cell Line					
	PC3			DU145			HT29			Colo205		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydroalcoholic	>80	>80	>80	>80	>80	>80	>80	>80	62.4	>80	>80	>80
Ethanolic	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Methanolic	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Petroleum ether	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80

Table 5: *In vitro* anticancer activity of extracts of seeds of *Abrus precatorius* L. using SRB Assay

Cell Lines / Extracts	Human Lung Cancer Cell Line			Human Hepatoma Cell Line			Human Oral Cancer Cell Line		
	A549			HEPG2			AW13516		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydroalcoholic	>80	>80	62.3	>80	>80	>80	>80	>80	>80
Ethanolic	>80	>80	>80	>80	>80	>80	>80	>80	>80
Methanolic	>80	>80	>80	>80	>80	>80	>80	>80	>80
Petroleum ether	>80	>80	>80	>80	>80	>80	>80	>80	>80

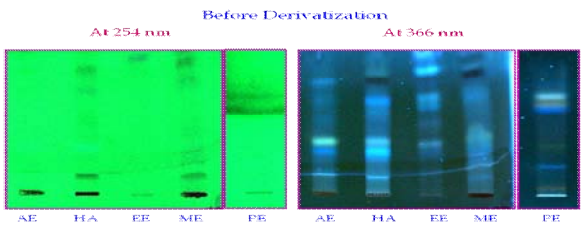

Plate 1: HPTLC fingerprint of extracts of seeds of *Abrus precatorius* L. under UV 254 nm and 366 nm.

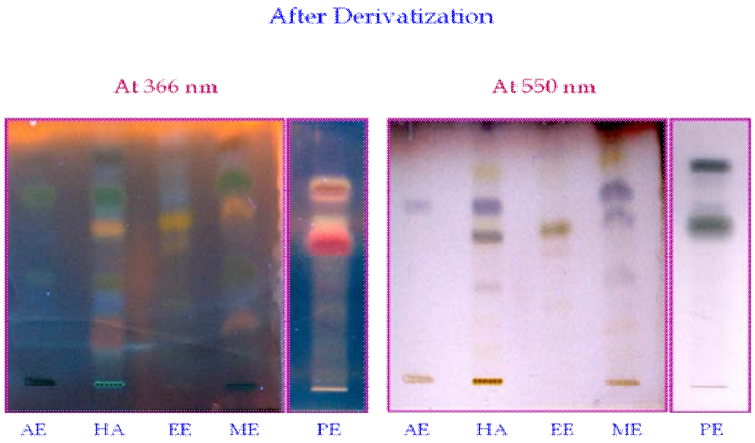

Plate 2: HPTLC fingerprint of extracts of seeds of *Abrus precatorius* L. under UV 366 nm and visible light 550 nm.

After spraying the plates with Anisaldehyde-Sulphuric acid reagent different coloured bands were observed under UV 366 nm and visible light.

DISCUSSION

Interest has revived recently in the investigation of medicinal plants to identify novel active phytochemicals that might lead to drug developments as anticancer drugs derived from research on plant antitumor agents.[63] Chemoprevention is recognized as an important approach to control malignancy and recent studies have focused on the search for desirable chemopreventive agents. Natural products, particularly, dietary substances, have played an important role in creating new chemopreventive agents.[64] Interesting patterns of differential cytotoxicity have been associated with known classes of compounds, such as cardenolides, lignans or quassinoids.[65] In any cancer drug discovery program, a paradigm based on Ethnobotanical and Ethnopharmacological data would be more economical and beneficial in identifying potential anticancer molecules than mass screening of plant species.[66] Natural products have been regarded as important sources of potential chemotherapeutic agents and many anticancer drugs have originated from natural sources.[67]

Plant derived natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and anticancer activity. One of their main properties in this regard is their antioxidant activity, which enables them to attenuate the development of tumour and inflammatory disease.[30]

Flavonoids have attracted a great deal of attention in relation to their potential beneficial effects on health. Flavonoids have been shown to possess antimalignant effects.[68,69] Ethanol extract of *Abrus precatorius* leaves exhibits greater cytotoxic effect against hepG2 (liver cancer cell line) as compared to cytotoxicity of chloroform extract against HeLa (Cervical cancer cell line). According to Manoharan,[70] the cytotoxic activity may be due to the presence of triterpenoids and flavonoids. Also the members of cycloartane, lupine, cursane, oleanane and friedelane (especially quinine methides) dammarane and limonoid Triterpenoids have demonstrated antiproliferative activity on various cancer cells.

