RESEARCH ARTICLE

Phytochemical Analysis and Anti Oxidant Activity of White, Red and Black *Abrus precatorius* L. (*Gunja*): Comparative Studies

Goli Penchala Prasad[1], Goli Penchala Pratap[2], Shyam Baboo Prasad*[3], M Srinivasan[4], SD Pawar [5], AK Mangal[6], N Srikanth[7]

[1]*Assistant Director Incharge, Regional Ayurveda Institute for Fundamental Research, Pune.*
[2]*Research Assistant (Botany), Central Research Institute of Unani Medicine, Hyderabad.*
[3]*Research officer (Pharmacognosy), Regional Ayurveda Institute for Fundamental Research, Pune.*
[4]*Research officer (Biochemistry), Regional Ayurveda Institute for Fundamental Research, Pune.*
[5]*Research officer (Pharmacology), Regional Ayurveda Institute for Fundamental Research, Pune.*
[6]*Assistant Director(Pharmacognosy) Central Council for Research in Ayurvedic Sciences, New Delhi.*
[7]*Deputy Director General, Central Council for Research in Ayurvedic Sciences, New Delhi.*

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ABSTRACT

Objective

To find the chemical and biological (Antioxidant action) variability of three verities of Gunja (*Abrus precatorius* L.) leaf based on its fruit colour i.e. White, Red and Black.

Methods

The aqueous and alcoholic extracts of all varieties were screened for phytoconstituents using preliminary chemical test, Chromatographic fingerprinting using LC-MS, possible antioxidant activities was screened by free radical scavenging activity (DPPH), FRAP inhibition method.

Results

The results showed that the three varieties vary little bit in their phytoconstituents. All the extract possessed antioxidant in dose dependent manner.

Conclusions

This study suggests that aqueous extract of leaves of red veriety of *A. precatorius* extracts exhibit great potential for antioxidant activity and may be useful for their nutritional and medicinal functions.

Keywords: *Abrus precatorius* L, Antioxidant, DPPH, Gunja, Oxidative stress.

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INTRODUCTION

A precatorius is known as Gunja in traditional Ayurvedic medicine. It is categorised in three major categories like white, black and red based in its fruit colour (Figure 1).

Free radicals/ Reactive oxygen species (ROS)/ have been reported to cause several diseases including, inflammation, cancer, diabetes, obesity, neurodegenerative disorders, atherosclerosis and liver cirrhosis. Antioxidant defence mechanisms of body protect against oxidative damage.[1] Though, the natural antioxidant defence mechanisms can be insufficient and hence dietary intake of antioxidant components is important and recommended. Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal products to replace synthetic antioxidants, which are being restricted due to their adverse reaction. Plants therefore constitute the main source of natural antioxidant molecules which have the capacity to eliminate or neutralize the deleterious ROS. The importance of medicinal plants to prevent or control diseases has been attributed to the antioxidant properties of their constituents, commonly associated with a large number of molecules such as phenols and flavonoids. The secondary metabolites compounds from higher plants like phenolics and flavonoids have been reported to be a potent free radical scavenger. *A. precatorius* is

