International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1926-1938 www.ijprajournal.com ISSN: 2249-7781

Anticancer Activity of Rati Plant: A Review

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Submitted: 15-05-2023 Accepted: 30-05-2023

ABSTRACT
Cell mortality was estimated by utilizing an MTT assay. Abrus precatorius revealed very striking inhibition on MDA-MB-231 cells. MTT assay indicated more than 75 % inhibition of the cells and treated cells showed a visible laddering pattern with thick compact band. The antiproliferative characteristics of seed extracts (ethyl acetate and 70% ethanol) produced from Soxhlet and Maceration extraction technique against Hep2C and HeLa Cells were examined. APE (Maceration) had excellent antiproliferative activity against Hep2C cells, while APA (Soxhlet) had excellent antiproliferative activity against HeLa cells. In this research, the antioxidant and antiproliferative features of the various leaf extracts (hexane, ethyl acetate, ethanol, and water) from Abrus precatorius were examined. We investigated the inhibitory effects of Abrus precatorius extracts on the proliferation of four different human tumor cell lines human colon adenocarcinoma cells (Colo-205), human retinoblastoma cancer cells (Y79), human hepatocellular carcinoma cells (HepG2) and Leukemia cells (SupT1). Ethanol extract (APA) and ethyl acetate extract (APE) of Abrus precatorius had apparent capacities of inhibiting the survival of tested human cancer cell lines. Five different extracts (Aqueous, Hydroalcoholic, Ethanolic, Methanolic & Petroleum ether) of seeds of Abrus precatorius L. were examined for in vitro anticancer activity. Sulforhodamine B (SRB) assay was done for in vitro anticancer activity for five different extracts (Aqueous, Hydroalcoholic, Ethanolic, Methanolic & Petroleum ether). In vitro, anticancer activity was examined against 19 Human Cancer Cell Lines namely Cervix, Leukaemia, Ovarian, Breast Prostate, Colon, Lung, Hepatoma, and Oral. Out of the five extracts and nineteen cell lines utilized for investigating anticancer activity, the hydroalcoholic & petroleum ether extracts of seeds of Abrus precatorius L were active on human breast cancer cell lines MCF-7 & Zr-75-1 correspondingly. The anticancer activity of hydroalcoholic & petroleum ether extracts of seeds of Abrus precatorius L. may be connected to its flavonoid, terpenoid, and alkaloid, and protein compositions. This research investigated the anti-proliferative activities of fractions of methanol root extract of Abrus precatorius on breast and cervical cancer cells. Abrus precatorius fractions revealed various chemical groups and were somewhat selective in antiproliferative activity against investigated cancer cells. Ethyl acetate fraction indicated the most significant antiproliferative activity with IC_{50} values of 18.10 µg/ml and 11.89 µg/ml against AU565 and HeLa cells correspondingly. Hexane fraction significantly ($p < 0.05$) inhibited HeLa cells (IC_{50} 18.24 ± 0.16 µg/ml), whereas aqueous fraction revealed mild inhibition (IC50 46.46 ± 0.14 µg/ml) on AU565 cell proliferation. All fractions revealed no cytotoxicity against NIH-3 T3 murine fibroblast normal cells. The current study was undertaken to examine the anticancer potential of Abrus precatorius. Bioassay-guided fractionation led to 2 active fractions, APH-11 and APM-3 containing IC50 values of 14.64±1.84 and 20.90±3.58 µg/ml, correspondingly against the human acute monocytic leukemia cell line. In vitro, cytotoxicity of APH-11 and APM-3 against the human acute monocytic leukemia cell line was correlated with that against peritoneal macrophages and HEK 293 cells. The cytotoxicity investigation with cancer cell line A-549 revealed that the methanol insoluble fraction of crude red forms of Abrus precatorius is toxic (CTC50 175,100 µg/ml) in microculture tetrazolium assay and sulphorhodamine B assays, whereas crude and fractions of the red and white forms contained toxicity at still higher concentrations. However, an antitumor screening by the short-term toxicity investigation with DLA cells revealed the extracts to be comparatively less toxic. This research plans to investigate the in vitro anticancer activity of aqueous extract of Abrus precatorius leaves on the murine mastocytoma cancer cell line (P815). In vitro, the anticancer effect was investigated by the cellular cytotoxicity against the murine mastocytoma cell line (P815).

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Volume 8, Issue 3 May-June 2023, pp: 1926-1938 www.ijprajournal.com ISSN: 2249-7781

Cellular cytotoxicity was estimated by the MTT assay. The in vitro anticancer effect, revealed a dose-dependent cytotoxic effect. It was examined, that the maximum activity of the extract at the largest concentration tested (200µg/ml) was 50% (±1.5). Further smaller doses of 3.12; 6.25; 12.15; 25 and 50 µg/ml % growth inhibition obtained by the extract was between 43 and 47 %, the IC_{50} value of the extract (200µg/ml). Our results reveal that the aqueous extract of A. precatorius leaves consists of several chemical groups and contains a weak in vitro anticancer effect against the P815 tumor cell line. A. precatorius leaves were extracted sequentially with hexane, ethyl acetate, and methanol by Soxhlet extraction. The aqueous extract was produced by decoction at 50 ˚C. Extracts of A. precatorius leaves were utilized to treat selected cancer and normal cell lines for 72 hours. Furthermore, 3-(4, 5-dimethyl thiazol-2-yl) 2, 5-diphenyl tetrazolium bromide assay was done to determine cell viability. Methanolic extract of Abrus precatorius leaves revealed the lowest IC_{50} on MDA-MB-231 cells at (26.40±5.40) µg/ml. This research work plans to investigate the in vitro cytotoxic effects of ethanol extract (AHE) and ethyl acetate (AEE) against MCF-7 cell lines by utilizing an MTT assay. Tamoxifen was utilized as a standard to compare the cytotoxic activity of the extracts. Both extracts are investigated on MCF-7 cell lines to examine the in vitro cytotoxic activity. The IC_{50} concentration of tamoxifen, ethanol extracts (AHE), and ethyl acetate (AEE) is 37.79 µg/ml, 60.89µg/ml, and 143.8µg/ml correspondingly. The IC_{50} concentration of ethanol and ethyl acetate extract revealed lower activity when compared with standard tamoxifen; this may be because of its crude nature. Based on the previous reports on the cytotoxic properties of Abrus precatorius seeds with that of the current reports it indicates that Abrus precatorius seeds have potential cytotoxic features and can be utilized as an origin of antitumor agents.

