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Phytochemical analysis, *In-vitro* antioxidant, anticancer and enzyme hydrolysis activity of *Abrus precatorius* seeds

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Abstract

Abrus precatorius is commonly known as Indian liquorice. It is an effective medicinal herb that comes under the Fabaceae (Leguminosae) - pea family. The objective of the current study was to determine whether the methanol extract of *Abrus precatorius* seeds and its separated components have antioxidant, anticancer, and enzyme hydrolysis activities. The crude extract of the seed was obtained by maceration with methanol for 7 days. Column chromatography was used to isolate the components from the crude extract using Silica gel with a 60–120 mesh as an adsorbent and methanol as solvent. The phytochemical investigation of the methanol extract of the seeds was achieved. The methanol extracts of the seeds contain many bioactive chemical constituents like Alkaloids, Glycosides, Flavonoids, Steroids, Saponins, Phytosterols, Terpenoids, Tannins, and Phenols. Using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical species assays, the antioxidant activity was measured. Superoxide-free radical scavenging activity was determined by the alkaline DMSO method. The result indicates strong antioxidant activity. The anticancer activity for breast cancer and colon cancer was determined by MTT cell viability assay. The enzyme hydrolysis activity was determined for the papain, alpha-chymotrypsin by the gelatin well plate method.

Keywords: *Abrus precatorius* seeds; DPPH; MTT assay; IC_{50}; Enzyme hydrolysis

1. Introduction

Abrus precatorius Linn is a climbing shrub present in subtropical regions of India commonly called Indian liquorice and crab's eye [1, 2]. It is a lovely deciduous climbing plant that belongs to the Fabaceae family [3, 4]. Its seeds have a very consistent weight of 1/10th of a gram, therefore in the past, goldsmiths used them as standard weights for measuring gold and silver [5]. These seeds are used to make Necklaces and other ornaments [6]. *Abrus precatorius* is recognized for its medicinal properties [7]. Alanine, serine, choline, valine, and methyl ester are among the necessary amino acids found in seeds [8]. Diabetes and chronic nephritis are treated with the use of seeds [9]. As boiling seeds are consumed in some regions of India, seeds are nutrient-dense. Cooking supposedly destroys the seeds' toxicity [10]. Seeds are poisonous and therefore are used after mitigation. The seeds are used to clear up digestive problems and enhance appetite and taste. Indianliquorice seeds consist of alkaloids abrine, hypaphorine, choline and precatorine, trigonelline[11]. Only 2.5% of a seed's weight is oil, which is made up of oleic and linoleic acids. The seeds include -sitosterol, stigmasterol, 5-cholanic acid, abricin, and cholesterol among other steroids. The glycosides of abranin, pelargonidin, cyaniding, and delphinidin are what give the seed its colour. Other components of the seeds include sapogenin, abrisapogenol J, sophoradiol, its 22-O-acetate, hederagenin methyl ester, kaikasaponin III methyl ester, and flavones including abrectorin and aknone [12]. The main components of the seeds are lectins, with abrin serving as the primary one. Both toxic (like abrin) and non-toxic lectins exist (abrus agglutinin). A long-polypeptide chain (MW 35,000) and a short-polypeptide chain connected by a disulfide bond make up abrins, which are represented by the letters abrin a, b, c, and d. The seeds also contain a small amount of abrusin and its 2'oapioside [13]. Antioxidants,

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Antiplatelet, and Anticancer are the major activity reported for the seed [14,15]. The oil obtained from the Indian liquorice seeds is used to stimulate hair growth [16]. *Abrus precatorius* leaf is used for breast cancer [17]. A Sodhita Gunja seed does not have any bactericidal effect on bacterial strains [18]. Phytochemical screening of the methanol extracts of the seeds showed the presence of Alkaloids, Glycosides, Flavonoids, Steroids, Saponins, Phytosterols, Terpenoids, Tannins, and Phenols [19].