**Author for Correspondence: b.shyam2@gmail.com*

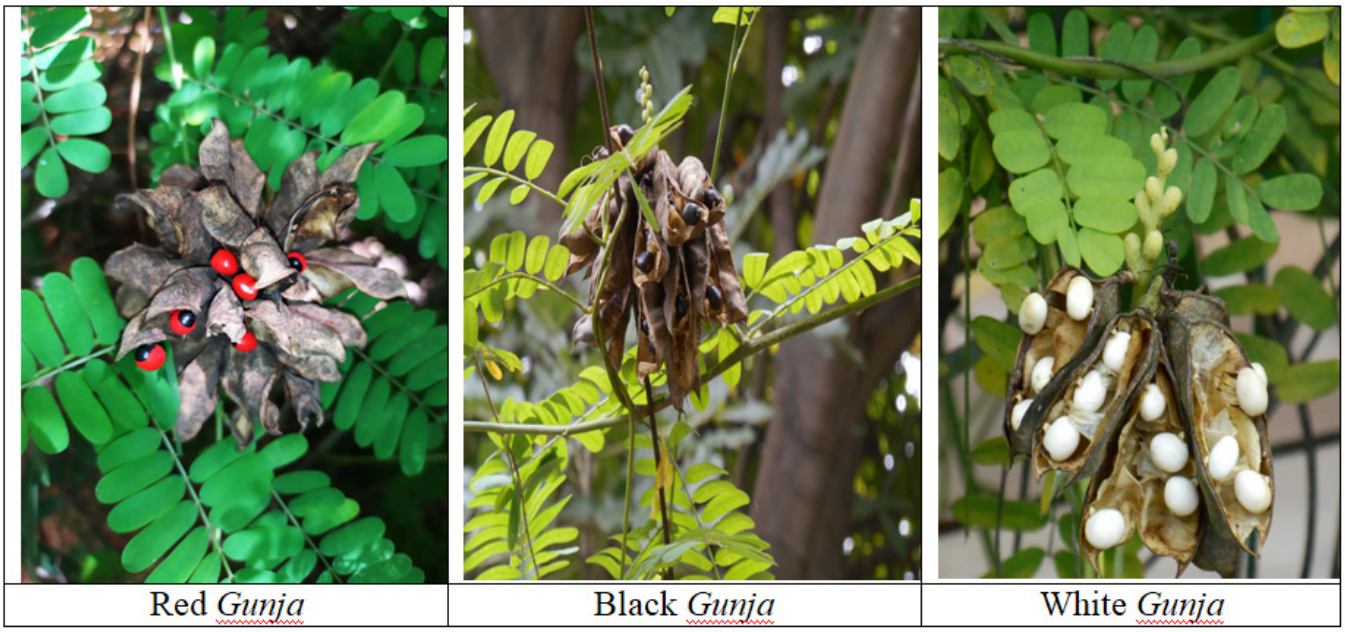


Figure 1

commonly known as Gunja or Jequirity and abundantly found throughout the plains of India. The roots, leaves and seeds of this plant are used for different medicinal purpose.[2]

In the Ayurvedic medicine leaves of *A. precatorius* are laxative, expectorant and aphrodisiac medicines and are used in urticaria, eczema, stomatitis, conjunctivitis, alopecia areata, migraine, lymphomas/leukemia and dysmenorrhoea Leaves principally contains phenolics, flavonoids, triterpene glycosides, abrin and alkaloids.[3] Hence, the current study was designed to evaluate the antioxidant activity of aqueous and alcoholic extracts of leaves of *A precatorius* using DPPH scavenging assay, FRAP inhibition assay, determination of total phenolics and flavonoids content for three verities of Gunja (*Swet, Rakta* and *Krishna).*

MATERIALS AND METHODS

Plant material

Leaves of *Swet,* and *Krishna Gunja* were collected from Talakona forest Chinturu and east Godavari Andhra Pradesh. Leaves of *Rakta Gunja* were collected from Sri Venkateswara University, Tirupati.

Identification and authentication of plant material

Plant material was identified and authenticated with the help of the Flora.[4] Plant material was also compared with the herbarium specimen available in Sri Venkateswara University, Tirupati. The herbarium accession number of *Swet, Krishna* and *rakta Gunja* are SVUBS1215, SVUBS 1672 and SVUBS 738 respectively.

