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Research Article

IN -VITRO ANTIOXIDATIVE ACTIVITY OF PHENOLIC AND FLAVONOID COMPOUNDS EXTRACTED FROM SEEDS OF *ABRUS PRECATORIUS*

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ABSTRACT

The aim of this work was to estimate the total phenolic and flavonoid content, and to evaluate in-vitro antioxidant activity of ethanolic seeds extract of *Abrus Precatorius* (ASEt). The raw, dry seeds powder was extracted with 99.9% of ethanol. Phytochemical test shows that extract contains higher level of total phenol and flavonoids. Total phenolic compound in ethanolic seeds extract of *Abrus precatorius* was found to be 95 mg/g of extract calculated as gallic acid equivalent (r^2=0.9976) and total flavonoids compound was found to be 21 mg/g of extract calculated as rutin equivalent (r^2=0.9985). The extract was screened for its potential antioxidant activities using tests such as hydroxyl radical-scavenging activity, reducing power activity, and hydrogen peroxide-scavenging activity.

The in-vitro antioxidant assay showed ASEt posses potent antioxidant activity when compared with reference compound butylated hydroxytoluene (BHT). ASEt could be useful for preparation of neutraceuticals as potent antioxidant to treat various human diseases and its complications.

Key words: *In vitro* antioxidant activity, Phenol, Flavonoid, Reducing power activity, Hydrogen peroxide-scavenging activity, *Abrus precatorius.*

INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons. and involved in many pathological conditions[1]. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants[2]. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods have side effect and are carcinogenic[3]. Plant polyphenolic compounds, such as flavonoids are described as scavengers of reactive oxygen species[4]. Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated[5]. Most sources of natural antioxidants originate from plant materials, but the content of polyphenolic compounds in the seeds and pericarp of tropical and sub-tropical flora have sparsely reported[6].

The plant *Abrus precatorius* belongs to family Fabaceae popularly known as Rati in Hindi, Crab's eye in English and Gunja in Sanskrit. It is native to India, from the Himalayas down to southern India and Sri Lanka, but now grows in all tropical regions throughout the world. The plant has been used in Hindu medicines from very early times, as well as in china and other ancient cultures. In ayurveda the plant is considered beneficial for the hair and the seeds extract is used in the treatment of ulcer and skin

affection. The major activity of the plant seeds till now reported are antitumour, immunomodulating, antiplatelate, anti-inflammatory, antiallergic, molluscicidal, insecticidal, antibacterial, antidiarrhoeal, antifertility activity in male and abortifacient activity in female. Thus, present study was undertaken to evaluate the *in vitro* antioxidant effect of ethanolic extract of *Abrus precatorius* seeds.

The main constituent present in the seeds are isoflavonoids, flavonoids, proteins, alkaloids, carbohydrates and triterpinoids[7].

MATERIAL AND METHODS

Phytochemical evaluations

Plant material

Seeds were collected from the Walni, Nagpur and authenticated by G. V. S. Moorthy, Botanical Survey of India (BSI), Southern circle, Coimbatore, Tamil nadu.

Extraction procedure

The fresh seeds were separated from matured fruits, shade dried, broken into small pieces and powered coarsely. About 600 gm of air dried powdered material was extracted with 99.9% of ethanol in a soxhlet extractor for 7 days. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50^{o} C) using rotary evaporator. The ethanolic extract yielded an brown sticky mass weighing 9.2g (10.3% w/w) and extractive value was found to be 3.326% w/w. the extract was used directly for total phenol and flavonoid content and also for assessment of antioxidant capacity through various chemical assays[8-9].

Phytochemical evaluation

The ethanolic extract of *Abrus precatorius* seeds (ASEt) was subjected to the following chemical tests for the identification of various active constituents.

S. No.	Phytochemical constituents	Ethanolic extract of *Abrus precatorius* seeds *(ASEt)*
1	Carbohydrates	+ve
2	Alkaloids	+ve
3	Steroids and sterols	+ve
4	Glycosides	-ve
5	Saponins	-ve
6	Flavanoids	+ve
7	Tannins and phenolic compound	+ve
8	Proteins and Amino acids	+ve
9	Fixed oil	+ve
10	Anthraquinone	-ve

Estimation of total phenolic content

The total phenolic content of ASEt was estimated according to the method of Makkar et.al (1997)[10]. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after overtexing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. Using gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml. using the standard curve, the total phenolic content was calculated and expressed as gallic acid equivalent in mg/g of extract.