Kew words: Rati plant, MTT Assay, SRB Assay, Cytotoxicity, IC_{50} value.

I. INTRODUCTION

Cancer is one of the most dreaded ailments. It is determined that by 2030, 26.4 million people a year may be identified with cancer and 17 million people will die from it (W.H.O 2010). Among cancers, breast cancer is the most generally identified cancer and is a leading cause of death among females worldwide, with 23 % (1.38 million) of the total new cancer cases and 14 % (458,400) of the total cancer fatality recorded in 2008. It is determined that half of the breast cancer cases and 60 % of mortality owing to cancer have been determined to occur in economically growing countries. Breast cancer, like many other cancers, tends to metastasize to other parts of the body, ideally to the lungs and bones. This method of neoplastic transformation, progression, and metastasis involves changes in the normal apoptotic pathway. Abnormal apoptosis is related to a broad variety of human ailments like cancer, auto-immune ailment, and neurodegenerative ailment [1]. Human epidermal growth factor receptor 2 (HER2), Vascular endothelial growth factor (VEGF), estrogens, and progesterone receptors are documented to be related to the progression of cervical cancer [2]. The human body contains various antioxidant defenses and repair mechanisms against oxidative stress. However, these mechanisms are insufficient to stop the damage entirely as the production of reactive oxygen species (ROS) is certain to act multiple crucial roles in tissue damage and loss of function in several tissues and organs. Free radicals and ROS have been elaborated as endogenous initiators in the etiology of cancer and several other degenerative or pathologic processes of numerous serious ailments, as well as in aging processes. Oxidative damage to DNA is declared as a critical step in cancer development. Over the past decade or so, various experimental and epidemiological studies have revealed that a broad variety of phytochemicals such as phenolics, flavonoids, isoflavone, flavones, anthocyanins, catechin, is catechin and carotenoids can stop or slow down oxidative stress-induced damage paramount to carcinogenesis by upsetting the molecular events in the initiation, promotion or progression states. Recent studies exhibited that a more dietary intake of fruits and vegetables could be correlated with lower cancer pervasiveness in humans [3]. Cancer is a chief public health concern in both developed and developing countries. Cancer is a significant worldwide health problem commonly because of the lack of widespread and comprehensive early identification techniques, the connected poor prognosis of patients identified in later stages of the ailment, and its growing incidence on a global scale. Indeed, the struggle to battle cancer is one of the largest challenges of mankind. American Cancer Society and International Union against Cancer estimated that 12 million cases of cancer were determined in 1997, with 7 million deaths worldwide; these numbers are expected to double

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by 2030[4]. Breast cancer is the largest identified cancer in several sub-Saharan African countries, a shift from a trend in which cervical cancer was the paramount cause of cancer-related mortalities among women in Africa over the past decade. Nitric oxide (NO) is a short-lived pleiotropic regulator that acts crucial roles in various physiological as well as pathological procedures. Its role in tumor production is somewhat complex. However, documented roles of NO such as genotoxic mechanisms, antiapoptotic effects, induction, and advancement of angiogenesis, limitation of host immune response against the tumor, and promotion of metastasis has been implicated in a different category of cancer and NO tumor-promoting effect appears to be both time and concentration-dependent. Overproduction of reactive oxygen (ROS) and nitrogen (RNS) species by phagocytes, called neutrophils, may report in chronic inflammation and initiation of the multistage procedure of different cancer development including breast and cervical cancer [5]. Plant-derived compounds contain an essential source of anticancer agents due to their structural diversity, drug ability, and biological compatibility. A plethora of antineoplastic drugs such as taxoids, camptothecin, vinca alkaloids, and podophyllotoxin derivatives have appeared from the anticancer screening of ethnopharmacological essential therapeutic plants [6]. The roots, leaves, and seeds of Abrus precatorius are utilized for therapeutic purposes in Ayurveda, Siddha, and Unani. The seeds are utilized in eye ailments, jaundice, pain, poisoning, fainting, arthritis, and leucoderma [7]. The World Health Organization has classified cancer as a non-communicable ailment, which is responsible for 63% of mortality worldwide. The World Bank income groups investigated that the incidence of 12.7 million new cancer cases in 2008 will increase to 21.4 million by 2030, and low or middle-income countries will be the large affected with nearly two-thirds of all cancer diagnoses. West Africa is contained of mostly poor countries where cancer is an emergent ailment. In 2008, men in the African Region had a higher than double rate of liver cancer while women in this region had the largest incidence of cancer of the cervix uteri worldwide [8]. Apoptosis is a characterized form of cell mortality and is largely studied. Known generally as programmed cell death, apoptosis is the packaging of dying cells into fragments that are easily consumed and removed by phagocytes without disturbing the regular function of surrounding tissues. Equilibrium between cell mortality and cell proliferation is essential to avoid disruption of the cellular balance. Excessive apoptosis or deficient apoptosis is the production of many clinical ailments including cancer. Apoptosis can be initiated through 2 separate pathways, the intrinsic or mitochondrial pathway, and the extrinsic or death receptor pathway. In cancer treatment, apoptosis has become an essential tool as a target by potent apoptosis-inducing agents, including both chemical and biological [9]. The plant is commercially utilized for the management of sore tongue and also has diaphoretic action. Seeds of Abrus precatorius are generally utilized as purgative, emetic, and aphrodisiac and for treating numerous disorders in traditional medicine [10]. In India it is known by several names such as Gunj; Ganchi, Gunchi, Rati; Chunhali, Kunch; Chanothi, Gunja; Ganji, Gul-Ganju, Guluganji, Madhuka; Kunni, Guruginia, Guruvenda; Kunnikuru; Chanoti, Gunchi, Gunja; Gundumani, Kuntumani; Gunja, Runji, Liluwani, Raturmani; Labrigunchi, Ratak [11]. The plant was examined as beneficial for the hair and the seeds extract is utilized in the management of ulcers and skin infections. Seeds of the plant are very much attractive, and utilized in ornaments, but are highly poisonous. Seeds are utilized for antidiabetic activity, antioxidative activity, antibacterial, and anti-inflammatory analgesic activity. Other applications of the plant are examined in cancer and malaria[12]. Abrus precatorius (red black seeded form) is distributed almost throughout the country and occurs in diverse habitats including village thickets, natural forests, and several protected areas of Bangladesh [13]. The most toxic part of the plant is the seed which consists of the toxic poison abrin responsible for the inhibition of cell protein preparation. It is one of the most potent plant poisons with a determined human fatal dose of 0.1-1 µg/kg and has produced death after accidental and intentional poisoning. It is classified as a potential chemical warfare substance [14]. The bioactive constituents are mostly plant secondary metabolites, which become drugs after processing to pure constituents; some are very helpful dietary supplements and many helpful commercial products. Further modification of the active constituents leads to stimulating the biological profiles and a large number of such constituents which are approved or undergoing clinical trials for clinical applications against different ailments like pulmonary illness, cancer, HIV/AIDS, malaria, Alzheimer's, and other ailments[15]. The roots, leaves, and seeds of the plant are utilized therapeutically. Abrus precatorius