Powder preparation

Shade dried leaves was made in to powder using grinding mill; passed through #60 sieve and kept in airtight container for further studies.[5]

Preparation of extract

Transferred 5 g of the air dried coarsely powdered drug in 250 ml conical flask 100 ml of solvent was added. Mixture was Shaken frequently for first 6 hours and allowed to stand for next 18 hours. Filtered rapidly, taking precautions against loss of solvent. Evaporated filtrate to dryness in a tared flat bottomed shallow dish at 50° C.[6]

Phytochemical screening

Phytochemical screenings of extracts were performed as per standard method.[7-10]

Chromatographic fingerprinting using LC-MS

Chromatographic fingerprinting was carried out using LC-MS. The column used was $C_{18.}$

The acetonitrile and water was used as mobile phase in gradient mode. MS was used as detector. The sample was allowed to run for 44 min (Run time). The peak with Rt were compared for variation in phytoconstituents.[11]

Antioxidant activity

DPPH radical scavenging assay:

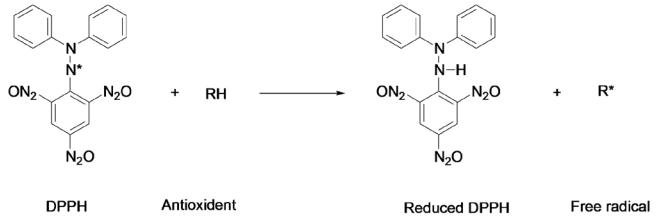
The free radical scavenging activity of plant extracts was measured by 1; 1-diphenyl-2-picrylhydrazil (DPPH) by using the method of Blois (1958) and Gomez-Alonso *et al*., (2003). The hydrogen donating ability of extracts was examined in the presence of DPPH stable radical. 150 µl of 0.3 mM DPPH methanol solution was added to 50 µl of sample/standard solution of different concentration and allowed to react at room temperature in dark. After 30 minutes, the absorbance was measured at 517 nm. Methanol was used as a blank. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Butylated

Hydroxy Anisole (BHA) was used as the reference material.[12-13]

Inhibitory percentage was calculated using the following formula-

$$\%\text{inhibitory effect} = \frac{\text{absorbance}_{(\text{control})} - \text{absorbance}_{(\text{sample})}}{\text{Absorbance}_{(\text{control})}} \times 100$$

Reaction of DPPH with antioxidant compound



Determination of reducing power

Ferric reducing antioxidant power (FRAP) assay:

The reducing power of plant extracts and standard was determined according to the method of Oyaizu (1986) with modifications. In 96-well plate, about 30 µl of plant extract or positive control was mixed with phosphate buffer (30 µl, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (30 µl, 1%). The mixture was incubated at 50°C for 20 min. A portion (30 µl) of trichloroacetic acid (10% in PBS) was added to the mixture, then 120 µl distilled water and Ferric chloride ($FeCl_3$) (25 µl, 0.1%) was added. The reaction was mixed well and allowed for incubation at room temperature for 30 min. The absorbance was measured at 700nm using Micro plate reader. Butylated Hydroxy Toluene (BHT) was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the average of three observations.[14]

RESULTS AND DISCUSSION

Phytochemical screening of ethenolic extract (E.E.) and Aqueous extract (W.E.) indicates the presence / absence of phytoconstituents as shown in Table 1. On the basis of

Table 1: Phytochemical screening

Qualitative tests of leaves of white, black and red *A precatorius* L (leaves of white, black and red Gunja).

