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In-Vitro Anti Tumour and Brine Shrimp Lethality Assay of Abrus Precatorius Seeds

S.Dharmaraj Santhosam[1]*, P.Selvam[2] and Abhinandan Danodia[1]

[1]Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan-333001
[2]Yazhini Biotech Research Laboratory, Rajapalayam-626117

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Abstract

Abrus precatorius seeds are generally called Crab's eye seeds. The research work aims to determine the In-vitro Anticancer and the Brine shrimp lethality assay for the methanolic crude extract CMME and its isolated compounds CMME I, CMME II, and CMME III. The methanolic crude extract was prepared by the maceration method for 7 days. The compounds from the crude extract were isolated by column chromatography using silica gel 60-120 mesh as the adsorbent. The isolated compounds are further purified by Thin Layer Chromatography. The In-vitro anticancer activity of CMME and its isolated compounds are determined against the skin cancer B16F10 cell line by the MTT assay method using 5fluoro uracil as the standard drug. The isolated compound CMME I shows more % inhibition against the skin cancer B16F10 cell line compared to CMME, CMME II, CMME III, and the standard. It has an IC_{50} value of 40µg/ml. The In- Vitro Antitumor activity was determined for the crude extract CMME and its isolated compounds against the Lung cancer cell line A549 by MTT assay method using 5fluoro uracil as the standard. The crude extract CMME and the isolated compound CMME III showed more % of inhibition against the Lung cancer A549 cell line compared to CMME I, CMME II, and the standard 5fluoro uracil. The crude extract CMME and the isolated compound CMME III have the IC_{50} value of 17µg/ml and 23µg/ml respectively. A brine shrimp lethality assay was done for the crude extracts and their isolated compounds. The crude extract CMME has more cytotoxicity against the Brine shrimp compared to CMME I, CMME II, and CMME III. The crude extract CMME has an IC_{50} value of 106µg/ml.

Keywords: Crab's eye seeds, Anti-tumour, MTT assay Brine shrimp.

1. Introduction

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria, and plant virus infections and also consumption by insects and other animals. The name comes from Greek (phyton-plant). Some phytochemicals have been used as a poison and others as traditional medicine.

***Corresponding Author:**
S. Dharmaraj Santhosam
Institute of Pharmacy,
SJJT, University,
Jhunjunu, Rajasthan, India

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Phytochemicals are found in all plant foods, including fruits, vegetables, legumes, nuts, grains, tea, wine, spices, and more. Phytochemicals are protective for plants against fungi, bacteria, viral infection, ultraviolet light, predators, insects, and disease. Many phytochemicals have antioxidant, anticarcinogenic, neuroprotective, or anti-inflammatory properties. *Abrus precatorius* seeds are generally called Crab's eye seeds, jequirity seeds, rosary pea, and guja seeds. The plant is native to Indonesia and breeds in tropical and subtropical areas of the world where it has been launched. It has the propensity to become weedy and aggressive where it has been launched. *Abrus precatorius* is a high-climbing, twining, or trailing woody vine. It is introduced as an agricultural weed, environmental weed, weed, and naturalized in the Global Compendium of Weeds. It has been announced as a category I noxious weed in the US state of Florida. The species is examined as native to the old world tropics and launched to the Neotropics and has been appreciated in different cultures, mainly its seeds and roots, for a broad range of benefits including medicine, food, beverage sweetener, licorice substitute, ornamental plant, jewellery, and beads, weighing unit, and for traditional cultural and spiritual reasons. The species is also well familiar with the high poison of its seeds, as a single seed is powerful sufficient to kill a human. *Abrus precatorius* proliferates by these bright red seeds, which are grown by both biotic and abiotic factors. It is an Herbaceous, Perennial, Seed Propagated, Vine/Climb type of plant. It obtained a high score of 16 in the PIER Risk Assessment. A score of 6 and above confirms rejecting the plant for import to Australia. In Florida Abrus precatorius is recorded as a noxious Category I aggressive plant species, defined as an aggressive exotic that is creating major ecological harm in Florida. The species has a wide dissemination kind, governs native flora, and makes seeds creatively with 3-8 seeds per pod, which can continue viable for more than a year and are distributed by human and bird actions. It is known to be aggressive in Cuba as well as Marianas Islands, Ecuador, Micronesia, French Polynesia, Hawaii, New Caledonia, Niue, and the Pitcairn Islands and is accepted in many parts of the tropics including Hawaii, parts of the Marquesmas, and Singapore. Chromosome calculation for Abrus precatorius is 2n=22. Abrus precatorius develops in the subtropical moist forest (1000 to 2000 mm of precipitation), and subtropical dry forest (below 1000 mm of precipitation), and prefers well-drained soil but can accept most types. In India, all kinds of forests (below 1000 mm of precipitation), and favor well-drained soil but can accept maximum kinds. In India, all kinds of topography are populated from near sea level to 1000 m in the promotion. The species challenges well with weeds and brush in unused farmland, distributed areas, and early secondary forests. It needs disturbance to support itself in dense, closed stands. The plant is also utilized in some traditional medicine to cure scratches and sores, and wounds caused by dogs, cats, and mice, and are also utilized with other ingredients to cure leucoderma. The leaves are utilized for their antisuppurative possessions. They are ground with lime and enforced on acne sores, boils, and abscesses. The plant is also traditionally utilized to cure tetanus and to stop rabies. Various African tribes utilize powdered seeds as oral contraceptives. Boiled seeds of Abrus precatorius are consumed in certain places in India. The seeds were also utilized to cure diabetes and chronic nephritis. Abrus precatorius is flexible to most soils but favors a tropical climate with fairly high rainfall and a sunny location. Proliferation is by scarified seed sown in spring. Abrus precatorius seeds grow more conveniently if scarified. The seeds are entombed with very hot water and immersed overnight or as far as they swell. Pick out those that didn't swell and return the procedure with them. The swollen seeds are instantly sown in a seeding mix, casing with two to three times their thickness. Overwater or extreme dryness should be examined and supply fine drainage and bright light to the plant. The plant should grow within a few weeks with pre-treatment or unevenly without. The poison present in Abrus precatorius is close analogous to ricin called abrin. It is a dimer containing two protein subunits, termed abrin A and abrin B [1]. In modern times, HMs produced and used in dissimilar forms, which also influence their activity result. The dosage form of herbal medicines differs commonly depending on such parts as the type of illness to be managed, pathway of application, patient, culture, and even philosophical backgrounds. In homes and traditional medicine clinics, HMs are prepared often from fresh or dried herbs which are commonly made into infusions, decoctions, poultices, and powders to be poured into