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leaves are utilized as aphrodisiac; tonic removes biliousness, useful in eye ailments, and cures leukoderma, itching, skin ailments, and wounds. The leaves are also utilized as diuretics and in diarrhea, gastritis, heart ailments, kidney ailments, insomnia, Cancer, and as CNS sedatives. Powdered leaves mixed with sugar are given in case of leukoderma and menorrhagia. The roots of this plant are consumed for sore throat and rheumatism. The seeds are utilized as purgative, but in large doses are acrid poison, giving rise to symptoms resembling those of cholera, taken internally by women, the seed disturbs the uterine functions and stops conception. Water decoction of the seeds of this plant concentrated into a paste is utilized for contusion and inflammation [16]. People of this subcontinent have been utilizing the Ayurvedic systems of therapeutic for many generations. Medicinal plants are very crucial for the health care of human beings concerning ancient therapeutic systems. Most of the traditional therapeutics are based on herbs, which are utilized by almost 80% of the world's population [17]. Natural antioxidant that exists in food and other biological materials have attracted larger interest due to their presumed safety and potential nutritional and therapeutic effects. In Ayurveda roots and seeds of Abrus precatorius L are utilized for the control of cervical adenitis, dental caries, baldness, and defect of vision and to eliminate dandruff [18]. There was 458,000 mortality per year from breast cancer worldwide being the paramount cause of female death in both developed and developing worlds [19]. The roots and leaves of this plant contain sweet-tasting glycyrrhizin [20].This review article focuses on the anticancer activity of the various parts of the Abrus precatorius plant by utilizing the different solvents

II. MATERIAL AND METHOD

Mohammed Shafi Sofi et al, (2013)[1]

Plant materials: Fresh leaves of the Abrus precatorius were gathered from, cleaned thoroughly 2–3 times with tap water and once with sterile distilled water, shade dried then powdered and utilized for extraction. Preparation of plant extract: Dried plant sample was reduced to a fine powder in a mixer-grinder and sieved. The powder was suspended in double distilled water at the rate of 5 grams in 30 ml, sonicated for 15 minutes, and shaken with a magnetic stirrer overnight. Each extract was transferred through double layered cheese cloth. The filtrate was then centrifuged at 5,000 rotations per minute for 30 minutes. Speedvac was used for the lyophilization of the mother solution. The lyophilized aqueous extract was mixed in PBS (sterile) to a stock concentration of 50 mg/ml and then passed through a 0.2 µm filter for sterilization and was then utilized for cytotoxicity assays. **Cell lines and culturing of cells:** Human breast cancer cell line MDA-MB-231 was obtained from the Indian Institute of Science, Bangalore (India). Cells were kept in Dulbecco's Modified Eagle's medium (DMEM) with 10 % FBS, 100 U/ml penicillin, and 100 mcg/ml streptomycin, in a humidified atmosphere of 95 percent air and 5 percent CO_2 at 37 ˚C. Once the cells had enveloped about 80 percent of the surface, they were trypsinized and the titer was altered utilizing a haematocytometer for antiproliferative and cytotoxicity determination. **Analyses of cell viability:** The effect of the aqueous extract of Abrus precatorius on the viability of cells was determined utilizing the standard colorimetric MTT assay utilizing the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-dimethyl tetrazolium bromide dye. This assay is grounded on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Human breast cancer cells (5×10^3 cells/well) were seeded to 96-well microtiter plates. After 24 hours of plating, cells were serum starved for 24 hours. Corresponding concentrations of Abrus precatorius extracts were mixed in a serum-free medium and the assay was concluded after 48 hours. The medium was removed and 200 µl DMSO was mixed and the amount of formazan produced was determined at 595 nm on a Model 680 Microplate Reader.

Amritpal Kaur et al, (2022)[2]

Material: Abrus precatorius seeds extract was synthesized by Ethyl acetate and 70% ethanol by Maceration and Soxhlet extraction separately. The extract prepared by ethyl acetate is named APE and the extract prepared by ethanol is APA. **Cytotoxic activity of seed extracts against cervical cancer cells:** Abrus precatorius seed extracts were investigated for their antiproliferative capacity utilizing MTT assay. Doxorubicin, tannic acid, ruin, and Abrus precatorius seed extracts all contained varying antiproliferative efficacy, with a dose-dependent reduction in the cell viability of cervical cancer cells (Hep2C and HeLa). IC50 values were also examined, with lower IC_{50} values showing greater antiproliferative activity.

Mir Z Gul et al (2013)[3]

Preparation of extracts: The air-dried leaves of the plant were reduced in size with a mechanical

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grinder to get a coarse powder, which was then utilized for successive extraction in a soxhlet apparatus utilizing hexane, ethyl acetate, ethanol, and water. Each time before extracting with the next solvent, the material was dried in a hot air oven at 40 ˚C. Extracts were then filtered through a Whatman No.1 paper filter and concentrated to the dry mass with the aid of a rotary evaporator. The extraction procedure was repeated 3 times at various periods. It was noticed that there was no significant variation in the % yield and content of phytoconstituents that are believed to act an essential role in biological activities. The yield of each extract was determined and residues were stored in dark glass tubes for further investigation. The various extracts were termed APH (hexane extract), APE (ethyl acetate extract), APA (ethanol extract), and APW (water extract). The dried extracts were mixed in dimethyl sulfoxide (DMSO) at 20 mg/ml and diluted with phosphate-buffered saline (PBS, pH 7.4) to produce final concentrations. **Anti-proliferative activity:** A panel of 4 human cell lines namely human colon adenocarcinoma cells - Colo-205, human retinoblastoma cancer cells - Y79, human hepatocellular carcinoma cells - HepG2, Leukaemia cells - SupT1 were utilized to examine antiproliferative activity. The cell lines HepG2 & Colo-205 were cultured in Dulbecco's modified Eagle's medium (DMEM) and Y79 & SupT1 in RPMI 1640 consisting of 10% (v/v) FBS, 100 units/ml penicillin and 100 µg/ml streptomycin. Cells were kept in a humidified incubator with 5percentage CO_2 for 24 hours at 37 ˚C and seeded onto 75 cm^2 culture flasks. In vitro, the response to extracts and the standard drug was investigated through a growth inhibition utilizing the MTT assay. The cells were seeded at a density of ~5×10^3 per well utilizing brief trypsinization. Furthermore, Doxorubicin and sample extracts (25–200 µg/ml) mixed in dimethylsulfoxide (DMSO; not exceeding the concentration of 2 percent), and further diluted in a cell culture medium were added into a 96-well plate. After 48 hours of incubation, 20 µl of MTT reagent (5 mg/ml) were mixed and mixtures were reincubated for 4 hours. The optical density of formazan was determined at 550 nm. Doxorubicin was also assayed as a (+) ve control at the concentration of 0.5–10 µg/ml. The resulting growth data indicates the net outcome of cell proliferation and cell mortality. The cell viability (%) was obtained by comparing the optical density between the samples and a (-) ve control.

