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Estimation of Total Alkaloid, Polyphenol, Flavonoid Contents, and Antioxidant Activity of *Saga - Saga* Seeds

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Abstract

The research work aims to estimate the Total Alkaloid content, Total flavonoid content, and Total polyphenol content of the methanol crude extract (CMME) of the *Abrus precatorius* seeds and to determine the Antioxidant power of the methanol crude extract CMME and its isolated compounds CMME I, CMME II, and CMME III. The methanol crude extract was prepared from the dried powdered *Abrus precatorius* seeds by the maceration method for 7 days. The compounds from the crude extract CMME 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 total alkaloid content of CMME was found to be 88 mg Atropine equivalent per gram of dried crude extract. The total flavonoid content of the crude extract CMME was found to be 415 mg Quercetin equivalent per gram of dried crude extract. The total phenol content of the crude extract CMME was found to be 239 mg Gallic acid equivalent per gram of dried crude extract. The antioxidant activity was determined by FRAP assay and ABTS assay. The isolated compound CMME II has more Ferric reducing antioxidant power compared to the CMME, CMME I, CMME III, and the standard Ascorbic acid. The isolated compound CMME II and CMME III have more ABTS radical scavenging activity compared to the CMME and CMME I. The isolated compounds CMME II and CMME III have IC_{50} values of 15mcg/ml and 11mcg/ml correspondingly.

Keywords: Flavonoid, Alkaloid, Polyphenol, UV-VIS spectrophotometer.

1. Introduction

Abrus precatorius, provincially specified as saga-saga, rosary pea, and crab's eye, is one of the most deadly plants understood to man. The seeds consist of the protein toxin, abrin which is deadly when consumed even at a small dose. It was revealed that as little as 0.00015% of toxin per body can produce mortality in humans. [1].The latest studies illustrated that the high dietary intake of fruits and vegetables could be related to lower cancer pervasiveness in humans. Natural products mostly from the plant kingdom

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offer a broad range of biologically active compounds that behave as natural antioxidants with accepted potential in drug discovery and development. [2].There are enlarging confirmations that free radicals caused molecular changes that are connected with various deterioration human ailments such as atherosclerosis, cancers, Alzheimer's disease, Parkinson's disease, diabetes, asthma, arthritis, immune deficiency ailments, and aging. Antioxidants are materials that eliminate free radicals and stop them from producing cell damage. Plants consist of antioxidant compounds that role as free radical scavengers, reducing agents, and quenchers of singlet oxygen emergence. [3].Bioactive ingredients obtained from plant extracts have been described experimentally for pharmacological actions. Plants cause these ingredients to preserve themselves, but current researches show that extremely of them can also be utilized against numerous human ailments and disorders. The most principal of these pharmacologically active ingredients are alkaloids, flavonoids, steroids, glycosides, terpenes, and tannins. These constituents can be extracted and utilized in the construction of useful drugs. [4].The requests for plant-based drugs are enlarging very fast in India. From this, we can say that exploration is going on for major plants which can give medicinal action in the therapy of numerous ailments. Between the conventional systems of therapeutics Abrus precatorius Linn is one of the principal herbs. [5].Paste of Abrus precatorius seeds was enforced locally in sciatica, stiffness or shoulder joint, and paralysis, said to be effective in dysentery, and paste was utilized against skin ailments. Half-boiled seeds are given as tonic. It also manifests anti-cancerous activity. [6]. *Abrus precatorius* species is generally present in Garhwal place up to a height of 1200 meters above sea level. A compost mixture containing two parts of loamy soil, one part each of leaf mold, cow dung, and sand is ideal. Plants are to be watered freely during the time of the dry season. The leaves, seeds, and roots are helpful. The leaf juice is sweet and utilized to heal hoar senses. The leaf juice mixed with oil is enforced on painful swelling of the body. Seeds are utilized as beads for rosaries. The root is utilized as an alternative to liquorice. [7]. Abrus precatorius plant has been utilized in Hindu therapeutic from very previous times, as well as in China and other classical cultures [8]. The World Health Organization [WHO] has described that 80% of the population of growing countries use traditional therapeutics for their healthcare needs [9]. The principal ingredients of the seeds of this plant are isoflavonoids, flavonoids, proteins, alkaloids, carbohydrates, and triterpenoids [10].