Estimation of total flavonoid content

Flavones and flavonols in the ethanolic extracts of *Abrus precatorius* seeds were estimated as rutin equivalent. Rutin was used to make the calibration curve [10, 20, 30, 40, 50, 60, 70, 80, 90, 100 in 99.9% ethanol (v/v)]. The standard solutions or extracts (0.5 ml) were mixed with 1.5 ml 95% ethanol (v/v), 0.1 ml 10% aluminum chloride 42(w/v), 0.1 ml of 1 mol/l sodium acetate and 2.8 ml water. The volume of 10% aluminum chloride was substitued by the same volume of distilled water in blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm.

Evaluation of in vitro antioxidant activity

Hydroxyl radical-scavenging activity

Hydroxyl radical scavenging activity of extract was measured according to the method of Halliwell et al. (1987)[11]. One milliliter of the final reaction solution consisted of aliquots (500 µL) of various concentrations of the extract, 1 mM $FeCl_3$, 1 mM EDTA, 20 mM H_2O_2, 1 mM L-ascorbic acid, and 30 mM deoxyribose in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 h at 37 °C, and further heated in a boiling water bath for 15 min after addition of 1 mL of 2.8% (w/v) trichloroacetic acid and 1 mL of 1% (w/w) 2-thiobarbituric acid. The color development was measured of 532 nm against a blank containing phosphate buffer.

Reducing power activity

The reducing power of extract was determined by the method of Yen and Duh (1993)[12]. Different concentrations of extracts were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated for 20 min at 50°C. After incubation, 2.5 mL of 10% trichloroacetic acid were added to the mixtures, followed by centrifugation at 650×*g* for 10 min. The upper layer (5 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm.

Hydrogen peroxide-scavenging activity

The Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch et al., (1989)[13]. The extract was dissolved in 3.4 mL of 0.1M phosphate buffer (pH 7.4) and mixed with 600 µL of 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min. for each concentration, a separate blank sample was used for background substraction.

RESULTS AND DISCUSSION

Total phenol content

Total phenolic compound in ethanolic seeds extract of *Abrus precatorius* was found to be 95 mg/g of extract calculated as gallic acid equivalent. (r^2=0.9976).

Total flavonoid content

Total flavonoids compound in ethanolic seeds extract of *Abrus precatorius* was found to be 21 mg/g of extract calculated as rutin equivalent. (r^2=0.9985).

Hydroxyl radical scavenging activity

BHT and ASEt showed hydroxyl radical scavenging activity with about 36.68-59.36% and 39.72-64.79% at concentration of 500 µg/mL (Table 1). A concentration dependent inhibition against hydroxyl radical induced deoxyribose degradation was observed in the deoxyribose assay. Because the ASEt was high in its phenol and flavonoid content, its antioxidant compounds may well act as antioxidant and scavenge hydroxyl radical generated from the Fenton reagent.

Table 1 : Shows hydroxyl radical-scavenging activity of ethanolic seeds extract of *Abrus precatorius*

Sample	Concentration (µg/mL)		
	50	500	1000
BHT	36.68	59.36	58.10
ASEt	39.72	64.79	79.20

All the values are means of three independent determinations, n=3, analyzed in triplicate.

Hydrogen peroxide-scavenging activity

Scavenging activity of hydrogen peroxide in ASEt (10 µg) and BHT (10 µg) as reference compound was not remarkably different and shown to be 86 and 92 % at initial time respectively (Table 2). The composition of hydrogen peroxide into water may occur according to the antioxidant compounds as the antioxidant components present in the extract are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O.

Table 2 : Shows hydrogen peroxide-scavenging activity of ASEt (10 µg/mL)

Sample	Time (min)		
	0	10	20
BHT	97	82	76
ASEt	86	79	74

All the values are means of three independent determinations, n=3, analyzed in triplicate.

Reducing power activity

At concentration 500 µg/mL, BHT (Reference) and ASEt showed absorbance with about 0.36 and 0.43 respectively (Table 3). Thus ASEt exhibited reducing activity. The reducing power might be due to hydrogen donating ability.

Table 3 : Shows reducing power activity of ethanolic seeds extract of *Abrus precatorius*

Sample	Concentration (µg/mL)		
	50	500	1000
BHT	0.138	0.361	0.809
ASEt	0.279	0.438	1.019

All the values are means of three independent determinations, n=3, analyzed in triplicate.

CONCLUSION

Based on the results obtained ASEt showed antioxidant and free radical scavenging activity not remarkably different than reference compound butylated hydroxyl

toluene (BHT) and major antioxidative component seems to be phenolic and flavonoids. Therefore, it can be concluded that the ethanolic extract of *Abrus precatorius* seeds could be considered for prevention and treatment of human diseases and its complications as potent antioxidant.

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