Avinash Patil et al, (2015)[4]

Drying: The seeds gathered were washed under running tap water, blotted dry, and placed for drying in an oven at a temperature of 40 ± 2° C for 5 days. The dried seeds were powdered and kept in an airtight container. **Preparation of Aqueous extract:** Distilled water was mixed with the powder in a ratio of 6:1. It was shaken thoroughly and refluxed for two hrs at 80 ˚C. The above step was repeated three times. The extract was filtered and concentrated utilizing Vacuum Rotary Evaporator. The extract generated was kept in an airtight amber-colored bottle. Preparation of extracts using different solvents: Different Solvents (Hydroalcoholic extract (50:50), Ethanol, Methanol, and Petroleum Ether) were mixed separately to the powder in a ratio of 4:1. The mixture was shaken thoroughly and macerated for four hours. The mixture was refluxed for two hours. The above step was repeated three times. The extract was filtered and concentrated utilizing Vacuum Rotary Evaporator. Extracts produced were stored in airtight amber-colored bottles and placed in the refrigerator. Human Cancer Cell Lines: Different human cancer cell lines utilized for in vitro SRB Assay are Cervix Cancer Cell Lines, Leukaemia Cell Lines, Lung Cancer Cell Lines, Breast Cancer Cell Lines, Prostate Cancer Cell Lines, Hepatoma Cell Lines, Colon Cancer Cell Lines, and Ovarian Cancer Cell Lines & Oral Cancer Cell Line. Sulforhodamine B (SRB) Assay: In vitro SRB Assay of the prepared extracts was done on the various Human Cancer Cell Lines. The antiproliferative SRB assay was done to investigate growth inhibition. This is a colorimetric assay that determines cell number indirectly by staining total cellular protein with the SRB dye. The microtiter plates were taken out after 48 hours of incubation of the cells with test materials and gently layered with chilled 50% Trichloroacetic acid (TCA) in all the wells to obtain 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 removed. The plates were washed five times with distilled water to eliminate TCA, growth medium, low molecular weight metabolites, serum proteins, etc. Plates were air dried; SRB dye was mixed with each well of the plates and incubated at room temperature for 30 minutes. The unbound SRB was discarded quickly by washing the wells 5 times with 1 % Acetic acid and then air dried. 100µl of Tris buffer (0.01 M, pH 10.4) was mixed and shaken softly for 5 min on a

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Volume 8, Issue 3 May-June 2023, pp: 1926-1938 www.ijprajournal.com ISSN: 2249-7781

mechanical shaker. O.D was documented on ELISA reader at 540 nm.

E. E. Okoro et al (2019)[5]

Extract preparation: Young fresh Abrus precatorius roots were cleaned, oven-dried at 40 ˚C for 96 hours to a constant weight, and pulverized. Methanol (70%) (1: 10) crude extraction of pulverized Abrus precatorius roots was done for 48 hours utilizing a shaker water bath at 40 ˚C and filtered through Whatman No.1110 mm. The filtrate was concentrated utilizing a Rotary evaporator to get the crude methanol extract, reconstituted in distilled water (1:5), and subjected to liquid-liquid partitioning utilizing solvents of increasing polarity to get partially purified – n-hexane, ethyl acetate, n-butanol, and aqueous fractions correspondingly. The fractions were further concentrated to a constant weight under decreased pressure utilizing a Rotary evaporator at 40 ˚C and freeze-drying for the aqueous fraction**.** **Cytotoxicity screening:** Cytotoxic activity of extract and fractions of Abrus precatorius roots, was investigated in 96-well flat-bottomed microplates by utilizing the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, cell lines were cultured in Minimum Essential Medium Eagle, supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin, and 100 µg/ml of streptomycin in 75cm^2 flasks, and kept in 5% CO_2 incubator at 37 ˚C. 200 µl of cell suspension were seeded in round bottom 96 well plates at the density of 10,000 cells/well and incubated at 37 ˚C in a 5% CO_2 incubator for 24 hours. Exponentially growing cells were harvested, counted with a hemocytometer, and diluted with the medium. Cell culture with the concentration of 6×10^4 cells/ml was prepared and introduced (100 µl/well) into 96-well plates. After overnight incubation, the medium was discarded and 200 µl of fresh medium was added with various concentrations of 10–100 µg/ml of fractions and 0.23–30 µg/ml for 3 T3 cells. After 48 hours 200 µl MTT (0.5 mg/ml) was mixed in each well and incubated further for 4 hours. Subsequently, 100 µl of DMSO was mixed in each well. The extent of MTT reduction to formazan within cells was determined by measuring the optical density at 570 nm, utilizing a microplate reader. The cytotoxicity was documented as a concentration producing 50% growth inhibition (IC_{50}) for cell lines. Doxorubicin and Cyclohexamide served as standard drugs. The percent inhibition was determined by utilizing the following formula: %Inhibition = 100− [{mean OD of test compound−mean OD of negative control} / {mean OD of positive control−mean OD of negative control}] 100.