2. Material and methods

2.1. Collection of sample and Identification

The *Abrus precatorius* sample for this research work was collected from the forest present in Srivilliputtur. The seeds are identified and authenticated by the Department of Botany, Ayya Nadar Janaki ammal college, Sivakasi The seeds are washed with water and dried at room temperature in the absence of sunlight, and after drying it was uniformly grounded using a mechanical grinder to make a coarse powder. The powdered material was stored in an air-tight container.

2.2. Method of preparation of sample

1000 ml of the round bottom flask was taken and washed with methanol and allowed to dry. After drying 60 grams of coarse powder of *Abrus precatorius* seeds was taken in the round bottom flask and 600 ml of methanol was added and shaken well and allowed to cold maceration for 7 days. To improve the extraction, the RB flask was shaken several times. It was then filtered using Whatman Filter Paper No. 1 and allowed to evaporate at room temperature. [20].

2.3. Phytochemical Analysis

A qualitative examination was conducted to determine whether the methanol extract included alkaloids, glycosides, flavonoids, steroids, saponins, phytosterols, terpenoids, tannins, or phenols. The result was present in the table 1.

2.3.1. Test for alkaloids

Mayer's test

A test tube was filled with a 2 ml solution of both the extraction and 0.2 ml of diluted hydrochloric acid. Mayer's reagent was then combined in 1 ml. The occurrence of alkaloids was confirmed by the yellow color of the precipitate that was produced.

Dragendroff's test

In a test tube, 0.2 ml of diluted hydrochloric acid and 2 ml of the extract's solution were added. Dragendroff's reagent was then blended in 1 ml. The existence of alkaloids was confirmed by the orange-brown precipitate that was produced.

2.3.2. Test for glycosides

Noticed reddish-brown coloring at the intersections of the two layers and the appearance of bluish-green color in the upper layer after adding 5 ml of the extract with glacial acetic acid several drops of ferric chloride, and strong sulphuric acid. It verifies that glycoside is present.

2.3.3. Test for Flavonoids

Alkaline Reagent Test

A few drops of a solution of sodium hydroxide were added to the extract. The existence of flavonoids is indicated by the appearance of a strong yellow color that fades to colourlessness when diluted hydrochloric acid is added.

Lead acetate test

Several drops of lead acetate solution were added to the extract. Precipitate with a yellow tint indicates the presence of flavonoids.

2.3.4. Test for saponins

Froth test

A graduated cylinder containing the extract was shaken for 15 minutes after being diluted with deionized to a volume of 20 ml. Saponins are present because a 1 cm layer of "honeycomb" foam forms.

2.3.5. Test for steroids

A test tube containing the crude extracts (1 mg) and chloroform (10 mL) was then filled halfway with concentrated sulfuric acid in an equal proportion. The test tube's upper layer turned red, while the layer of sulphuric acid displayed yellow with green fluorescence.

2.3.6. Test for phytosterols

Salkowski's test

In a test tube, the extract was combined with 2 ml of chloroform. Sulfuric acid was carefully applied to the test tube's wall to create a lower layer. It is confirmed that a glycoside steroid ring, or reddish brown color, has formed at the contact.

Liebermann Burchard's test

Chloroform was added to the extract, and then it was filtered. The filtrate was heated and then cooled after acetic anhydride was added in little amounts. Sulfuric acid was added, conc. Phytosterols are present as evidenced by the brown ring that forms at the junction.

2.3.7. Test for phenols

Ferric chloride test

Three drops of newly made 1% ferric chloride and potassium Ferro cyanide were added to the extract's aqueous solution. The presence of a bluish-green hue was regarded favorably. The water had the methanol extract added to it. The mixture was supplemented with a few ferric sulfate crystals. The occurrence of phenolic chemicals is confirmed by the rich violet color.