2. Material & Method

2.1 Collection and Identification of Sample

The *Abrus precatorius* seeds are obtained from the Meenakshi sundharanar shop placed in the Rajapalayam and identified and authenticated by the botany department of Ayya Nadar Janaki Ammal College, Savakis. 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 with 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 and named CMME. The total yield of the crude extract was found to be 4 grams. From the crude extract, the compounds are isolated by column chromatography by using silica gel 60-120 mesh as the adsorbent. The isolated compounds are further purified by Thin Layer Chromatography. The first eluted compound was named CMME, the second eluted compound was named CMME I, and the third eluted compound was named CMME III. Compounds that have the same RF value are mixed and the solvents are evaporated at room temperature and then dried under a vacuum.

2.3 Estimation of Total Alkaloid content: [11]

Procedure

Bromo Cresol Green solution (BCG): 69.8 mg of bromocresol green was warmed with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and diluted to 1000 ml with distilled water.

Phosphate buffer (pH 4.7): pH of 2M Sodium phosphate (weighing 71.6g Na_2HPO4 and made up to 1000 ml with distilled water) was adjusted to pH 4.7 with 0.2 Molar citric acids (weighing 42.02g citric acid and made up to 1000 ml with distilled water).

Atropine standard solution: 1mg pure atropine (sigma chemical co.) was dissolved in 10ml distilled water.

Preparation of standard curve: Accurately measured aliquots (0.4, 0.6, 0.8,1, and 1.2ml) of atropine standard solutions were transferred to different separatory funnels. 5ml phosphate buffer (pH4.7) and 5ml BCG solution were added. The mixture was shaken with 1, 2, 3, and 4 ml of chloroform. The extracts were collected in five different 10 ml volumetric flasks and diluted to volume with chloroform. The optical density of the complex in chloroform was determined at 470 nm against blank processed as above but without atropine.

Sample Extraction: 10 mg of the crude extract CMME was dissolved in 2N HCl and then filtered. 1 ml of this solution was transferred to a separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1N NaOH. Then 5ml of BCG solution and 5ml of phosphate buffer was added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4ml chloroform by vigorous shaking. The extracts were collected in a 10ml volumetric flask and diluted to a volume of 10ml with chloroform. The optical density of the complex in chloroform was determined at 417nm with a blank prepared by the same method by omitting the sample crude extract. The result was presented in tables 1 and 2. The Linearity curve for Atropine was present in fig 1.

2.4 Estimation of Total Flavonoid Content [12, 13]

Procedure:

The total flavonoid content in the plant extract was determined by the Aluminium chloride method. 1 ml of the crude extract CMME (1mg/ml) in methanol and 1ml of the standard quercetin (200,400,600,800, and 1000µg/ml) in methanol were taken in a different 10 ml of the volumetric flask, and then 4ml of distilled water was added and then 0.3 ml of 5% sodium nitrite was added and after 5minutes 0.3ml of 10% Aluminium chloride was added. Two ml of 1Molar NaOH was added after five minutes and diluted to 10 ml with distilled water. The absorbance of the crude extract and standard quercetin were determined against the reagent blank at 510 nm with a UV-VIS spectrophotometer. The total flavonoid content was expressed as mg of quercetin equivalents per gram of dried crude extracts. The absorbance of the test sample was performed in triplicate. The result was presented in Tables 3 and 4. The Linearity curve for the quercetin was present in fig 2.

2.5 Estimation of Total Polyphenol Content. [12, 13]

Procedure:

The total polyphenol content of the crude extract was determined by the Folin-Ciocalteu method using a UV-VIS spectrophotometer. 1ml of the crude extract CMME (1mg/ml) in methanol and 1ml of the standard Gallic acid (200, 400, 600, 800, 1000µg/ml) in methanol were taken in a different 25ml volumetric flask. 9ml of the distilled water was added to each volumetric flask and mixed well. 1 ml of the Folin-Ciocalteu phenol reagent was added to the mixture and shaken vigorously. After 5 minutes 10ml of 7% sodium carbonate solution was added to the mixture. The volume was adjusted to 25ml with distilled water. After the incubation period of 90 minutes at room temperature, the absorbance of the test and standard was measured at 550nm using a reagent blank by UV-VIS spectrophotometer. The absorbance of the test sample was performed in triplicate. The result was presented in Tables 5 and 6. The Linearity curve for the Gallic acid was present in fig 3.