M. Z. Gul et al (2018)[6]

Preparation of crude extract: The air-dried leaves of the plant were reduced in size with a mechanical grinder to get a coarse powder, which was then utilized for successive extraction in a soxhlet apparatus utilizing hexane, ethyl acetate, ethanol, and water. Each time before extracting with the next solvent, the material was dried in a hot air oven at 40 ˚C. Extracts were then filtered through a Whatman No.1 paper filter and concentrated to the dry mass with the aid of a rotary evaporator. The extraction procedure was repeated 3 times at various periods. It was noticed that there was no significant variation in the % yield and content of phytoconstituents that are believed to act an essential role in biological activities. The yield of each extract was determined and residues were stored in dark glass tubes for further investigation. The crude extracts were termed APE (ethyl acetate extract), and APA (ethanol extract) and stored at – 20 ˚C until further use. The dried extracts were mixed in dimethyl sulfoxide (DMSO) at 20 mg/ml and diluted with phosphate-buffered saline (PBS, pH 7.4) to produce final concentrations. Cell lines and culture conditions: THP-1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium, whereas HEK-293 cells were grown in Dulbecco's modified Eagle's medium. Mouse peritoneal macrophages were harvested from female BALB/c mice following intraperitoneal injection of 3 ml of 4 % thioglycollate medium. The cells (THP- 1, HEK-293, and macrophages) were suspended in recommended media supplemented with 10 % (v/v) heat-inactivated fetal bovine serum, 100 units/ml of penicillin and 0.1 mg/ml of streptomycin sulfate and incubated in a humidified atmosphere of a 5 % CO_2 at 37 ˚C. Before the experiments, a culture medium was utilized for diluting the test samples to make the final concentration of dimethyl sulphoxide (DMSO) in culture to be ≤0.1 %. **Antiproliferative activity:** Antiproliferative activity of plant extracts and their fractions was determined utilizing 3-(4,5-dimethyl-thiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT assay was undertaken in 3 stages. In the 1st stage, 50-200 µg/ml of crude extracts were tested against THP-1 cells. The crude plant extracts that revealed >50 % inhibition of proliferation of cells were chosen for

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further analysis in stage II. In the 2nd stage, various fractions received from column chromatography of active crude extracts were again examined for their antiproliferative activity. The fractions that displayed >50 % inhibition of growth were chosen for IC_{50} determination at stage III. In the 3rd stage, 5 concentrations (10, 25, 50, 75, and 100 µg/ml) were produced from each fraction and further established against THP-1 cells. To estimate whether the inhibitory effects of crude extracts and fractions were specific for THP-1 cells, the effect of active fractions on the proliferation of peritoneal macrophages and HEK-293 were subsequently examined.

R. Sivakumar et al (2008)[7]

Preparation of extract: Shade-dried, seed powder of red and white form was extracted with 50% aqueous ethanol in cold maceration technique at room temperature, separately. After filtration, the marc was extracted twice in the same state. Ethanol was eliminated under vacuum and the aqueous residue was lyophilized to dryness. Extracts (crude 50% ethanol extract) were fractioned in petroleum ether, chloroform, and methanol. The crude (50% ethanol extract of red form and white form) and methanol soluble and insoluble fractions of crude (red form and white form) were kept in desiccators for pharmacological experiments. **Determination of total cell protein content by sulphorhodamine B (SRB) assay:** Sulphorhodamine B (SRB) is a bright pink amino xanthene dye with 2 sulfonic groups. Under mildly acidic conditions, sulphorhodamine B binds to the protein's basic amino acid residues in TCA (trichloroacetic acid) fixed cells to give a sensitive index and cellular protein content that is linear over a cell density range of at least two orders of magnitude. Color formation in sulphorhodamine B assay is rapid, stable, and visible. The formed color can be measured over a broad range of visible wavelengths in either a spectrophotometer or a 96-well plate reader. When TCA-fixed and sulphorhodamine B stained samples are air-dried, they can be kept indefinitely without deterioration. The monolayer cell culture was trypsinized and the cell count was altered to 1.0 $x10^5$ cells /ml with medium containing 10% newborn calf serum. For each of the 96 wells of a microtiter plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was mixed. After 24 hours, when a partial monolayer was produced, the supernatant was flicked off, washed once with medium and 100 µl of various drug concentrations were mixed into the cells in microtiter plates. The plates were then incubated at 37 ˚C for 72 hours in a 5% CO_2 atmosphere. The microscopical investigation was performed and observations are documented every 24 hours. After 72 hours, 25 µl of 50% trichloroacetic acid was softly mixed in the wells such that it forms a thin layer over the drug dilutions to produce an overall concentration of 10%. The plates were incubated at 4 ˚C for 1 hour. The plates were flicked and cleaned 5 times with tap water to eliminate traces of medium, drug, and serum, and were then air-dried. The air-dried plates were stained with sulphorhodamine B for 30 minutes. The unbound dye was then discarded by rapidly cleaning it 4 times with 1% acetic acid then the plates were then air-dried. 100 µl of 10 mM tris base was then mixed in the wells to solubilize the dye. The plates were shaken vigorously for five minutes. The optical density was determined utilizing a microplate reader at a wavelength of 540 nm. The % growth inhibition was determined. Anti-tumor screening: The tests rely on a breakdown in membrane integrity determined by the uptake of a dye such as (Tryphan blue, erythromycin, and nigrosin) to which the cell is regularly impermeable. DLA cells were cultured in the peritoneal cavity of mice by injecting a suspension of DLA cells (1.0 x10 5 cells/ml) intraperitoneally. The DLA cells were withdrawn from the peritoneal cavity of the mice between 15 - 20 days with the help of a sterile syringe. The cells were washed with HBSS and centrifuged for 10 - 15 min at 10,000 rpm. The procedure was duplicated 3 times. The cells were suspended in a known quantity of HBSS and the cell count was adjusted to 2 x 10^6 cells /ml. The cell suspension was distributed into Eppendorf tubes (0.1 ml containing 2 lakh cells). The cells were exposed to drug dilutions and incubated at 37 ˚C for 3 hours. After a 3-hour dye exclusion test, that is, equal quality of the drug-treated cells were added with trypan blue (0.4%) and left for 1 minute. It was then loaded in a hemocytometer and the viable and non-viable count was documented within 2 minutes. Viable cells do not take up color, whereas dead cells take up color. However, if placed longer, live cells also generate and take up the color. The percentage of growth inhibition was calculated using the following formula: Growth inhibition (%) = 100 – {(Total cells – Dead cells) / (Total cells)} X 100

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M. Lebri et al, (2015)[8]