Sr. No.	*Phyto-constituent tested*	*Tests Performed*	*Observations*					
			White		*Black*		*Red*	
			E..E.	*W.E.*	*E.E.*	*W.E.*	*E.E.*	*W.E.*
1	Carbohydrates	Molish's Test	-ve	+ve	-ve	+ve	-ve	+ve
2	Reducing sugar	a) Fehling's Test	+ve	+ve	+ve	+ve	+ve	+ve
		b) Benedict's Test	-ve	+ve	+ve	+ve	+ve	+ve
3	Pentose sugar	Phloroglucinol Reag. Test	+ve	+ve	+ve	+ve	+ve	+ve
4	Hexose sugar	a)Tollen's Phloroglucinol Test	+ve	+ve	+ve	+ve	+ve	+ve
		b)Cobalt Chloride Test	+ve	+ve	+ve	+ve	+ve	+ve
5	Protein	a) Biuret Test	-ve	-ve	-ve	-ve	-ve	-ve
6	Amino acid	a)Ninhydrin Test	-ve	-ve	-ve	+ve	-ve	+ve
		b)Test for cysteine	-ve	+ve	-ve	-ve	-ve	-ve
7	Steroids	a)Libermann-Burchard Test	+ve	+ve	+ve	-ve	+ve	-ve
		b)Salkowaski reaction	+ve	+ve	+ve	-ve	+ve	+ve
8	Glycoside	General test	+ve	+ve	+ve	-ve	+ve	+ve
9	Cardiac Glycosides	a) Legal Test	+ve	+ve	-ve	+ve	-ve	+ve
		b) Keller-Killiani Test	+ve	-ve	+ve	-ve	+ve	-ve
10	Anthroquinone Glycoside	a)Borntrager's Test	-ve	-ve	-ve	-ve	-ve	-ve
		b) Modified Borntrager's Test	-ve	-ve	-ve	-ve	-ve	-ve
11	Saponins	a)Foam Test	-ve	+ve	-ve	+ve	-ve	+ve
		b) Lead Acetate Solution Test	-ve	+ve	-ve	+ve	-ve	+ve
12	Coumarin Glycoside	a)Aromatic odour Test	-ve	-ve	-ve	-ve	-ve	-ve
		b) Fluorescence Test	+ve	-ve	+ve	-ve	+ve	-ve
13	Flavonoids	a) Shinoda test	-ve	-ve	-ve	-ve	-ve	-ve
		b) Lead acetate test	-ve	+ve	-ve	+ve	-ve	+ve
14	Alkaloids	a) Dragendorff's test	-ve	+ve	-ve	+ve	-ve	+ve
		b) Mayer's test	-ve	+ve	-ve	+ve	-ve	+ve
		c) Wagner's test	+ve	+ve	+ve	+ve	-ve	+ve
15	Tannins	a) Lead acetate solution Test	-ve	-ve	-ve	-ve	-ve	-ve
		b) Gelatine sol. Test	-ve	-ve	-ve	-ve	-ve	-ve
16	Phenol	a) Neutral $Fecl_3$ Test	+ve	-ve	+ve	-ve	+ve	-ve
17	Starch	Iodine test	-ve	-ve	-ve	-ve	-ve	-ve

phytochemical screening it is clear that three varieties of gunja differ in phytoconstituents. The finding of Phytochemical screening of ethenolic extract (E.E.) and Aqueous extract (W.E.) of three verities of Gunja is shown in Table 1.

LC-MS Chromatographic fingerprinting:

It indicates that the three varieties of Gunja are varying in their some chemical constituent as the peak Rt is different as shown in Figure 1-4.