open wounds or incorporated into native beverages, puddings, and so on. The presentation of HMs in pharmaceutical dosage forms is expected to enhance accurate dosing, aesthetics as well as compliance by enticing usage. Safety and efficacy are other important factors overriding the use and commercialization of HMs. The quality of herbal products is essentially dependent on the safety and efficacy of the herbal material concerning the intrinsic chemical components, type of contaminants as well as production processing. The chemical compounds that are contained in herbal materials have shown a wide range of benefits in the management of various diseases including challenging diseases/conditions such as HIV/AIDS, cancer, sickle cell disease, malaria, and other infectious diseases as well as non-infectious diseases such as diabetes, obesity, infertility, and so on. Despite the wide acceptance, benefits, and sometimes misconceptions: there is a compelling need for decisive control of HMs to ensure that enough and correct information on herbal materials and herbal products is always available to especially healthcare providers and the general public, particularly on subjects such as identification, quality, safety and efficacy of the HM. Herbal medicines (HM) include herbs, herbal materials, herbal preparations, and finished herbal products that contain as active constituents parts of plants, or other plant materials, or combinations and are applied chiefly for the prevention and treatment of ailments [2]. HM has been used as protection for the passive maintenance of health as well as for radical treatment of diversification of light to major sickness [3, 4, 5, 6]. Abrus precatorius is generally called a saga-saga. [7]. Abrus precatorius seeds consist of significant HIV-1 PR inhibitory action. [8]. Native traditional herbal specialists utilize aqueous infusion or extracts (cold or hot) of leaf, seed, and root of Abrus precatorius for the therapy of intestinal diseases that could be of bacterial, viral, or protozoan origins.[9]. Abrus precatorius was first reported as a medicinal plant by William Boericke in the Homoeopathic Materia Medica entitled Jequirity. Abrus precatorius seeds extract can be accepted orally to treat malaria. [10]. Abrus precatorius extracts have been accepting awareness as antitumor agents as it has been revealed that numerous phytochemicals from Abrus precatorius have the characteristic to induce cell death in numerous types of cancers [11]. Abrus precatorius plants and seeds are present in Fig 1.

2. Material & Method

2.1. Collection and Identification of Sample

The Abrus precatorius seeds are purchased from the Meenakshi sundharanar shop present in the Rajapalayam and identified and authenticated by the botany department of Ayya Nadar Janaki Ammal College, Sivakasi. The seeds are washed with distilled water and dried in the absence of sunlight. The dried seeds are converted into coarse powder by using a mechanical grinder and then stored in an air-tight container.