Anti-tumor agents, which can modulate apoptosis, may be able to affect the steady state of cell populations that are helpful in the management and therapy of cancer. It has been suggested that Abrin could induce tumor cell death both by physiological or pathological means.[71] The antineoplastic effect of a protein extract isolated from the seeds of *A. precatorius* has direct cytoctoxic effect on the Yoshida sarcoma (Solid and ascites form) in rats and fibrosarcoma in mice.[72-74] Other study reported the high anti-tumor activity of agglutinin protein purified extract from the seeds of *Abrus precatorius* L. About 90% of the tumor growth was inhibited with Abrin A than Abrin B after 1ng administered into mice. Binding inhibition studies with sugars suggested that abrins A and B have different binding sites to inhibit sarcoma in mice.[28] Abrin was effective in reducing solid tumour mass development induced by Dalton's Lymphoma Ascites (DLA).[75] It has been reported that Abrus abrin, isolated from the seeds of *Abrus precatorius*, showed *in vitro* and *in vivo* antitumor properties by the induction of apoptosis.[76] Abrin from *A. precatorius* seeds after being purified on sepharose 4B affinity column brought significant reduction in tumor volume of mice and increased the life span of ascites tumor bearing mice.[75] HPTLC reports suggested that presence of Abrin (Alkaloids) in *Abrus* seed[77-78] may be responsible for this antitumor activity.[49]

Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical. Some herbs protect the body from cancer by enhancing detoxification functions of the body. Certain phytoconstituents derived from herbs are known to inhibit growth of cancer by modulating the activity of specific hormones and enzymes. Some herbs reduce toxic side effects of chemotherapy and radiotherapy.[6]

In the present study, the anticancer activity of hydroalcoholic and petroleum ether extracts of seeds of

Abrus precatorius L. on Human Breast Cancer Cell Line MCF-7 and Zr-75-1 respectively may be related to its flavonoid, terpenoid, alkaloid and protein contents.

The chromatographic screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. TLC enables reliable separation and analysis of compounds from a wide variety of classes in many types of biological samples.[79]

High performance Thin Layer Chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby reducing analytical time. With HPTLC, the same analysis can be viewed collectively in different wavelengths of light thereby providing a complete profile of the plant.[80] Fingerprint analysis approach using chromatography has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drugs. Hence a characteristic HPTLC fingerprint profile developed of 5 extracts submitted for *in vitro* SRB Assay is unique to extracts of seeds of *Abrus precatorius* L.

CONCLUSION

From the present study it was concluded that out of the 5 extracts (*viz.* Aqueous, Hydroalcoholic, Ethanolic, Methanolic and Petroleum ether) and 19 Human Cancer Cell lines namely Cervix (ME180, SiHa), Leukemia (HL60, K562), Ovarian (A2780, Ovkar-3), Breast (MCF-7, MDA-MB-468, MDA-MB-435, MDA-MB-231, ZR-75-1, BT-474), Prostate (PC3, DU145), Colon (HT29, Colo205), Lung (A549), Hepatoma (HEPG2) and Oral (AW13516) used, Hydroalcoholic and Petroleum ether extracts of seeds of *Abrus precatorius* L. exhibited anticancer activity on Human Breast Cancer Cell Lines MCF-7 and Zr-75-1 respectively which may be due to effect of the secondary metabolites present in the extracts.

However, further studies are required to assess the molecular mechanism of anticancer activity of the *Abrus precatorius* L. seeds.

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