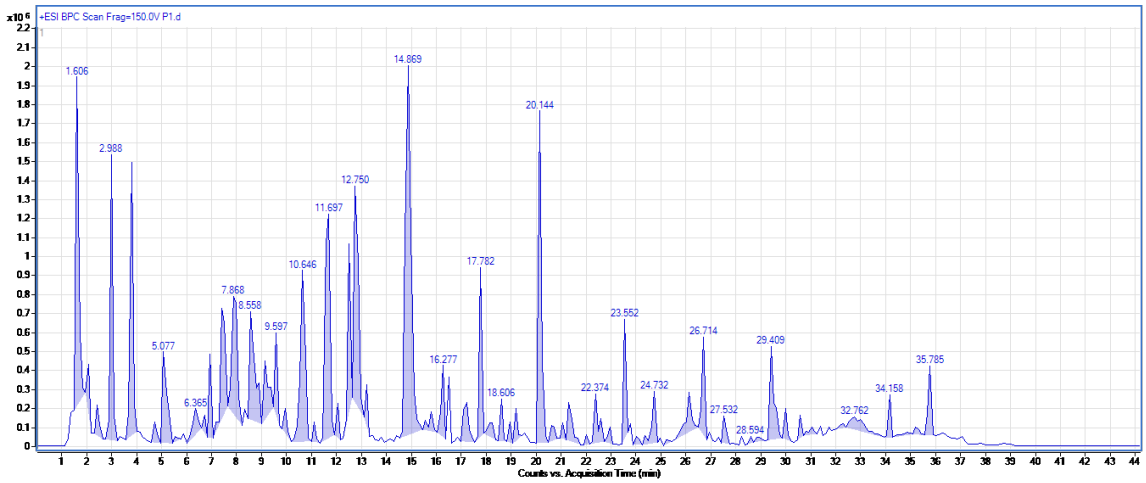


Figure 1: LC-MS chromatogram of Krishna gunja

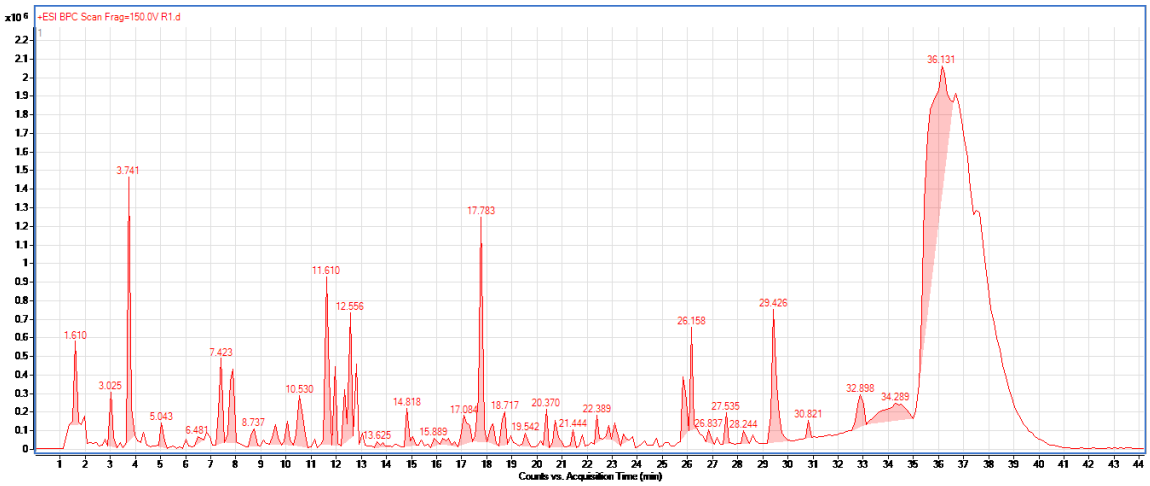


Figure 2: LC-MS chromatogram of rakta gunja

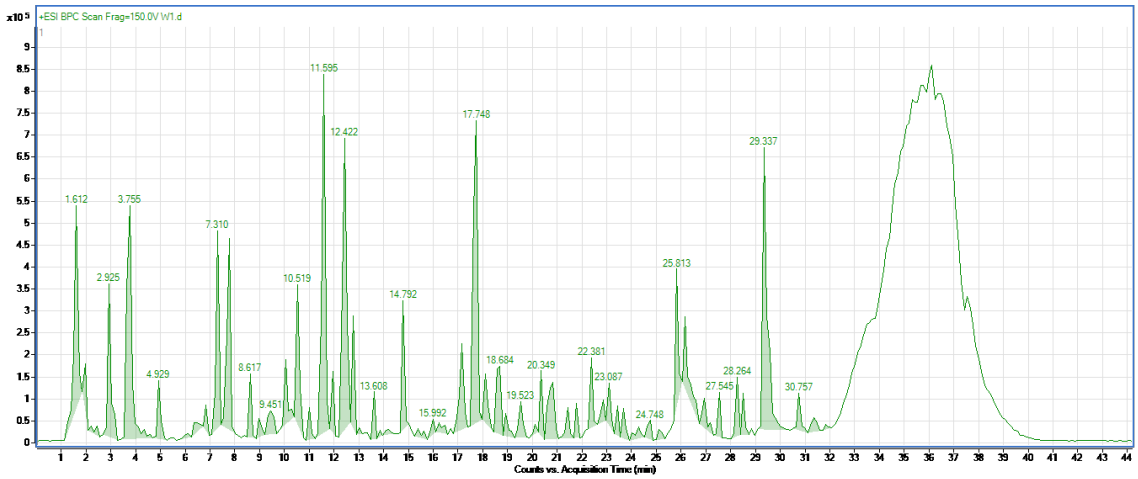


Figure 3: LC-MS chromatogram of swet gunja

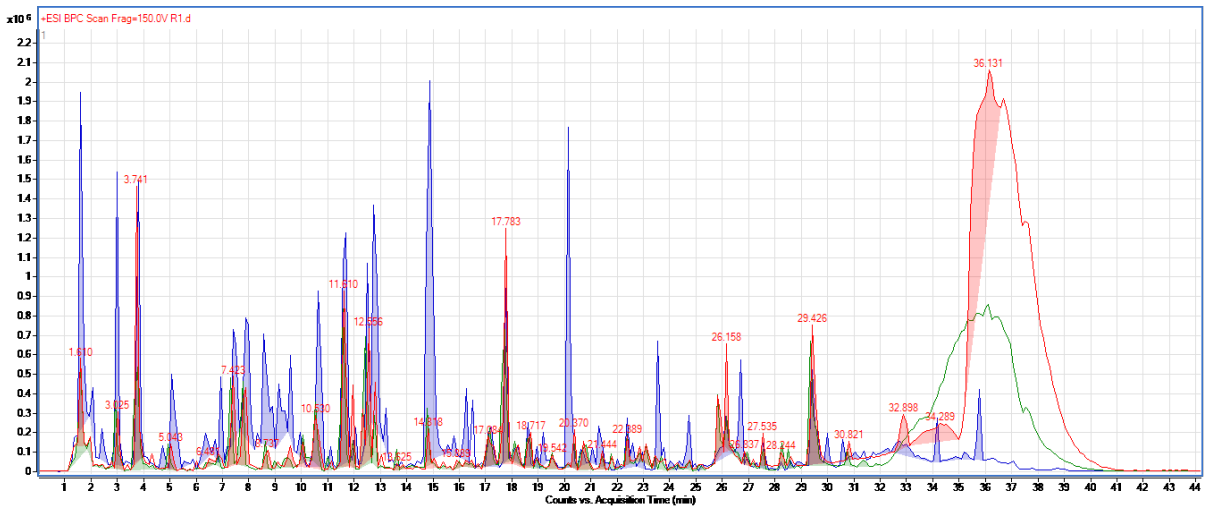


Figure 4: LC-MS chromatogram Overlap graph of krishana, swet and rakta gunja

Antioxidant activity

DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry stable antioxidant drug can be able to reduce the free radical generated by this assay, so it can be useful to assess various products at a time. In our study we found that all the six extracts were screened for DPPH radical scavenging activity with percentage inhibition ranges from 13.14 to 72.43 % in which the highest activity was detected in aqueous extract of red *A precatorius* shown highest activity of inhibition when compared with standard antioxidant ascorbic acid. Other extracts of white and black *A precatorius* shown radical scavenging activity. The aqueous extracts of white, black and red *A precatorius* shown highest activity of DPPH radical inhibition when compared with ethanolic extract. Percentage inhibition of extracts on DPPH radical scavenging assay of ethanolic and aqueous extracts of *A precatorius* is shown in table 2.

The total antioxidant activity can be measured by the ferric reducing antioxidant power assay (FRAP). The flavonoids and phenolic acids are present in the medicinal plant extracts exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper. This method is based on the principle of increase in the absorbance of the reaction mixtures, the absorbance increases the antioxidant activity increases. In our study we reported that all the six extracts were screened for FRAP radical scavenging activity which showed the reducing power (as indicated by absorbance at 700 nm) of ethanolic and aqueous extracts which increased with increasing concentration to certain extent did not produce any significant increasing reducing power. A strong reducing power was noted for aqueous extract of red *A precatorius* values are comparable with standard antioxidant butylated hydroxytoluene (BHT). The aqueous extracts of white, black and red *A precatorius* shown highest activity of reducing activity when compared with ethanolic extract. Ferric reducing antioxidant power (FRAP) radical scavenging assay of ethanolic and aqueous extracts of *A precatorius* is shown in table 3.