Preparation of extracts: Decoction is the technique recommended in traditional medicine. In our case, this decoction was realized from already powder-dried leaves in the laboratory environment. 100 g of powder of leaves were introduced into a triple-neck round-bottom of 250 ml; 100 ml of distilled water were mixed. A round-bottom was topped with a cooler connected to a faucet opened by pipe. The round-bottom is put down into a warm balloon kept in a constant temperature of heating during one hour. After cooling, the mixture is filtered with cotton wool three times and the obtained filtrate was moved in the stove at 55 ˚C during 24 hour. The extract was dried and the aqueous extract (ETA) was generated. Extraction was repeated many times to generate a sufficient quantity. Different extracts of the leaf were produced for a comparative phytochemical analysis. These different extracts were obtained utilizing different solvents polarities (ethanol, acetate diethyl and hexane). 25 g of powder of leaves was subject to maceration under magnetic agitation for 48 hours in 1250 ml of ethanol. Ethanolic mixture was filtered once on cotton wool and then filtered on filter paper (whatman). The filtrate was concentrated utilizing a rotary evaporator at 65 ˚C. The concentrate was totally dried in the steam room at 55 ˚C during 24 hours. Then the ethanol extract was obtained (EEAP). To get acetic extract (EAAP), 25 g of powder of leaves was subject to maceration under magnetic agitation for 48 hours in 1250 ml of acetate diethyl. Acetatic mixture was filtered twice with cotton wool. The filtrate is left evaporate in the room temperature during 24 hours and dried totally in the steam room at 55 ˚C during 24 hours. 25 g of powder of leaves was macerated by magnetic agitation for 48 hours in 1250 ml of hexanic mixture to receive hexanic extract. After that the extract was filtered twice with cotton wool. The filtrate is left evaporate in the laboratory temperature during 24 hours and dried totally in the stove at 55 ˚C during 24 hours. Tumor cell line and culture: The mastocytoma tumor cell line, were grown in RPMI1640 (Sigama-Aldrich) supplemented with 10% heat inactivated Fetal Bovine Serum (FBS), 1% penicillin-streptomycin, and 0.2% sodium bicarbonate, under a fully humidified atmosphere of 95% air and 5% CO_2 at 37 ˚C. **Cytotoxicity assay:** Cellular cytotoxicity was examined by the MTT reduction assay. This Colorimetric assay is based on the power of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Growing concentrations of the tested extract (solubilised in Dimethyl sulfoxide (DMSO): 3, 12; 6, 25; 12, 5; 25; 50; 100 and 200 µg/ml) were applied to the wells of a 96-well plate containing the confluent cell monolayer (106 cells per well) in duplicate. Methotrexate as positive control drug was added in the same concentrations and conditions. After 48 h of incubation, 20 µl of the MTT solution [5 mg/ml in Phosphate buffered saline (PBS)] was added. After incubation in the same conditions for 4 h, the plates were treated with a mixture of HCl / Isopropanol (24:1) to dissolve the blue intracellular formazan product. One hour later, the plates were read on a MicroELISA reader utilizing two wavelengths (540 and 630 nm). DMSO was utilized as negative control. The median inhibitory concentration (IC50) was estimated as the concentration of the sample that leads to 50% of cell lysis comparatively to the negative (positive) control. The relative inhibition of cell proliferation was calculated by the formula: % inhibition =100 x (1- A / A0), where A0 and A, are the optical densities of negative control and ETA extract or methotrexate treated cells, respectively.

Wan Suriyani Wan-Ibrahim et al, (2019)[9]

Extract preparation: About 22 g of ground Abrus precatorius were subjected to consecutive Soxhlet extraction with hexane, ethyl acetate and methanol. Aqueous extract was produced by decoction of the ground leaves at 50 C˚ in 500 ml of distilled water until the water was reduced to a 1/3 of the initial volume. All extracts were kept at -20 ˚C until utilize. Cells were seeded in 25 cm^2 tissue culture and grown at 37 ˚C under humidified 5% CO_2 in Dulbecco's Modified Eagle's medium supplemented with 5% fetal bovine serum and 1% penicillin-streptomycin. Confluent cells were harvested by trypsinization (0.25%). **Anti-proliferative activity by 3-(4,5-dimethyl thiazol 2-yl) 2,5-diphenyl tetrazolium bromide (MTT) assay:** To determine the anti-proliferative activity of Abrus precatorius, human breast cancer cell lines, MDA-MB-231 and MCF-7; human liver cancer cell lines, HepG2; human colon cancer cell lines, SW480; human cervical cancer cell line, SiHa and HeLa; human normal breast cell, MCF-10a and mouse normal fibroblast cell, NIH were added with all extracts. The anti-proliferative activities were examined by MTT assay. IC_{50}

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values were calculated, with lower IC_{50} values revealing greater anti-proliferative activity. Cytotoxicity of plant extracts against cancer cell line was classified based on US National Cancer Institute and Geran Protocol as follows: highly cytotoxic ($IC_{50} \leq 20$ µg/ml), moderately cytotoxic (21 µg/ml$\leq IC_{50} \leq 200$ µg/ml), weakly cytotoxic (201 µg/ml $\leq IC_{50} \leq 500$ µg/ml), and, no cytotoxicity ($IC_{50} \geq 501$ µg/ml). Cells were seeded into 60 wells at the centre of a 96-wells plate with the concentration of 5 X 10^4 cells/ml per well. Extracts of Abrus precatorius leaves were mixed following a serial dilution starting from 99 µg/ml until 0.39 µg/ml in each well. Anti-proliferative activity of Abrus precatorius extracts was calculated by the MTT assay, which was performed after a 72-hour incubation post treatment with the extracts, tamoxifen (positive control) and dimethyl sulfoxide (negative control). Optical density was measured at wavelength of 570 nm. The optical density value at this wavelength directly represents the relative cell numbers in comparison with the control group. The percentage of cell viability was estimated according to the following equation: Percentage of cell viability (%) = OD of treated cells (extracts or tamoxifen)/OD of treated cells (dimethyl sulfoxide) X 100. Anti-proliferative activity of Abrus precatorius leaves extract was screened on selected cancer and normal cells. Extract that exhibited the lowest IC_{50} value with its respective cell was utilized for subsequent investigation in this research work.