Ferric Reducing Antioxidant Power Assay (Frap Assay). [15]

Procedure:

The methanol crude extract CMME and its isolated compounds CMME I, CMMME II, and CMME III at a concentration of 1mg/ml by using methanol as a solvent were taken in a different test tube and then 2.5ml of phosphate buffer (0.1M, pH 6.7) & 2.5 ml of potassium ferricyanide (1%w/v by using distilled water) were added and was incubated at 50^{o}C for 20min and then 2.5ml of 10% trichloroacetic acid (TCA) was added. 2.5ml of these solutions were mixed with 2.5ml of distilled water and 0.5ml of $Fecl_3$ (0.1% w/v) and incubated for 30 minutes. After 30 minutes of incubation, the absorbance was read at 700nm. Ascorbic acid at the concentration of 1mg/ml is used as a positive control and instead of the sample 1ml of distilled water is taken in the test tube is act as the negative control. The absorbance value was present in table 7.

2.7. In-vitro Antioxidant activity by ABTS ASSAY method [16].

In a 96-well plate, Different concentrations(10,20,40,80, and 160µg/ml) of Trolox standard solution (10µl), sample CMME, CMME I, CMME II, and CMME III(10µl) and control DMSO solvent (10µl) were thoroughly mixed with ABTS working solution (290µl) in the assay wells. The micro plate was subsequently incubated in the dark at 37ºC for 6 minutes. The absorbance of the solution in each assay well was determined using a micro plate reader at a wavelength of 630 nm as presented in table 8. The comparison of IC_{50} value of CMME, CMME I, CMMME II, CMME III, and standard Trolox in ABTS assay was present in fig 5.

Preparation of ABTS solution:

The ABTS solution was prepared by mixing an equal volume of 7 m.mol /L ABTS stock solution with a 2.45 mmol /L potassium persulfate solution. The mixture was then stored in the dark at room temperature for 12-16 hours.

Preparation of ABTS working solution:

Take 1 ml of the above solution and made up to 50 ml with PBS.

3. Result and Discussion

The total Alkaloid was determined by the Bromo Cresol Green method. The total alkaloid of CMME was found to be 88 mg Atropine equivalent per gram of dried crude extract. The total flavonoid content was determined by the Aluminium chloride method. The total flavonoid content of the crude extract CMME was found to be 415 mg quercetin equivalent per gram of the dried crude extract. The total phenol content was determined by the Folin-ciocalteu reagent method. The total phenol content of the crude extract CMME was found to be 239 mg Gallic acid equivalent per gram of dried crude extract. The comparison of the total content of Alkaloid, Flavonoid, and Polyphenol of crude extract CMME was present in fig 4. The antioxidant power of the crude extract CMME and its isolated compounds CMME I, CMME II, and CMME III was determined by FRAP assay using ascorbic acid as the positive control and distilled water as the negative control. The more absorbance value indicates that the sample has more ferric-reducing antioxidant power. The less absorbance value indicates that the sample has less ferric-reducing antioxidant power. The crude extract CMME and the isolated compound CMME II have more ferric-reducing antioxidant power when compared to CMME I, CMME III, and the standard Ascorbic acid. The antioxidant power of the crude extract and the isolated compound was determined by the ABTS assay method. The compounds CMME II and CMME III have more % Inhibition of ABTS radical scavenging compared to the standard Trolox. The IC_{50} value of CMME II and CMME III was found to be 14mcg/ml and 11mcg/ml correspondingly.