2.2. Method of Preparation of Sample

60 grams of the powdered Abrus precatorius seeds were taken in a 1000 ml volumetric flask previously washed with methanol. 600 ml of the methanol was added and then shaken well by closing the lid and then allowed to cold maceration for 7 days. During the process of maceration, the volumetric flask was shaken several times to get a better extraction. After 7 days the extracted solvent is filtered through man filter paper no 1 and evaporated at room temperature. Finally, the crude extracts are dried under a vacuum. The crude extract was named CMME. The three isolates were isolated from the crude extract CMME of *Abrus precatorius* seeds by column chromatography using silica gel 60-120 mesh as the adsorbent and methanol as the mobile phase. The isolated compounds are further purified by TLC. The compounds that have the same RF value are combined. The crude extract was named CMME. The isolated compounds were named CMME I, CMME II, and CMME III.

2.3. In-Vitro Anti tumour activity against skin cancer B16F10 and Lung cancer A549 Cell Line by MTT Assay method.

Cancer Cells were incubated at a concentration of 1×10^4 cells/ml in culture medium DMEM with high glucose, FBS, and Antibiotic-Antimycotic 100X solution for 24 h at 37ºC and 5% CO2. Cancer cells

were seeded at a concentration (70µl) 1 x10^4 cells/well in 100 µl culture medium with 100µl of samples CMME, CMME I, CMME II, CMME III, and standard 5-fluoro uracil at different concentrations into micro plates (tissue culture grade, and 96 wells). Control wells were incubated with 100 µl DMSO (0.2% in PBS) and cell line. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37ºC and 5% CO2 in a CO2 incubator. The medium was completely removed after incubation and 20 µl of MTT reagent was added (5mg/ml PBS). After the addition of MTT, cells were incubated for 4 hrs at 37ºC in a CO2incubator. Observed the wells for formazan crystal formation under a microscope. The viable cells will reduce yellowish MTT to dark-colored formazan. 200µl of DMSO was added after removing the medium completely (kept for 10 min) and incubated at 37ºC (wrapped with aluminum foil). Samples were analyzed by measuring the absorbance of each sample with a micro plate reader at a wavelength of 550 nm. The % inhibition of the Skin cancer cell line B16F10 and Lung cancer A549 cell line was presented in Tables 1 & 2 respectively. The IC_{50} value comparison in the Sin cancer B16F10 cancer cell line was present in fig 2. The IC50 value comparison in the Lung cancer A549 cell line was present in fig 3.

2.4. Brine Shrimp Lethality Assay

Sample Preparation

10mg of the dried crude extract CMME and the dried isolated compounds CMME I, CMME II, and CMME III were dissolved in 20µl of DMSO and made up to volume 10ml with distilled water in different 10ml volumetric flask to get 1000µg/ml stock solution. From this stock solution 250µl, 500µl, 2500µl, and 5000µl were taken and volume was made up to 5ml with distilled water to get final drug concentrations of 50µg/ml, 100µg/ml, 500µg/ml, and 1000µg/ml respectively. Control vials were prepared by adding an equal volume of distilled water.

Preparation of Seawater

The crude sea salt of 25 grams/litter was dissolved in distilled water. The dried Brewer's yeast 6 mg/litter was added for food of Brine shrimp. It was filtered through filter paper before use.

Hatching of Brine Shrimp Eggs:

2 litter of seawater was added to the special chamber and then 40mg of eggs were washed with water and then these eggs were sprinkled into a compartment that was darkened. After 48 hours the phototropic nauplii were collected by capillary from the lighter side and used for bioassay.

Bioassay Procedure [12, 13, 14]

Nauplii were drawn in a glass capillary along with water, and ten of such shrimps were transferred to each sample vial containing 4.5 ml brine solution (specific volume brine and yeast suspension) after they are counted in the stem of capillary against the lighted background. In every experiment, 0.5 ml of the solution of samples CMME, CMME I, CMME II, and CMME III were added to 4.5 ml of artificial seawater and 0.5 ml of artificial seawater with 0.2% DMSO water are added. The survivors were counted after 24 hours by using 3x magnifying glasses or against a light background, and the percent death was calculated by using a formula. The % Mortality for the samples CMME, CMME I, CMME II, and CMME III was presented in Table 3. An IC_{50} value comparison in Brine Shrimp Lethality Assay was present in fig 4.