Pusapati Madan Ranjit et al (2013)[10]

Preparation of extracts: Abrus precatorius seeds were conquered to soxhlet extraction with 70% ethanol and ethyl acetate for 48 hours, the extracts were gathered and evaporated to dryness and stored at 4 ˚C until use. The % yield of ethanol extracts was 12.5% and ethyl acetate was 5.5%. Cell culture: Carcinoma of breast cancer [Michigan Cancer Foundation (MCF-7)], cell lines utilized in this research were obtained from National Centre for Cell Science, Pune. This cell line was kept in Dulbecco's modified essential medium (DMEM) supplemented with in minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/l glucose, 2 mM L-glutamine, antibiotics (50U/ml of Benzyl pencillin, 50µg/ml of streptomycin and 50µg/ml of amphotericin-B) and 5% fetal bovine serum (FBS) (growth medium) at 37 ˚C in 5% CO_2 incubator. **In Vitro Cytotoxic Activity by MTT assay:** The trypsinized cells from T-25 flask were seeded in each well of 96-well flat bottomed tissue culture plate not the similar concentration but minimum of 5000 cells per well were seeded in growth medium and cultured at 37 ˚C in 5% CO_2 to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were consequently added with various concentrations of extract (12.5, 25, 50, 100, and 200 µg/ml) in triplicates to get a final volume of 100 µl and then cultured for 48 hours. The compound was produced as 1.0 mg/ml concentration stock solutions in PBS. Each well then received 5 µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2 hours at 37 ˚C. The supernatant growth medium was discarded from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. Tamoxifen is taken as positive control in order to correlate IC_{50} of extract against the standard drug utilized. Culture medium and solvent utilized as negative controls. After 30 minutes incubation, the OD of the culture plate was read at a wavelength of 570 nm on an ELISA reader. The percent cell viability was measured with respect to control, is estimated utilizing the formula. % Viability = corrected OD of sample /Control OD * 100 and % of inhibition was calculated by utilizing formula, % Inhibition = 100 - %viability.

III. RESULT & DISCUSSION

Aqueous extract from leaves of Abrus precatorius contains strong dose and time-dependent anticancer activity against human breast cancer cells, with the greater inhibition of cell growth (>75 %) obtained at 600 µg/ml after 48 hour of incubation, with respect to the control. Microscopic studies reveal significant morphological changes like shrinking of cytoplasm, condensation of nucleus and causing of membrane bound vesicles (apoptotic bodies) in treated MDA-MB-231 cells (1×10^6 cells/ml) with 600 µg/ml of the aqueous extract of Abrus precarious for 48 hour as compared to control cells.

In Hep2C cells, the order of cytotoxicity was APE (Mac) > APE (Sox) > APA (Sox) > APA (Mac). APE (Mac) with an IC_{50} value of 85.91±6.7 µg/ml, revealed particularly high antiproliferative activity among other extracts. Additionally, Doxorubicin, rutin and tannic acid demonstrated IC_{50} values of 5.60±0.29 µg/ml, 35.18±1.46 µg/ml an 26.40±1.85 µg/ml, correspondingly. Similarly, in HeLa cells, the order of cytotoxicity was determined to be APA (Sox) > APA (Mac) > APE (Mac) > APE (Sox). APA (Sox) with an IC_{50} value of 26.26±1.09 µg/ml, revealed particularly more

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antiproliferative activity among other extracts. Further, Doxorubicin, rutin and tannic acid demonstrated IC_{50} values of 1.10±0.07 µg/ml, 36.81±0.17 µg/ml and 12.02±1.82 µg/ml, respectively. Morphological changes in Hep2C and HeLa were observed after 48 hour of treatment with the IC_{50} values of Abrus precatorius seed extracts. In comparison to untreated cells, most of the Hep2C and HeLa cells altered from spindle to star-shaped, with some becoming damaged and shrunk. Meanwhile, their growth was decreased in a concentration-dependent manner.

Deregulation of cell proliferation, together with suppressed apoptosis, is a minimal, general platform for all cancer evolution and progression. Uncontrolled cell division is the main key in the progression of cancer tumours. In order to evaluate Abrus precatorius as a potential treatment for cancer, various extracts were assayed against a panel of 4 human cancer cell lines: Colo-205, Y79, HepG2 and SupT1. The antiproliferative effects were determined in terms of cytotoxicity (percentage inhibition) and IC_{50} values were also calculated with lower IC_{50} values revealing a greater antiproliferative activity. Out of 4 extracts tested, only APA and APE demonstrated significantly effective antiproliferative activities in a concentration dependent manner, whereas APH and APW extracts did not decrease the proliferation of tumour cells, thus revealing their non - cytotoxic properties. In fact, APA was by far the greatest inhibitor of tumour cell proliferation with above 85% growth inhibition of all tested cell lines, while as APE was slightly slower inhibitor of growth of cell lines than APA. APA exerted the greatest cytotoxicity at a concentration of 200 µg/ml against Colo-205 (92.25 ± 2.05%) and Y79 (92.80 ± 6.34%) cells with an IC_{50} value of 18.91 ± 1.06 and 26.74 ± 1.34 µg/ml correspondingly. Whereas APE decreases growth up to 68.33 ± 1.41% and 66.40 ± 5.44% against Colo-205 and Y79 cells correspondingly at the similar concentration of 200 µg/ml with higher IC_{50} values of 29.57 ± 2.02 and 35.94 ± 2.10 µg/ml correspondingly. In addition, APA also revealed significant inhibition activity on other two human cancer cell lines HepG2 (88.52 ± 3.04%) and SupT1 (94.12 ± 3.34%) at 200 µg/ml with the IC_{50} value 27.03 ± 1.03 and 26.89 ± 3.24 µg/ml respectively. The APE revealed moderate ability to inhibit cancer cell growth in a concentration-dependent manner with IC_{50} value of 44.31 ± 3.07 µg/ml for HepG2 and 37.00 ± 2.38 µg/ml for SupT1.

The results revealing anti-cancer activity of five extracts of A. precatorius L. on 19 various Human Cancer Cell Lines. Out of the five extracts and 19 cell lines utilized for investigating anticancer activity, the Hydro alcoholic and Petroleum ether extracts of seeds of A. precatorius L. were active against Human Breast Cancer Cell Lines MCF-7 and Zr-75-1 correspondingly.

The results of the cytotoxicity of Abrus precatorius fractions against breast adenocarcinoma (AU565) cell line revealed that ethyl acetate and aqueous fraction significantly ($p < 0.05$) decreased breast adenocarcinoma proliferation (IC_{50} 18.10 ± 2.68 µg/ml, IC_{50} 46.46 ± 0.14 µg/ ml correspondingly). Crude extract, hexane, and butanol fractions were determined to be inactive against breast adenocarcinoma. The ethyl acetate and hexane fractions also revealed significant ($p < 0.05$) inhibition of cervical cancer (HeLa) cell proliferation (IC_{50} 11.89 ± 0.63, 18.24 ± 0.16 µg/ml correspondingly). Crude extract, butanol, and aqueous fractions were determined to be inactive against cervical cancer cells.