Table 1: Determination of Total Alkaloid

S.NO	Concentration (µg/ml)	O.D
1	20	0.18
2	40	0.21
3	60	0.31
4	80	0.42
5	100	0.49
6	120	0.57

Table 2: Total Alkaloid content of CMME

Sample Name	Conc. (µg/ml)	O.D	Total Alkaloid Content(µg Atropine Equivalent/mg Dried Crude Extract)	Total Alkaloid Content(mg Atropine Equivalent/ g Dried Crude Extract)
CMME	1000	0.44	88 µg	88 mg

Table 3: Standard Quercetin Linearity

S.No	Sample Name	Concentration (µg/ml)	Absorbance
1	Quercetin	200	0.086
		400	0.232
		600	0.358
		800	0.450
		1000	0.596

Table 4: Total Flavonoid Content

Sample Name	Conc. (µg/ml)	O.D	Total Flavonoid Content(µg Quercetin Equivalent/mg Dried Crude Extract)	Total Flavonoid Content(mg Quercetin Equivalent/ g Dried Crude Extract)
CMME	1000	0.23	415	415

Table 5: Standard Gallic acid Linearity

S.NO	Sample Name	Concentration (µg/ml)	O.D
1	Gallic acid	200	0.102
		400	0.241
		600	0.332
		800	0.421
		1000	0.520

Table 6: Total Poly Phenol Content

Sample Name	Conc (µg/ml)	O.D	Mean	Total Poly Phenol Content (µg Gallic acid equivalent / mg dried crude extract)	Total Poly Phenol Content (mg Gallic acid equivalent / g dried crude extract)
CMME	1000	0.14	0.14	239	239
		0.18			
		0.11			

Table 7: Frap Assay

S.No	Sample	Absorbance	Average
1	Vitamin C	0.02	0.03
		0.03	
		0.04	
2	CMME	0.07	0.09
		0.10	
		0.11	
3	CMME I	0.05	0.08
		0.09	
		0.10	
4	CMME II	0.12	0.12
		0.10	
		0.15	
5	CMME III	0.06	0.07
		0.07	
		0.08	

Table 8: Antioxidant activity by ABTS assay

Compound	Conc (µg/ml)	O.D	% of ABTS Radical Scavenging	IC_{50} (µg/ml)
Control		0.436		
Std Trolox	10	0.069	84.23	7
	20	0.061	86.01	
	40	0.054	87.61	
	80	0.048	88.99	
	160	0.042	90.36	
CMME	10	0.211	51.60	23
	20	0.185	57.56	
	40	0.154	64.67	
	80	0.132	69.72	
	160	0.110	74.77	
CMME I	10	0.315	27.75	25
	20	0.289	33.71	
	40	0.265	39.11	
	80	0.239	45.10	
	160	0.217	50.24	
CMME II	10	0.113	74.08	15
	20	0.098	77.52	
	40	0.086	80.27	
	80	0.074	83.02	
	160	0.059	86.36	
CMME III	10	0.142	67.43	11
	20	0.132	69.72	
	40	0.123	71.79	
	80	0.114	73.85	
	160	0.106	75.69	