2.3.8. Test for Tannins

Ferric chloride test

Water was then mixed with the extract. Filtration was used to clarify the mixture, and the clear filtrate was then mixed with a 10% ferric chloride solution. This was noticed in a shift to blue-black.

Lead acetate test

Water and a 10% lead acetate solution were combined with the extract. The presence of tannins is indicated by the presence of a yellow precipitate.

2.3.9. Test for Terpenoids

Chloroform (2 ml) was mixed with 2 ml of methanol extract, evaporating to dryness. Then, 2 ml of concentrated sulfuric acid was added, and it was warmed for about 2 minutes. The existence of terpenoids was confirmed by the greyish color that was acquired.

2.4. Isolation of Compounds from Crude Extracts

The crude extract obtained by the maceration method is mixed with a small amount of methanol and introduced into the top of the column. The column was packed by wet packing method by using the adsorbent Silica gel 60-120 mesh and the solvent methanol. The compounds were isolated from the crude extract by using methanol as a mobile phase. The compounds that have more affinity towards the stationary phase travel slower. The compounds that have less affinity towards the stationary phase travel faster. The solvent eluted from the bottom of the column is collected. The fractions that have the same Rf value are mixed. The solvents are evaporated and the isolated compounds are dried

under a vacuum. The first eluted compound is CMME I, The second eluted compound is CMME II, Third eluted compound is CMME III.

2.5. Invitro Antioxidant Activity by DPPH Assay Method

Using DPPH (1,1-Diphenyl-2, Picryl - Hydrazyl) free radicals, the antioxidant activity of the compounds CMME, CMME I, CMME II, and CMME III was estimated [20]. In the microtiter plate, 100 L of the CMME, CMME I, CMME II, and CMME III (1 mg/mL) compounds were added. The samples were covered with 100 L of 0.1% methanolic DPPH, which was then left to incubate for 30 minutes in the dark. After that, the samples were examined for discoloration; any shades of purple, yellow, or pale pink were deemed to be strongly and weakly positive, respectively. The plate was then read using an Elisa plate reader at 490 nm. The crude extract's and its separated components' percentages of DPPH free radical scavenging were shown in Table -2.

The following equation was used to compute Free Radical Scavenging activity:

DPPH radical scavenging activity (%) = [(Absorbance of control – Absorbance of test sample) / (Absorbance of control)] x 100

2.6. Scavenging of Super Oxide Radical by Alkaline DMSO Method

2.6.1. Procedure

0.3 ml of the drug sample in concentration (100 micrograms per ml) and 0.1 mL of NBT (100 micrograms per mL) were added to the reaction mixture that contained 1 milliliter of alkaline DMSO, resulting in a final volume of 1.4 ml. At a wavelength of 560 nm, the absorbance was measured. Similarly, in place of the drug sample, 0.3 mL of ascorbic acid at a concentration of one hundred micrograms per milliliter was added to one milliliter of alkaline DMSO, and then 0.1 mL of NBT at the same concentration was added. At a wavelength of 560 nm, the absorbance was measured. Table 3 displayed the percentage of inhibition that was achieved.

2.7. Invitro Anti-Cancer Activity by MTT Assay method

2.7.1. Experimental Procedure

Cells were incubated at a concentration of 1 X 10^4 cells/ml in a culture medium for 24 hours at 37 °C and 5% CO2. Cells were seeded at a concentration (70µl) 10^4 cells / well in 100µl culture medium and 100 µl sample of CMME, CMME I, CMME II CMME III, and standard 5 Fluorouracil in different concentrations into micro plates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and a cell line. Controls were maintained to determine the control cell survival and the percentage of cancer cells after culture. Cell cultures were incubated for 24 hours at 37 °C and 5% CO_2 in a CO_2 incubator (Thermo scientific BB150). After incubation, the medium was completely removed and added 20 µl of MTT reagent (5mg/ml PBS) was. After the addition of MTT, cells were incubated for 4 hours at 37 °C in a CO_2 incubator. Observed the wells for formazan crystal formation under a microscope. The yellowish MTT was reduced to dark-colored formazan by viable cells only. After removing the medium completely. Added 200 µl of DMSO (kept for 10 min) and incubate at 37 °C (wrapped with aluminum foil). Samples were analyzed by measuring the absorbance of each sample with an Elisa microplate reader (Benesphera E21) at a wavelength of 570 nm. The results were compared and discussed and presented in Tables 4, and 5 respectively for Breast cancer, Colon Cancer respectively.