The preliminary research undertaken to analyse the antiproliferative nature of extracts of Abrus precatorius revealed that the extracts are cytotoxic to THP-1 cells in a concentration-dependent manner. APE and APA contained a significant antiproliferative activity in a concentration-dependent manner against THP-1 cells. APA revealed the maximum inhibition of cell growth (78.11 ± 0.66 %) with 200 µg/ml of extracts ($p<0.05$) with an IC_{50} value of 298.68 ± 11.17 µg/ml. The APE also contained significant cytotoxic activity and decreased the cell growth (58.30 ± 6.16 %) at 200 µg/ml concentration ($p<0.05$) with IC_{50} values of 116.84 ± 7.09 µg/ml. Furthermore, sub-fractions of these extracts revealed significant cytotoxicity to THP-1 cells, with different IC_{50} values, when correlated to parent extracts. The fraction; APH- 11, got from bioassay-guided fractionation of APE produced superior cytotoxic effects correlated to original extract (APE), with an IC_{50} value of 14.64 ± 1.84 µg/ml and 87.32 ± 0.17 % suppression at the greatest concentration of 100 µg/ml. Similarly, the APM-3 produced from APA presented better cytotoxic effects correlated to parent extract (APA) with the IC_{50} value of 20.90 ± 3.58 µg/ml with % inhibition of 85.84 ± 7.52.

The cytotoxicity study revealed that the methanol insoluble fraction of crude red forms is toxic (CTC50 175, 100 µg/ml) to the cell in both assay (cancer cell line A549 - small cell lung

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carcinoma; whereas all the other extracts revealed toxicity at a higher concentration only, The cytotoxicity study was done by MTT and SRB assays with the crude and its fractions within the cancer cell line A-549 (small cell lung carcinoma). This reveals that the methanol insoluble fraction of crude red forms is toxic to the cell in both assays, whereas all the other extracts revealed toxicity at a greater concentration only. In antitumor screening by the short term toxicity studies utilized by Dalton's lymphoma ascities (DLA) cells all the extracts showed less toxic (CTC_{50} 740, >1000, 860, 940, 490, 510 µg/ml).

The In vitro anticancer activity of the ETA extract was examined at 3.12; 6.25; 12.15; 25; 50; 100 and 200 µg/ml against P815 tumour cell. It was also noticed, that the greatest activity of the extract at the greatest concentration examined (200µg/ml) was 50% (±1.5) of lysis. Further, on smaller doses of 3.12; 6.25; 12.15; 25 and 50 µg/ml percent growth inhibition noticed by the extract was between 43.35 and 47.50 %. The IC_{50} value of the extract (200µg/ml) is greater compared with the Methotrexate utilized as positive control which exhibits IC_{50} value of 2.5µg/ml. These values of lysis % describe that aqueous extract of Abrus precatorius leaves have a smaller effect on P815 cancer cell line.

Methanol extract had the smallest IC_{50} value on MDA-MB-231 cells at (26.40 + 5.40) µg/ml, which could be categorized to moderate toxicity. IC50 values of all extracts were examined by plotting the graph of concentration of the extract or tamoxifen versus % of cell viability, which represents the data for MDA-MB-231 cells.

Both the ethyl acetate and ethanol extracts were examined on MCF-7 cell lines to observe in vitro cytotoxic activity. Ethanol extract (AHE) revealed good activity on MCF-7 cell lines, when correlated with Ethyl acetate extract

IV. CONCLUSION

The results of the current research revealed that the extract of Abrus precatorius had a distinct inhibitory action on the proliferation of breast cancer cell line (MDA-MB-231). A. precatorius extracts markedly decreased cell viability in a concentration dependent manner.

Te present study concludes that both the extraction techniques (Maceration & Soxhlet) were effective in obtaining a largest amount of biologically active constituents. We are among few studies that have looked at the anticancer activity of Abrus precatorius seed extracts on Hep2C and HeLa cells.

It has been described that the Abrus precatorius leaf extracts (APA and APE) are potential antiproliferative agents.

From the present study it was concluded that out of the five 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, MDAMB-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. contained anticancer activity on Human Breast Cancer Cell Lines MCF-7 and Zr-75-1 correspondingly. This may be due to effect of the secondary metabolites present in the extracts. This work provides experimental proof that methanol root extract of Abrus precatorius contains bioactive compounds that exhibit anticancer activity. The present study demonstrated for the first time the antiproliferative effect of fractions of Abrus precatorius leaves that induced programmed cell death via induction of DNA fragmentation, alteration of cell cycle and activating a caspase cascade. In addition, we could hypothesize that these fractions might be preferably involved in the intrinsic apoptotic pathway. The cytotoxicity study was done by MTT and SRB assays with the crude and its fractions within the cancer cell line A-549 (small cell lung carcinoma). This reveals that the methanol insoluble fraction of crude red forms is toxic to the cell in both assays, whereas all the other extracts revealed toxicity at a larger concentration only. In antitumor screening by short term toxicity studies utilized by Dalton's lymphoma ascities (DLA) cells all the extracts revealed less toxic. This current research confirmed that aqueous extract of Abrus precatorius leaves could be potentially helpful for the development of medicinal agents against cancer. Anti-proliferative study gives an insight on the possibility of an occurrence of cell death of selected cancer cells stimulated by the extract. Our study described that the methanol leaves extract had the best anti-proliferative activity at 26.4 µg/ml against the human breast cancer cells, MDA-MB-231, an androgen-independent human breast cancer cells that express wild-type . However, these values are not comparable to the control, tamoxifen, which described the IC50 values of 2.27 µg/ml in MDA-

MB-231. Abrus precatorius seeds have significant cytotoxic features and can be utilized as a source of antitumor agent.

V. ACKNOWLEDGMENT

The authors are very much thankful for the management, **Director Bro.Baiju Valuparampil**, **General Manager – Mr. Jacob Kora** and **The principal Dr.Shaji Selvin.M.pharm, PhD.** of **St.John's College of Pharmaceutical Sciences and Research, Kattappana, Idukki district, Kerala, India**, for providing us with the facilities to carry out the research work.

Disclosure of conflict of Interest

We state that we have no conflicts of interest. We alone are responsible for the content and writing of this article.

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