% Mortality = (Total nauplii – Alive nauplii) / Total nauplii X 100

Fig: 1 *Abrus precatorius* plant and seeds

Table: 1 Anti Tumour activity against skin cancer B16F10 cell line

Sample Name	Conc (µg/ml)	O.D	% Inhibition	IC_{50} (µg/ml)
Control	-	1.723	-	-
5 FLUORO URACIL	10	0.427	75.21	47
	40	0.345	79.97	
	100	0.156	90.94	
CMME	10	1.443	16.25	32
	40	1.311	23.91	
	100	1.276	25.94	
CMME I	10	0.351	79.63	40
	40	0.231	86.60	
	100	0.109	93.67	
CMME II	10	1.431	16.95	41
	40	1.332	22.70	
	100	1.214	29.54	
CMME III	10	1.011	41.32	73
	40	1.005	41.67	
	100	0.912	47.07	

Table: 2 Anti Tumour activity Against Lung Cancer A549 Cell Line

Sample Name	Conc (µg/ml)	O.D	% Inhibition	IC_{50} (µg/ml)
CONTROL	-	0.914	-	-
5 Fluoro Uracil	10	0.351	61.59	20
	20	0.231	74.72	
	40	0.109	88.07	
	80	0.089	90.26	
	100	0.074	91.90	
CMME	10	0.468	48.79	17
	20	0.328	64.11	
	40	0.236	74.17	
	80	0.221	75.82	
	100	0.218	76.14	
CMME I	10	0.735	19.58	145
	20	0.718	21.44	
	40	0.656	28.22	
	80	0.618	32.38	
	100	0.578	36.76	
CMME II	10	0.701	23.30	40
	20	0.687	24.83	
	40	0.600	34.35	
	80	0.518	43.32	
	100	0.509	44.31	
CMME III	10	0.400	56.23	23
	20	0.331	63.78	
	40	0.221	75.82	
	80	0.207	77.35	
	100	0.186	79.64	

Table3: Brine Shrimp Lethality Assay

Sample Name	Conc. (µg/ml)	Total no of shrimp Used	Shrimp Survived			Total No of Shrimp Survived	% Mortality	IC_{50} (µg/ml)
			T_1	T_2	T_3			
Control	-	10	10	10	10	30	-	-
CMME	50	10	5	4	3	12	60	106
	100	10	2	3	2	07	76.67	
	500	10	0	0	0	0	100	
	1000	10	0	0	0	0	100	
CMME I	50	10	5	4	4	13	56.67	806
	100	10	5	4	4	13	56.67	
	500	10	5	3	3	11	63.33	
	1000	10	0	0	0	0	100	
CMME II	50	10	4	5	4	13	53.57	678
	100	10	4	5	4	13	53.57	
	500	10	3	4	3	10	64.28	
	1000	10	0	0	0	0	100	
CMME III	50	10	5	4	4	13	53.57	224
	100	10	5	4	4	13	53.57	
	500	10	3	4	3	10	64.28	
	1000	10	3	4	3	10	64.28	