CONCLUSION

Antioxidant activity of aqueous extract of rakta gunja was found to be more effective as compared to other extracts. Though gunja seed is considered as toxic but such report is not reported for leaf. Although leaves of gunja having potent antioxidant action its uses as nutrient as well as therapeutic moiety are recommended after its toxicological evaluation.

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Table 2: Percentage inhibition of extracts on DPPH radical scavenging assay of ethanolic and aqueous extracts of *A precatorius*

	White A precatorius		*Black A precatorius*		*Red A precatorius*		
Concentration (µg/mL)	*% inhibition of ethanolic extract*	*% inhibition of aqueous extract*	*% inhibition of ethanolic extract*	*% inhibition of aqueous extract*	*% inhibition of ethanolic extract*	*% inhibition of aqueous extract*	*Std. Ascorbic acid % inhibition*
25 µg/mL	13.14 ± 1.00	25.47 ± 1.94	11.69 ± 0.89	23.38 ± 1.78	21.46 ± 1.63	49.22 ± 3.75	54.67 ± 4.16
50 µg/mL	18.15 ± 1.38	45.90 ± 3.49	14.36 ± 1.09	24.43 ± 1.86	23.73 ± 1.81	59.17 ± 4.51	62.84 ± 4.79
100 µg/mL	27.22 ± 2.07	61.09 ± 4.66	15.70 ± 1.20	35.95 ± 2.74	34.38 ± 2.62	64.12 ± 4.88	72.00 ± 5.48
200 µg/mL	33.34 ± 2.54	62.66 ± 4.77	25.47 ± 1.94	61.26 ± 4.66	43.45 ± 3.31	68.24 ± 5.20	84.34 ± 6.42
400 µg/mL	37.00 ± 2.02	67.72 ± 5.16	27.84 ± 2.12	69.81 ± 5.32	54.45 ± 4.15	72.43 ± 5.52	90.50 ± 6.89

Data are given as Mean ± SEM (n = 3)

Table 3: Ferric reducing antioxidant power (FRAP) radical scavenging assay of ethanolic and aqueous extracts of *A precatorius*

	White A precatorius		*Black Aprecatorius*		*Red A precatorius*		
Concentration (µg/mL)	*% O.D. value at 700 nm of ethanolic extract*	*% O.D. value at 700 nm of aqueous extract*	*% O.D. value at 700 nm of ethanolic extract*	*% O.D. value at 700 nm of aqueous extract*	*% O.D. value at 700 nm of ethanolic extract*	*% O.D. value at 700 nm of aqueous extract*	*STD. BHT (µg/mL) % O.D. value at 700 nm*
25 µg/mL	0.11 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.21 ± 0.02	0.12 ± 0.01	0.23 ± 0.02	0.15 ± 0.01
50 µg/mL	0.20 ± 0.02	0.25 ± 0.02	0.19 ± 0.01	0.29 ± 0.02	0.28 ± 0.02	0.30 ± 0.02	0.22 ± 0.02
100 µg/mL	0.23 ± 0.02	0.29 ± 0.02	0.22 ± 0.02	0.34 ± 0.03	0.31 ± 0.02	0.34 ± 0.03	0.39 ± 0.03
200 µg/mL	0.27 ± 0.02	0.35 ± 0.03	0.30 ± 0.02	0.42 ± 0.03	0.42 ± 0.03	0.41 ± 0.03	0.47 ± 0.04
400 µg/mL	0.36 ± 0.03	0.46 ± 0.04	0.35 ± 0.03	0.57 ± 0.04	0.48 ± 0.04	0.58 ± 0.04	0.62 ± 0.05

Data are given as Mean ± SEM (n = 3)

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