2.8. Studies on Enzyme Hydrolysis (α-Chymotrypsin and Papain)

The gelatine well plate method is used to study the α-chymotrypsin and papain hydrolysis activity (Fig. 1). If the zone of inhibition diameter is reduced it indicates that the plant compounds were not hydrolyzed by the enzyme. If the zone of inhibition diameter increases it indicates that the plant compounds were hydrolyzed by the enzyme. Here the plant compounds CMME, CMME I, CMME II, and CMME III are mixed with the alpha-chymotrypsin and papain separately in the presence of the tris buffer and kept aside for 12 hours at room temperature.100µL of the mixture samples were then applied in the well of diameter 6mm made by the sterile corn borer.100µL of the tris buffer was added to the well, which acts as the control. The Zone of inhibition was measured in mm QuPath and is presented in Table -6.

3. Results and Discussion

The methanol extract of *Abrus precatorius* seeds were shown to include alkaloids, tannins, glycosides, Flavonoids, Steroids, Saponins, Phytosterols, Terpenoids, Tannins, and Phenols after undergoing a variety of chemical tests for the identification of significant classes of therapeutically relevant substances.

Invitro antioxidant activity was determined by the 1,1-Diphenyl-2, Picryl – Hydrazyl as the control, and ascorbic acid as the standard. CMME and its isolated compounds have good antioxidant activity.

Scavenging of Super Oxide Radical was done by Alkaline DMSO Method. In this method, the reaction mixture containing alkaline DMSO and NBT is act as a control and Ascorbic acid is act as the standard. The isolated compounds have good superoxide scavenging activity compared to the standard.