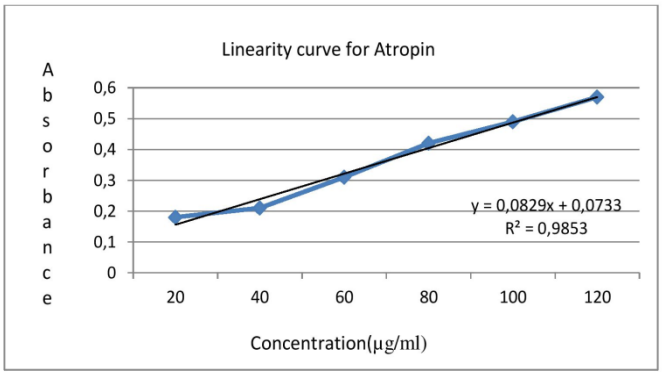

Figure 1: Linearity curve for Total Alkaloid

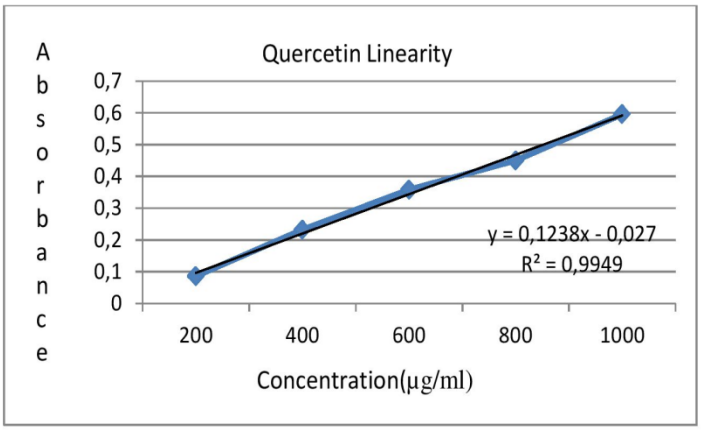

Figure 2: Linearity Curve of Quercetin

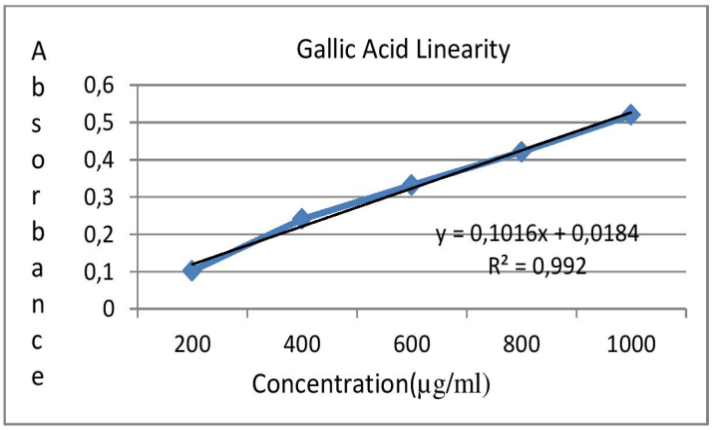

Figure 3: Linearity Curve for Gallic acid

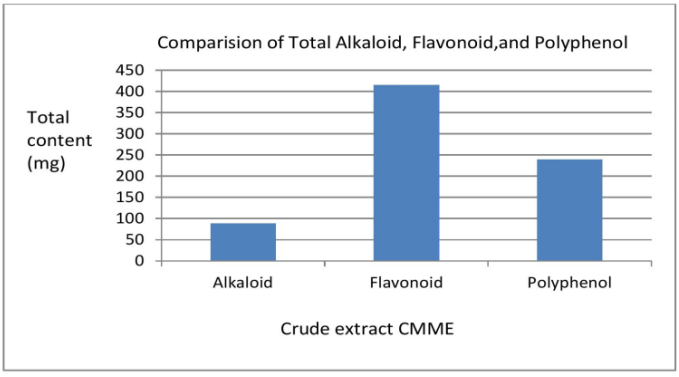

Figure 4: Comparison of Total Alkaloid, Flavonoid & Polyphenol

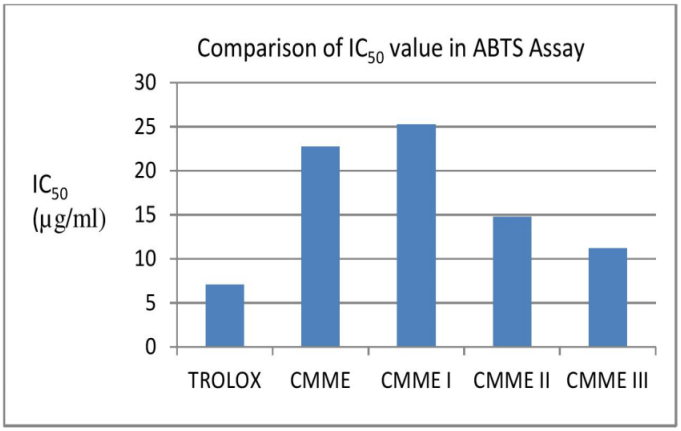

Figure 5: Comparison of IC_{50} value in ABTS Assay

4. Conclusion

The total Alkaloid content of CMME was found to be 88 mg atropine equivalent per gram of dried extract. The total flavonoid content of the crude extract CMME was found to be 415 mg quercetin equivalent per gram of dried extract. The total phenol content of the crude extract CMME was found to be 239 mg Gallic acid equivalent per gram of dried extract. The isolated compound CMME II has more Ferric reducing antioxidant power compared to the CMME, CMME I, CMME III, and the standard Ascorbic acid. The isolated compound CMME II and CMME III have more ABTS radical scavenging power compared to CMME and CMME I. The isolated compounds CMME II and CMME III have IC_{50} values of 15 mcg/ml and 11mcg/ml correspondingly.

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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