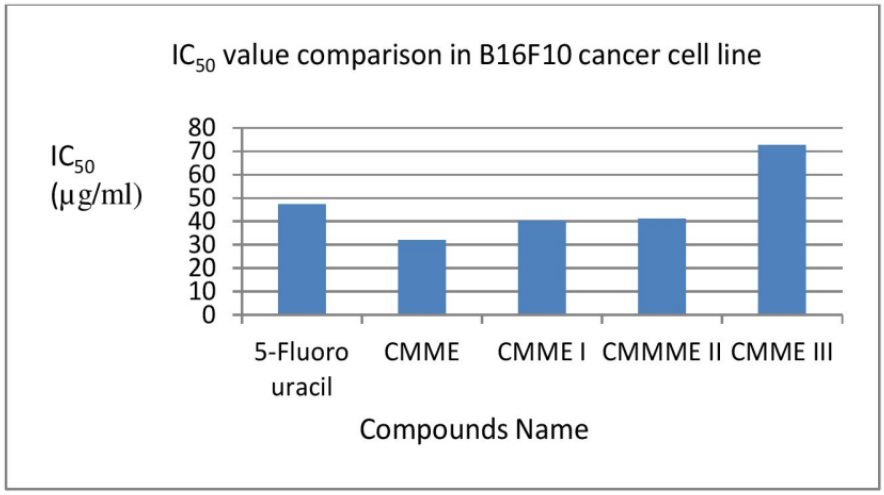

Fig 2: IC_{50} value comparison in Skin cancer B16F10 cancer cell line

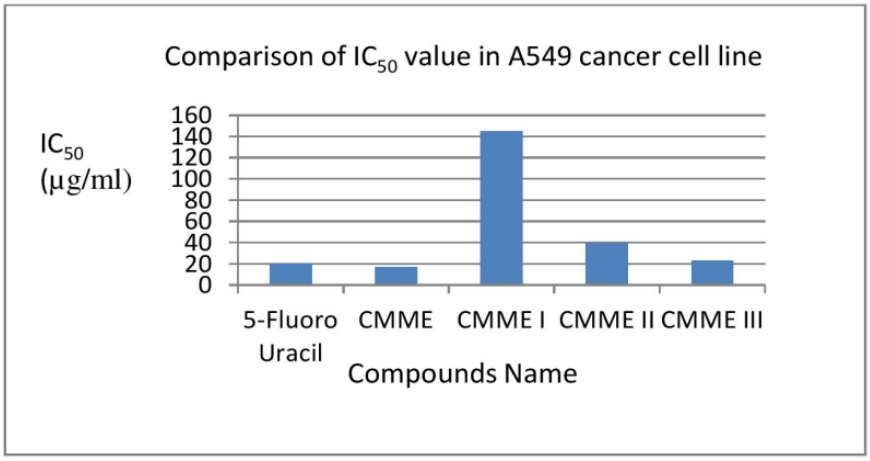

Fig 3: IC_{50} value comparison in Lung cancer A549 cell line

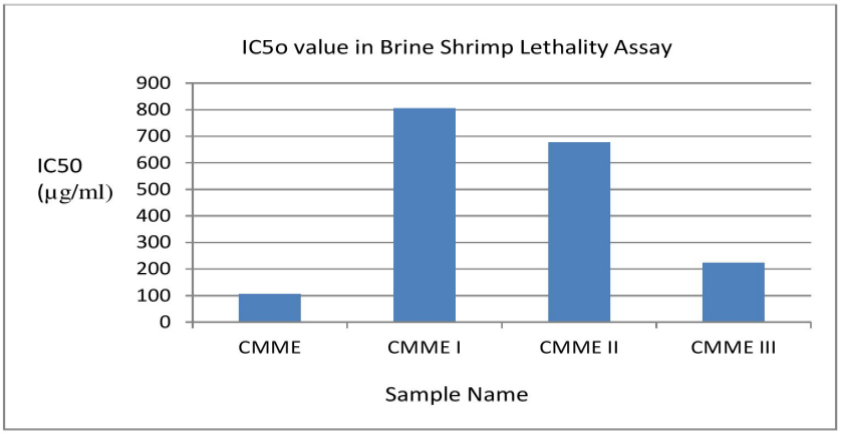

Fig 4: IC_{50} value comparisons in Brine Shrimp Lethality Assay

Results and Discussion

The In-vitro anti tumour activity of CMME and its isolated compounds are determined against the skin cancer B16F10 cell line by the MTT assay method using 5-fluoro uracil as the standard drug. The isolated compound CMME I shows a more % inhibition against the skin cancer B16F10 cell line compared to the other isolated compounds and crude extracts and the standard. It has an IC_{50} value of 40µg/ml. The In-vitro anti tumour activity of CMME and its isolated compounds are determined against the Lung cancer

A549 cell line. The crude extract CMME and the isolated compound CMME III showed more % of inhibition against the Lung cancer A549 cell line compared to the CMME I, CMME II, and Standard 5fluoro uracil. The crude extract CMME and the isolated compound CMME III have the IC_{50} value of 17mcg/ml and 23mcg/ml correspondingly. The brine shrimp lethality assay has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity of brine shrimp, which could also indicate possible cytotoxicity properties of the test materials. This significant lethality of the presence of potent cytotoxicity and probably insecticidal compounds warrants further investigation. This bioassay is a good correlation with human solid tumour cell lines. At the different concentrations, the crude extract CMME has more cytotoxicity activity against Brine shrimp. The crude extract CMME has an IC_{50} value of 106µg/ml.

Conclusion

The isolated compound CMME I shows a more % inhibition against the skin cancer B16F10 cell line compared to CMME, CMME II, CMME III, and the standard 5fluoro uracil. It has an IC_{50} value of 40µg/ml. The crude extract CMME and the isolated compound CMME III showed more % of inhibition against the Lung cancer A549 cell line compared to the CMME I, CMME II, and the standard 5fluoro uracil. The crude extract CMME and the isolated compound CMME III have the IC_{50} value of 17µg/ml and 24µg/ml correspondingly. Crude extracts resulting in an LC_{50} value of fewer than 250µg/ml were considered significantly active and had the potential for further investigation (Rieser et.al., 1996). A brine shrimp lethality assay was done for the crude extract CMME and its isolated compounds. The crude extract CMME has more cytotoxicity against the Brine shrimp. The IC_{50} value for the CMME was found to be 106 mcg/ml.

Declaration of Interest

The author reports no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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