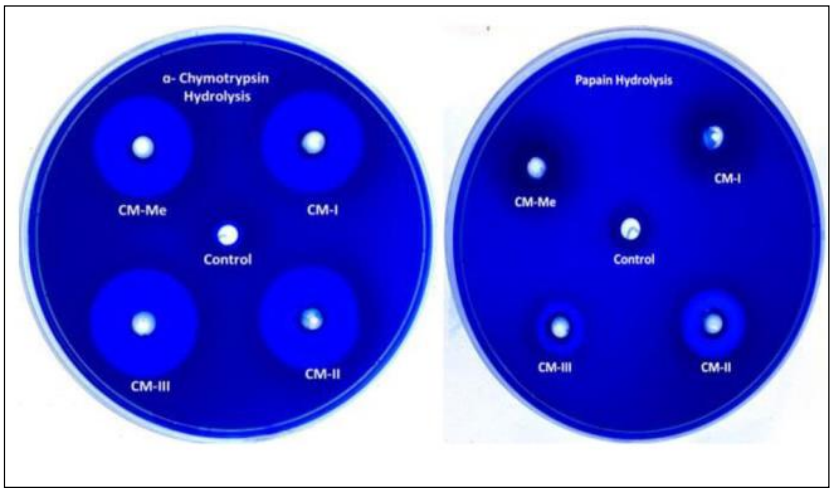

Figure 1 Enzyme hydrolysis of α-chymotrypsin and papain

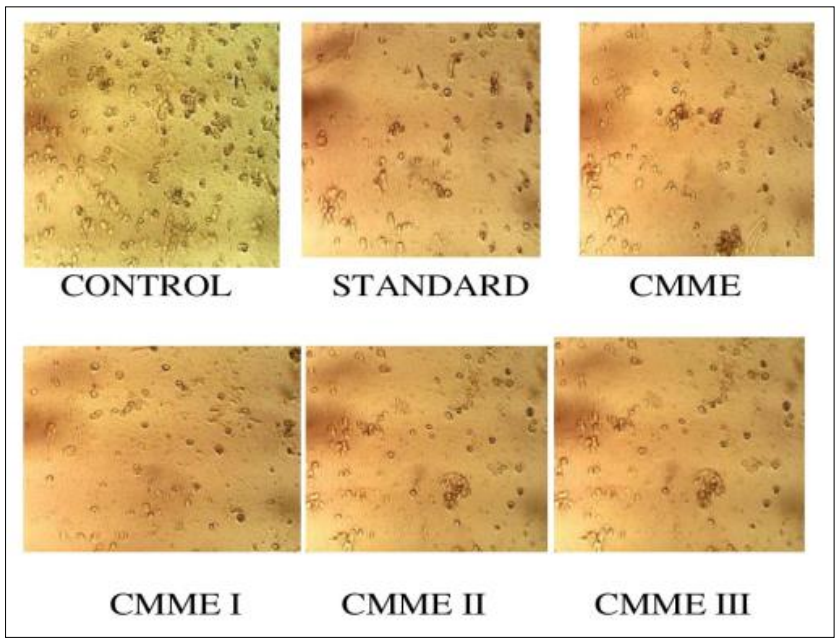

Figure 2 Effect of compounds against COLO 205 M cell line

In-vitro Breast cancer activity was determined by using the MCF-7 Breast cancer cell line, by using the media DMEM with high glucose and the Antibiotic– Antimycotic100Xsolution, 5-fluorouracil is the standard. The crude extract CMME

and the isolated compounds CMME I, CMME II, and CMME III have good anticancer activity against the MCF-7 breast cancer cell line when compared to standard.

In-vitro colon cancer activity was determined by using COLO 205M Cancer Cell Line, by using the Media DMEM with high glucose, FBS, Antibiotic – Antimycotic 100X solution, and 5 fluorouracil as the standard. The crude extract CMME and the isolated compounds CMME I, CMME II, and CMME III have good anticancer activity against the COLO 205M cancer cell line when compared to the standard .

α-Chymotrypsin is a protease with proteolytic as well as esterolytic activity of the CMME, CMME-I, CMME-II, AND CMME-III plant extracts. Besides processes based on stereo-specific hydrolysis, α-chymotrypsin has been used for the synthesis of peptides. In contrast, in papain hydrolysis of CMME, CMME-I, CMME-II, and CMME-III plant extracts during the incubation, only CMME-II, and CMME-III only active in Proteolytic enzymes help break proteins down into smaller protein fragments called peptides and amino acids because of meat tenderizer. In this conclusion of CMME-II and CMME-III is very active compounds found in the samples for both enzymes were hydrolysis.

Table 1 Phytochemical Screening of of *Abrusprecatorius* seeds

S.No.	Test for Secondary metabolites	Extraction solvent - Methanol
1	Alkaloids	
	Mayer's Test	+
	Wagners's Test	+
	Dragendroff's	+
2	Glycosides	+
3	Flavonoids	++
4	Steroids	++
5	Saponins	+
6	Phytosterols	+
7	Terpenoids	+
8	Tannins	+
9	Phenols	+

++: Moderate amount, +: Trace amount

Table 2 *In-vitro* Antioxidant Activity by DPPH Assay Method

COMPOUNDS (1mg/ml)	O.D	MEAN	% OF DPPH FREE RADICAL SCAVENGING
CONTROL	0.815	1.014	
	1.087		
	1.140		
ASCORBIC ACID	0.288	0.28	72.38
	0.269		
	0.283		
CMME	0.362	0.403	60.26
	0.389		
	0.458		
CMME I	0.469	0.529	47.83

	0.546		
	0.574		
CMME II	0.443	0.494	51.28
	0.506		
	0.533		
CMME III	0.435	0.429	57.69
	0.423		
	0.431		

Table 3 Scavenging of Super oxide Radical Activity

SAMPLE	O.D	% INHIBITION
CONTROL	1.38	
ASCORBIC ACID	0.52	62.31
CMME	1.04	24.63
CMME I	0.17	87.68
CMME II	0.56	59.42
CMME III	0.17	87.68

Table 4 % inhibition of MCF-7Breastcancercellline

SAMPLE	CONC (µg/ml)	OD	MEAN	% INHIBITION	IC_{50} (µg/ml)
CONTROL		0.935	0.946		
		0.953			
		0.950			
Std 5FU	10	0.365	0.450	51.56	51.66
		0.468			
		0.518			
	40	0.436	0.423	54.46	
		0.464			
		0.369			
	100	0.223	0.265	71.47	
		0.315			
		0.259			
CMME	10	0.555	0.566	40.16	36.98
		0.576			
		0.568			
	40	0.558	0.536	43.34	
		0.462			
		0.588			

	100	0.532 0.448 0.571	0.517	45.34	
CMME I	10	0.649 0.597 0.699	0.648	31.510	46.82
	40	0.582 0.661 0.651	0.631	33.22	
	100	0.515 0.655 0.606	0.592	37.42	
CMME II	10	0.604 0.556 0.586	0.579	38.79	36.23
	40	0.497 0..580 0.446	0.507	46.40	
	100	0.464 0.468 0.469	0.467	50.63	
CMME III	10	0.595 0.526 0.586	0.567	40.6	42.55
	40	0.503 0.536 0.526	0.522	44.82	
	100	0.487 0.438 0.477	0.467	50.63	

Table 5 % Inhibition of COLO 205 M cell line

SAMPLE	**CONC (µg/ml)**	**O.D**	**% INHIBITION**	**IC_{50}(µg/ml)**
CONTROL		0.973		
STD 5FU	10	0.287	70.50	19.01
	40	0.240	74.71	
	100	0.115	88.00	
CMME	10	0.356	63.41	38.76
	40	0.333	65.77	
	100	0.314	67.72	

CMME I	10	0.724	25.59	31.02
	40	0.631	35.14	
	100	0.611	37.20	
CMME II	10	0.629	35.35	34.96
	40	0.578	40.59	
	100	0.555	42.95	
CMM III	10	0.469	51.79	32.95
	40	0.431	55.70	
	100	0.419	56.93	

Table 6 Enzyme Hydrolysis of alpha-Chymotrypsin and Papain

Sr. No	Enzyme	ZONE OF INHIBITION (mm)				
		CMME	CMME I	CMME II	CMME III	CONTROL
1	α-Chymotrypsin	7.85	7.79	8.06	8.53	1.40
2	Papain	1.57	1.82	6.03	4.38	1.85

4. Conclusion

This research work was intended and aimed to determine the first steps of the discovery of phytochemicals with promising pharmacological activity. The methanol extract of Abrus precatorius seeds consists of many photochemical constituents like alkaloids, flavonoids, phenols, saponins, glycosides, tannins, steroids, phytosterols, phenols, and terpenoids. The antioxidant activity of the methanol extract of Abrus precatorius seeds and its isolated compound was determined by DPPH assay and Superoxide scavenging activity by alkaline DMSO method. At Concentration 10, 40 100µg/ mL, The samples CMME, CMME I, CMME II and CMME III show good % inhibition of the MCF-7 Breast cancer cell line as compared to the standard drug. At Concentration 10, 40, and 100µg/ mL, The samples CMME, CMME I, CMME IIand CMME III show good % inhibition of the COLO 205M cell line as compared to the standard drug. Papain, α-Chymotrypsin enzyme hydrolysis was determined by the gelatine well plate method.

Compliance with ethical standards

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Disclosure of conflict of interest

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

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